Abstracts accepted for publication only

Pathogenesis

R2105 Gram-positive and Gram-negative bacteria induce neutrophil oxygen burst augmented by platelets; role of platelet-derived TXA2 in cellular host response

J. Miedzobrodzki, T. Panz, R. Jaworek, S. Chlopicki (Krakow, PL)

Objectives: The non-self particles and bacteria invading the bloodstream (which circulate in the blood) trigger the inflammatory response e.g. oxygen burst in neutrophils. This study was aimed to test the ability of neutrophils to respond to variety of bacteria species and to latex particles in absence and in presence of platelets. The second goal was to investigate the role of platelet–derived TXA2 in the regulation of neutrophil-response to latex. The following bacteria species were used as stimulus generators: Gram+ bacteria such as *Staphylococcus aureus* and *Bacillus subtilis*, Gram– bacteria as *Escherichia coli* and *Klebsiella pneumoniae*.
Methods: The oxygen burst in neutrophils * platelets was investigated with luminol chemiluminescence (CL). The role of TXA2 was studied with the use of furegrelate, the TXA2 synthase inhibitor. All CL experiments were performed with MicroLumat LB 96P Berthold apparatus and WINGLOW programme.
Results: The Gram+ bacteria activate neutrophils. Neutrophils interacting with platelets increase the intensity of oxygen burst when compared with control neutrophils without platelets. There was no difference in neutrophile stimulation between killed and live bacteria. In presence of TXA2 inhibitor furegrelate, (bacteria induced and) latex-induced oxygen burst of neutrophils, was completely blocked. The activation of neutrophils with opsonised bacteria induces stronger oxygen burst than stimulation with non-opsonised bacteria. The neutrophile activating factor in Gram+ bacteria is connected with bacteria cell walls, and not with their extracellular metabolites. In Gram– bacteria LPS inhibited neutrophile response, the same effect was with polysaccharide capsule of *Klebsiella pneumoniae.*
Conclusion: The stronger CL recorded in experiments with neutrophils in the presence of platelets indicates the cooperation between these cells during inflammation. TXA2 was the key mediator in platelet-dependent amplification of reactive oxygen species production by neutrophils.

R2106 Profiling the acute inflammatory response to heat-killed menigococci and pneumococci in cerebrospinal fluid

M. Wittwer, C. Bellac, S. Leib (Berne, CH)

Objectives: The two most common aetiological agents of bacterial meningitis (BM) *Streptococcus pneumoniae* and *Neisseria meningitidis* differ considerably with respect to mortality (approx. 30% vs. 10%) and the incidence of neurological sequelae (approx. 40% vs. 10%). Here we assessed the cytokine response to the two pathogens in the cerebrospinal fluid (CSF) after intracisternal challenge with heat killed bacteria in infant rats with the aim to investigate the mechanisms for the observed differences in clinical outcome.
Methods: Eleven days old Wistar rats were randomised for intracisternal challenge with either 10 microL of saline containing heat killed *S. pneumoniae* (serogroup 3; 5.00E+10 cfu/mL; n = 15) or heat killed *N. meningitidis* (5.00E+10 cfu/mL; n = 15). Microsphere-based multiplex assays (Lincoplex, Linco Research Inc., St Charles, MA, USA) was used to assess the concentrations of IFN-gamma, TNF-alpha, IL-1alpha, IL-1beta, IL-2, IL-4, IL-6, IL-10 and GM-CSF in CSF samples at 0 h, 3 h, 6 h, 9 h and 12 h after infection (n = 3 for each experimental group and time).
Results: The inflammatory reaction to the two pathogens showed a similar expression pattern over time for the assessed CSF parameters. The presence of heat killed bacteria led to a pronounced increase (~1000 fold) in the CSF concentration of the pro-inflammatory cytokines IL-1beta, TNF-alpha and IL-6 over 12 h with peak levels at 3 h to 6 h after challenge. Parallel to the pro-inflammatory cytokines the concentration of the anti-inflammatory cytokine IL-10 was increased (~10 fold) starting at 3 h after challenge.
Conclusion: Based on the assessed CSF parameters and time period, we found no marked difference in the host reaction to the two heat killed bacteria in the cerebrospinal fluid. Thus, other mechanisms likely including bacterial virulence factors may contribute to the observed differences in clinical outcome of BM due to the two pathogens.

R2107 The effect of endocannabinoids on apoptosis in "experimental sepsis model" on mice

D. Kilic, T.R. Aydos, Z. Keskil, O. Bozdogan, F. Andic, O. Korkut, M. Edremitlioglu (Kirikkale, TR)

Purpose: To investigate the apoptotic changes in renal tissues of septic mice and the effects of cannabinoid receptor antagonist (AM 630) and agonists (anadamid and WIN 55,212-2) on these changes.
Materials and Method: In this study, 56 healthy Swiss albino mice weighing 25–35 g. The mice were randomly divided into 8 groups: control, sham, sepsis, sepsis treated with anandamide or WIN 55,212-2 or AM 630 and sepsis treated with ethanol or DMSO. Sepsis was induced by cecal ligation and puncture (CLP) under ketamine/xylazine anaesthesia. After 48 hours, the mice were treated intraperitoneally with anandamide (5 mg/kg) or its solvent ethanol, WIN 55,212-2 (5 mg/kg), AM 630 (1 mg/kg) or DMSO which is used as the solvent of both. Ten minutes later, the abdomen was incised and periton culture specimen was taken to confirm the existence of sepsis. The kidneys were harvested and fixed in 10% neutral formolin solution. The apoptotic changes in glomerulus, proximal and distal tubulus, and collecting ducts were determined using TUNEL method.
Findings: Neither one of sepsis, anandamide, WIN 55,212-2, AM 630, ethanol or DMSO caused any apoptotic effects on glomerulus. The induced sepsis caused an increase of apoptosis on proximal and distal tubulus. In these parts of kidney, while anandamide, WIN 55,212-2, ethanol or DMSO did not make any changes, AM 630 caused a decline in apoptosis. In the collecting ducts; groups of sepsis, anandamide, WIN 55,212-2 and AM 630 did not show any differences than the sham group.
Results: In consistency with other studies indicating the inducement of apoptosis by endotoxin in some tissues; sepsis, induced by CLP in mice renal proximal and distal tubulus, showed apoptotic effects in this study. While anadamide and WIN 55,212-2 did not cause any changes in this effect, CB2 cannabinoid receptor antagonist AM 630 caused a decline in apoptosis. In renal glomerulus, apoptotic changes did not increase.

R2108 The prevalence of *Clostridium difficile* toxin as a cause of acute or chronic diarrhoea in a high HIV prevalence population in sub-Saharan Africa

M.B.J. Beadsworth, A.M. Perez, L. van Lieshout, A. Watson, C.A. Hart, E. Zijlstra, N.J. Beeching (Liverpool, UK; Blantyre, MW; Leiden, NL)

Background: There have been relatively few studies on the prevalence of *Clostridium difficile* (CD) in developing countries, and even less on the importance of CD in diarrhoea related to HIV in the tropics.

Objective: To study the prevalence of CD toxin (CDT) in adults hospitalised in Malawi with bowel disturbance related to HIV, compared to controls.
Methods: All consenting adult patients with diarrhoea and controls were recruited on every Tuesday and Wednesday for one year. Alongside demographic data, HIV testing (and CD4 if HIV-1 positive) were carried out. *Clostridium* Difficile toxin A/B were tested using Techlab® Elisa. HIV-1, Unigold® and Determine® Spot tests. CD4 Becton Dickinson FACScount®. Confirmation of toxins using Techlab® Elisa and spectrometry is on-going.
Results: Over one year 398 were recruited, 84.4% of those were HIV positive (n = 305). Approximately, 12% of those invited declined to enroll over the year.
Acute diarrhoea was seen in 48.9%, chronic diarrhoea 32.5% and 19% were controls. 96.4% of those with chronic diarrhoea were HIV-1 positive ($p < 0.0001$). Of the 137 so far tested for CDT in Malawi and the UK, 23.4% (n = 32) are positive and 76.6% negative (n = 105). Of the 32 positive samples 34.4% are male and 62.5% are female. 80.6% had diarrhoea, 19.4% were control patients. Of those with diarrhoea 72% had non-bloody and 28% bloody.
Recent antibiotic use was described in 46.9%, denied in 46.9% and in 6.3% unknown. Complete analysis will be presented.
Conclusions: Thus far:
1. A quarter of recently hospitalised adults in Malawi have detectable CD Toxin in faeces.
2. Over 80% of sequentially admitted adults are HIV positive, rising to >95% of patients with chronic diarrhoea.
3. Preliminary analysis does not support a strong link between the presence of CD toxin and diarrhoea or dysentery in this setting.

R2109 The role of *Chlamydophila pneumoniae* and human herpes virus 6 in aetiopathogenesis of multiple sclerosis and the relation of multiple sclerosis with apolipoprotein E genotypes

B. Kocazeybek, B. Karatoka, M. Aslan, S. Saribas, A. Altintas, S. Ergin, V. Koksal, A. Dirican, S. Saib (Istanbul, TR)

MS (multiple sclerosis) is an disease of central nervous system (CNS) which act on teenagers and adults. Some studies showed that several infectious agents can play a role as the aetiological factor in MS progression. ln the last three decades, investigators suggested that microorganisms can be related with MS progression. The purpose of this study was to investigate the relationship between MS and *C. pneumoniae* with HHV-6, and also to determine the Apo E genotype distrubution in MS patients and its relationship with *C. pneumoniae* and HHV-6.
39 MS patients, 42 healthy controls and 10 patients with other neurologic diseases (OND) were included in this study. We detected *C. pneumoniae* IgG, IgM and IgA with HHV-6 IgG and IgM in sera taken from MS patients and healthy controls with microimmunfluorescence method and we detected *C. pneumoniae* and HHV-6 DNA in cerebrospinal fluid (SCF) specimens taken from MS patients and OND patients with Polymerase Chain Reaction (PCR). Apo E genotyping was applied on whole blood samples (taken in EDTA tubes) of 36 MS patients and 30 healthy controls.
35 MS patients (89.7%), 32 healthy controls (76.2%) and 10 OND patients (100%) were previously infected with *C. pneumoniae*. According to these results, there was no difference between these groups when MS patients were compared, with control group. Furthermore, *C. pneumoniae* or HHV-6 DNA was not detected in any of MS nor OND patients' CSF (cerebro spinal fluid) samples. In 26, 8 and 2 of MS patients, e3e3 (72.2%), e2e3 (22.2%) and e3e4 (%5.6) were detected, respectively and Apo E genotype distrubution was found similiar with the normal population when these results were compared with control group. Furthermore, no relationship between Apo E e4 allele and MS clinical findings were observed.
As a result of this study, we suggest that there is no triggering or distrubing effect of *C. pneumoniae* or HHV-6 on MS which was thought to be as multifactoriel ethiologic agents of MS progression according to our findings. We suggest that Apo E genotype distrubutions in MS patients is similar with normal population and there is no relation between apo E alleles with disease duration and disability progression in MS patients. We think that it is early to say something definite and further studies were needed for relationship between Apo E genotype with *C. peumoniae* and HHV-6.

R2110 The effect of variceal bleeding on T-cell subsets in patients with cirrhosis

M. Lagadinou, E. Kaissi, M. Karakantza, H. Bassaris, C. Gogos (Patras, GR)

Aim: Variceal bleeding may lead to infectious complications in patients with liver failure. The aim of the present study was to investigate the effect of variceal bleeding on T-cell supsets of patients with cirrhosis.
Patients and Methods: The study included 21 patients with biopsy proven liver cirrhosis, that were admitted to the hospital. Six of them were admitted to the Hospital due to variceal bleeding (group 2) while the rest 15 were free of bleeding (group 1) and were admitted due to another cause. T-cell subsets (Total T-cells [CD3], helper T-cells [CD4], suppressor T-cells [CD8] and Natural Killer cells [CD56]) were measured upon admission and day 3, by using flow cytometry.
Results: The absolute number of T-cell subsets in group 1 vs group 2 were the following (mean ± SEM): Admission = CD3: 565±88 vs 507±197, CD56: 160±37 vs 95±14 ($P < 0.05$), CD4: 379±60 vs 311±143, CD8: 192±35 vs 172±56, Day 3 = CD3: 565±88 vs 259±62 ($P < 0.05$), CD56: 160±37 vs 60±13, CD4: 379±60 vs 178±36 ($P < 0.05$), CD8: 192±35 vs 111±62.
Conclusions: It seems that variceal bleeding may cause immunosuppression through a significant decrease of Natural Killer cells, as well as a decrease in total and helper T-cells. This may compromise immune response and may be associated with the observed increased in the frequency of infectious complications in cirrhotic patients, complicated with variceal bleeding.

Biofilm

R2111 Influence of organic and inorganic acids on biofilm formation by *Salmonella* spp.

I. Cirkovic, M. Svabic Vlahovic, S. Stepanovic (Belgrade, RS)

Bacterial biofilms in food processing environments is of special importance as it has the potential to act as the chronic source of microbial contamination that may lead to food spoilage or transmission of diseases. Biofilms by pathogenic bacteria such as *Salmonella* spp. have been reported. Numerous studies have shown that these bacteria are capable of forming biofilm under different environmental conditions. The objective of the present study was to investigate the influence of organic and inorganic acids on biofilm formation by *Salmonella* spp.
The quantification of biofilm formation by 30 *Salmonella* spp. strains was performed by the modified microtiter-plate test. After overnight incubation in Trypcase-soy broth, which pH was adjusted by HCl and acetic acid to 7; 6; 5 or 4, poured in 96-well flat-bottomed plastic microplates, bacterial biofilms were fixed with methanol and stained with crystal violet. The bound dye was released with 33% glacial acetic acid, and optical density was measured at 570 nm by using an automated microtiter-plate reader. Upon the optical densities of bacterial biofilms, all strains were classified into the following categories: no biofilm producers, weak, moderate or strong biofilm producers. Differences in the quantity of produced biofilm were examined by the Friedman test, followed by the Wilcoxon signed ranks test. P values of <0.05 were considered significant.
The number of biofilm producing strains were highest in broth at pH 7, adjusted by HCl or acetic acid (26, 86.7%). In the media with HCl 14 (46.7%) strains produced biofilm at pH 6, 7 (23.3%) strains at pH 5 and none at pH 4. In the media with acetic acid only 7 (23.3%) strains produced biofilm at pH 6, 1 (3.3%) strain at pH 5 and none at pH 4. The quantity of biofilm produced by tested strains was significantly

larger ($p < 0.05$) in medium at pH 7 than in other media. Organic acid significantly reduced ($p < 0.05$) biofilm formation compare to inorganic acid.
The obtained results showed that acid pH reduces the ability of *Salmonella* spp. to produce biofilm. The stronger influence of organic compare to inorganic acid on biofilm formation could have a great implication in food industry.

Antimicrobial pharmacokinetics, pharmacodynamics, general pharmacology

R2112 Pharmacokinetic study of ertapenem in the biliary tract

A. Skiada, D. Chardaloubas, D. Dimitroulis, A. Smyrnis, J. Anyfantis, M. Grammatikou, E. Petrikkou, A. Papachristodoulou, G. Petrikkos (Athens, GR)

Objectives: Ertapenem has been licensed for the treatment of community acquired pneumonia, acute gynaecological infections and intra-abdominal infections, including biliary tract infections. However, pharmacokinetic data on biliary tract tissues are limited. The purpose of this study was to measure the levels of ertapenem in the bile, the gallbladder wall and the surrounding tissues of the biliary tract as well as the kinetics of the drug.
Patients and Methods: Adult patients undergoing elective cholecystectomy were included in the study, after giving informed consent. Patients who had renal, hepatic or cardiac failure, as well as patients with an active infection, were excluded. Two doses of ertapenem of 1gr were administered via intravenous infusion of 30 minutes duration to all patients, divided by a 24 h interval. Specimen of the bile, the wall of the gall-bladder as well as of other tissues of the biliary tract were taken intraoperatively. The specimen were obtained at various time intervals (0, 2, 4, 8, 12, 18 post administration of the second dose of the drug). Serum levels of the drug were measured simultaneously. Agar well microbiology diffusion method was used for determination of drug level.
Results: Twenty-six patients were initially enrolled in the study, but eight were dropped out. Eighteen patients, 11 female and 7 male, with an average age of 54 years (range 28 to 65) were evaluated. After administration of ertapenem no serious adverse experiences were observed. Serum samples were taken from 16 patients, bile samples from 18 and gallbladder tissue from 7. The concentrations of ertapenem in the gallbladder tissues were noted to be lower as the time interval between administration of the second dose of ertapenem and tissue sampling increased. However, no such trend emerged regarding the concentrations in bile and serum. The measured ertapenem concentrations in the bile and the bile-to-plasma concentration ratio showed a broad variation. The concentrations of ertapenem in serum ranged from 0.07 to 181 mg/L, with no clear correlation with the times of infusion of ertapenem. The same was true for concentrations in the bile, which ranged from 0.31 to 389 mg/L.
Conclusions: Concentrations of ertapenem in bile and serum vary considerably from patient to patient. Since the sample was relatively small, it would be interesting to extend it to include more patients in order to better analyse the results.

R2113 Do high doses of quinolones decrease the emergence of antibacterial resistance? A systematic review of data from comparative clinical trials

M. Falagas, I. Bliziotis, P. Rafailidis (Athens, GR)

Background: The use of high doses of antimicrobial agents is considered a therapeutic approach that may reduce the development of antimicrobial resistance.
Methods: We performed a systematic review of the available data from comparative clinical studies reporting on the emergence of resistance when using different daily doses of quinolones.
Results: Relevant studies were identified from PubMed and the Cochrane Central Register of Controlled Trials (until June 2006). Twelve studies reported comparative data regarding the emergence of antimicrobial resistance. Development of resistance occurred in patients of 5/12 studies included in the review, with no statistical difference between the compared arms.
Conclusions: Although data from laboratory studies are indicative of a benefit from using high daily doses of quinolones in order to minimise the emergence of antimicrobial resistance, the limited data from the reviewed trials do not support this. Further comparative clinical studies focusing on this issue are justified.

R2114 Adverse events and β-lactam trough plasma concentrations during the treatment of endocarditis

P. Tattevin, O. Tribut, E. Polard, F. Gouaillier, M. Mercier, C. Kervegant, J. Chapplain, C. Arvieux, C. Michelet (Rennes, FR)

Objectives: High doses of Beta-Lactam (BL) are recommended for the treatment of infective endocarditis (IE). Guidelines have not significantly changed since 1960's although IE is now frequently diagnosed in elderly patients with comorbidities, which may affect BL pharmacokinetics. We investigated the range of plasma BL concentrations achieved during the treatment of IE and their association to potential adverse events (AE).
Methods: Prospective study of 30 consecutive patients with definite IE (Duke criteria) treated with BL according to guidelines. Trough BL plasma concentrations were determined by high performance liquid chromatography at least twice during treatment. Potential AE were defined as events occurring after the initiation of BL and were retrospectively extracted from medical chart and nurses reports by 2 investigators unaware of their BL concentrations.
Results: There were 21 men and 9 women. Mean age was 65.5 years (SD 17.9). In 19 patients (63.3%), at least one AE was suspected including sleep disorders (n = 11), confusion (n = 8), diarrhoea (n = 8), vomiting (n = 6) and mood disorders (n = 7). Mean ± SD amoxicillin concentrations were 33.6±22.7 mg/L (31 measures) in patients without AE and 92.7±80.1 (38 measures) in patients with AE ($P < 0.001$).

Through concentration	Amoxicillin	Cloxacillin	Ceftriaxone
No. of measures	69	14	4
Mean (mg/L)	66.9	43.2	85.8
Standard deviation (SD)	68.4	37.4	36.6

Conclusions: The incidence of potential AE is high during the treatment of IE with currently recommended doses of BL and is associated with high trough BL plasma concentrations.

Mechanisms of action and resistance

R2115 Frequency of mutations in rpoB and katG gene of rifampicine and isoniazid-resistant strains isolated from primary and secondary tuberculosis infection in Iran

S. Zaker Bostanabad, L. Titov, A.R. Bahrmand, M. Taghikhani (Tehran, IR; Minsk, BY)

Background: Multidrug-resistant *M. tuberculosis* is an emerging problem of great importance to public health of Iran. The aim was to determine resistance-associated mutation in 81 bp region of the rpo B gene in Rif-r MBT strains. Resistance to izoniazid in *M. tuberculosis* is associated with single nucleotide polymorphism resulting in amino acid substitution in kat G gene.
Methods: 39 cultures of Rif-r Mycobacterium spp. were isolated from 160 sputum specimens collected in Iran, 34 Rif-r isolates (87%) were identified as *Mycobacterium tuberculosis*. 28 IZN-R MBT were isolated from patients with active pulmonary tuberculosis in Iran. 411 bp fragments of rpo B gene and 209 bp fragments of kat G gene were

PCR-autosequenced and analysed with computer programmes MEGA and DNAMAN.
Results: For rpoB: It was detected 60 mutations and 13 microdeletions in 29 RIF-r MBT (85%). No mutations were found in the core region of the rpoB gene in 5 RIF-r MBT (15%). Of 60 found mutations 6 were silent, 54 were missense. Most of detected deletions were identified in codon 510. All silent mutations were localised in codon 507, missense mutations produced 23 types of amino acid substitutions. Alterations in these triplets displayed 50 and 35% tested isolates. Mutations in codons 510, 507, 531 were registered in 27, 24, 21% of isolates correspondingly. We observed 5 alleles in codon 526, 3 alleles in triplets 507, 508, 513. 6 strains (19%) harboured single mutations placed in 526, 510 codons while the rest of isolates had multiple mutations: double, triple and quadruple mutations present in 34; 22, 3% of strains. 12% of strains harboured 5 mutations.
For katG: 2 strain has not any mutation. In 20 strains mutation was proved in codon 315 by three type of mutation consist of AGC → ACC (Ser → Thr) (80%), AGC → AGG (Ser → Arg) (5%) and AGC → AAC (Ser → Asn) (15%). Furthermore most mutations were proved in 311, 299, and 322 codons by one type of mutation. In 12 strains one mutation in codon 315 was proved. In 7 strains 2, in 5 isolate 3, and in 2 strains 4 mutations were proved. Nine silent mutations was demonstrated in codon 311 (GAC → TAC) with anything changes in amino acid. In 2 strains with any drug resistances any mutations was demonstrated too.
Thus, all IZN-r MBT had resistance-associated nucleotide changes mostly in codons 315. Hence RIF-r MBT had resistance-associated mutations predominantly in rpo B gene codons 523 and 510.

R2116 The rate of horizontal transmission of antibiotic resistant plasmids is increased in food preservation-stressed bacteria

M.A.S. McMahon, I.S. Blair, J.E. Moore, D.A. McDowell (Newtownabbey, Belfast, UK)

Objectives: Many modern food preservation processes involve the continuous application of one or more bacteriostatic (sub-lethal) stresses to slow or prevent bacterial growth in food within the food chain. This is very different from the approach used in traditional preservation systems, which usually apply single, brief, severe, bactericidal (lethal) treatments, with the aim of killing or permanently inactivating most of the bacteria present in the food, prior to its release into the food chain. This study investigated the possibility that sub-lethal food preservation stresses (high/low temperature, osmotic and pH stress) can alter the rate of horizontal transmission of antibiotic resistance (ABR) plasmids between *E. coli* strains and between *E. coli* and *Salmonella typhimurium*.
Methods: Plasmid-bearing *E. coli* donor cultures (NCTC 50021 – F1 plasmid R386 or NCTC 50338 – Inc I1 plasmid TP307) and *E. coli* recipient cultures (NCTC 35695 & NCTC 33694) and *S. typhimurium* (wild type st11) recipient cultures were mated under a range of sub-lethal environmental stress conditions (low temperature, high temperature, low pH and high NaCl). A transfer rate was determined for each donor/recipient/stress combination.
Results: The study found that the horizontal transmission rate of F1 plasmid R386 and Inc I1 plasmid TP307 is significantly increased ($p < 0.05$) when pre-stressed donor and recipient cells are mated under conditions of environmental stress (high/low temperature, osmotic and pH stress).
Conclusions: These results indicate that increased use of bacteriostatic (sub-lethal), rather than bacteriocidal (lethal) food preservation systems, may be contributing to the dissemination of ABR among important food borne pathogens.

R2117 QRDR mutations and efflux activity of 87 clinical *S. pneumoniae* isolates collected during the MOTIV Study (2004–2005)

I. Knezevic, G. Weskott, S. Doerlemann, P. Surdakowski, E. Operschall, P. Arvis, S.H. Choudhri, K. Ehlert, J. Ambler (Wuppertal, DE; Puteaux, FR; West Haven, US)

Objectives: This study explored the mutation pattern of 87 *S. pneumoniae* isolates collected during the MOTIV study before antibiotic therapy was started. Hospitalised patients with CAP (PSI classes, III, IV or V) received 400 mg IV/PO moxifloxacin (MXF) q.d. or high dose ceftriaxone (CTX) 2 g q.d. and IV/PO levofloxacin (LFX) 500 mg b.i.d. for 7–14 days. MICs were determined for MXF, LFX, CFX, clarithromycin (CLAR) and penicillin (PEN). Quinolone resistance determining regions (QRDRs) of topoisomerase II and IV and efflux of MXF, LFX and CFX were analysed.
Methods: MICs were determined by broth microdilution method (CLSI methods). The QRDRs were amplified and sequenced. Strains were tested for active efflux by the broth microdilution method with or without 10 μg/mL reserpine. Strains for which there was ⩾4-fold decrease in MIC in the presence of reserpine were considered positive for reserpine-inhibited efflux.
Results: All *S. pneumoniae* isolates were susceptible to MXF, LFX and CFX. MXF showed the lowest MIC_{90} (0.25 mg/L). 15% of isolates were resistant to CLAR and 17% were intermediate or resistant to PEN. No *S. pneumoniae* isolate possessed a first-step mutation in Ser79 of parC or Ser81 of gyrA. New mutations were found in gyrA (Asn137Ile) and gyrB (Arg571Cys, Tyr525His and Val355Ile). Mutations were found most frequently in parE (Ile460Val) and parC (Lys137Asn). 20 (23%) strains were observed to have efflux activity; 17 of these were active on CFX, of which 4 were also active on LFX and/or MXF. 2 strains had efflux that was active on LFX but not on CFX, and one strain had efflux with activity on LFX and MXF but not on CFX. All but one isolate with efflux had the Ile460Val substitution in the parE gene.
Conclusion: No fluoroquinolone resistance or Ser79/Ser81 substitutions in parC/gyrA could be detected among 87 *S. pneumoniae* isolates collected during the international MOTIV study. However, 4 new mutations were found in the QRDR of gyrA and gyrB but these mutations did not influence susceptibility to fluoroquinolones. Efflux pump activity was most prominent for CXF.

R2118 mef gene can be not enough for macrolide resistance in Viridans streptococci clinical isolates

M. Hernández Iglesias, J. García Rodríguez, J. Muñoz Bellido (Salamanca, ES)

Background: Our initial purpose was to know the role of pharyngeal viridans streptococci (VS) as a resistance reservoir for pneumococci. Thus, we determined the frequency of macrolide resistance, and macrolide resistance genes among pharyngeal VS in healthy individuals, and their capacity to be transformed to *S. pneumoniae*.
Methods: Pharyngeal swabs from 89 non-selected, healthy individuals were spread onto antibiotic-free blood agar plates, and onto blood agar plates with 0.5 μg/mL of erythromycin (ERY). Alpha-haemolytic Gram positive cocci growing in ERY plates, and 3–4 colonies among beta-haemolytic Gram positive cocci growing in antibiotic-free plates, were identified by usual methods, and by the API 20 STREP system. MIC of ERY was determined by the agar dilution method. The presence of mef, ermA, ermB and ermTR genes was determined by PCR. Transformation to *S. pneumoniae* R6 was performed according previously described methods.
Results: We isolated macrolide-resistant viridans streptococci (MRVS) in 48 individuals (53.9%). 42 MRVS harboured mef genes (87.5%), one ermB (2.1%), and 5 both mef and ermB genes (10.4%). No isolates harboured ermA or ermTR genes. ERY MIC range of MRVS was 1–128 μg/mL (MIC_{50}: 4 μg/mL, MIC_{90}: >28 μg/mL). ERY MIC range of macrolide-non-resistant (MSVS) isolates was 0.02–0.5 μg/mL (MIC_{50}: 0.1 μg/mL, MIC_{90}: 0.2 μg/mL). Among MSVS, we found

4 *S. mitis* isolates (9.7%) harbouring a mef gene. Nevertheless, their MICs of ERY were similar to mef(−) MSVS isolates (range: 0.05–0.5 μg/mL). The genetic environment study suggests mef gene might be included in a genetic element similar to MEGA. When we performed transformation experiments, we got mef(+) *S. pneumoniae* R6 transformants. Nevertheless, these transformants were ERY-resistant, with ERY MICs similar to mef(+), MRVS isolates.
Conclusions: mef gene may be not enough for determining ERY-resistance in VS clinical isolates. Nevertheless, their DNA seems to be able to confer ERY-resistance to pneumococci.

R2119 Carbapenem resistance of *Escherichia coli* strains

R. Schaumann, D. Adler, A.C. Rodloff (Leipzig, DE)

Objectives: Carbapenem (CP) resistance of *Escherichia coli* strains is extremely rare and seldomly discussed. The aim of the present study was to investigate the resistance mechanisms of three recent clinical *E. coli* isolates that were CP resistant.
Methods: MICs for imipenem (IMP), meropenem (MER), and ertapenem (ERT) as well as ESBL or MBL production were determined by Etest. Furthermore, the three strains were analysed for blaTEM, blaSHV, blaampC, blaACC, blaVIM, and blaIMP by PCR and isoelectric focusing and visualisation by nitrocefin and coomassie blue was carried out. In addition, the strains were serially passaged on antibiotic free columbia agar plates and MICs re-determined. Conversely the three strains and – for control purposes – eight susceptible *E. coli* strains were incubated on solid media containing IMP, MER, and ERT, respectively, at twice their MICs. After incubation growing bacteria were harvested and incubated at four times MICs. This procedure was repeated with increasing antibiotic concentrations. The resulting MICs were confirmed by Etest.
Results: The MICs for the three resistant strains ranged from 4 to >32 mg/L. The other *E. coli* strains showed MICs between 0.004 and 0.38 mg/L. After passages on antibiotic free medium, the MICs for the three strains decreased to 0.38 to 16 mg/L. However, after passages on antibiotic containing agar plates the MICs were >32 mg/L for all three CPs tested. The resistant strains contained blaampC and blaTEM. Isoelectric focusing demonstrated β-lactamases of various isoelectric points while the MBL and ESBL tests were negative or not calculable except for one strain. Furthermore, elevated MICs were inducible with ERT and IMP in the susceptible strains but not with MER.
Conclusion: *E. coli* strains may posses various mechanisms such as blaampC and blaTEM that in combination cause CP resistance. They may loose their phenotypic resistance after several passages on antibiotic free medium. Conversely, employing passages on antibiotic containing medium led to reappearance of the resistant phenotype.

R2120 Characterisation of unusual patterns of MLS resistance among *Streptococcus pyogenes* clinical isolates

L. Vitali, S. Rombini, D. Petrelli, S. D'Ercole, G. Pasquantonio, C. Ripa, M. Prenna (Camerino, Rome, Ancona, IT)

Objectives: In Gram-positive cocci, macrolide, lincosamide, and streptogramin B (MLS) resistance can be expressed either constitutively (cMLS) or inducibly (iMLS) and is mediated by the presence of erm genes. The present work sought to investigate the iMLS and cMLS phenotypes of a group of erm(B)-positive *Streptococcus pyogenes* clinical isolates showing non-standard behaviours in the erythromycin (ERY)–clindamycin (CLI) double disk diffusion testing.
Methods: Eight clinical isolates coming from patients with pharyngitis and two reference strains were analysed. Their ERY resistance phenotype was determined by the double disk diffusion testing and by growth in liquid medium using cells induced by preincubation with ERY (0.1 mg/L) followed by challenge with 50 mg/L of CLI. Inoculum preparation was accomplished by the two alternative procedures suggested by CLSI. Muller–Hinton II (MH) and Brain-Heart-Infusion (BHI), both supplemented with 5% sheep blood, were used as test media. Cultures were incubated at 35ºC in an atmosphere of 5% CO_2 for a maximum of 24 h.
Results: Double disk diffusion testing, with the inoculum prepared by direct colony suspension, assigned 50% of the strains to the cMLS phenotype. Within this group three strains showed an inner hazy D-shaped inhibition zone around the CLI disk (HD). Among the four iMLS strains, three presented small colonies growing proximal to CLI disk in otherwise essentially clear zone (D+). Induction experiments in liquid medium showed that HD strains responded to some extent to induction but only during the logarithmic phase of growth, while in the D+ group even non-induced samples became resistant when cells reached the stationary phase. To test weather the efficiency in the control of the iMLS resistance was growth rate dependent, MH was replaced with BHI and the inoculum prepared following the growth method. In these conditions, D+ strains reverted to a plain iMLS and HD to a cMLS, both in agar and in liquid. These effects were even more evident after an incubation time of 6–8 h.
Conclusion: These results indicate that double disk diffusion testing for MLS phenotype determination in *S. pyogenes* should be performed starting with an inoculum prepared by the growth method and results read also during the course of the experiment (e.g. at 6–8 h). The use of BHI instead of MH further diminish difficulties in the assignment of some *S. pyogenes* strains to the correct MLS phenotype.

R2121 In vitro activity of tigecycline and comparators against *Acinetobacter baumannii* isolated from severe infections in Italy

M. Mezzatesta, M. Trovato, V. Nicolosi, D. Nicolosi, G. Nicoletti, A. Cassone, G. Fadda, G. Schito, S. Stefani (Catania, Rome, Genoa, IT)

Objectives: In a recent multi-centre survey (2003–2004), conducted in 45 laboratories throughout Italy with the aim of monitoring microrganisms responsible for severe infections and their antibiotic resistance, *Acinetobacter baumannii* was isolated from the ICU of 12 centres as the third most frequent pathogen. Due to the increasing importance of this microrganism in hospital epidemiology, which is partly due to its success in acquiring resistance to carbapenem or showing a multi-drug-resistance (MDR) phenotype, it is mandatory to look at the activity of new antimicrobial agents.
Methods: Included in the study were 88 clinically significant strains of *A. baumannii*. Tigecycline and comparators were tested by MICs following the CLSI guidelines. For the carbapenem resistant strains, a preliminary phenotypic screening for the presence of metallo-enzymes (MBL) was performed by Etest, and PCR was used to investigate the presence of blaOXA, blaIMP and blaVIM genes. PFGE was performed to test clonality.
Results: *A. baumannii* was a frequent cause of ventilator-associated pneumonia and bacteraemia. Resistance to ceftazidime, ciprofloxacin and aztreonam was widespread in almost 90% of strains; resistance to imipenem was 51% and resistance to meropenem was 64%, amikacin and gentamicin were active against 30% of strains and colistin was very active.
Tigecycline had a MIC_{90} value of 2 mg/L and our strains showed a unimodal distribution of susceptibility, demonstrating that this new drug is more active than other tetracyclines. Among the 45 imipenem-resistant isolates (MIC gt; 16 mg/L) only 17 strains showed a reduction in the imipenem MIC with EDTA indicating MBL activity, but no PCR products for blaIMP and blaVIM were obtained from the strains analysed. Further studies are in progress for the characterisation of the blaOXA resistance determinants. PFGE analyses showed the existence of a multiresistant *A. baumannii* clone, widespread in different hospitals.
Conclusion: In conclusion, tigecycline showed a potent activity against the MDR *A. baumannii* strains maintaining the same MIC_{90} of 2 mg/L against the majority of carbapenem-resistant strains. The use of additional molecular techniques to fingerprint isolates will provide further information on these clinically important MDR *A. baumannii*.

R2122 Macrolide resistance by ribosomal mutation detected in clinical isolates of *Streptococcus pneumoniae* isolated from PROTEKT, 2000–2005

J.G.E. Northwood, L.R. Cossins, S.M. Forrest, D.J. Farrell (London, Bollington, Macclesfield, UK)

Objectives: PROTEKT is a global, longitudinal study of the antimicrobial susceptibility of bacterial pathogens associated with community-acquired lower respiratory tract infections. We have been screening clinical isolates from PROTEKT 2000–2005 for the genetic determinants of macrolide resistance (Macr) (total of 9927 isolates) and have discovered 166 isolates negative for known efflux or rRNA methylase mechanisms. The aim of this study was to determine the molecular basis of Macr in these strains.
Methods: Segments of the L4 & L22 riboprotein genes and the 4 copies of the 23S rRNA (domains V and II) gene were amplified, sequenced and mutations determined.
Results: [23s mutation, n/4 alleles, n isolates, erythromycin MIC mg/L]. A2058G, 2, 5, 128; A2058G, 3, 5, 32–128; A2058G, 4, 4, 128; A2059C, 4, 2, 128; A2059G, 1, 3, 1–16; A2059G, 2, 7, 4–64; A2059G, 3, 10, 16–128; A2059G, 4, 24, 16–128; C2611A, 4, 1, 1; C2611G, 3, 1, 128; G2057A, 4, 1, 0.5. [L4 riboprotein substitution and 23s mutation, n/4 alleles, n isolates, erythromycin MIC mg/L] G95D, A2058G, 2, 1, 32, 128; G95D, A2059G, 4, 5, 32–128; A73T, A2059C, 4, 1, 128. [L4 riboprotein substitutions, n isolates, ERY, CLARI, AZI MIC mg/L] 66_69InsRQKG, 1, 1, 0.5, 1; 68_69InsGK, 2, 0.5, 0.25, 4; 68–72 Del KGTGR, 1, 1, 1, 4; G69E, T70A, 1, 0.25, 0.25, 1; E30Q, G69T, T70P, G71S, V88I, 2, 128, 64, 128; G69T, T70P, G71S, V88I, 1, 128, 64, 128. Resistance mechanisms other than known efflux or rRNA methylase mechanisms for Years 1, 2, 3, 4, and 5 were 1.7%, 1.6%, 1.5%, 1.6% and 2.0% respectively. Isolates without a detectable mechanism of resistance for Years 1, 2, 3, 4 and 5 were 0.2%, 0.9%, 1.0%, 0.9% and 1.4% respectively. The telithromycin MIC range for the 166 isolates was 0.004–1 mg/L.
Conclusions: This study shows that the percentage of Macr *S. pneumoniae* with resistance mechanisms other than known efflux or rRNA methylase mechanisms has not increased over the period of the study. Resistance due to undetectable resistance mechanisms have increased from 0.2% to 1.4%. The isolates are global in distribution. Telithromycin MIC values remain low for all 166 isolates suggesting that riboprotein mutations and the undetectable mechanisms of resistance do not have a major effect on this new ketolide antimicrobial.

R2123 Carbapenem resistance among clinical isolates of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* from hospitals in Cochabamba, Bolivia

E. Fernández, J. Zamora, M. Torrez, I. Rosales, C. Valderrey, E. Sevillano, L. Gallego (Bilbao, ES; Cochabamba, Anzaldo, BO; Leioa, ES)

Objectives: The aim of this work was to study the level of resistance to carbapenems among clinical isolates of *P. aeruginosa* and *A. baumannii* obtained from patients attended at different hospitals of the region of Cochabamba, Bolivia.
Methods: The study included 58 isolates of *P. aeruginosa* and 5 *A. baumannii* obtained in the Microbiology Service of the Instituto Gastroenterológico Boliviano–Japonés from Cochabamba, Bolivia during 2006 (January to October). Susceptibility to antimicrobial agents was determined by the disc-diffusion method following the NCCLS recommendations. The antibiotics tested were ceftazidime, piperacillin, ceftriaxone imipenem, meropenem, amikacin, gentamicin and ciprofloxacin. The presence of carbapenemases was detected by Hodge test, Hodge+ZnSO4 test, double-disc synergy test (DDST) and PCR analysis searching for blaOXA-40, blaIMP, blaVIM, blaSPM and blaGIM genes.
Results: Total percentage of resistance was 11% to meropenem, 21% to imipenem, 59% to amikacin, 62% to ciprofloxacin, 68% to gentamicin and piperacillin and 97% to ceftriaxone and 98% to ceftazidime. Nine *P. aeruginosa* and four *A. baumannii* isolates were resistant to carbapenems and some of them showed carbapenemase activity. Table 1 shows patient data and results obtained in the carbapenem-resistant isolates.

Code	Source	Patient[a]	Resistance								Carbapenemases	
			IPM	MEM	CAZ	PIP	CRO	AMK	GEN	CIP	OXA-type	MBLs
Pseudomonas aeruginosa												
PB1	Wound	F, 17	I	–	R	I	R	R	R	R	–	–
PB2	Wound	M, 35	I	–	R	S	I	R	R	S	–	–
PB3	Ulcer	M, 30	S	I	I	R	R	I	R	R	+	–
PB4	Pharsec	M	R	R	S	S	R	S	S	I	–	–
PB5	Urine	M, 30	I	S	R	R	R	R	R	R	+	–
PB6	Trasec	M, 0.17	I	R	I	S	R	S	I	S	–	–
PB7	Sputum	F, 60	I	I	R	I	R	R	R	R	–	–
PB8	Trasec	F, 3	I	S	R	S	S	R	R	R	–	–
PB9	Brsec	F, 2	I	I	R	S	I	S	S	S	–	–
Acinetobacter baumannii												
AB1	Brsec	M, 68	I	–	R	R	R	R	R	R	+	–
AB2	Brsec	F, 39	R	–	R	R	R	R	R	R	–	–
AB3	Sputum	F, 39	I	S	R	R	R	R	R	R	+	–
AB4	Trasec	M, 68M	R	R	R	R	R	R	R	R	+	–

[a]Patient data: gender, age. [b]Brsec, bronchial secretion; Phrsec, pharingeal secretion; Trasec, tracheal secretion.

Conclusions: Imipenem and meropenem showed the best activity against the isolates tested. The proporcional resistance to carbapenems was greater in *A. baumannii* (4/5) than in *P. aeruginosa* (9/58). This is the first study which analyses the carbapenem resistance and carbapenemases among Bolivian isolates.

R2124 Characterisation of the wild type AmpC β-lactamase CHE

A. Fernea, P. Mercuri, G. Arlet, M. Galleni (Liège, BE; Paris, FR)

Objectives: AmpC-CHE is a chromosomal class C β-lactamase produced by *Enterobacter cloacae*3. The most remarkable feature of this enzyme is a 6 amino acid deletion (SKVALA, position 289–298) corresponding to a deletion of 18 nucleotides in the gene. Furthermore the alignments with other AmpC β-lactamase sequences shows that the 6 amino acid sequence is conserved in the AmpC β-lactamases of *Citrobacter* freundii and in the plasmid-mediated β-lactamases derived from. Our study is focused on the determination of the influence of this deletion by producing, purifying and undertaking kinetic characterisation of wild type AmpC-CHE.
Methods: AmpC-CHE was isolated from a culture of *Enterobacter cloacae*. The bacteria were grown at 37ºC in BHI media in the presence of amoxicillin 20 µg/mL. At an OD of 1.2 at 600 nm, cefoxitin (5 µg/mL final concentration) was added to the media and the culture was incubated for 4 additional hours. The bacteria were collected by centrifugation, resuspended in 15 mM sodium cacodylate pH 6 (buffer A) and lysed by sonication. The crude extract was loaded on a SP sepharose equilibrated in buffer A. The β-lactamase was eluted with a linear salt gradient. The active fractions were collected, dialysed against 10 mM sodium phosphate buffer pH 6.8 (buffer B) and loaded on a hydroxyapatite column equilibrated in buffer B. The AmpC enzyme was recovered by elution of the column with a linear gradient (10–400 mM sodium phosphate pH 6.8). The N-terminal sequence and the exact molecular mass of CHE were determined.
Finally, the kinetic profile of the enzyme was characterised.

Results: The purification yield was estimated at 10%. The N-terminal sequence of AmpC was in good agreement with the predicted cleavage site of the pre-β-lactamase (TPSVE-). The mass of the mature form was also in good agreement with the molecular mass determined by mass spectrometry (38725 Da). The activity of AmpC-CHE was measured against nitrocefin, cephalothin, benzylpenicillin, cefotaxime, cefepime and cefpirome. The enzyme exhibited a broad spectrum of activity where benzylpenicillin and nitrocefin were the best substrates.
Conclusion: No major difference in the catalytic properties could be found when compared with the other enzymes of class C β-lactamases. The impact of the deletion on the stability of the enzyme is in progress.

Resistance surveillance

R2125 Susceptibility of glycopeptide-resistant *Enterococcus faecium* to tigecycline, dapto-mycin, linezolid, and quinupristin-dalfopristin

J. Abb (Ludwigsburg, DE)

Objective: To study the in vitro activity of the glycylcycline tigecycline, the lipopeptide daptomy-cin, the oxazolidinone linezolid and the streptogramin quinupristin-dalfopristin against glycopeptide-resistant *Enterococcus faecium* (GRE).
Methods: Sixty primary GRE isolates from hospitalised patients were collected between January and December 2004. Minimum inhibitory concentrations of vancomycin, tei-coplanin, tigecycline, daptomycin, linezolid and quinupristin-dalfopristin against *E. faecium* strains were determined by the E test. *E. faecalis* ATCC 29212 was used as a control strain.
Results: MIC 50, MIC 90, and MIC range values are demonstrated in the table. According to CLSI standard MS 100–S15 criteria all GRE isolates were fully susceptible to tigecy-cline, daptomycin, and quinupristin-dalfopristin. GRE strains, however, demonstrated a high rate of only intermediate sensitivity (82 percent) against linezolid (MIC: 3.0 to 4.0 mg/L), but none of the isolates was fully resistant to the oxazolidinone.

	MIC (mg/L)		
Antimicrobial agents	MIC_{50}	MIC_{90}	Range
Tigecycline	0.047	0.094	0.032–0.125
Daptomycin	1.5	1.5	0.5–3.0
Linezolid	3.0	4.0	0.75–4.0
Quinupristin–Dalfopristin	1.0	1.5	0.38–2.0
Teicoplanin	48	128	16–>256
Vancomycin	>256	>256	128–>256

Conclusion: The present and previous in vitro studies show that tigecycline, daptomycin, quin-upristin-dalfopristin, and linezolid may be useful alternative agents for the treatment of patients with GRE infections. The rapid emergence of *E. faecium* isolates with re-sistance to the novel compounds, however, underlines the need for careful pre-therapy susceptibility testing.

R2126 Microbial keratitis and antimicrobial resistance of the isolates during a nine-year period

D. Mylona-Petropoulou, G. Ganteris, K. Tzanetou, E. Ziva, D. Miltsakakis, D. Droutsas, H. Malamou-Lada (Athens, GR)

Objectives: To study the incidence and the aetiology of the microbial keratitis and to examine the susceptibility of the isolates to antibiotics.
Methods: A retrospective study was performed of all isolates from cases of keratitis between January 1, 1998 until August 31, 2006 at Dept of Ophthalmology, Reference Center, Athens University, General Hospital of Athens "G. Gennimatas". Corneal scraping was performed and processed for direct microscopy and culture for bacterial and fungal isolates. MICs were determined using the VITEK II (boiMérieux) and the E test (Biotest, Solna).
Results: During the nine years period 876 consecutive corneal samples were obtained and 625 isolates were recovered in 515 positive cultures (58.7%). Of these 429 (83%) had only bacterial growth (1–6 isolates), 56 (11%) had fungal growth (1–2 isolates) and 30 (6%) had mixed fungal and bacterial growth. Polymicrobial infection was found in 90 (17.5%). Gram-positive bacteria dominated (447/625, 71%) followed by Gram-negative bacteria (15%) and fungi (14%). *Staphylococcus* species (*Staphylococcus epidermidis* was the predominant) and *Pseudomonas aeruginosa* represented 36% and 6% respectively of the total bacterial isolates. The commonest fungal pathogen was *Fusarium* spp. 20/86 (23%), followed by *Aspergillus fumigatus* 17/86 (20%), *Acremonium* spp. 12/86 (14%) and *Candida albicans* 14/86 (16%). Although the number of scraped corneal ulcers varied from 92 (2005) to 162 (2002) positive cultures, recovered bacteria and proportion of Gram-positive, Gram-negative and fungal isolates per year remained constant during the study period. There has not been an increase in the proportion of isolates resistant to fluoroquinolones and to gentamicin and tobramycin from 2000, (9.5% and 14%), to 2006 (7% and 17%) respectively. *Staphylococcus* species were resistant to methicillin in 19%, *Pseudomonas aeruginosa* isolates were sensitive to all tested antibiotics during the study period. *Streptococcus pneumoniae* isolates were penicillin resistant in 29%. In addition, voriconazole was the most active antifungal agent.
Conclusion: The incidence, aetiology and susceptibility of the isolates remained almost unchanged during the study period. *Staphylococcus* spp. and *Pseudomonas aeruginosa* were the predominant bacterial pathogens followed by fungi, with *Fusarium* spp. being the most common fungus. Continued surveillance of the microbial keratitis is warranted.

R2127 Isotretinoin treatment in acne vulgaris has no influence on the incidence of antibiotic-resistant *Propionibacterium acnes*

C. Oprica, L. Emtestam, L. Hagströmer, C.E. Nord (Stockholm, SE)

Objectives: Isotretinoin is a synthetic retinoid and is a very effective agent in the treatment of severe inflammatory acne, but also in moderate acne in the presence of scars or psycho-social problems, affecting all factors involved in its pathogenicity: sebum production, follicular hyperkeratinisation, microbial colonisation by *Propionibacterium acnes* and release of inflammatory mediators into dermis. The purpose was to investigate the density and the antimicrobial susceptibility of P. acnes skin strains isolated before, during and after treatment in patients with moderate/severe acne with isotretinoin.
Methods: Twenty-four patients (17 men and 7 women, 15–35 years) with moderate or severe inflammatory acne vulgaris participated in the study. The patients received oral isotretinoin 1 mg/kg/day in two doses for 6 months. The drug is a potent teratogen and for women treated with this drug a pregnancy test was performed and oral contraception was started before and continued during the treatment period and 6 weeks post-therapy. Skin samples were taken at baseline, after 2, 4, 6 months of treatment and 2 months after the treatment had stopped from five areas by using a soft gelatine with a cross-sectional area of approximately 1 cm2 which was pressed against the skin surface for 10 seconds without rotation. Then a slice of the soft gelatine with the bacterial bearing surface was cut off, put into a sterile glass tube with pre-reduced peptone-yeast extract medium and immediately transported to the laboratory for microbial cultivation, identification, quantification and antibiotic susceptibility. The strains were tested for tetracycline, clindamycin, erythromycin and linezolid and the breakpoints were according to EUCAST.
Results: The treatment produced a significant reduction in the total *P. acnes* counts but tetracycline-resistant strains were acquired during the 6-month treatment period. Two patients lost their resistant strains 2 months after the treatment had stopped. After 6 months of treatment, patients carrying tetracycline-resistant *P. acnes* were found to be

colonised with clindamycin-erythromycin-resistant strains more often than patients with no tetracycline-resistant strains. No strain was found to be linezolid resistant.
Conclusion: There is a complex relationship between antibiotic-resistant *P. acnes* and treatment of acne vulgaris. Isotretinoin treatment has no influence on the incidence of resistant *P. acnes* strains.

R2128 Antibiotic resistance amongst cutaneous propionibacteria from Egyptian acne patients

T.S.M. El-Mahdy, M.M. Abd-Elaziz, M.H. El-Sayed, J.I. Ross, S. Abd-Allah, H. Radwan, A.M. Snelling (Bradford, UK; Cairo, Ismailia, EG)

Objectives: Antibiotics are widely used to treat acne, with the aim of reducing levels of *Propionibacterium acnes* on the skin. Unfortunately, this species is rarely cultured and sensitivity profiles determined, before prescriptions are written. Little work has been done in Egypt on drug resistance in cutaneous propionibacteria, and there is no surveillance data to inform clinical decisions. Anti-acne drugs are widely available, including over the counter. This study aimed to 1) investigate antibiotic-resistant propionibacteria amongst acne patients and controls in Cairo and 2) screen for any resistance to triclosan, which is being increasingly used in skin cleansers.
Methods: 52 acne patients (attending two dermatology clinics), 36 age-matched controls from the community (no antibiotics taken in past 3mths), and 13 dermatology staff were sampled. Swabs of the face were used to inoculate plates of tryptone yeast extract glucose (TYEg) agar containing 6 mg/L furazolidone ± breakpoint concentrations of tetracycline (TET 5 mg/L), erythromycin (ERY 0.5 mg/L), clindamycin (CLIN 0.5 mg/L) and triclosan (TRIC 4 mg/L). After anaerobic incubation at 37ºC for 7 days, growth of colonies morphologically resembling propionibacteria was scored.
Results: Propionibacteria were cultivated from the skin of all participants except one control. For all three cohorts, CLIN was the most common resistance encountered, being detected amongst 26.9% patients, 38.5% staff, 41.7% controls. For ERY, the prevalences were 13.5, 23.1 and 33.3% respectively. Controls were more likely than patients to carry ERY resistant strains ($p = 0.016$). In total, 18.8% of people had both ERY and CLIN resistant strains. TET resistant strains were isolated from just 2 (3.8%) patients, compared to 7.7% of staff and 11.1% of controls. Patients taking antibiotics were no more likely to carry resistant strains than those who were not. Amongst the staff, 5 of 13 were colonised with strains having at least one resistance, including all those who specialised in treating acne. No resistance to TRIC was detected.
Conclusions: Commensal carriage of propionibacteria resistant to drugs used to treat acne was common in this Egyptian survey. The finding of CLIN resistance in the absence of ERY resistance for propionibacteria is unusual, and the mechanism remains to be elucidated. The slightly lower incidence of drug resistant strains amongst patients merits further investigation of treatment practices in this area.

R2129 Antimicrobial resistance of clinical isolates *Acinetobacter baumannii* in a university hospital

T. Stoeva, E. Savov, K. Bojkova (Varna, Sofia, BG)

Objective: to evaluate the antimicrobial resistance of 126 strains *A. baumannii*, isolated from ICUs and non ICUs in Varna University Hospital, Bulgaria.
Methods: for the period 2005–2006, 126 nonduplicate *A. baumannii* strains, isolated from different clinical specimens, were studied: 44.6% from respiratory system, 39.8% from wounds, 11.6% from urinary tract and 6% from blood. The identification was done by conventional, BD BBL Crystal ID System and Mini Api System (bioMérieux, France). The strains were tested to Ampicillin/sulbactam (A/S), Piperacillin (PIP), Cefoperazone/sulbactam (SCF), Ceftazidime (CAZ), Cefepime (FEP), Gentamicin (G), Amikacin (AM), Ciprofloxacin (CIP), Aztreonam (ATM), Imipenem (IMP) and Meropenem (MEM), using Disk Diffusion Method. CAZ and carbapenem resistant isolates from ICUs (51) were tested for production of Metallo Beta Lactamases, using Hodge test and E test with Imipenem (IP) and Imipenem/EDTA (IPI).
Results: The antimicrobials with the lowest level of resistance for the studied period were: IMP (9.5%), MEM (13.4%) and SCF (44.4%). The other groups of antibiotics were with resistance rates more than 50%. Isolates from ICUs were more resistant than the other isolates: IMP (20%), MEM (23.6), A/S (69%), AM (70.9%), SCF (74.7%), FEP (76.3%), CIP (83.6%), G (85.4%), PIP (92.7%), CAZ (92.7%), ATM (92.7%). Multi-resistant (resistance to at least 4 of the tested drugs) were 83.3% of the strains. The agent with the lowest level of resistance among multi-drug resistant isolates was IMP (11.4%). G, CIP, and FEP resistant isolates showed high levels of cross-resistance to other groups of antimicrobials. All CAZ and carbapenem resistant strains from ICUs (51), tested by the Hodge test, gave negative results. The E test detected the presence of MBLs in 10 IMP resistant strains, for which the MIC ratio of IP/IPI was >10.
Conclusions: the isolates showed considerable resistance to most of the tested agents. Resistance rates to IMP and MEM were relatively low. We observed high levels of multi-drug and cross resistance. The MBL positive strains (19%) will be studied further, using PCR for detection of bla IMP gene.

R2130 Antimicrobial resistance patterns of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* isolates in a hospital in Algiers, Algeria

N. Ramdani-Bouguessa, F. Djennane, M. Neggazi, M. Tazir (Algiers, DZ)

Objectives: *Acinetobacter baumannii* and *Pseudomonas aeruginosa* are a commonly isolated pathogens from nosocomial infections in intensive care units (ICU). The aim of the study was to establish the frequency of drug resistance of these pathogens and to prevent nosocomial infections.
Methods: A total of 355 *A. baumannii* and 638 *P. aeruginosa* strains were collected between January 2004 to June 2006 from different specimens and wards. Identification was performed by the API 20 NE system (bioMérieux, Marcy l'Etoile, France). Antimicrobial susceptibility was determined by disc diffusion according to the CLSI guidelines. Strains that were not susceptible to ceftazidime and imipenem were tested by E test strips (AB Biodisk, Slona, Sweden).
Results: *A. baumannii* and *P. aeruginosa* isolates were originated from pus, bronchial aspirates and urine in 79.4% to 78.5% respectively; and from sterile sites, bloodculture and cerebrospinal fluid, in 9.3% to 6% respectively. The distribution frequency regarding wards for *A. baumannii* and *P. aeruginosa* was as follows: 37.2% to 21% in medical ICU and in 32.4% to 47% in surgery wards respectively.
Resistance rates are shown in the table. The highest rate of resistance to ceftazidime (67%) for *A. baumannii* was observed in ICU and to imipenem (43%) for P.aeruginosa in thoracic surgery. MICs 50 and 90 to ceftazidime and imipenem were 16 μg/mL and 256 μg/mL for non susceptible *A. baumannii* and *P. aeruginosa*.

Species	Ticarcillin	Piperacillin	Ceftazidime	Imipenem	Gentamicin	Amikacin	Tobramycin	Ofloxacin
A. baumannii (n = 355)	84%	90%	69%	2%	83%	66%	52%	48%
P. aeruginosa (n = 638)	12.5%	10%	4%	13.4%	22.6%	10%	12%	22%

Conclusions: *A. baumannii* show a multidrug resistance reducing dramatically the therapeutic options and imipenem resistant *P. aeruginosa* increased. Failure of control measures enhanced the dissemination of such highly resistant strains. Prevention of nosocomial infection is needed by implementation of control measures and the rational use of antibiotics.

R2131 Antimicrobial activity of polymyxin B against non-fermentative Gram-negative bacteria in Georgia

A. Nanuashvili, M. Kereselidze, T. Davitashvili (Tbilisi, GE)

Objectives: The polymyxins have activity against a wide variety of Gram-negative bacilli, including non-fermentative isolates. Polymyxins B and E were introduced into clinical practice during the 1950s for the treatment of Gram-negative infections. However, the parenteral use of these compounds was abandoned during the 1970s when better-tolerated anti-pseudomonal agents became available. The emergence of multidrug-resistant (MDR) isolates of *Pseudomonas aeruginosa* and *Acinetobacter* spp. has required the expanded systemic use of these polymyxins. The main objective of this study was to assess the activity and spectrum of polymyxin B against the non-fermentative Gram-negative bacilli isolated in Georgia.
Methods: In total, 257 isolates of *Pseudomonas aeruginosa* and 100 isolates of *Acinetobacter* spp. were collected between January 2000 and December 2005. All isolates were identified at the Microbiological Laboratory, "Cito". For the cultivation, microbiological identification and antimicrobial susceptibility testing "Bio Merioux" (France) test systems were used. Isolates exhibiting resistance to piperacillin/tazobactam, ceftazidime, cefepime, imipenem, ciprofloxacin and amikacin were categorised as MDR.
Results: Susceptibility rates for the antimicrobial agents tested ranged between 46.5% (ciprofloxacin), 64.5% (amikacyn), 69.7% (piperacillin/tazobactam), 74.5% (ceftazidime), 77.3% (cefepime) and 89.5% (imipenem) for *P. aeruginosa*, and between 16% (ciprofloxacin), 34% (piperacillin/tazobactam), 40% (ceftazidime), 44% (amikacin), 67% (cefepime) and 95% (imipenem) for *Acinetobacter* spp. Only polymyxin B demonstrated reasonable potency against *Acinetobacter* spp. (100% susceptible) and *P. aeruginosa* (98.9% susceptible).
Conclusion: Polymyxin B remains very active against clinical isolates of *P. aeruginosa* and *Acinetobacter* spp., including isolates resistant to carbapenems. Polymyxin resistance has been documented rarely, but the emergence of polymyxin-resistant *P. aeruginosa* and *Acinetobacter* spp. would pose a serious therapeutic problem, since no new antimicrobial agents are available currently for treatment of infections caused by MDR Gram-negative bacilli.

R2132 Epidemiology of evolution and antibiotic resistance profile of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* in a Greek tertiary care hospital

E. Protonotariou, D. Vitti, M. Tsivitanidou, D. Sofianou (Thessaloniki, GR)

Objectives: Infections with *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Acinetobacter baumannii* (*A. baumannii*) are of great concern for hospitalised patients, especially with multidrug-resistant strains. The aim of the present study was to determine the evolution of infections caused by these bacteria and to define current and microbial susceptibility profile for a period of 7 year in our hospital.
Methods: Data was collected from patients admitted in Hippokration General Hospital and infected either with *A. baumannii* or *P. aeruginosa*. Bacterial identification and susceptibility testing were achieved using the VITEK 1 automated system (bioMérieux, France) for the period 1999–2001 and VITEK 2 for the period 2002–2005. Ceftazidime (CAZ), imipenem (IMP), ciprofloxacin (CIP), amikacin (AN) and the combination piperacillin/tazobactam (PIP/TAZ) were tested.
Results: During the 7 year-time period a total of 7148 strains were reviewed. The study comprised of 2382 *A. baumannii* and 4766 of *P. aeruginosa* strains collected from wounds, bronchial secretions, blood, urine, catheter tips and other clinical specimens. About *A. baumannii*, the number of strains isolated ranged from 165 to 526 during 1999–2005. In the same period, the antibiotic resistance rates (ARR) of CAZ, CIP, PIP/TAZ and AN were quite high, from 77 to 90%. The resistance rate of IMP increased significantly from 22% in 1999 to 74% in 2005 ($p < 0.001$).
The number of *P. aeruginosa* isolates was between 513 and 840 and the ARR to the tested antimicrobial agents ranged from 11 to 40%. About IMP, the ARR never exceeded 30%.
Conclusions: Our study showed quite high resistance rates of CAZ, CIP, PIP/TAZ and AN to *A. baumannii* throughout our study's period. During the same period the ARR to IMP increased significantly. On the other hand, the resistance of the above agents to *P. aeruginosa* increased slightly and in some cases like in piperacillin/tazobactam even decreased. The high rates of antimicrobial resistance, especially, of *A. baumannii* to the tested antibiotics indicate that a) the broad use of these drugs needs to be revised b) continuous surveillance studies in order to observe changes in resistance of isolates which might help therapeutic choices are necessary.

R2133 Antimicrobial resistance in *Escherichia coli* from pigs in relation to incidence of post-weaning diarrhoea and antimicrobial use

U.G. Andersson, B. Bengtsson, A. Franklin, S. Thyselius (Uppsala, SE)

Objectives: Post-weaning diarrhoea in pigs (PWD) is a multifactorial disease comprising husbandry factors and where overgrowth of *Escherichia coli* in the intestinal tract plays an important role. The objectives of the study were to investigate the association between antimicrobial resistance in *E. coli* from clinically healthy pigs and incidence of PWD and use of antimicrobials.
Methods: From herds with more than 100 sows, 200 herds were randomly selected and two growers in each herd (pigs < 16 weeks) were sampled using rectal swabs. The herd veterinarian collected the samples. Information on incidence of PWD (0%, <25%, <25%) of PWD, and use of antimicrobials were recorded.
Results: Data presented are preliminary results from 101 herds sampled. The herds were distributed according to incidence of PWD as follows: 0%: 31 herds, <25%: 60 herds and >25%: 10 herds. In 71 herds PWD was treated with antimicrobials, among these 48% used trimethoprim-sulphonamides, 28% tylosin, 20%, fluoroquinolones and 20% used colistin.
Resistance (%) in *E. coli* (190 isolates): ampicillin 13.2, cefotaxime 0, ceftiofur 0 chloramphenicol 7.5, ciprofloxacin 3.4, florfenicol 0, gentamicin 0, nalidixic acid 3.8 sulphamethoxazole 21.8, tetracycline 15.3, trimethoprim 15.9. Microbiological cut-off values are according to the Swedish veterinary antimicrobials resistance monitoring programme (SVARM) [1].
No association between resistance patterns and herd incidence of PWD or use of antimicrobials were found.
Conclusion: Sweden has a favourable with regard to resistance in bacteria from animals (SVARM). Occurrence of resistance in this study more common than in data presented for *E. coli* from pigs in SVARM 2005. One explanation could be that in SVARM older pigs are sampled and intestinal bacteria in younger animals usually display higher antimicrobials resistance [2,3].

Reference(s)

[1] www.sva.se
[2] Mathew AG, Saxton AM, Upchurch WG et al. Multiple antibiotic resistance patterns of *Escherichia coli* isolates from swine farms. Appl Environ Microbiol 1999; 65: 2770–2.
[3] Khachatryan AR, Hancock DD, Besser TE et al. Role of calf-adapted *Escherichia coli* in maintenance of antimicrobial drug resistance in dairy calves. Appl Environ Microbiol 2004; 70:752–7.

R2134 Resistance dynamics and multidrug resistance among *Klebsiella* spp. and *Pseudomonas aeruginosa* in severe sepsis

A. Slavcovici, M. Flonta, M. Lupse, N. Hagau, A. Almas, A. Dicea, C. Cismaru (Cluj-Napoca, RO)

Objective: The aim of this study is to assess the prevalence and trends of antibiotics resistance for *Klebsiella* spp. and *P. aeruginosa* strains isolated during 8 years period from medical and surgical hospitals.

Methods: There were isolated 288 *Klebsiella* spp. strains and 285 *P. aeruginosa* strains from patients with severe sepsis admitted on medical and surgical wards between 1999–2002 and 2003–2006 in Cluj-Napoca. Identification was performed with API 20E and API 20NE. Antibiotic susceptibility testing was performed by disk diffusion method and API ATB. Expression according to CLSI Guidelines. Strains with resistance at more than 3 antibiotic groups were considered multidrug resistant strains (MDR).
Results: On that two periods, resistance of *Klebsiella* spp. strains to ceftazidim and ciprofloxacin increased from 33% to 54% ($p = 0.007$) respectively from 26% to 30.3%. Amikacin, carbapenems and colistin resistance was constantly low. The frequency of ESBL strains significantly increased from 21% to 34.8% ($p = 0.017$). MDR strains were detected in 21.8% respectively in 23% of cases. 50.6% of these strains were isolated in nosocomial infections. There was significantly increasing resistance to ceftazidim and amikacin from 63% to 73% and from 25% to 37.5% ($p = 0.03$) in *P. aeruginosa* strains. Colistin and carbapenems resistance remain low. Comparing the two periods we detected significant increasing of MDR strains from 23.7% to 43.75% ($p = 0.0003$). 77.3% of these strains were isolated in nosocomial infections.
Conclusions: *Klebsiella* spp. and *P. aeruginosa* strains isolated from patients with severe sepsis demonstrate increasing trends of resistance to ceftazidim, ciprofloxacin and amikacin correlated with nosocomial infections. Carbapenems and colistin remains the only reliable antibiotics against MDR strains.

R2135 Investigation of the gentamicin MIC values by e test method in the Gram-negative bacteria isolated from different clinic samples

F. Yildirim, G. Sengoz, K. Kart Yasar, O. Nazlican (Istanbul, TR)

Objectives: *Pseudomonas* and *Acinetobacter* species and enteric Gram-negative bacilli isolated from various materials sent from intensive care unit (ICU) and other clinics of our hospital were examined for their gentamicin resistance.
Methods: The 100 bacteria examined within this study were identified using conventional methods and API-ID 32E. Gentamicin resistance was studied with E test according to the recommendations of CLSI.
Results: 48 of them were enteric Gram-negative bacilli (6 *Klebsiella* spp., 7 *Enterobacter* spp. and 35 *E. coli*), and 40 of them were *Acinetobacter*, and 12 of them were *Pseudomonas*. 51 of the strains were isolated from the materials sent from ICU and 49 from other clinics. 85% of the *Acinetobacter* strains were isolated from ICU. But the ratio of ICU strains was 20% in enteric Gram-negative bacilli.
Pseudomonas strains isolated from the ICU and other clinics were approximately the same rate. 29% of the *E. coli* strains were found from urine and 20% of the *Acinetobacter* strains were isolated from sputum and abscess. The resistance in *Pseudomonas* and *Acinetobacter* strains was over 40%. In the Enterobacteriaceae family, the resistance was 14.5%. Among *Pseudomonas* strains, resistance ratio was higher in materials sent from ICU (57%) than materials obtained from other clinics (20%). However in *Acinetobacter* strains there was an opposite situation: the resistance in ICU samples was 38% and in other clinical materials was 80%. These resistant strains from other clinics were taken from diabetic foot wounds. Among the enteric Gram-negative bacilli, the resistance rates were similarly low in strains isolated from ICU and other clinics. (10% and 15.7% respectively)
Conclusion: In our hospital Since 1994 gentamicin has been administered freely whereas usage of other agents have always been under control of microbiologists. However almost for the last 10 years aminoglycoside resistance declined considerably due to wide usage of quinolones.

R2136 Antibiotic resistance of *Stenotrophomonas maltophilia* in children with cystic fibrosis to the currently used antibiotics

H. Alexandrou-Athanasoulis, S. Doudounakis, K. Samantas, J. Papavasiliou, C. Baclakou, A. Pangalis (Athens, GR)

Stenotrophomonas maltophilia (SM) is an important emerging pathogen which has been cultured with increasing prevalence from the sputum of patients with cystic fibrosis (CF).
The aim of our study is to determine the resistance to currently used antibiotics for the treatment of these patients.
Material and Method: Eighty seven *Stenotrophomonas maltophilia* isolates from sputum and deep throat cultures, which were obtained from 87 patients with cystic fibrosis, during 2000–2006, were examined. The isolates were stored at −70ºC, thawed and subcultured twice on blood agar. The identification of the isolates was made by the API 20NE identification system (bioMérieux, France) and the antibiotic resistance was determined with the E test strips (AB Biodisk, Sweden) on Muller Hinton agar according to CLSI recommendations.
Results: (1) The resistance (%) to ticarcillin/clavulanic acid, co-trimoxazole, minocycline, ciprofloxacin, levofloxacin and colistin were 25.3, 36.0, 1.6, 22.5, 7.8, and 48.3 respectively.
(2) The MIC_{50} and MIC_{90} of ticarcillin/clavulanic, co-trimoxazole, minocycline, and levofloxacin are seen in Table 1.

Table 1

Antibiotic	MIC (mg/L)		
	Range	MIC_{50}	MIC_{90}
Ticarcillin/clavulanic acid	0.5–256	8	128
Co-trimoxazole	0.125–32	0.75	24
Minocycline	0.125–16	0.75	2
Levofloxacin	0.125–32	1	6

Conclusions: (1) The most effective antibiotic for our isolates of Sm is minocycline, followed by levofloxacin, co-trimoxazole and ticarcillin/clavulanic acid. (2) The multidrug resistant isolates were 3.4%.

R2137 First report of methicillin-resistant *Staphylococcus aureus* strain of animal provenience in the Czech Republic

J. Bardon, M. Kolar, P. Sauer, D. Koukalova, J. Petrzelova, L. Cekanova (Olomouc, CZ)

Objectives: Bacterial resistance to antimicrobial agents has become a serious problem both in human and veterinary medicine. It is not unrealistic to presume that multiresistant bacteria may spread from animal to human populations by foods of animal origin as well by persons working e.g. in livestock breeding. Therefore, the study aimed at determining the susceptibility of *Staphylococcus* sp., *Enterococcus* sp. and *Escherichia coli* strains to antimicrobial agents in pigs bred in one of the regions of the Czech Republic.
Methods: The tested strains were isolated from bred pigs (piglets aged 7 to 30 days). Bacterial species were identified by standard microbiological techniques and susceptibility to antibiotics was determined quantitatively by the standard microdilution method. Resistance of the *Staphylococcus aureus* strain to oxacillin was confirmed by detection of the mecA gene and PBP2a. Susceptibility of staphylococci to penicillin was determined by production of B-lactamase, using the chromogenic cephalosporin method. Susceptibility to clindamycin, if simultaneous with resistance to erythromycin, was verified by modified disk diffusion test.
Results: From the piglet rectal swabs, a total of 115 strains of *Staphylococcus* sp., 61 strains of *Enterococcus* sp. and 111 strains of *Escherichia coli* were isolated. In the case of staphylococci, the methicillin-resistant *Staphylococcus aureus* strain (MRSA) was identified. Moreover, higher frequency of coagulase-negative staphylococci with minimum inhibitory concentration of oxacillin $\pm$ 0.5 mg/L was noticed. Inducible

resistance to clindamycin in the *Staphylococcus* hominis strain was also detected. The strains of *Enterococcus* sp. exhibited high resistance to tetracycline (98.5%), erythromycin (86.8%) and chloramphenicol (54.4%). Vancomycin-resistant enterococci were not isolated. In the case of *Escherichia coli* strains, higher frequency of resistant strains to tetracycline (81.1%) and ampicillin (62.2%) was documented. Resistance to fluoroquinolones and production of broad-spectrum B-lactamases was not noticed.

Conclusion: This is the first described case of MRSA being detected in pigs, and apparently in animals generally, in the Czech Republic.

The study was supported by the Czech Ministry of Health Grant Agency project no.1A/8258-3.

R2138 Resistance to antibiotics in coagulase-negative staphylococci in an infectious diseases hospital, Iasi, Romania

O. Dorneanu, E. Miftode, T. Vremera, O. Filip, E. Nastasa, V. Luca (Iasi, RO)

Objectives: 1. To evaluate the resistance of coagulase-negative staphylococci (CoNS) with clinical significance, isolated in the Infectious Diseases Hospital, Iasi, Romania. 2. To assess minimum inhibitory concentration (MIC) of CoNS to oxacillin and vancomycin. 3. To evaluate the accuracy of ATB STAPH 5 (bioMérieux, France) strips in detection of susceptibility of CoNS to oxacillin and vancomycin.

Methods: We have investigated 98 CoNS strains isolated from blood cultures (38 strains), catheters (8 strains), cerebro-spinal fluid (4 strains), pus (8 strains) and urine (40 strains), between 1.01.2004 and 1.11.2006. Clinical significance was supported by presence of prosthetic and indwelling devices, immunocompromised patients' status, isolation of the strain in pure culture from the clinical specimen and the repeated isolation of the same strain over the course of infection. Identification to species level was performed using ID 32 STAPH (bioMérieux, France) strips and susceptibility testing with ATB STAPH 5 (bioMérieux, France). MICs to oxacillin and vancomycin were detected using E test (AB Biodisk, Sweden).

Results: The most frequently encountered CoNS species were *S. epidermidis* (51.3%) and *S. haemolyticus* (33.3%). The overall resistance to oxacillin was high, 63.2% (55% in *S. epidermidis* and 76.9% in *S. haemolyticus*). Methicillin-resistant CoNS (60 strains) were also resistant to fluoroquinolones (60%), gentamicin (60%), erythromycin (63.3%), tetracycline (60%). For *S. epidermidis* MIC_{50} of oxacillin was 0.5 μg/mL and MIC_{90} was 256 μg/mL. For *S. haemolyticus* MIC_{50} of oxacillin was 2 μg/mL and MIC_{90} was >256 μg/mL. We have also detected 2 CoNS strains with modified susceptibility to vancomycin (4 μg/mL). There were 10 disagreements between the susceptibility testing to oxacillin by ATB STAPH 5 strips and E test, thus the accuracy of the commercial method is 89.7%. None of the 2 strains with modified susceptibility to vancomycin was correctly identified by ATB STAPH 5.

Conclusions: Resistance to antibiotics, especially to oxacillin represents a problem in the management of CoNS infections. Vancomycin and quinupristin-dalfopristin remain excellent alternatives for the therapy of severe infections produced by methicillin-resistant CoNS strains. Accuracy of ATB STAPH 5 (bioMérieux, France) strips is only satisfactory for the detection of oxacillin resistance.

R2139 Diversity of bla-type genes in extended-spectrum β-lactamases producing *Klebsiella pneumoniae* strains, isolated in 2003 and 2004 at a hospital in Pretoria, South Africa

L. Meyer, M.M. Ehlers, M.G. Dove, G.F. Weldhagen (Pretoria, ZA)

Multidrug resistance is emerging in many Gram-negative bacteria like *Klebsiella pneumoniae*, which is an important cause of severe nosocomial infections. Since the first extended-spectrum β-lactamases (ESBLs) were reported in *K. pneumoniae* in 1983, the increasing production of ESBLs has become a growing concern worldwide, because of the association with significant longer duration of hospital stay and greater hospital costs. The β-lactamases produced by *K. pneumoniae* has a broad spectrum of antibiotic resistance, and are typically resistant to β-lactam antibiotics commonly used in the hospital setting. Only a few studies have investigated ESBL production in bacterial isolates collected in Africa while only Essack (2001) included *K. pneumoniae*. The aim of this study was to determine the prevalence of the β-lactamases TEM, SHV and CTX-M detected in *K. pneumoniae* isolated from patients with nosocomial infections at Pretoria Academic Hospital. Fifty *K. pneumoniae* isolates obtained from blood cultures reported to be resistant to one or more of the oxyimino-cephalosporins, ceftazidime and cefotaxime, were examined. Detection of the gene sequences coding for TEM, SHV and CTX-M enzymes were performed with genomic DNA extracted from the *K. pneumoniae* isolates. Polymerase chain reaction (PCR) amplification of the target DNA was performed and the resulting gel-electrophoresis patterns were examined. The ESBLs for only the TEM type β-lactamase of *K. pneumoniae* isolates represented 10% and those positive for only the SHV type β-lactamase represented 18%. The ESBLs that were negative for both TEM and SHV type β-lactamase were 6%. None of the 50 isolates examined were positive for the CTX-M type β-lactamase. Since this is a pilot study which included only 50 isolates, it can only give a limited indication of the ESBLs present in the *K. pneumoniae* isolates that may be circulating in the specific nosocomial setting. A larger sample size will enable one to determine the prevalence of the different ESBLs expressed by *K. pneumoniae* in the Pretoria Academic Hospital.

R2140 Antibiotic resistance of *Helicobacter pylori* strains isolated from gastric biopsy specimens in Iran

A. Mohabbati Mobares, F. Haghi Tomatari, M. Amini, R. Hosseini Doust (Tehran, IR)

Helicobacter pylori is associated with gastritis, peptic ulcer, gastric adenocarsinoma and mucosa-associated lymphoid tissue (MALT) lymphoma. Prevalence of high primary and secondary antibiotic resistance of *Helicobacter pylori* is the most common reason for failure in its eradication.

The aim of this study was to determine the incidence of resistance of *H. pylori* to metronidazole, clarithromycin, amoxicillin, tetracycline and cephtazidim.

Forty isolates of *H. pylori* obtained from gastric biopsy specimens. After the culture of biopsy specimens, *H. pylori* identified by Gram stain, colony morphology, and biochemical tests. Susceptibility tests were performed with the MDDM (Modified Disk Diffusion Method) for all antibiotics and E test for determines metronidazol MIC. Resistance rates to metronidazole, clarithromycin, amoxicillin, tetracycline, cephtazidim were 63%, 23%, 3%, 0% and 7% respectively.

Seventy two percent of metronidazole resistance strains had high-level resistance (MIC >256 mg/mL).

In conclusion our finding underlines the importance of local surveillance to detect changing patterns of antibiotic susceptibility in *H. pylori* isolates.

Molecular epidemiology of resistance and resistance genes, strains or serotypes

R2141 Emergence of PER-1 extended-spectrum β-lactamase-producing *Acinetobacter baumannii* clinical isolate in Bulgaria

T. Strateva, V. Ouzounova-Raykova, A. Todorova, I. Mitov (Sofia, BG)

Objectives: Strains of *Acinetobacter baumannii* producing the extended-spectrum β-lactamase (ESBL) PER-1 are widespread in Turkey and Korea, and have also been reported from France and Belgium. Here we describe the emergence of strain of *A. baumannii* producing PER-1 ESBL in Bulgaria.

Methods: A strain of *A. baumannii* 255–00 was isolated on 11 February 2000 from the wound of a male patient aged 65 years in a Clinic of

surgery in Sofia. Antimicrobial susceptibilities were detected by the disk diffusion method and Etest (AB Biodisk, Solna, Sweden) and interpreted according to the NCCLS-2004 recommendations. The ESBL-production was evaluated by the double-disk synergy test. Polymerase chain reaction (PCR) amplification and sequencing of blaPER-1 were performed.
Results: The studied strain of *A. baumannii* was resistant to ampicillin/sulbactam, carbenicillin, piperacillin, ceftazidime, cefoperazone, cefepime, amikacin and gentamicin, but remained susceptible to imipenem, meropenem, ciprofloxacin and polymyxin B. A synergic "ghost zone" was observed between the ceftazidime 30 μg disk and the amoxicillin/clavulanic acid 20/10 μg disk. Using PCR, this *A. baumannii* isolate was identified as PER-1-positive. PER-1 enzyme-mediated resistance was confirmed by sequencing.
Conclusions: The present work illustrates the inter-country spread of PER-1-producing *A. baumannii* isolates. To our knowledge, this is the first report of PER-1 in clinical isolate of *A. baumannii* in Bulgaria.

R2142 Inducible clindamycin resistance in *Staphylococcus aureus* isolates causing bacteraemia at a university hospital in southern Taiwan

R. Janapatla, J. Yan, A. Huang, H. Chen, H. Wu, J. Wu (Tainan, TW)

Background: A major concern while prescribing clindamycin to treat infections caused by inducible macrolide, lincosamide and group B streptogramin (iMLSB) resistant strains is clinical therapy failure.
Objective: To determine the prevalence, mechanism and clonality of the iMLSB phenotype in oxacillin resistant *S. aureus* (ORSA) and oxacillin-susceptible *S. aureus* (OSSA).
Results: Among the 729 OSSA isolates collected from July 1995 to March 2006, 72 (10%) were clindamycin sensitive (Clis) and erythromycin resistant (Ermr), and 55 (8%) had the iMLSB phenotype. In the 709 ORSA isolates collected from January 1997 to March 2006, 31 (4%) were Clis and Ermr and 29 (4%) isolates demonstrated the iMLSB phenotype. In OSSA ermC was the predominant (51 of 55 isolates) genetic determinant responsible for the iMLSB phenotype, while in ORSA ermA was predominant (27 of 29). Pulsed-field gel electrophoresis showed that in ORSA isolates (n = 27) 8 pulsed types (RA to RH) were present and in the OSSA (n = 24) isolates 14 different pulsed types (SA to SN) were identified.
Conclusion: These results indicate that in Taiwan, the incidence of iMLSB resistance phenotype is very high in Clis and Ermr *S. aureus* isolates and the genetic determinants responsible for the iMLSB phenotype vary in OSSA and ORSA.

R2143 Occurrence of CTX-M-3 extended-spectrum β-lactamase producing *Salmonella* Brandenburg in Bulgaria

P. Petrov, G. Asseva, T. Kantardjiev, P. Padeshky, N. Vlahova, I. Ivanov, A. Ivanov, M. Nikolova (Sofia, BG)

Objectives: to study the genetic profile and resistance to antibiotics of a rare serotype of *Salmonella* causing a cluster of cases among infants and children in Bulgaria.
Methods: nine strains of *Salmonella* Brandenburg were isolated from infants and children with acute enterocolitis in the beginning of 2005. Culture, identification, serotyping and antimicrobial susceptibility testing to fourteen antibiotics have been performed. PCR with primers detecting bla-CTX-M genes and PFGE after restriction with XbaI were applied.
Results: a single PFGE type of strains confirmed the occurrence of a cluster of cases caused by *Salmonella* Brandenburg among infants and children. The strain was found to be multidrug-resistant. It produced a CTX-M-3 extended-spectrum β-lactamase.
Conclusion: our findings proved the ethiological role of an unique genetic variant of *Salmonella* Brandenburg in a cluster of cases among infants and children. This is the first report for Bulgaria of *Salmonella* Brandenburg producing a CTX-M-3 extended-spectrum β-lactamase.

R2144 Molecular study of methicillin-resistant *Staphylococcus aureus* bacteraemia in a tertiary care hospital

E. Platsouka, E. Kraniotaki, E. Belesiotou, A. Argyropoulou, E. Perivolioti, O. Paniara (Athens, GR)

Objectives: Bacteraemia is a serious complication in hospitalised patients and nosocomial *Staphylococcus aureus* bacteraemia, in particular with methicillin resistant *S. aureus* (MRSA) is a major problem with treatment difficulties. A molecular analysis of representative MRSA blood isolates of patients was carried out in a Greek tertiary care hospital.
Methods: Between March 2004 and October 2006 a total of 232 *S. aureus* blood isolates were collected, one isolate per patient. Blood bottles were incubated in BACTEC-NR 9240 automatic system according to standard recommendations. They were identified by standard methods and MICs were determined by the broth microdilution method, according to CLSI guidelines. Molecular typing was performed by using PFGE technique, with digestion of genomic DNAs from the staphylococcal isolates by the restriction endonuclease SmaI.
Results: Of 232 *S. aureus* blood isolates, 158 in Medical wards, 36 in Surgical wards and 38 in ICUs, 121 (52.15%) were MRSA and 111 (47.85%) MSSA. The distribution of MRSA bacteraemia in the hospital was: Medical wards 67 (42.40%), Surgical wards 23 (63.89%), ICUs 31 (81.58%). The resulting restriction endonuclease digestion patterns showed four different genotypes with several common bands suggesting a common epidemiologic background, according to Tenover criteria (Tenover et al., 1995).
Conclusion: The incidence of MRSA bacteraemia is higher in ICUs than in the medical and surgical wards. MRSA isolates in ICU patients seems to belong to a common clone. Other typing techniques apart from PFGE, will be performed in order to characterise completely the MRSA clones in our hospital, since the presenting results are preliminary.

R2145 Clonality among high-level gentamicin-resistant *Enterococcus faecalis* and *Enterococcus faecium* isolated from clinical samples

M. Soltan Dalal, M. Hasibi, S. Aghaei, B. Mohajer Iravani, M. Pourshafie (Tehran, IR)

Objectives: During recent years, nosocomial infections caused by enterococci have increased in different countries. Unfortunately enterococci which acquire high level gentamicin resistance (HLGR) become non curable by using the common effective antibiotictherapy protocoles.
Methods: We determined the prevalence of HLGR among clinical enterococcal isolates with disc diffusion method by using Gm (120μg) disk. 726 enterococcal strains were collected from different hospitals and clinical laboratories. Clonality relation between HLGR strains were determined by PFGE and Ribotyping techniques and AAC (6′)-APH(2″) genotype detection was performed by PCR method.
Results: The species distribution percents was 8.5% for *E. faecium* and 91.5% for *E. faecalis*. These figures in HLGR strain were 61.6% and 38.4 for *E. faecalis* and *E. faecium* respectively. PFGE, ribotyping and PCR techniques classified these isolates in different genotypes.
Conclusion: Our results suggest dissemination of genetically related HLGR strains in nosocomial infection and indicate that high prevalence of HLGR enterococcal colonisation is an important problem in our medical centres.

R2146 Detection of extended-spectrum β-lactamases, including the CTX-M-type enzymes, among Enterobacteriaceae using VITEK 2 system and the Advanced Expert system

S. Ferreira, A. Paradela, A. Ribau, E. Ramalheira, S. Mendo (Aveiro, PT)

Objectives: The aims of the present study were to assess if the VITEK 2 AES could accurately detect ESBL in Enterobacteriaceae isolates, comparing the final data report with the results of phenotypic and genotypic based methods. The susceptibility of ESBL producers to

Ertapenem was also investigated. A special relevance was given to a new emergent CTX-M-type ESBL.
Methods: From April 2006 to October 2006, 35 isolates of Enterobacteriaceae were recovered from 35 inpatients of different wards of the Hospital Infante D. Pedro de Aveiro, Portugal, and identified as extended-spectrum β-lactamases (ESBL) producers by the automatic VITEK 2 system and Advanced Expert System (VITEK 2 AES) (BioMérieux, Marcy L'Etoile, France). The antibiotic susceptibilities were determined by VITEK 2 and the susceptibilities reports were edited after interpretation by AES of the inferred resistance phenotype. ESBL were also detected by Etest® (AB Biodisk) ESBL with Cefotaxime/Cefotaxime + Clavulanic acid and Ceftazidime/Ceftazidime + Clavulanic acid strips, according to manufacturer's instructions. Susceptibility to Ertapenem was tested using disc diffusion method (DDM) (10 ug, Oxoid). PCR, sequencing and sequence analysis was used to assess β-lactamase encoding sequences. Sequences obtained were compared with others deposited in the GeneBank database.
Results: VITEK 2 determined that: for CAZ, 32 isolates were resistant and 3 were susceptible, for CTX, the same result was obtained. AES edited these data as resistance phenotypes, suggesting the production of ESBL. Using the Etest, 34 isolates presumable produced ESBL. One isolate was indeterminate, according to the manufacturer's guidelines. All the isolates identified as ESBL producers were sensitive to Ertapenem. PCR specific for CTX-M encoding sequences showed the presence of this gene in 14.3% of the isolates bearing an ESBL.
Conclusion: This study demonstrated the capacity of VITEK 2 AES to detect and interpret resistance mechanisms, inferring with a high level of confidence the presence of isolates ESBL producers. Also shows that Ertapenem is effective against ESBL producers. CTX-M-1 and CTX-M-15 were the CTX-M-type enzymes found among the Enterobacteriaceae population studied.

R2147 First detection of VIM-1 type metallo-β-lactamase in a multidrug-resistant *Klebsiella pneumoniae* clinical isolate from Turkey also producing the CTX-M-15 extended-spectrum β-lactamase

I. Yildirim, M. Ceyhan, D. Gur, C. Mugnaioli, G.M. Rossolini (Ankara, TR; Siena, IT)

Currently the most prevalent and widespread acquired MBLs are the IMP- and VIM-type enzymes, although other types (SPM, GIM and SIM) have recently been identified.
In Turkey, the only MBL thus far reported is VIM-5, a variant of the VIM-1-like sublineage, in clinical isolates of *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Enterobacter cloacae*. During a survey to detect acquired MBLs in 127 nonreplicate Gram-negative isolates that were resistant or intermediate to carbapenems and/or resistant to ceftazidime in a tertiary centre in, Ankara, Turkey, during the period March 2005-March 2006, a single *K. pneumoniae* isolate was found to be positive to the MBL phenotypic screening using the IPM-EDTA combo disk test and the 2-MPA double-disk synergy test. Production of MBL activity was confirmed by spectrophotometric assay as described previously. This isolate (*K. pneumoniae* HU41) was cultured from the urine of a 3-year-old girl in May 2005. *K. pneumoniae* isolate HU41 had also an ESBL-positive phenotype, as detected by CLSI recommendations. The HU41 isolate was also resistant to carbapenems (the MIC of imipenem was 128 mg/L while the MICs of meropenem and ertapenem were 16 mg/L) Enterobacteriaceae producing MBLs have often been reported to remain susceptible to carbapenems, despite increased carbapenem MICs. This isolate, however, exhibited a frank resistant phenotype to all carbapenems suggesting the presence of additional resistance mechanisms such as decreased outer membrane permeability.
PCR analysis, performed with primers specific for blaVIM and blaIMP genes, yielded a 523 bp-amplification product with the blaVIM-specific primers. The presence of a class 1 integron was detected by PCR amplification with primers designed on 5′- and 3′-conserved segments as described previously. An amplification product of 2.3 kb was obtained. Direct sequencing revealed the presence of two inserted gene cassettes: blaVIM-1 and aac(6′)Iic with the same integron variable region in an *Enterobacter cloacae* from Greece, which is currently the European country most affected by dissemination of the VIM-1 MBL in Enterobacteriaceae, suggesting a regional spread of the element carrying the MBL gene. To our knowledge; this is the first report of a VIM-1 type MBL enzyme in Turkey. PCR and direct sequencing for detection of common ESBL genes (TEM-, SHV- and CTX-M-type) revealed the presence of a blaCTM-15 gene that was consistent with the ESBL phenotype.

R2148 Prevalence of low-level quinolone resistance mediating genes in extended-spectrum β-lactamase producing *Klebsiella* spp. and *Escherichia coli* strains from Slovenia

J. Ambrozic Avgustin, R. Keber, K. Žerjavic, T. Oražem, M. Grabnar (Ljubljana, Maribor, SI)

The occurrence of plasmid-mediated low-level quinolone resistance genes among uropathogenic extended-spectrum β-lactamase (ESBL) producing *Klebsiella* sp. and *Escherichia coli* strains was studied. 74 non-repetitive *Klebsiella* sp.and 27 *E. coli* strains from various sources in the Ljubljana region in Slovenia were screened by PCR and conjugation. None of the known low-level quinolone resistance mediating qnr genes was found, although the MICs of ciprofloxacin for several transconjugants were almost uniformly elevated when compared with the recipient strain J53Azr. The resistance determinant was cloned, sequenced and identified as the aac(6′)-Ib-cr variant of the aminoglycoside acetyltransferase gene aac(6′)-Ib.
Two single nucleotide substitutions in the wild type allele enable the aac(6′)-Ib-cr gene product to confer low-level quinolone resistance as it was described recently. A combined PCR-RFLP protocol was prepared and used for detecting either the wild type or mutant allele in the strain collection. The aac(6′)-Ib-cr allele was found in 50% of the ESBL *Klebsiella* sp. strains and in 40% of the ESBL *E. coli* strains. The emergence of the aac(6′)-Ib-cr gene coincides with increased number of fluoroquinolone resistant ESBL strains and the extinction of the intermediate resistance phenotype according to the CLSI standards. This indicates a possible role of low-level plasmid-mediated quinolone resistance determinants enhancing the selection of chromosomal mutations and resulting in the occurrence and dissemination of clinically relevant resistance levels. Thus, it is tempting to speculate, that the aac(6′)-Ib-cr gene, being present with ESBL genes in the same integron, may be at least one of the reasons for arising flouroquinolone resistance of ESBL strains in Enterobacteriaceae.

R2149 Clonal dissemination of vancomycin-resistant *Enterococcus faecium* isolates in two Russian centres over a 3-year period

A. Mironova, G. Kliasova, A. Brilliantova, S. Sidorenko (Moscow, RU)

Background: The purpose of this study was to evaluate the molecular relatedness of clinical isolates of vancomycin-resistant *Enterococcus faecium* (VREF) collected from two haematological centres in Russia.
Methods: A total of 123 VREF strains were isolated from two centres between January 2004 and September 2006. VREF included 119 strains from centre I and 4 strains from centre II. Isolates were from stool (108), urine (7), throat (3), blood (1), and other samples (4). Resistance genes were detected by PCR. Macrorestriction analysis of SmaI digests was performed by pulsed-field gel electrophoresis. PFGE patterns were then compared using BioNumerics 3.0.
Results: PFGE typing revealed 21 strain types of VREF. Among VREF included in the study isolates carrying vanA genes were predominant 114 (93%), they belonged to 19 clonal types. Two PFGE types (A and F) were most prevalent 69% and 11% respectively. Type A isolates were detected in centre I in 2004, and appeared in centre II in 2006. Type F isolates were detected in centre II in 2004 and appeared in centre I in 2005. Clinically significant infections caused by VREF were observed in three patients in centre I, one caused by type A isolate and two – by type F

isolates. In centre I strains of VREF were prevalent in two departments – intensive care and haematology. Isolates from intensive care department were more heterogeneous than from haematology department. vanB genes were detected in 9 (7%) of VREF isolates. Eight of them belonged to two PFGE types and were detected only in centre I, one remaining isolate was clonally unrelated and was isolated in centre II. Two isolates of the same PFGE type were carrying two different resistance genes (one – vanA, and one – vanB).
Conclusion: The majority of VREF isolates belonged to the two predominant PFGE types. Clonal inter- and intrahospital dissemination was responsible for most of the spread of VREF.

R2150 Extended-spectrum β-lactamases and plasmid-mediated AmpC enzymes among *Enterobacter cloacae* strains isolated from cardiovascular prosthetic devices associated infections

R. Cernat, C. Balotescu, V. Lazar, O. Banu, D. Ivanescu, M. Bucur (Bucharest, RO)

Objective: The purpose of this study was to establish the frequency of resistance to broad-spectrum cephalosporins of *E. cloacae* strains isolated from patients with cardiovascular prosthetic implants hospitalised in a Romanian institution and to characterise the types of ESBL produced.
Material and Methods: During 2003–2006, 120 *E. cloacae* strains were recovered. ESBL-producing enzymes were screened by double-disk synergy test or positive ceftazidime and cefotaxime clavulanic combination discs tests. MICs of 18 antibiotics were performed by microdilution broth method against ESBL-positive strains. Isoeletrofocusing (IEF) and PCR with specific primers were used to confirm and type β-lactamase genes in ESBL producers: blaTEM, blaOXA, blaSHV, blaCTX-M and ampC. When IEF and PCR indicated concordant results, final identification of ESBL enzymes was obtained by sequence analysis. PFG with XbaI-digested genomic DNA established the diversity of ESBL-positive clones.
Results: 46 of 120 (38.3%) strains were confirmed as ESBL producers and showed a multiresistance phenotype. All strains had the ubiquitary blaCTX-M, blaTEM genes plus AmpC gene. Sequencing of blaCTX-M, blaTEM amplicons identified that all the strains encode the TEM-1A enzyme, 20 the CTX-M-15 and 5 the CTX-M-3; these enzymes were characterised with pIs of 5.4 and between 8.0 and 9.0 respectively. PFGE profiles of these strains indicated that only five clones were related.
Conclusion: This study illustrates a high frequency of polyclonal dissemination of resistance to broad-spectrum β-lactams in a hospital in Romania through CTX-M ESBL, most probably facilitated by mobile elements such as gene cassettes for which preliminary results indicated to be carried by the class 1 integrons. Our results suggest that in Romania also the therapeutic options may be dramatically diminished if CTX-M enzymes continue spreading among Enterobacteriaceae implicated in the aetiology of bacterial infections occurred in hospitalised patients with cardiovascular prosthetic devices.

R2151 Prevalence of mutations in PBP2x of *Streptococcus pneumoniae*

S.A. Grudinina, T.A. Savinova, E.N. Ilina, S.V. Sidorenko (Moscow, RU)

Objectives: Mutations in penicillin-binding proteins (PBP) are the main mechanisms of *Streptococcus pneumoniae* (Spn) resistance to β-lactams, and PBP2x is a primary determinant. There are two groups of PBP2x amino acid substitutions (AAS) in clinical strains: one group is characterised by T338A substitution; other group composed of sequences containing the Q552E substitution. The two groups of proteins display reduced reactivity towards β-lactam owing to two different mechanisms which may sometimes be combined as a few sequences can harbour mutations at both positions 338 and 552. We evaluate the prevalence of these and other AAS's in clinical Spn isolates from Russia.
Methods: Spn isolates (n = 75) with different MIC's of β-lactams were studied. Mutations in PBP2x genes were detected in thermocyclic primer extension reaction, followed by mass-spectrometric detection of the products.
Results: Results of susceptibility testing and detection of AAS are shown in the Table. All isolates lacking AAS of interest were susceptible to cefotaxime (according to the current CLSI meningitis break-points), but 5 of them were intermediate to penicillin. PBP2x harbouring T338A substitution in combinataion with other AAS's are the most prevalent among Spn isolates in Russia. Presence of four AAS's (T338A, I371T, R384G, N605T) predicts nonsusceptibility to penicillin, but in 11 of 34 isolates with current genotype MIC's of cefotaxime was in susceptible range. MIC's of β-lactams against isolates with other combinations of AAS's including T338A were significantly lower. MIC's of β-lactams against isolates with Q552E AAS's were in susceptible/intermediate range. We were able to find only one isolate carrying both T338A and Q552E AAS's simultaneously.

AAS in PBP2x	Distribution of isolates according to Cefotaxime/penicillin MIC (mg/L)			
	⩽0.06	0.125–0.5	1.0	2.0–4.0
No AAS (n = 17)	14/12	3/5	0/0	0/0
T338A, I371T, R384G, N605T (n = 34)	2/0	9/6	16/3	7/25
T338G, I371T, R384S, Q552E, N605T (n = 1)	0/0	0/0	0/0	1/1
T338A, I371T, R384G (n = 1)	1/0	0/1	0/0	0/0
T338A, R348G, N605T (n = 3)	1/1	2/1	0/0	0/1
T338A, R384G (n = 10)	10/1	0/9	0/0	0/0
I371T, N605T (n = 1)	0/0	1/1	0/0	0/0
R384G, Q552E (n = 5)	2/0	3/5	0/0	0/0
Q552E (n = 3)	1/0	2/3	0/0	0/0

Conclusions: Detection of a limited number of AAS's in PBP2x can be used for the prediction of susceptibility to cefotaxime Spn meningitis isolates.

R2152 Detection of class I integrons of *Aeromonas* ssp. strains isolated from diarrhogenic disease

A. Pérez-Valdespino, E. Fernandez Rendon, M. Sanchez Mendoza, E. Curiel Quesada (Distrito Federal, Pachuca, MX)

Introduction: Since the early 90's an increase to antimicrobial resistance in *Aeromonas* sp. has been observed worldwide. *Aeromonas* sp. has showed to contain several resistance determinants like transposons, plasmids and integrons. We have searched for these genetic elements in strains isolated from patients with diarrhogenic diasease, in order to characterise and relate them to those found in environmental and food-born strains.
Methods: We have studied 41 *Aeromonas* sp. strains isolated from diarrhogenic faeces. They were identified by 16S/rDNA PCR-RFLP. Susceptibility tests for 28 antibiotics were performed by the Kirby Bauer method (see Table 1). Plasmid DNA was extracted according to Birmboin and Doly et al. Primers for PCR for 5′ and 3′ portions of class I integrons were those described by Zhang et al. 2004. A set of primers were designated to variable region of integron class I.
Results: From a total 41 strains we identified *A. caviae* (n = 27), *A. hydrophila* (n = 10), *A. veronii* (n = 3), A. media (n = 1). All strains were resistant to: ampicillin, amikacin, vancomycin, bacitracin, oxacyllin, sulfadiazine and penicillin but sensitive to: cefotaxime, norfloxacin, aztreonam, gentamycin, neomycin. The results of other susceptibility tests are shown on table 1. 36% (15/41) strains yielded the 920bp product of 5′CS region of the class I integrase gene. The 800bp product from the 3′CS of the integron was amplified only in 9 out of these 15 strains. Amplification of the variable region yielded bands ranging from 1800bp to 3700bp. 32% (13/41) strains contained plasmids, all of 13 strains with plasmids showed different electrophoretic patterns. 36.6% (15/41).
Conclusions: A third of the strains possess plasmids and class I integrons was detected in nine strains. Currently, these genetics elements have been characterising in order to know their relationship with the antimicrobial resistance.

Table 1. Resistance percentage in *Aeromonas* spp.

Antibiotic	% Resistance (n = 41)
Imipenem	2%
Kanamicin	2%
Ciprofloxacin	3%
Azitromicin	5%
Chloramphenicol	5%
Erythromicin	12%
Piperacillin	12%
Streptomycin	17%
Tetracycline	39%
Nalidixic acid	41%
Trimetropim	53%
Rifampicin	88%
Mercurio	54%

In vitro antibacterial susceptibility & drug interaction studies

R2153 Inhibitory effect of probiotic and non-probiotic micro-organisms against *Helicobacter pylori* clinical isolates

J. Diaz-Regañon, T. Alarcon, D. Domingo, D. Monclus, M.J. Moreno, M. Lopez-Brea (Madrid, ES)

Objective: To determine the effect of 15 probiotic, and 17 non-probiotic (8 Gram-positive and 9 Gram-negative microorganisms) against *Helicobacter pylori* clinical isolates.
Material and Methods: *H. pylori* strains were isolated from 35 gastric biopsies and processed by standard microbiological methodology. Several probiotic and non-probiotic bacteria were obtained from several specimens following standard microbiological procedures and were identified by Gram stain and MicroScan (Dade–Behring). The following bacteria were tested: probiotics: 3 *Lactobacillus* spp., 2 *Lactococcus lactis lactis*, 4 *Streptococcus* spp., 2 *Bacillus* spp., 3 *Enterococcus* spp. and 1 *Saccharomyces cerevisiae*, and non-probiotics: 8 *Staphylococcus* sp., 2 *E. coli*, 2 *Klebsiella* spp., 1 *P. aeruginosa*, 1 *Salmonella* GDF9, 1 *A. baumannii*, 1 *E. cloacae* and 1 *S. maltophilia*.
The effect of these microorganisms on *H. pylori* was determined by the "drop" method: A blood agar plate was completely inoculated with *H. pylori* and a drop was deposited containing 10 μL of the microorganism after 0.5 McFarland concentrations. Plates were incubated at 37ºC under microaerobic atmosphere for 3–5 days. Lecture was made by the diameter of the inhibition zone observed after incubation.
Results: Among probiotics 2 *Lactobacillus* spp. showed inhibitory activity against 5 and 6 out of the 30 *H. pylori* strains tested, *Bacillus* spp. inhibited 11 out of 27 tested, 1 *E. faecium* inhibited 1 out of 27 tested and *S. cerevisiae* inhibited 7 out of 27 *H. pylori* strains tested. *Staphylococcus* spp. showed inhibitory effect against *H. pylori* strains: *S. hominis* against 5 out of 35, 1 *S. auricularis* against 7 out of 27, 1 *S. auricularis* against the 37 strains tested, 2 *S. epidermidis* against the 27 and 35 strains tested, *S. warneri* against 1 out of 35, *S. cohni-cohni* against 1 out of 35 and *S. aureus* against 6 out of 35. Among Gram-negative bacteria one *E. coli* strain was able to inhibit the growth of 1 out of 25 *H. pylori* clinical isolate tested. *K. pneumoniae*, *A. baumannii* and *Salmonella* GDF9 were able to inhibit the growth of 4 out 25 strains each. *E. cloacae* and *S. maltophilia* were able to inhibit the growth of 14 out 25 *H. pylori* isolates each.
Conclusions: 3 *Staphylococcus* spp. strains (1 *S. auricularis* and 2 *S. epidermidis*) were able to inhibit the growth of all *H. pylori* strains tested. Several probiotic and non-probiotics bacteria were able to inhibit some *H. pylori* strains.
Acknowledgement: The study was supported by FIS PI 052442.

R2154 ESBL-producing Enterobecteriaceae and in vitro evaluation of tigecycline

E. Chinou, P. Davlouros, M. Georgouli, E. Skoutelli, A. Kamagiani, P. Golemati (Athens, GR)

Objectives: This study was performed to investigate the prevalence of ESBL producers in Enterobecteriaceae isolated from oncology patients and to examine the in vitro activity of a new class of agents, tigecycline against these strains.
Materials and Methods: We studied 156 nonreplicate isolates of Enterobecteriaceae recovered over four months period (June to October 2006) from clinical specimens. Isolates were identified using Api 20E. ESBL production was screened for by MIC analysis (Microscan Gram negative) and confirmed by the double-disk synergy test. To examine the activity of tigecycline, we used the disk diffusion test on Mueller Hinton agar.
Results: A total of 102 *E. coli*, 38 *K. pneumoniae*, 10 *K. oxytoca* and 6 *E. cloacae* strains were detected among 156 Enterobecteriaceae isolates. 33.3% of clinical isolates were identified as ESBL producers (42 *E. coli* and 10 *K. pneumoniae*). The most efficient antibiotics for ESBLs were the carbapenems imipenem and meropenem (94.2% sensitive), amicacin (90.5%) and ciprofloxacin (62%). Tigecycline was active against all the tested strains of *E. coli*, *K. oxytoca* and *E. cloacae*. 5 strains of *K. pneumoniae* were resistant to tigecycline and sensitive only to carbapenems.
Conclusions: We detect an increase of ESBLs isolates. Their expression was usually associated with multidrug resistance. The invitro activity of tigecycline against Enterobecteriaceae and especially against ESBL producers was encouraging.

R2155 Antibiotic susceptibility of Yersinia strains in Kazakhstan

L. Nekrassova, T. Meka-Mechenko, A. Aikimbayev, G. Temiralieyva (Almaty, KZ)

Objectives: Study of the regional peculiarities of sensitivity to antibiotics of Yersinia strains may to serve the considerable requisite for the rational using of antibacterial preparations in the treatment of Yersinioses.
Methods: The method of serial dilution on the agar was applied to comparative assay of antibiotic sensitivity. It was determined antibiotic susceptibility 200 strains different kinds of Yersinia.
Results: High sensitivity *Y. pseudotuberculosis*, *Y. enterocolitica*, *Y. kristensenii*, *Y. intermedia*, *Y. frederiksenii* to streptomycin and to a lesser degree to chloramphenicol is established. On the literary data of significant distinctions in sensitivity Yersinia to these antibiotics is not present. Probably, this fact also specifies territorial feature of the activators of Yersinioses circulating in Kazakhstan. Now there is no though some an appreciable tendency to development resistance to streptomycin, chloramphenicol and tetracycline in strains of *Y. pseudotuberculosis*, *Y. enterocolitica*.
However, the quantity resistant forms to these antibiotics in strains, isolated in natural landscapes are a little bit lower. In number of areas of Kazakhstan which anthropogenous influence has affected are found out in strains of *Y. pseudotuberculosis* and *Y. enterocolitica* with resistance to streptomycin, tetracycline. These strains were isolated from wild rodents. Other species of Yersinia (*Y. kristensenii*, *Y. frederiksenii*, *Y. intermedia*) were differed by the big number of resistance forms, irrespective of source of isolation. Occurrence of a plenty resistance to streptomycin, chloramphenicol and tetracycline forms of these species Yersinia can be specific and territorial features of these species. It is impossible to exclude penetration of resistance variants in the natural foci from anthropurgical.
Conclusion: There are distinctions by quantity antibiotics resistance forms in epizootic foci exposed and not exposed anthropogenous influence. In the foci exposed to anthropogenous influence the quantity of such strains is much higher.

R2156 Analysis of tigecycline activity against *Staphylococcus aureus* based on clinical specimen source

D. Draghi, M. Dowzicky, N. Brown, M. Jones, C. Thornsberry, D. Sahm (Herndon, Collegeville, US; Breda, NL)

Objective: Tigecycline (TIG), the first in class glycylcycline available for clinical use, has been approved in the US (2005) and EU (2006) for complicated skin and soft tissue infections and for complicated intra-abdominal infections. Because the susceptibility patterns of target pathogens can vary with the clinical specimen sources, the current activity of TIG was analysed according to specimen source (SPEC) from which *S. aureus* (SA) isolates were obtained.
Methods: SA isolates were collected from 64 hospital sites distributed among all nine US Bureau of Census regions and 11 hospital sites distributed across 4 countries in Europe (EU; Germany, Italy, Spain, and France). All SA were isolated from clinical specimens in 2005–2006 and centrally tested against TIG and comparators by broth microdilution (CLSI guidelines M7-A7). The SPECs studied were blood [BD], respiratory [RP], and skin and soft tissue [SST]). Additionally, TIG activity was assessed according to oxacillin-susceptible (OX S) and -resistant (OX R) populations. US FDA breakpoints (BPs) were applied to results from isolates that originated from the US and EUCAST BPs were applied to results from isolates that originated from EU.
Results: see the table.

SPEC	Phenotype	USA				EU			
		Total	MIC (mg/L)		%S	Total	MIC (mg/L)		%S
			Range	MIC_{90}			Range	MIC_{90}	
SST	All	1004	⩽0.008–1	0.25	99.9	233	0.03–0.5	0.25	100
	OX S	475	⩽0.008–0.5	0.25	100	196	0.03–0.5	0.25	100
	OX R	529	0.06–1	0.25	99.8	37	0.03–0.25	0.25	100
RP	All	451	0.06–0.5	0.25	100	86	0.015–0.5	0.25	100
	OX S	200	0.06–0.5	0.25	100	62	0.015–0.5	0.25	100
	OX R	251	0.06–0.5	0.25	100	24	0.06–0.5	0.25	100
BL	All	347	0.06–0.5	0.25	100	66	0.03–0.5	0.25	100
	OX S	162	0.06–0.5	0.25	100	57	0.03–0.5	0.25	100
	OX R	185	0.06–0.5	0.25	100	9	0.06–0.25	100	

Conclusions: Based on susceptibility patterns, TIG demonstrated a high level of activity against SA including oxacillin-resistant isolates. This level of activity did not vary with either the geographic (US or EU) or clinical source of the isolates. While TIG currently exhibits potent activity against SA, the ability of this organism to develop resistance to a variety of antimicrobials warrants continued surveillance as TIG use to treat infections increases.

R2157 Antimicrobial susceptibility of 2,254 isolates from different body sites: Tigecycline Evaluation Surveillance Trial (TEST) in South America, 2004–2006

R. Badal, S. Bouchillon, B. Johnson, M. Hackel, J. Johnson, D. Hoban, M. Dowzicky (Schaumburg, Collegeville, US)

Background: Efficacy of antimicrobials continues to be eroded by development and spread of bacterial resistance. Surveillance studies can help guide appropriate use of antimicrobials by understanding current trends in susceptibility. The Tigecycline Evaluation Surveillance Trial (TEST) is an ongoing global study that can serve to help recognize these trends on many levels. This report evaluates differences in susceptibility of strains collected from different body by investigators in South America 2004–2006.
Methods: 2,254 isolates were collected and identified from 2004–2006 at 12 hospitals in Argentina, Chile and Brazil. MICs for each strain were determined per CLSI guidelines at each facility using broth microdilution. MIC_{50}/90 values were analysed to identify any significant differences in antibiograms from different sources.
Results: Minimal differences were seen in susceptibility patterns of evaluated pathogens and specimen sources. MIC_{50}/90 values for all organism/specimen source pairings were usually ± 2 dilutions of each other, but statistical differences were noted between body site groups for all drugs ($p < 0.001$) and the patterns varied by drug.
Conclusions: Antibiograms of bacteria isolated from different body locations were generally similar, with significant differences of sensitivity patterns that varied by drug. TIG's demonstrated broad spectrum of activity and consistently low MIC_{90}/MIC_{50} ratios, including strains resistant to other drugs should establish its role as an important addition to hospital formularies.

R2158 Antimicrobial susceptibility of *Streptococcus pneumoniae* isolated from lower respiratory tract infections – Therapeutic consequences

C. Popescu, G.A. Popescu, E. Benea, M. Popoiu, M. Ivan, L. Gavriliu, A. Paise (Bucharest, RO)

We analysed the resistance rate of pneumococcal strains, isolated from the patients with lower respiratory tract infections.
Methods: retrospective study of lower respiratory tract infections admitted in Matei Bals Infectious Diseases Institute between 1.01.2004 and 31.03.2005.
Results: 47 cases: 37 cases of pneumonia and 10 cases of acute exacerbation of chronic bronchitis; 5 cases had bacteraemia with *Streptococcus pneumoniae*. The rate of antimicrobial susceptibility were: 100% for moxifloxacin (95% CI: 86.7–100%), ceftriaxone 92.3% (95% CI: 79.7–97.5%), clindamycin 78.6% (95% CI: 64.1–88.3%), erythromycin 69.4% (95% CI: 55–81.4%), tetracycline 68.4% (95% CI: 52.5–80.9%), penicillin 67.4% (95% CI: 53–79.1%), TMP/SMX 40% (95% CI: 25.5–56.4%).
Conclusions: The resistance rate for co-trimoxazole, penicillin, erythromycin and tetracyclin indicates that these antimicrobial cannot be used like first choice therapy in lower respiratory tract infections. The failure risk for respiratory fluorquinolones and third generation cephalosporines is low.

R2159 A survey of multi-drug resistance clinical pathogens in Turkey – T.E.S.T. Program, 2006

S. Bouchillon, R. Badal, M. Hackel, J. Johnson, D. Hoban, B. Johnson, M. Dowzicky (Schaumburg, Collegeville, US)

Background: The T.E.S.T. Program determined the in vitro activity of tigecycline compared to broad spectrum antimicrobials against Gram-negative and Gram-positive species collected from two hospitals within Turkey throughout 2004–2006.
Methods: Clinical isolates were identified to the species level and confirmed by a reference laboratory. Minimum Inhibitory Concentration (MICs) were determined by the local laboratory using supplied broth microdilution panels and interpreted according to CLSI guidelines.
Results: Out of 347 isolates collect, 128 (37%) were determined to be multi-drug resistant (MDR) as defined by resistant to 3 or more antimicrobial drug classes. *A. baumannii* had the highest percentage of MDR strains with 26/30 (86%) followed in order by *K. pneumoniae* 22/43 (51%); *E. cloacae* 9/18 (50%); *E. coli* 25/54 (46%); *P. aeruginosa* 16/40 (40%) *S. marcescens* 9/23 (39%) and *S. aureus* 15/50 (30%). Of these, 80% or better were nosocomial infections in adults over 30 years age. Tigecycline inhibited >98% of the Gram-negative MDR strains (excluding *P. aeruginosa*) at 2 μg/mL, equal to imipenem. All 16 MDR Gram-positive strains (13 MRSA, 3 *E. faecium*) were inhibited by linezolide at 4 μg/mL, vancomycin at 32 μg/mL and tigecycline at 0.5 μg/mL.
Conclusions: Turkey had the highest level of MDR strains (37%) of any country seen to date in the T.E.S.T. Program. Tigecycline remained active against more than 98% of all the MDR strains (excluding *Pseudomonas*) from these sites with activity similar to linezolid and vancomycin against Gram-positive strains and equal to or more potent than imipenem against Gram-negative strains.

R2160 Tigecycline antibacterial activity in current (2004–2006) global population – A Gender Population Analysis – T.E.S.T. Program 2006

S. Bouchillon, M. Hackel, J. Johnson, D. Hoban, B. Johnson, R. Badal, M. Dowzicky (Schaumburg, Collegeville, US)

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 comparators against respective Gram-positive/negative species. Isolates were collected from 272 hospital sites in 34 countries from 2004 to 2006.
Methods: A total of 48,068 clinically significant isolates collected worldwide were analysed 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 (formerly NCCLS) guidelines.
Results: Selected pathogens tested against tigecycline are shown in the table.

Organisms[a]	Male				Female			
	n	%S	MIC_{50}	MIC_{90}	n	%S	MIC_{50}	MIC_{90}
Acinetobacter spp.	1,906	na	0.25	1	1,416	na	0.25	1
EcKoKp	5,720	97.6	0.25	1	7,143	98.2	0.25	1
Enterococcus spp.	1,942	100	0.06	0.12	1,969	100	0.06	0.12
H. influenzae	1,738	na	0.12	0.5	1,293	na	0.12	0.5
S. aureus	3,331	100	0.12	0.25	2,536	100	0.12	0.25
S. pneumoniae	1,883	na	0.03	0.25	1,366	na	0.03	0.25

EcKoKp: *E. coli*, *K. pneumoniae* and *K. oxytoca*; na, breakpoint not available.

Conclusions: Tigecycline showed excellent inhibitory activity against all pathogens and species groups regardless of gender. Tigecycline MIC_{90} of 0.25 μg/mL, 0.12 μg/mL and ⩽0.25 μg/mL against *S. aureus*, *Enterococcus*, and *S. pneumoniae*, respectively, and MIC_{90} of 1 μg/mL against selected Enterobacteriaceae and *Acinetobacter* spp. validate the potent inhibitory activity of TIG against pathogens without respect to gender.

R2161 In vitro activity of tigecycline in an outpatient vs. inpatient Western European population

B. Johnson, R. Badal, S. Bouchillon, M. Hackel, J. Johnson, D. Hoban, M. Dowzicky (Schaumburg, Collegeville, US)

Background: Tigecycline 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 tigecycline compared to most commonly prescribed broad spectrum antimicrobials against Gram-negative and Gram-positive species collected during 2004 to 2006.
Methods: A total of 8,900 clinical isolates from five Western European testing sites were identified to the species level. Minimum Inhibitory Concentration (MICs) were determined by each site using supplied broth microdilution panels and interpreted according to EUCAST guidelines.
Results: Selective results are presented in the tables.

	E. coli and *Klebsiella* spp.				*Acinetobacter* spp.			
	In-patients (n = 1,913)		Out-patients (n = 474)		In-patients (n = 531)		Out-patients (n = 88)	
	%S	MIC_{90}	%S	MIC_{90}	%S	MIC_{90}	%S	MIC_{90}
Tigecycline	95.0	1	98.1	0.5	na[a]	1	na	1
Amikacin	95.6	4	95.8	4	72.1	64	78.4	64
Cefepime	84.7	4	86.2	4	17.9	32	25.0	>32
Imipenem	99.6	0.5	100	0.5	83.4	>16	81.8	4
Levofloxacin	80.7	8	80.2	8	60.3	>8	63.6	8
Pip-Tazo	na	64	na	16	na	>128	na	>128

[a]na, breakpoints not yet available.

	S. aureus				*Enterococcus* spp.			
	In-patients (n = 810)		Out-patients (n = 248)		In-patients (n = 643)		Out-patients (n = 88)	
	%S	MIC_{90}	%S	MIC_{90}	%S	MIC_{90}	%S	MIC_{90}
Tigecycline	100	0.25	100	0.25	100	0.25	100	0.12
Levofloxacin	69.8	16	82.7	8	54.6	>32	66.3	>32
Linezolid	100	4	100	2	95.5	2	97.5	2
Minocycline	na	0.5	na	0.5	na	8	na	8
Vancomycin	100	1	100	1	95.0	2	97.5	2

*na = breakpoints not yet available

Conclusion: Tigecycline's in vitro activity was comparable to or greater than most commonly prescribed broad spectrum antimicrobials without any demonstrable change in activity between in- and out-patient bacterial study strains. Tigecycline's inhibitory against *E. coli*, *Klebsiella* spp., and *Acinetobacter* spp. was comparable to imipenem. Against Gram positive organisms, Tigecycline's activity was similar to linezolid and vancomycin.

R2162 Tigecycline antibacterial activity in current (2004–2006) European population – An Age Population Analysis – T.E.S.T. Program 2006

R. Badal, S. Bouchillon, M. Hackel, J. Johnson, D. Hoban, B. Johnson, M. Dowzicky (Schaumburg, Collegeville, US)

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 272 hospital sites in 34 countries from 2004 to 2006.
Methods: A total of 11,281 clinically significant isolates collected across Europe were analysed 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 EUCAST guidelines.
Results: Selected pathogens tested against tigecycline are shown in the table.

Organisms	n	%Sus	MIC_{50}	MIC_{90}
Paeds (NB-13)				
Acinetobacter spp.	52	na[a]	0.12	0.5
E. coli/Klebsiella spp.	277	96.8	0.25	1
S. aureus	150	100	0.12	0.25
S. pneumoniae	178	na	0.03	0.5
Young adult (14–29)				
Acinetobacter spp.	56	na	0.25	1
E. coli/Klebsiella spp.	164	98.2	0.25	0.5
S. aureus	118	100	0.12	0.25
S. pneumoniae	43	na	0.03	0.5
Adult (30–64)				
Acinetobacter spp.	337	na	0.25	1
E. coli/Klebsiella spp.	1,146	95.3	0.25	1
S. aureus	500	100	0.12	0.25
S. pneumoniae	264	na	0.03	0.5
Geriatric (65+)				
Acinetobacter spp.	337	na	0.25	1
E. coli/Klebsiella spp.	1,405	95.1	0.25	1
S. aureus	542	100	0.12	0.25
S. pneumoniae	258	na	0.06	0.5

[a]na, breakpoint not available.

Conclusions: Tigecycline showed excellent inhibitory activity against all groups of pathogens regardless of age group. Tigecycline MIC_{90} of

$\leqslant$0.25 μg/mL and $\leqslant$0.5 μg/mL against *S. aureus* and *S. pneumoniae*, respectively, and MIC_{90} of $\leqslant$1 μg/mL against Enterobacteriaceae and *Acinetobacter* spp. validate the potent inhibitory activity of TIG against community/hospital pathogens in all age populations.

R2163 In vitro activity of tigecycline against common pathogens – Eastern European Data – T.E.S.T. Program 2006

S. Bouchillon, R. Badal, M. Hackel, J. Johnson, D. Hoban, B. Johnson, M. Dowzicky (Schaumburg, Collegeville, US)

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 205 hospital sites in 30 countries from 2004 to 2006.
Methods: Clinically significant isolates from East European testing sites (Czech Republic, Poland, Hungary, and Latvia) were analysed 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 EUCAST guidelines.
Results: TIG activity against selected pathogens and body sites are shown in the table.
Conclusions: Tigecycline showed excellent inhibitory activity against all groups of pathogens regardless of isolation site. Tigecycline MIC_{90} of $\leqslant$0.5 μg/mL against Gram-positive pathogens (including resistant phenotypes) and MIC_{90} of $\leqslant$1 μg/mL against Enterobacteriaceae and *Acinetobacter* spp. validate the potent inhibitory activity of TIG against Eastern European community/hospital pathogens.

Organism (#)	Tigecycline		% inhibited at MIC			
	MIC_{50}	MIC_{90}	$\leqslant$0.5	1	2	4
A baumannii (73)	0.05	1	74.0	95.9	100	
E. faecalis (51)	0.12	0.12	100			
EC, KO, KP[a] (225)	0.25	1	88.4	95.6	97.3	99.6
ESBL[b] (16)	0.5	4	62.5	68.8	75.0	93.8
Enterobacter spp. (118)	0.5	1	86.4	94.1	96.6	100
H. influenzae (59)	0.12	0.25	100			
P. auruginosa (96)	16	>16	0	2.1	9.4	
S. agalactiae (47)	0.03	0.25	100			
S. aureus (MR) (23)	0.12	0.25	100			
S. pneumoniae (58)	0.12	0.5	100			

[a]*E. coli*, *K. oxytoca*, *K. pneumoniae*. [b]Extended-spectrum β-lactamase producers.

R2164 In vitro interaction of imipenem, sulbactam, colistin and rifampin on 18 imipenem-resistant *Acinetobacter baumannii* isolates

H. Antonopoulou, D. Kavatha, I. Galani, Z. Chryssouli, M. Lelekis, H. Giamarellou (Athens, GR)

Objective: Imipenem-resistant strains of *Acinetobacter baumannii* (Ab) are increasingly recognized worldwide, leaving colistin and, sometimes, sulbactam, as the only alternative treatment. However, the use of colistin, is limited, due to its adverse effects and poor pharmacokinetics so that other therapeutic options are needed. The aim of this study was to investigate, the in vitro activity of the interaction between imipenem (IMP), colistin (CL), sulbactam (SUL) and rifampin (RIF), on IMP-resistant isolates of Ab.
Methods: Eighteen clinical multidrug-resistant isolates from 12 hospitals of Athens were selected. MICs were determined by broth microdilution and E-tests, according to CLSI guidelines. All isolates were sensitive only to colistin. Genetic analysis was performed by pulse-field gel electrophoresis (PFGE). Time-kill assays were performed for each antibiotic and for the following combinations: CL+SUL, CL+RIF, IMP+SUL, CL+SUL+RIF and CL+SUL+RIF. The concentrations of antibiotics were selected according to their mean serum levels in clinical practice; IMP: 0.02 mg/L, SUL: 0. 03 mg/L, RIF: 0. 0075 mg/L and for CL: 1×MIC. Bacterial growth was determined at 0, 1, 3, 5 and 24 hours of incubation in shaking water bath at 37ºC. Synergy between tested antibiotics was defined as any more than 2 log10 decrease of viable cells, compared to log10 decrease achieved by the most active single agent.
Results: Seven strains belonged to one major clone. Other seven strains were possibly related to it. Two other different clones were detected with three and one representatives respectively. Synergy between tested antibiotics, in any time of growth, is shown in table 1. In all, except one isolate, no regrowth was detected after synergy was appeared.

Table 1. Synergy between the tested antibiotics at 1, 3, 5 and 24 h of incubation on the total of 18 isolates

Combination	Incubation time				Indifferent result
	1 h	3 h	5 h	24 h	
CL+IMP	–	9 (50%)	2 (11.1%)	6 (33.3%)	1
CL+SUL	–	4 (22.2%)	7 (38.8%)	5 (27.7%)	2
CL+RIF	10 (55.5%)	6 (33.3%)	–	2 (11.1%)	–
IMP+SUL	–	1 (5.5%)	–	15 (83.3%)	2
CL+SUL+RIF	9 (50%)	4 (22.2%)	4 (22.2%)	1 (5.5%)	–
CL+SUL+IMP	–	8 (44.4%)	6 (33.3%)	3 (16.6%)	1

Conclusions: All tested combinations showed synergistic effect although combinations with RIF seamed to express a faster synergistic effect than those without RIF. All combinations were bactericidal. The triple combinations tested, were not more effective than the double ones. Further, other studies are required to determine the interaction of the tested antibiotics in vivo.

R2165 In vitro activity of tigecycline and commonly-used antimicrobials against clinical isolates collected from 2004 to 2006 in Spain and Portugal

R. Badal, S. Bouchillon, B. Johnson, M. Hackel, J. Johnson, D. Hoban, M. Dowzicky (Schaumburg, Collegeville, US)

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 Spain and Portugal from 2004 to 2006 were evaluated for susceptibility to several antimicrobials.
Methods: Strains were collected and identified at 8 sites in Spain and Portugal. MICs were determined at each site using microdilution panels following EUCAST guidelines.

	MIC_{90}		
	S. aureus n = 215	Enterococci n = 132	*S. pneumoniae* n = 118
Amoc/Clav	>8	>8	1
Ampicillin	>16	>16	2
Ceftriaxone	>64	>64	0.5
Imipenem	4	>16	0.5
Levofloxacin	8	>32	1
Linezolid	2	21	
Minocycline	0.5	>8	4
Penicillin	>8	>8	1
Pip/Tazo	>16	>16	2
Tig	0.25	0.12	0.5
Vancomycin	1	2	0.5

	E. coli/Kleb n = 439	*Enterobacter* n = 205	*Acinetobacter* n = 116
Amikacin	4	2	>64
Amox/Clav	32	>32	>32
Ampicillin	>32	>32	>32
Cefepime	4	2	>32
Ceftazidime	⩽8	32	>32
Ceftriaxone	16	32	>64
Imipenem	0.5	1	>16
Levofloxacin	8	0.25	>8
Minocycline	8	4	16
Pip/Tazo	16	32	>128
Tig	0.5	1	1

Results: The tables summarise results for all isolates, and for specific key pathogens.
Conclusions: Tig had the lowest MIC_{90} vs. Gram-positive strains (incl. MRSA, PRSP), and was essentially as active as imipenem vs. Gram-negative strains (incl. ESBL+). It was also significantly more active than any comparators (other than linezolid) vs. enterococci and *Acinetobacter*.

R2166 Tigecycline in vitro activity against carbapenem-non-susceptible *Acinetobacter baumannii*

J. Komarnicka, L. Naumiuk, A. Samet (Gdansk, PL)

Objectives: To evaluate tigecycline activity against carbapenem-resistant and carbapenem intermediate susceptible *Acinetobacter baumannii* isolated from patients of a university hospital in Poland.
Methods: Twenty two multidrug-resistant *A. baumannii* isolates which were non-susceptible to carbapenems by disk-diffusion method were selected for tigecycline activity determination. MICs to tigecycline (TGC), imipenem (IMP) and meropenem (MEM) were determined by E test.
EUCAST Enterobacteriaceae TGC susceptibility/resistance breakpoints were used due to lack of official *Acinetobacter* specific breakpoints and were as follows: susceptible ⩽1 and resistant >2 mg/L.
Results: Eleven isolates were intermediate susceptible to TGC (MIC from 1.5 to 2 mg/L), 10 were susceptible (MIC from 0.016 to 1 mg/L) and 1 was resistant (MIC=4 mg/L) according to choosen breakpoints. In 13 isolates resistant to both IMP and MEM (MIC ⩾16 mg/L) TGC retained full activity in only 4, further 9 isolates remained intermediate susceptible to TGC. There were 6 isolates with IMP-I and MEM-R phenotype, five of them were susceptible to TGC and one was intermediate. Each of 3 isolates which had IMP-I and MEM-I phenotype, displayed different TGC phenotype: resistant, intermediate and susceptible.
Conclusion: TGC was only partially active against carbapenem non-susceptible *A. baumannii* in our hospital. The lowest TGC activity was noted among isolates resistant to both IMP and MEM. TGC remains an interesting alternative in treating multidrug-resistant *Acinetobacter* but demands antimicrobial susceptibility testing in every case.

R2167 Antimicrobial susceptibility to third and fourth generation cephalosporins and the prevalence of extended-spectrum β-lactamase-producing isolates among Enterobacteriaceae from different clinical specimens

B. Kocic, S. Mladenovic-Antic, G. Randjelovic, P. Stojanovic, M. Dinic (Niš, RS)

Infections that are caused by multiple antibiotic resistant organisms cause significant morbidity and mortality. ESBL-producing aerobic Gram-negative rods are resistant to first, second and the third generation cephalosporins and penicilins, and may show simultaneous resistance to fluoroquinolones and aminoglicosides. They have rapidly emerged as major pathogens around the world, and have compromised therapy with β-lactam antibiotics, including third generation cephalosporins.
Objectives: To evaluate the antimicrobial susceptibility to third and fourth generation cephalosporins, and to comprise the frequency of ESBL-producing strains among the most common Gram negative rods collected from different clinical specimens from the Institute for Public Health in Niš during the year 2005.
Methods: 1112 strains of Enterobacteriaceae were studied. Their antimicrobial susceptibility was tested by the disk-diffusion method according the CLSI guidelines. Screening for the detection for ESBL from Enterobacteriaceae was carried out by the double-disk sinergy test (DDST). The E-test ESBL was used as a confirmation test.
Results: The species distribution as follow: *E. coli* 449 (40.02%), *Enterobacter* spp. 215 (19.16%), *Klebsiella* spp. 161 (14.35%), *Proteus mirabilis* 142 (12.66%), *Morganella morganii* 39 (3.48%), *Citrobacter* sp. 39 (3.48%), *Providencia* spp. 19 (1.69%), *Proteus vulgaris* 18 (1.6%). Antimicrobial resistance to β-lactam antibiotics was: to amoxicillin clavulanic acid 60%, to ceftazidime 57.1%, to ceftriaksone 47.1%, to cefotaksime 40.7%, and to cefepime 47.7%. ESBL production was observed in 17.8% of all the isolates. The highest rates were for *Klebsiella* spp.33%, *E. coli* 19.1% and *Enterobacter* spp. 27.4%. Seventy five percent of ESBL-positive strains were nosocomial.
Conclusion: In our country, most of the investigated strains showed high rates of resistance to third and fourth generation cephalosporins. In addition, we have found an increase in the ESBL-positive strains of Enterobacteriaceae, from 15.79% in 2002. to 17.8% in 2005.

New antimicrobials

R2168 Anti-giardial activity of phenolic essential oils

C. Cavaleiro, B. Gaspar, L. Salgueiro, J. Poiares-da-Silva, M.C. Sousa (Coimbra, PT)

Giardia lamblia, a parasitic flagellated protozoan, is the most common causative agent of diarrhoeal illness world-wide. Current therapy against *G. lamblia* infection (giardiasis) is unsatisfactory due to high incidence of undesirable side effects and a significant failure in clearing parasites from the gastrointestinal tract. Because of these problems, new compounds are being screened for antigiardial activity, namely plant extracts.
Aromatic plants and their essential oils, mixtures of natural volatile compounds isolated by distillation, have been used since antiquity to heal microbial infections. Previous works showed that several essential oils showed important antibacterial, antifungal and antiparasitic activity, predicting therapeutic benefits on diseases involving gastrointestinal, mucosal, cutaneous and respiratory infections.
In the present work, we study the effect of essential oils obtained from several aromatic plants (*Thymus zygis* ssp. *sylvestris*, *T. pulegioides*, *Thymbra capitata*, *Origanum virens* and *Lippia graveolens Thymus capitelatus*, *Thymus mastichina*, *Mentha cervina* and *Mentha piperita*) on *G. lamblia* growth.
Essential oils were obtained by hydrodistillation from fresh plant material and analysed by GC and GC-MS. Constituents were identified from their retention indices on two different phases GC columns (polydimethylsiloxane and polyethyleneglycol) and from their mass spectra, which were compared with reference data. Culture trophozoites of WB strain (ATCC 30957) were incubated in growth medium with different concentrations of essential oils for 48 h at 37ºC under anaerobic conditions. The inhibitory concentration (IC50) values were determined by cell counting. The plant activities were compared with metronidazol activity, considered a "gold standard" antibiotic used in therapy of giardiasis.
Oils from plants with non-phenolic compositions did not revealed anti-giardial activity. Contrarily, essentials oils from Thymus zygis ssp. *sylvestris*, *Thymus pulegioides*, *Thymbra capitata*, *Origanum virens* and *Lippia graveolens* inhibited trophozoites growth and were classified as "active". The IC50 values range from 0.07 microL/mL to 0.15 microL/mL. The compositions of these active oils are characterised

by the predominance of phenolic monoterpenes, carvacrol and or timol. These results suggest that phenolic compounds are responsible for the antigiardial activity and have potential for use as therapeutic agents against giardiasis.

R2169 Persistent suppression of bacterial growth after exposure to high-frequency alternated current

C. Cassanelli, S. Roveta, A. Marchese, E.A. Debbia (Genoa, IT)

Background: The exposure of a bacterial suspension to the hight frequency alternated current (HFAC) damages the bacterial envelope and delayes cell division. In this study was verified if the HFAC generated by Endox® Endodontic System, an instrument used in endodontic treatment for disinfection of the root canal, produced a phenomenon similar to postantibiotic effect.
Methods: *P. aeruginosa* and *E. faecalis* strains employed in this study were clinical isolated belonging to the collection of this Laboratory. A sample of 0.1 ml of a saline bacterial suspension (105 cell/mL) was exposed to HFAC (140 msec, 500 kHz, 1200 kV) for three times and then resuspended in 10 ml of Mueller–Hinton broth. A suspension not treated was employed as control. The suspensions were grown at 37ºC for 6–8 hours; plate counts were performed every 60 min. All experiments were repeated for five times.
Results: A persistent suppression of bacterial growth of about 3 hours was observed in the two bacterial species after exposure to HFAC. In the control strains no growth suppression was noted.
Conclusion: The present findings suggest that HFAC generated by Endox® Endodontic System produce a damage in the structure of bacterical cells membrane. This phenomen can be usefull to study the possible variations in the permeability of bacterial membranes and is probaly responsable of bactericidal effect of this instrument.

R2170 Assessment of susceptibilities of a range of antibiotic-resistant clinical isolates to *Aloe vera* inner gel

F.M. Habeeb, A.A. Dashti, D. Morrison, A.I. Gray, V.A. Ferro (Kuwait, KW; Glasgow, UK)

Since the discovery of penicillin in 1928, by Alexander Fleming, the emergence of antibiotic resistant bacterial strains has been a growing problem. This has led to an urgent global call for new antimicrobial drugs, particularly from natural resources. We investigated the therapeutic potential of *Aloe vera*, a plant known anecdotally for its anti-microbial properties. *Aloe vera*, has widespread use in health and beauty products, toiletries and disinfectants, however, we wished to determine if there was a scientific basis for its anti-microbial usage and whether this could be exploited for use against antibiotic resistant bacteria.
Objectives: To determine the effect of *Aloe vera* on a range of clinical isolates identified as antibiotic resistant, including Gram positive (Methicillin-Resistant *Staphylococcus aureus*, [MRSA] and *Enterococcus bovis*) and Gram negative (*Enterobacter cloacae*) strains.
Methods: A standard broth tube dilution method, as recommended by NCCLS was used to calculate the minimum inhibitory concentrations (MIC) of a preservative free *Aloe vera* inner gel powder (*Aloe vera* of America, Inc). The MIC is read as the lowest concentration of antimicrobial agent, which inhibited bacterial growth. Minimal bactericidal concentrations (MBC) were determined by inoculating 10 microlitres from each MIC broth tube without visible growth, on a Nutrient agar plate. Plates were then incubated in an inverted position at 37ºC. Following overnight incubation, the plates were examined for colony growth. Lack of growth indicates that the tested drug was bactericidal at that dilution and this was reported as MBC. Growth indicates that the drug was bacteriostatic at that dilution.
Results: The results showed that the MIC of *Aloe vera* on MRSA 9, MRSA 6, *E. cloacae*, and *E. bovis* was 25, 25, 25 and 12.5 mg/mL respectively. The MBC of *Aloe vera* on MRSA 9, MRSA 6, *E. cloacae*, and *E. bovis* was 62.5, 125, 62.5 and 125 mg/mL respectively.
Conclusion: *Aloe vera* has antimicrobial activity and it could be used in healthcare settings. If a product contains 250 mg/mL of *Aloe vera* this will result in 10×MIC for MRSA 9, MRSA 6, *E. cloacae*, 20×MIC for *E. bovis*, 4×MBC for MRSA 9 and *E. cloacae* and 2×MBC for MRSA 6 and *E. bovis*. This indicates that *Aloe vera* is active against these clinical isolates and has the potential to be a source for new antimicrobials.

Epidemiology of MRSA, VRE & other Gram-positives

R2171 Comparison of coagulase-positive and coagulase-negative variants of MRSA strains isolated from hospital specimens

K. Kopron, S. Giedrys-Kalemba (Szczecin, PL)

Objectives: The purpose of the study was to estimate biochemical characteristic, drug resistance and genotype patterns of coagulase-positive and coagulase-negative HA-MRSA isolated from the same hospital.
Methods: A total of 258 MRSA strains were determined as aetiological agent of different kind of infections in Clinical Hospital in Szczecin in the year 1999–2005. Isolates were collected from various materials (blood, bronchoalveolar washings, sputum, secretion from wounds, catheters, drains, liquids from pleura and peritoneum) and wards (intensive care unit, surgery, internal medicine, urology, dermatology, cardiology). The plasma coagulase tube test, the slide test for clumping factor, thermonuclease test, API–STAPH kit, mecA and coa genes were used for identification. Susceptibility tests according to CLSI guidelines were preformed for cefoxitin, erythromycin, clindamycin, tetracycline, gentamicin, rifampicin, ciprofloxacin, cotrimoxazole, chloramphenicol, fusidic acid, vancomycin. All isolates were subjected to the SmaI genomic DNA macrorestriction analysis (PFGE) and the obtained patterns were compared by software Molecular Analyst Fingerprinting Software.
Results: All coagulase-positive MRSA were positive in tests for thermonuclease but 19 isolates were CF negative (10%). HA-MRSA coagulase-negative strains were isolated from clinical specimens with a relatively high frequency – 29% (74 strains), mostly from intensive care unit – 52% (39 strains). All these strains were positive in tests for clumping factor and thermonuclease, most of them possess coa gen. The coagulase-positive HA-MRSA strains were more resistant to chloramphenicol and rifampicin than coagulase-negative, which were more resistant to cotrimoxazole; the level of resistance to other drugs was similar, about 90–100%. No vancomycin resistant HA-MRSA were found. HA-MRSA strains belonged to 13 genotypes (A-M), grouped 2–48 isolates, and 6 unique patterns. Coagulase-negative strains were identified in 3 types (C, F, G) only, however in those types coagulase-positive MRSA were also found.
Conclusion: Coagulase-negative MRSA strains are becoming more and more important problem in hospital infections. They belong to multidrug resistant strains and to the same epidemic/endemic genotypes as coagulase-positive MRSA. More attention should be given in their identification in routine diagnostic – phenotypic and genotypic methods are recommended.

R2172 Antimicrobial resistance of *Staphylococcus aureus* nosocomial blood isolates in Russian intensive care units

A. Dekhnich, E. Ryabkova, O. Kretchikova, N. Ivanchik (Smolensk, RU)

Objectives: To evaluate the susceptibility of nosocomial strains of *Staphylococcus aureus* isolated from blood of patients hospitalised in ICUs in different parts Russia.
Methods: A total of 143 strains of *S. aureus* isolated in 2004–2005 from patients with bacteraemia hospitalised in ICUs in 16 cities in different parts of Russia, were studied. Antimicrobials tested included chloramphenicol (CHL), ciprofloxacin (CIP), clindamycin (CLI), co-trimoxazole (CTX), erythromycin (ERY), fusidic acid (FUS), gentamicin

(GEN), levofloxacin (LEV), linezolid (LNZ), mupirocin (MUP), oxacillin (OXA), rifampin (RIF), tetracycline (TET), vancomycin (VAN). Antimicrobial susceptibility testing was performed by agar dilution method according to CLSI/NCCLS guidelines (2005–2006). *S. aureus* ATCC 29213 was used as a control strain.
Results: Results of susceptibility testing are presented in the table.

Antimicrobial	I, %	R, %	MIC (mg/L)		
			MIC_{50}	MIC_{90}	Range
CHL	0.7	62.9	64	64	4–128
CIP	4.9	62.9	16	64	0.125–128
CLI	0	47.6	0.06	512	0.03–512
CTX	0	8.4	0.06	2	0.03–8
ERY	2.1	60.8	512	512	0.125–512
FUS	0	0	0.125	0.25	0.015–0.25
GEN	0.7	58.7	128	512	0.25–512
LEV	11.2	36.4	2	8	0.125–16
LNZ	0	0	2	2	0.5–2
MUP	0	0	0.25	0.25	0.125–0.5
OXA	0	86.0	64	256	0.25–512
RIF	2.8	26.6	0.015	64	0.015–256
TET	0	54.6	64	256	0.125–256
VAN	0	0	1	1	0.25–2

Conclusions: (1) very high rate of MRSA (86%) was found that do not allow to use β-lactams in ICU patients with staphylococcal bacteraemia; (2) the most active antimicrobials with all strains susceptible were vancomycin and linezolid; (3) other antimicrobials with high in vitro activity were fusidic acid and sulfamethoxazole/trimethoprim with 0% and 8.4% of strains non-susceptible, respectively; (4) high rates of resistance to fluoroquinolones, lincosamydes, macrolides, tetracyclines, gentamicin and chloramphenicol were detected.

R2173 Prevalence and clinical impact of *Enterococcus* spp. in gynaecological department

V. Kolodjieva, R. Yafaev, A. Suvorov (Saint-Petersburg, RU)

Objectives: The aim of the study was to investigate features of enterococcal infections and comparative molecular analyses of *Enterococcus* virulence determinants of clinical strains obtained from the patients of gynecological hospital and antenatal centre.
Methods: We investigated 890 women from the gynecological department of the St. Mary Hospital and 180 women of antenatal centre. We used PCR to detect the virulence factor genes (VFG). Genes encoding for gelatinase (gelE), serine protease (sprÅ), extracellular surface protein (esp), regulator, controlling expression of gelatinase and serine protease (fsrA) and aggregation substances (asa) have been analysed. Assessment of antibiotic susceptibility of enterococcal strains has been performed.
Results: Enterococci represented the most common pathogen isolated from patients with genital infections in gynecological department. This group included patients with the endometritis (65.8%), uterine apparandages (28.5%). Enterococcal strains from the patients of gynecological department were isolated more often than from women supervised by antenatal centre ($p = 0.0001$). The VFG were represented more often in the *Enterococcus* strains isolated from women with infection than in the strains from the women without infection.
The rate of ampicillin resistance among clinical isolates of this species was 73.8%, streptomycin (high-level resistance [HLR]) 78.5%, HLR to gentamicin 58.9%, ciprofloxacin 63.2%, erythromycin 87.3%. Five isolates were intermediate to vancomicin (IIE 8 mg/mL).
Enterococcal infection sources include endogenous, as from the colon (>40%), and exogenous such as nosocomial transmission via gloves of personnel. Long-term (at 6 months) circulation of one clone using VFG-carriage typing, phage typing and antibiogram in gynecological department is shown.
Conclusion: *Enterococcus* spp. could cause NI. Clinical strains of enterococci vary substantially by the presence of VFG. The lability of properties allows them to adapt and to persist in conditions of a hospital on objects of an environment, and also to gain a resistance to the antibiotics used in the given hospital.

R2174 Screening for MRSA at hospital admission in Turkey

N. Baykam, H. Esener, P. Zarakolu, O. Ergonul, T. Cirkin, A. Celikbas, S. Eren, B. Dokuzoguz (Ankara, Istanbul, TR)

Objective: To determine the prevalence of Methicillin Resistant *Staphylococcus aureus* (MRSA) at the hospital admission and to predict the risk factors for MRSA carriage.
Methods: Anterior nare cultures were obtained at the time of hospital admission to identify patients colonised with *S. aureus*. To detect methicillin resistance, oxacillin disk charged with 1 μg was applied to Mueller Hinton agar containing 4% sodium chloride. Panton-Valentine leukocidin (PVL) gene was detected by PCR and Pulse Field Gel Electrophoresis (PFGE) was performed to examine the clonal divercity of the MRSA strains. A multivariate analysis was performed to detect the risk factors of MRSA. Age, smoking, use of antibiotics within last 6 months, healthcare worker within the family, chronic obstructive lung disease, history of hospitalisation within last year were included to the model.
Results: Nine hundred patients were screened. The rate of females was 46%, and the mean age was 43.5. Eleven (1.1%) MRSA strains were detected. All the MRSA strains were positive for the mecA and PVL gene. Three different clones of MRSA strains were detected in pulse field gel electrophoresis. The MRSA carrier patients were older ($p = 0.013$), were using antibiotic on the admission ($p = 0.009$), had diabetes ($p = 0.031$) and obstructive pulmonary lung disease ($p = 0.008$) more commonly. Antibiotic use within the last 6 months was more common among MRSA positive patients, but not statistically significant ($p = 0.100$). Intravenous drug use was not common among the patients (2%). Six out of 11 MRSA positive patients did not stay in the hospital within the last 12 months. However, 5 out of 11 PVL positive MRSA carriers had the history of hospitalisation within the last 6 months.
Conclusion: The rate of community acquired MRSA colonisation is low in Turkey. The PVL can not be defined as the indicator of community acquired MRSA. There is no strict and rigid line between hospital and community acquired MRSA infections.

R2175 Differences in the features of staphylococcal bacteraemias in adults with and without cancer

I. Kourbeti, J. Papadakas, G. Fanti, E. Kalavrytinou, E. Koutsounaki, M. Rovithi, A. Christidou, G. Samonis (Heraklion, GR)

Background: *Staphylococcus aureus* and coagulase-negative staphylococcal bacteraemias (SAB and CNSB) cause substantial morbidity and mortality. Between 2003 and 2005 we contacted a prospective study to investigate the differences in the features of SAB and CNSB in adults with (ON) and without cancer (IM). Our goal was to compare the outcome with emphasis on metastatic infections and the treatment and hospitalisation days.
Methods: The medical records of the patients >18 years old hospitalised in IM and ON words that were reported to have SAB or clinically significant CNSB were reviewed and the course of the patients was followed. Data were abstracted to a standard form and analysed using SPSS.
Results: During the aforementioned period a total of 33 SAB (27 in the IM and 6 in the ON group) and 38 CNSB patients (12 in the IM and 26 in the ON group) were identified. The groups were balanced for age and sex.
Significant observations from the analysis of the data include the increase in the percentage of MRSA in the IM Department from 18.2% in 2003, to 89% in 2004 and 86% in 2005

For the SAB the IM population required significantly more days of treatment ($p < 0.05$) and hospitalisation ($p < 0.05$). 66.7% of the SAB in the ON group were catheter associated (CAB) (statistically significant, $p < 0.001$) and there was a trend for higher methicillin resistance ($p = 0.065$). There were no differences in the clinical and laboratory features (fever, WBC), the survival, the association with treatment in the ICU, the metastatic infections and the mixed bacteraemias. The metastatic infections most commonly seen included abscesses (22.2%), endocarditis (18.5%) and septic arthritis (7.4%).
For the CNSB the IM population needed more days of treatment ($p < 0.05$) and there was an association with prior ICU treatment ($p < 0.05$). The ON population had more mixed bacteraemias ($p < 0.05$). There were no differences in the fever, the WBC, the survival and the metastatic infections.
Conclusions: The patients with cancer present with SAB as a result of their indwelling central venous catheters (CVCs). Leukopenia is not a predisposing factor. The length of stay was significantly longer for the IM group for both SAB and CNSB. The IM group required significantly longer antibiotic treatment for the SAB. SAB and CNSB are associated with significantly higher morbidity in the adults without cancer.

R2176 Prevalence of MRSA in a population of ambulatory patients

M. Szczyrek, A. Kopaczewska, A. Chudnicka (Lublin, PL)

Objectives: The aim of the study was to characterise drug resistance of the *Staphylococcus aureus* strains isolated from clinical materials obtained from ambulatory patients in Lublin, Poland, and to evaluate the problem of methicillin resistance in the analysed group.
Methods: The study comprised 302 randomly chosen individuals, both men (157) and women (145), who were infected with *S. aureus*. Materials included specimen from nose, pharynx, ears, skin changes and wounds. Specimens were collected with cotton swabs and cultured on Columbia blood agar. The organisms were identified by using API Staph identification system (bioMérieux, France). Antimicrobial susceptibility to several antibiotics (penicillin, doxycycline, erythromycin, Co-trimoxazole, clindamycin, ciprofloxacin, and oxacillin) was determined by Kirby Bauer disk diffusion method using Becton Dickinson disks, according to NCCLS (National Committee for Clinical Laboratory Standards).
Results: In the studied materials 305 catalase-positive bacterial strains belonging to the species *S. aureus* were found. In 20 cases (6.5%) isolated strains showed resistance against methicillin (MRSA). Among the MSSA strains, resistance occurred against penicillin (87%), doxycycline (39%), and erythromycin (23%). Most strains remain susceptible to Co-trimoxazole (only 8% resistant), clindamycin (12%) and ciprofloxacin (14%). Some of the isolated MRSA strains were additionally resistant to antistaphylococcal drugs, teicoplanin and mupirocin. No VRSA (Vankomycin Resistant *Staphylococcus aureus*) or VISA (Vancomycin Intermediate *Staphylococcus aureus*) strains have been found.
Conclusion: The study revealed that infections caused by MRSA in Poland do occur outside hospitals, and can cause therapeutical failures with empirical pharmacotherapy. Some of the isolated strains were additionally resistant to other antistaphylococcal drugs. Those results demonstrate the need for the aimed antimicrobial therapy in ambulatory patients.

R2177 Molecular epidemiology of bacteriophages in group A streptococcal strains belonging to serotype M49

E. Polyakova, S. Suvorov, B. Beall, J. Ferretti (St. Petersburg, RU; Atlanta, Oklahoma, US)

Objectives: Group A streptococci (GAS) are pathogenic bacteria causing various severe human diseases. It was determined that almost all GAS strains carry temperate bacteriophages in their genome which might be important for GAS biology including the pathogenic potential of the strain. Streptococcal phages can carry various toxin genes, genes of streptodornases and antibiotic resistances. Genetic analyses of temperate phages in GAS promote understanding "fast" evolution mechanisms of bacterial genomes. However at present very little is known regarding the association of phages and specific GAS serotypes.
Goals of investigation: Analysis of M49 GAS strains for the presence of bacteriophages carrying virulence factors genes.
Materials and Methods: 48 GAS (38 strains from serotype M49) clinical isolates had been studied employing PCR and macro array hybridisation. 27 phage genes encoding for toxins, integrases of phage structural proteins were used as a probes or PCR targets.
Results: It was determined that M49 GAS strains are characterised by the particular set of bacteriophages and virulence genes. The most common phage toxin genes for M49 were speA, speI and speH. SpeK, speM and speC were the rarest. Two types of integrases encoded by int5 and int7 were found to be common in M49 GAS. There were no strains from M49 serotype without the phage genes in the genome. M49 strains collected from one geographical area during the 6 year period were found to carry identical phage genes pattern. This finding allows considering phage gene typing as potent tool for molecular epidemiological analysis.

R2178 The clinical significance of Viridans streptococcal bacteraemia in patients at a district general hospital

L. Tan, S. Lacey, M. Melzer (London, Essex, UK)

Objectives: Viridans streptococci (VS) are low virulent commensals of the upper respiratory tract (URT). Community-acquired bacteraemia occurs commonly but its clinical significance in children and immuno-competent adults has not been well studied. Our aim was to determine the proportion of community-acquired bacteraemia caused by VS, their significance, sites of infection and 30-day mortality.
Methods: Patients with VS bacteraemia were identified from a database at a UK district general hospital from September 2003-October 2005. Available case notes were retrospectively reviewed for demographic and clinical data. Cultures were classified according to definite, probable or no clinical significance, depending on the number of positive blood cultures and compatible clinical syndrome. Patients with suspected infection were treated empirically based on likely site and local antibiotic policy. Following VS identification, antibiotics were altered according to site and sensitivities.
Results: VS caused 63/829 (7.6%) community-acquired bacteraemias, 50/723 (6.9%) in adults and 13/106 (12.3%) in children. 55 notes were available for review. There were no neutropenic patients. Among adults, four (9.3%) cultures were of definite clinical significance, 17 (39.5%) of probable significance and 22 (51.2%) not significant. There were four (9.3%) cases of infective endocarditis, one (2.3%) possible endocarditis, seven (16.3%) lower respiratory tract (LRT) infections and two (4.6%) female genital tract infections. Four (9.3%) patients had URT symptoms. Non-significant cultures included two (4.7%) patients with poor dentition, one (2.3%) post seizure, and 19 (44.2%) with no identifiable source. Among children, five (41.7%) cultures were of probable significance and seven (58.3%) were not significant. There were two (16.7%) LRT infections and three (25%) URT infections. Non-significant cultures included four (33.3%) bacteraemic episodes post seizure or febrile convulsion. Overall, one patient died as a consequence of infection (LRT). 20% of isolates were penicillin resistant, 25% erythromycin resistant and 4% amoxicillin resistant.
Conclusion: In adults, the lower respiratory tract is commonest site of infection but infective endocarditis also occurs. In children, the lower respiratory tract is the commonest site of infection and febrile convulsions are commonly associated with non-significant VS bacteraemia. Despite penicillin resistance, mortality was low and infections were easily treated.

R2179 Increased resistance to fluoroquinolones in *Streptococcus agalactiae* isolated from urinary tract infections acquired in the community

C. Freyre, M. Rodriguez-Iglesias (Cadiz, ES)

Objective: *Streptococcus agalactiae* is an important pathogen causing serious neonatal infections, characterised by sepsis and meningitis and also maternal infections. It is very important his detection in urine of pregnant women due to can be responsible of prematurity and/or pyelonephritis. Quinolones is a therapeutic option in allergic patients to β-lactams. We have studied the resistance to ciprofloxacin in *S. agalactiae* isolated from patients with urinary tract infections acquired in the community.
Methods: We have selected 531 *S. agalactiae* strains among urinary clinical isolates from 2000 to 2006. Clinical significance was considered with bacterial count >100,000 CFU/mL. Identification and conventional antimicrobial susceptibility test was done (Microscan, Dade–Behring). E-test was used as confirmatory test in selected strains. MIC >2 μg/mL was considered resistant and decreased sensitivity with MIC 1–2 μg/mL according to CLSI breakpoints.
Results: Between 2000 and 2006 it was found 13 strains resistant to ciprofloxacin (2.45%) and 24 presenting moderate sensitivity (4.52%). Statistical differences between years were not found with the exception of the last year. In 137 strains isolated from January to October we found similar proportion in resistant strains (2.19%) but a strong increase in moderate sensitivity (8.03%) was evident. Also, clindamycin has increased the resistance level from 15 to 20% in the last year.
Conclusions: The selection and proliferation of non-targeted microorganisms due to empirical use of antimicrobials, particularly quinolones, is a growing problem. We have detected a strong increase in moderate susceptibility to ciprofloxacin (MIC 1–2) although resistance levels are maintained constant in the last 6 years (2% aprox.). This increase can be the herald of future resistances and related with several mutations in GyrA and/or ParC that could be to cause high-level resistance. They are necessary more studies for to understand the importance of mutations in GyrB and to establish analogies between number and location of the mutation, similarly to other bacteria.

R2180 Effect of adequate empiric antibiotic therapy on survival of patients with methicillin-resistant *Staphylococcus aureus* bacteraemia

R. Hazzan, M. Paul, H. Shaked, M. Wattad, H. Konigsberger, B. Rubinovitch, L. Leibovici, J. Bishara (Petah Tikva, IL)

Objective: Glycopeptides are the treatment of choice for methicillin-resistant *Staphylococcus aureus* (MRSA) bacteraemia. We evaluated the effect of empirical treatment with glycopeptides for MRSA bacteraemia on mortality.
Methods: Retrospective chart review of prospectively identified patients with clinically-significant MRSA bacteraemia, in a primary and tertiary care hospital. Empirical treatment was defined as that initiated during the first 48 hours. Mortality was defined as 30-day all cause mortality. Variables evaluated included: age, sex, functional capacity, healthcare acquisition of infection, presence of intravascular catheters, urinary catheter, mechanical ventilation, recent surgery or invasive procedures, steroid or cytotoxic therapy, neutropenia, source of infection; Background conditions: diabetes, renal failure, hemodialysis, prosthetic valve, pacemaker, other prosthetic device, malignancy, lung disease, liver disease, wounds, heart and vascular disease, McCabe and Charlson score; Infection presentation: temperature, shock, leukocytes, platelets, creatinine, albumin, liver function tests. Univariate comparisons were performed using the chi-square test or t-test. Multivariate analysis was performed using forward conditional binary regression.
Results: We included 150 episodes of MRSA bacteraemia among 141 patients. Significant risk factors for mortality are shown in the table. Empirical glycopeptide (vancomycin) treatment was administered to 44.9% of patients that remained alive at 30 days compared to 29.5% of those that died (unadjusted $p = 0.06$). Patients who were given vancomycin empirically had significantly more frequent repeated episodes of bacteraemia, implanted catheters, diabetes, hemodialysis, prosthetic valves, recent angiography, higher albumin and no malignancy. Significant risk factors for 30-day all cause mortality on multivariate analysis included no empirical treatment with vancomycin ($p = 0.03$), septic shock ($p < 0.001$) and total leukocyte count ($p = 0.01$). The small number of cases evaluated limits our analysis.

Variable	Alive	Dead	P-value
N episodes	89	61	
Fuctional status (full unrestricted activity)	36 (41.4%)	14 (24.1%)	0.03
Decubitus ulcer, burns of chronic wounds	10 (11.2%)	20 (32.8%)	0.001
Septic shock	7 (8.4%)	27 (50%)	<0.001
Temperature, ºC, mean (SD)	38.5 (0.71)	38.1 (1.15)	0.03
White blood cells, K/micl, mean (SD)	13.2 (0.76)	15.9 (0.70)	0.04
Empirical glycopeptide treatment	40 (44.9%)	18 (29.5%)	0.06

Conclusion: Among patients with MRSA bacteraemia, empirical treatment including vancomycin reduced 30-day mortality.

Epidemiology of MDR-Gram-negatives

R2181 Risk factors for community-acquired urinary tract infection due to quinolone-resistant uropathogens

I. Kometiani, B. Chazan, R. Colodner, R. Raz (Afula, IL)

Background: *E. coli* remains the most common pathogen in Community Acquired Urinary Tract Infections (CAUTI).
The massive use of antimicrobials in general and quinolones in particular increased the frequency of multiresistant uropathogens. The aim of this study was to evaluate demographic and clinical risk factors associated with CAUTI due to quinolone-resistant *E. coli* (QREc) in Northern Israel.
Methods: During July – October 2005, clinical and demographic data of 150 consecutive urine cultures from outpatients which grew quinolone-resistant *E. coli* isolates and 150 with quinolone-sensivitive *E. coli* (QSEc) isolates at the Clinical Microbiology Department at Ha'Emek Medical Center, were screened and analysed.
Results: By univariate analysis the risk factors associated with QREc were: older patients, males, nursing home residents, bed ridden, dementia, diabetes, cardiovascular diseases, immunosupression, nephrolithiasis, recurrent UTI, invasive procedures, hospitalisation within prior 6 months and antibiotic use (not only quinolones) taken within previous 6 months. By multivariate analysis, recurrent UTI (O.R 4.7, 95% CI: 2.3–9.3; $p \leqslant 0.0001$), previous invasive procedure (6.6; 3.0–14.7; $p \leqslant 0.0001$), use of ofloxacin (7.5; 2.9–19.4; $p \leqslant 0.0001$), ciprofloxacin (20.6; 2.3–179.2; $p = 0.006$) and previous hospitalisation (2.9; 1.4–6; $p = 0.003$) were identified as independent risk factors.
Conclusions: In patients with one or more of the risk factors identified here, the empiric use of quinolones should be avoided.

R2182 Influence of doxycycline resistance on outcome in *Acinetobacter baumannii* bacteraemia in an intensive care unit

R. Zaragoza, J. Camarena, S. Sancho, R. González, A. Artero, J. Navarro, J. Nogueira (Valencia, ES)

Objective: The aims of this study were to identify the epidemiological and clinical differences between doxycycline resistant *Acinetobacter baumannii* bacteraemia (ABB) and doxycycline-susceptible episodes, to know their prognosis, and finally to define if doxycycline resistance is a factor independently associated to hospitality and related mortality in critically ill patients with ABB.
Methods: From 1996 to 2006, 341 patients with a clinically significant bacteraemia were prospectively evaluated in an intensive care unit of

a university hospital. Doxycycline resistant *Acinetobacter baumannii* (DR) was defined when MIC was ⩾μg/mL (E-test®). Clinical and microbiological variables were studied. A multivariate analysis was performed to determine the factors independently associated to hospitality and related to bacteraemia mortality in critically ill patients with ABB.

Results: Ninety-three (27.2%) of 341 ICU bacteraemias were due to ABB. A sixty-eight percent of them were DR episodes. The mean age of patients with ABB was 60.7 SD 16.2 years and the relation between men/women was 3.3. APACHE II score was 19.2 SD 7.6. The incidence of inadequate empirical antibiotic treatment was 54.7% in ABB patients. The principal origins of ABB were: respiratory (49.5%), unknown (23.2%) and catheter (12.6%). The global and related mortality rate for ABB was 54.7% and 22.1% respectively. There were no differences in presence of severe sepsis or septic shock, APACHE II or SOFA score (admission and at the onset of ABB) between DR and susceptible episodes. Although the incidence of inadequate empirical antimicrobial treatment (48.4% vs 72.4%; $p = 0.03$) was statistically lower in DR group, related mortality rates (28.1% vs 10.3%; $p = 0.04$) was significantly higher in DR group. Multivariate analysis confirmed DR (OR 8.2; 95% CI 1.5 – 42.9.9; $p = 0.01$) as an independent predictor of related mortality to ABB.

Conclusions: The prevalence of ABB is very high among critically ill patients, and majority of them are DR. Although DR did not imply a higher rate of inappropriate empirical antimicrobial treatment, DR was independent associated with an increased related mortality to ABB in critically ill patients.

R2183 Epidemiological study of infections caused by extended-spectrum β-lactamase producing *Escherichia coli* and *Klebsiella* spp. in a tertiary care hospital in Badajoz, Spain (2003–2005)

E. Garduño, M.A. Galán-Ladero, M.T. Muñoz-Lozano, M. Sánchez-González, J. Blanco-Palenciano (Badajoz, ES)

Objectives: The aim of this study is to determine the prevalence and antimicrobial resistance pattern of extended-spectrum β-lactamase (ESBL) *Escherichia coli*, *Klebsiella pneumoniae* and *Klebsiella oxytoca* producing isolates over a 3-year period at our institution.

Methods: A retrospective study was performed from January 2003 to December 2005. Identification and antimicrobial susceptibilities were determined using the WalkAway system (Dade–Behring), and ESBL-producing bacteria were confirmed by E-test method. Resistance rates are described as percentage and analysed using Chi2-test. Statistical significance has been considered when p value was <0.05.

Results: Out of the 4,137 isolates studied 227 (5.48%) were ESBLs. 13.21% were isolated during year 2003, 14.53% during 2004 and 72.24% during 2005. The distribution of the ESBL isolates was: 122 (53.74%) *E. coli*, 103 (45.37%) *K. pneumoniae* and 2 (0.88%) *K. oxytoca*. Species distribution by year was: 2003, 25 *E. coli* and 5 *K. pneumoniae*; 2004, 23 *E. coli*, 9 *K. pneumoniae* and 1 *K. oxytoca* and 2005, 74 *E. coli*, 89 *K. pneumoniae* and 1 *K. oxytoca*.

Overall, the infection was intrahospitalary in 85.91% of the cases, and most of them in medical areas (38.03%), followed by surgical areas (18.30%) and the ICUs (29.58%). *E. coli* was more frequently isolated from outpatients (19.82%), whereas *K. pneumoniae* was was more frequent in patients from ICUs (55.91%). The majority of *E. coli* was obtained from urine (68.1%), whereas *K. pneumoniae* from respiratory (39.8%) and invasive (24.7%) samples.

Sensitivity of ESBL-*E. coli* compared to non-ESBL *E. coli* was as follows: trimethoprim-sulfamethoxazole (45 and 61%; $p < 0.05$), fosfomycin (97 and 97%), gentamycin (87 and 92%; $p > 0.05$) and ciprofloxacin (40 and 69%; $p < 0.05$). ESBL-*K. pneumoniae* vs. non-ESBL *K. pneumoniae* was: trimethoprim-sulfamethoxazole (80 and 81%; $p > 0.05$), fosfomycin (92 and 87%; $p > 0.05$), gentamycin (100 and 94%; $p > 0.05$) and ciprofloxacin (80 and 86%; $p > 0.05$).

Conclusions: ESBLs have dramatically increased in our hospital over the last three years. In 2005, the more prevalent species was *K. pneumoniae* isolated from respiratory samples of patients in ICU, suggesting clonal origin (data under further investigation). ESBL-*E. coli* isolates showed higher and statistically significant resistance to trimethoprim-sulfamethoxazole and ciprofloxacin than non-ESBL *E. coli*.

Antibiotic usage

R2184 Antibiotic consumption in three hospitals, Latvia, 2005

S. Berzina, M. Ferech, L. Saule, D. Umbrasko, I. Jekabsone, H. Goosens for ESAC (European Society of Antimicrobial Consumption)

Objectives: To collect the data on antibiotic consumption in the three Latvian hospitals – Stradini Clinical University Hospital (CUH), which is a many profile hospital; Riga 1st hospital – also a many profile hospital; and Daugavpils hospital, which is a regional hospital in the eastern part of Latvia, also many profile hospital – according to the ESAC data collection protocol (European Surveillance of Antimicrobial Consumption, granted by DG SANCO of the EC).

Methods: The data on antibiotic consumption in the three hospitals for 2005 have been collected using ATC/DDD classification (WHO, version 2005) and expressed in Defined Daily Doses (DDDs) and in DDD per 100 bed days (DBD). Data were obtained from the hospitals pharmacies.

Results: The overall use of antibiotics in 2005 was – in Stradini CUH 109.0 DBD, in Daugavpils hospital 77.1 DBD, and in Riga 1st hospital – 81.7 DBD.

The most used antibiotics in Stradini CUH and in Daugavpils hospital were penicillins with extended spectrum (mainly amoxicillin) – 33.0% (104,745.0 DDDs) and 52.0% (87,187.5 DDDs), respectively, while in Riga 1st hospital cephalosporins (mainly cefazolin) were mostly used – 32% (74,800.0 DDDs).

Combinations of penicillins (mostly amoxiclav) represented the second mostly used antibiotic group in Stradini CUH – 28% (89,676.0 DDDs), whereas their use was very limited in Daugavpils and Riga 1st hospital – 5% (8,383.5 DDDs) and 1% (2,337.5 DDDs), respectively.

The quinolones were frequently used in Stradini CUH – 12.8% (40,664.9 DDDs) and in Riga 1st hospital – 16% (37,400.0 DDDs), but were not used in Daugavpils hospital at all.

From hospital antibiotics – cephalosporins – were used mostly – Stradini hospital, Daugavpils hospital and Riga 1st hospital – 14.8% (46,976.7 DDDs), 16% (26,826.9 DDDs) and 32% (74,800.0 DDDs), respectively, but cefazolin was the antibiotic which was used predominantely.

Monobactams (aztreonem) was not used, but other hospital, specific antibiotics (carbapenems, aminoglycosides, glycopeptides) were used much more less than those characterised before.

Conclusions: Three hospitals show very different patterns of AB use partially due to their different profiles. Nevertheless mention most striking variations not attributable to the hospital type, e.g., amoxiclav vs. quinolones in Riga 1st hospital and Daugavpils hospital, also the use of hospital-specific antibiotics have to be discussed.

R2185 Uniformity of antibiotic prophylaxis in surgical procedures is associated with improvement of timing

I. Willemsen, R. van den Broek, T. Bijsterveld, P. van Hattum, M. Winters, G. Andriesse, J. Kluytmans (Breda, NL)

Background: The Amphia Hospital is a 1370 bed teaching hospital that was founded in 2001 after a merger of three hospitals. In 2005 the initiative was taken to standardise perioperative antimicrobial prophylaxis (PAP) in surgical procedures.

Objectives: To implement a guideline for PAP, describing the choice of the agent, the dosage and the timing.

Methods: Before and after the implementation the application of PAP was measured. In the new policy cefazolin was the first choice agent, if necessary combined with metronidazol. Clindamycine was preferred as an alternative for patients reporting an allergy to β-lactams. The guideline was approved by all surgeons and anaesthetist and distributed within the

hospital. It included for each type of procedure, the antibiotic agent and the dosage.
Results: Before the intervention 153 procedures, from different specialities were observed (in 2 series). Eight different antibiotics in different dosages were used. In 22% of the procedures, PAP was given after the incision. Two months after the intervention 147 procedures were observed (in 3 series). Three different antibiotics, 135 times cefazoline (92%), 11 times metronidazol (7.5%) and one time cefuroxime, were given. All antibiotics were used in the correct dosage. In 12% of the procedures, PAP was given after the incision. This was a significant improvement ($p = 0.026$).
Conclusion: This project shows that a uniform, simple and clear protocol improves not only the choice and dosage of antibiotics but also the timing. Furthermore, the switch to cefazoline resulted in net savings of at least €40.000 a year.

R2186 Trends in antibiotic consumption in 5 Hellenic hospitals over a three-year period: results of the Hellenic Network for Nosocomial Antibiotic Consumption for the years 2003–2004–2005

M. Lelekis, C. Loupa, K. Katsimpris, I. Tzannou, G. Xylomenos, C. Anitsakis, A. Provis, C. Efthymiou, P. Gargalianos (Athens, Rhodes, Lesvos, Zakynthos, GR)

Objectives: Hellenic Network for Nosocomial Antibiotic Consumption (HENNAC) is a network of 5 hospitals located in different parts of Greece, established for monitoring antibiotic consumption in Hellenic hospitals: "G. Gennimatas" General Hospital (GGH) of Athens (tertiary care, 700 beds), "A. Fleming" General Hospital (AFH) of Athens (300 beds), "A. Papandreou" General Hospital of Rhodes Island (RH, Southeastern Greece, 335 beds), "Vostaneio" General Hospital of Lesvos Island (LH, Northeastern Greece, 225 beds) and Zakynthos Island General Hospital (ZH, Western Greece, 125 beds).
Methods: The antibiotic consumption for the years 2003, 2004 and 2005 was calculated in DDDs per 1000 patient days (ABC calc 3.0).
Results: Compared to 2003, in 2005: (1) Total consumption increased in AFH (from 710 to 892), LH (from 1148 to 1299) and GGH (from 906 to 1117), decreased in ZH (from 1268 to 1060) and remained practically stable in RH (from 1371 to 1346). (2) Concerning consumption of major classes of antibiotics: A. Penicillins: increase in AFH and LH, stable in RH and decrease in ZH, GGH. B. Cephalosporins + aztreonam: increase in AFH, RH, decrease in GGH and stable in ZH, LH. C. Carbapenems: increase in all but LH where it was stable. D. Penicillins+inhibitors: increase in RH, LH, GGH, decrease in AFH, ZH. E. Aminoglycosides: increase in LH, GGH, stable in AFH, decrease in ZH and RH. F. Macrolides: increase in all but ZH. G. Glycopeptides: increase in all but GGH. H. Quinolones: increase in AFH, GGH, decrease in ZH, RH, LH. (3) Cephalosporins + aztreonam were the most popular antibiotics for the year 2005 (being first in 3 hospitals and second in other 2) followed by penicillins + inhibitors, while for the year 2003 exactly the opposite was the case.
Conclusions: A. While there is no uniform trend for antibiotic consumption in the hospitals of the network, total consumption is high in all of them, compared to hospitals of other European countries. B. Cephalosporins + aztreonam are now the most popular antibiotic class. C. Further studies are under way in order to explain local peculiarities in antibiotic prescribing in the hospitals of the network.

R2187 Antibiotic usage and resistance development in an intensive care unit

M.S. Lapatschek, A. Hartinger (Munich, DE)

Objectives: SARI (Surveillance of antibiotic usage and bacterial resistance in intensive care units) is a German nationwide project for monitoring the usage of antibiotics in intensive care units (ICUs) and to compare it with the development of bacterial resistance to these antibiotics. The long-term goal is to avoid unnecessary usage of antibiotics and reduce the burden of highly resistant pathogens in these ICUs.
Methods: The antibiotic usage of the 44 participating ICUs is registered by the providing pharmacies and is send monthly to the study reference centre at the University of Freiburg. There, the density of antibiotic usage is calculated as defined daily doses (DDD) per 1000 patient days. As well, the microbiology labs are sending the resistance data for selected bacteria (selection is conform to §23 Infektionsschutzgesetz) to the reference centre.
The cumulated collected data is send back twice a year to the ICUs, so that trends over months and years can be visualised.
Results: In our ICU, we were able to detect a correlation between cephalosporin usage and emergence of ESBL-carrying *Klebsiella* and between carbapenem usage and carbapenem resistant *Pseudomonas aeruginosa*, which led to a change in antibiotic prescription policy.
Conclusions: The monitoring of antibiotic usage together with bacterial resistance data can help to identify reasons for the spread of highly resistant bacteria in the clinical setting and support awareness for the sensible use of antibiotics in ICUs.

R2188 Main trends of outpatient antimicrobial consumption in Russia, 2000–2005

A.A. Fokin, S.A. Ratchina, S.N. Kozlov, E.P. Shal, A.A. Ishmukhametov, M.N. Denisova, L.P. Abramenko (Smolensk, Moscow, RU)

Objectives: To assess main trends of outpatient (OP) antimicrobials (AM) consumption (Cons) in Russia in 2000–2005.
Methods: Data containing products names, ATC/DDD codes and values (WHO, version 2006), drug forms, dosages and number of packages were collected during pharmacy audit. The use of AM was expressed as a number of defined daily doses/1000 inhabitants/day (DID).
Results: From 2000 to 2005 OP AM Cons in Russia was 9.28, 8.28, 9.51, 9.57, 9.14 and 8.90DID, respectively. The leading groups were sulfonamides and trimethoprim (J01E) in 2000–2002 and penicillins (J01C) in 2003–2005. The proportions of J01C, quinolones (J01M), macrolides, lincosamides (J01F) and other β-lactam antibacterials (J01D) Cons increased during the study period by 7.2, 5.9, 1.3 and 1.3%, respectively, while J01E use decreased (10.3%). The latter was predominantly due to twofold decrease in co-trimoxazole use (from 1.38 in 2000 to 0.68DID in 2005). The J01C Cons was characterised by increasing in amoxicillin and amoxicillin/clavulanate and decreasing in ampicillin use (from 0.26, 0.02 and 0.91 DID in 2000 to 1.28, 0.28 and 0.52 DID in 2005, respectively). The J01M Cons increase was mainly due to cipro- and norfloxacin (from 0.46 and 0.15 in 2000 to 0.77 and 0.34 DID in 2005, respectively), similar trends were typical for newer fluoroquinolones (levo- and moxifloxacin) (from 0.00003 in 2000 to 0.01 DID in 2005, respectively). As for J01F Cons, erythromycin use decreased (from 0.27 in 2000 to 0.17 DID in 2005), whereas azithro-, clarithro- and josamycin Cons was characterised by the opposite trend (from 0.09, 0.02 and 0.012 in 2000 to 0.25, 0.14 and 0.03 DID in 2005, respectively). Among cephalosporins the proportion of first-, second-, third- and fourth-generation was 75.1%/3.67%/20.9%/0.33% in 2000, 78.2%/3.56%/18.1%/0.14% in 2001, 81.1%/2.64%/16.1%/0.16% in 2002, 81.4%/2.4%/16.0%/0.2% in 2003, 76.8%/2.38%/20.6%/0.22% in 2004 and 71.0%/3.67%/25.1%/0.%23% in 2005, respectively.
Conclusion: Total OP AM Cons in Russia in 2000–2005 was rather stable with the prevalence of the cheapest, old poor safety profile drugs. But in the dynamics some positive tendencies like the increasing of the proportion of the newer AM usage were observed.

R2189 Antibiotic usage and resistance patterns in a Saudi tertiary hospital

M. Al-Jamal, A. Al-Barrak, N. Elkhizzi (Riyadh, SA)

Background: The increasing difficulty in fighting off microbes leads to an increased risk of acquiring infections in hospitals or other settings. One of the most important factors that could affect the increasing patterns

of resistance in Saudi Arabia as well as the rest of the world is the inappropriate use of antibiotics.
Objective: To evaluate the resistance patterns of *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* in 2005 at RMH comparing with 1997–1998 antibiogram.
Methodology: All isolates that have been reported in 2005 have been reviewed and compared to 1997–1998. The isolates were classified to outpatient, inpatient and intensive care unit (ICU). The isolates have been identified at Riyadh Military Hospital (RMH) laboratory according to the standard of the National Committee for Clinical Laboratory Standards (NCCLS).
Results: A total of 11,492 isolates have been isolated from all sites from both inpatient and out patient setting in 2005. About 42% of these isolates were Gram-positive and 58% were Gram-negative. *Staphylococcus aureus* isolates were 43% of isolated Gram-positive. Of these, the Methicillin Sensitive *Staphylococcus aureus* (MSSA) was 73% and the Methicillin Resistant *Staphylococcus aureus* (MRSA) was 27%. *Streptococcus pneumoniae* isolates was 295 (16% were penicillin resistant and 34% were intermediate). *Escherichia coli* was isolated in 34% of Gram-negative isolates where *Pseudomonas aeruginosa* isolates were 26%. Inpatient isolates have resistant patterns higher than outpatients and the highest was in the ICU. These data were compared with 1997–1998 antibiogram where we found that the resistant patterns is significantly increasing ($p < 0.05$). The antibiotics usage was significantly higher in 2005 compared to 1997–1998 period ($p < 0.05$).
Conclusions: The antibiotic resistance has been a problem as long as we are overusing antibiotics. The resistant pattern is higher at hospitals with the highest reported in ICU. These data was supported by that reported in the literature. This might be due to poor compliance to infection control measures were applied in ICU and the relative small, crowded and busy space. Several measures have been applied in 2006 to control infections and to prevent resistance. These include antibiotic restriction protocol, in-service education programme, surgical prophylaxis policy and continuing of implementing a good infection control strategy.

Molecular bacteriology

R2190 Comparison of real-time PCR and BDProbeTec ET system for rapid diagnosis of tuberculosis patients

S. Bernal, C. Freyre, M.J. Torres, I.J. de la Calle, M. Rodríguez-Iglesias, J.C. Palomares, E. Martín-Mazuelos (Sevilla, ES)

Objective: To evaluate the efficacy of an "in house" real-time PCR technique using the LightCycler system (Roche Biochemicals) compared to the semiautomated BDProbeTec ET system (BD) (Becton–Dickinson) and to acid-fast bacilli stain and culture to detect and identify *M. tuberculosis* in DNA extracts from samples obtained from patients with high suspicious of having TB. Final diagnosis of TB determined on microbiological and clinical findings was used as "gold standard".
Methods: The real-time assays amplifies a region from the IS61110 sequence and the BD system amplifies a region of the mycobacterial 16S rDNA. Conventional mycobacterial culture was performed with Bactec MGIT 960 and Lowenstein–Jensen medium. A total of 93 clinical samples (77 pulmonary and 16 extrapulmonary specimens) from 81 patients with high clinical and/or radiological suspicious of having tuberculosis were processed by the three methods.
Results: TB diagnosis was confirmed in 33 patients. Both methods showed a 92.6% concordance. This concordance was of 84.4% for TB cases and of 97.9% for TB negative cases. Twenty-two TB cases were detected by both methods. There were five discrepancies, all of them in smear negative/culture positive patients and 6 TB cases that were missed by both methods. The global sensitivity, specificity, positive predictive value and negative predictive value were 72.7%, 100% 100%, and 84.2% respectively for real time PCR and 75.7%, 97.8%, 96.1% and 85.2% respectively for BD system. For smear negative specimens the results were 59%, 100%, 100%, and 84.2% for real time PCR and 63.6%, 97.9%, 93.3% and 85.4% for BD system.
Conclusions: (1) Both methods showed an excellent concordance. (2) The real time PCR diagnosed the 59% of smear negative patients in the same day of specimen's reception. (3) A negative result of PCR discard the TB diagnosis because the specificity was 100%.

R2191 Analysis of essential gene changes in MRSA and non-MRSA strains inhibited by methanolic extract correlated to membrane permeabilising peptide

M. Shamsudin, N. Alipiah, N. Vasantha, F. Yusoff, A. Arshad (Serdang, MY)

Objective: In this study, a methanolic extract derived from a marine organism was investigated for the effect on the growth of MRSA resistant to β-lactam antibiotics. The nucleotide sequence changes of essential genes inclusive of mecA, mecR1, 16sRNA, mprF and msR of the inhibited MRSA strains were determined. A potential antibacterial peptide with activity on permeabilising the membrane of MRSA strains was investigated through a cellular bioassay.
Methods: *S. aureus* isolates (MRSA and non-MRSA) obtained from patients of hospitals in Malaysia and environmental isolates were studied. Anti-MRSA activity was determined through MIC and disc diffusion assay. Membrane permeabilising property of the methanolic extract was investigated by the penetration of the fluorescence Sytox green dye into MRSA cells. The RT-PCR analysis and nucleotide sequencing of essential genes, followed by gene sequence analysis were conducted for determining molecular mechanism of inhibition of *S. aureus* isolates.
Results: The methanolic marine extract showed variation in nucleotide sequence changes of several essential genes namely the mprF membrane gene in *S. aureus* (gene encoding for peptides involved in transportation of lysine to phospholipids bilayer in membrane). The intensities of SYTOX green fluorescence dye in *S. aureus* cells treated with the extract at the MIC values increases with time, similar to treatment with polymyxin, the positive control antibiotic. The negative control fucidic acid accordingly did not affect bacterial cell membrane permeabilisation and thus showed constant fluorescence intensity even after 6 hour of incubation in the antibiotic. The fluorescence intensities increase drastically first 120 minutes treatments indicating the membrane permeabilising ability is affected and the dye coloured the nucleic acid.
Conclusions: This study demonstrates the anti-methicillin-resistant *Staphylococcus aureus* (MRSA) activity of methanolic marine extract by exhibiting substantial effect at both the molecular and cellular levels elucidated through nucleotide sequence changes and Sytox green dye penetration. Molecular correlation to cellular activity of extract is a viable strategy to explore alternative anti-MRSA agents.

R2192 Investigation and sequencing of urease gene of *H. pylori* cocoid forms after exposure to different environmental conditions

F. Can, Z.C. Karahan, I. Dolapci, M. Demirbilek, A. Tekeli, H. Arslan (Ankara, TR)

Objectives: The aim of this study was to investigate whether convertion to the coccoid form under different environmental conditions resulted in sequence differences in the Urease A gene in *H. pylori* strains
Methods: *Helicobacter pylori* standart strain NTCC 11637 was used in this study. Transformation of the bacteria from helical to coccoid form was carried out with different techniques; exposure to amoxicillin, cold starvation, aerobiosis and aging of the culture. Urease activity was measured by rapid urea test. DNA was extracted from all samples by using Nucleospin DNA extraction kit (Clontech, CA). Urease A gene was amplified by using the primers HPU1 (GCC AAT GGT AAA TTA GTT CC) and HPU2 (GTA AAA ACA ATT AAG GAG). Bi-directional sequence analysis was performed by using one of the primers in each run by ABI Prism 310 DNA sequencer.
Results: Urease activity was determined in spiral and coccoid forms of *H. pylori*. All samples yielded 411 bp amplimer by polymerase chain reaction. For all sequence comparisons, spiral form of *H. pylori*

11637 was used as the Standard. The sequence of the Standard strain was compared to the sequence in GenBank (accession number M60398). The standard strain had 6 silent mutations when compared to the Genbank sequence. All the sequences of the coccoid forms had the same silent mutations with the spiral shaped standard strain and the sequences of all the samples were identical.
Conclusion: Coccoid forms of *H. pylori* had urease activity. Convertion to coccoid form in various environmental conditions did not result in any change in the amplified region of the Urease A gene of the *H. pylori*.

Molecular virology

R2193 Efficacy of a commercial multiplex herpes viruses PCR in the viral diagnosis of different clinical samples

J. Reina, E. Ruiz de Gopegui, A. Mena, M. Macia (Palma de Mallorca, ES)

Objectives: To know the efficacy of a commercial nested PCR in the aetiological diagnosis of infections caused by the herpesviruses family in different clinical samples.
Material and Methods: The different studied samples have been analysed by means of a commercial herpesviruses (HSV, VZV, CMV, EBV and HHV-6) multiplex nested PCR (Real, Durvitz, Valencia, Spain) following the instructions of the manufacturer.
Results: During the period 2000–2006, 1,445 PCRs have been made; being considered as positive 170 (11.7%). The percentage of positivity have varied widely in the study period from 8% (2003) to 17% (2001), as the number of clinical samples studied, 115 in the 2000 to 316 in 2005 ($p < 0.05$). The viruses amplified were: citomegalovirus (CMV) 90 (52.9%) ($p < 0.05$), herpes simplex (HSV) 55 (32.5%), varicella-zoster (VZV) 19 (11.1%), Epstein-Barr (EBV) 4 (2.3%) and human herpesvirus type 6 (HHV-6) 2 (1.1%). The clinical sample most studied was the CSF with 1.087 (75.2%) ($p < 0.05$) from patients with the clinical diagnosis of aseptic meningoencephalitis; being positive 57 (5.2%), corresponding to 36 HSV (63.1%) ($p < 0.05$), 14 CMV (24.5%), 5 VZV (8.7%) and 2 HHV-6 (3.5%). The colon biopsies of patients with colitis or Crohn's disease have represented 117 samples (8.1%), being positive 53 (45.2%), corresponding 45 CMV (84.9%), 4 HSV and 4 EBV. 93 bloods have been studied (6.4%), being positive 13 (13.9%), corresponding to 12 CMV (92.3%) and 1 VZV. The ocular punctions (aqueous specimens) represented 44 samples (3.1%), being positive 14 (31%), corresponding to 7 CMV (50%), 4 HSV (28.5%) and 3 VZV. Other analysed samples were 29 ammniotic fluids (2%) with 4 positives (13.7%), 2 CMV and 2 VZV; 23 Bronchoalveolar lavage, BALs (1.5%) with 13 positives (56.5%), 7 CMV (53.8%), 3 HSV and 3 VVZ. In the other 52 (3.5%) diverse samples, 16 positive were detected (30.7%), corresponding to 8 HSV (50%), 5 VVZ (31%) and 3 CMV (19%).
Conclusions: The commercial nested PCR studied has shown a high diagnostic efficacy specially in the samples of central nervous system (CSF) and in the diagnosis of viral encephalitis. This method must be recommended in all those samples in which a low viral load exists.

R2194 Evidence of Epstein-Barr and cytomegalovirus co-infection in ulcerative colitis

M. Pape, K. Mandraveli, D. Papadopoulou, I. Sidiropoulos, D. Koliouskas, S. Alexiou-Daniel (Thessaloniki, GR)

Objective: The aim of our study was to emphasize the possible role of infectious agents (EBV, CMV) in persistent ulcerative colitis (UC).
Methods: We report a case of exacerbation of ulcerative colitis in a female patient who admitted to the gastroenterology clinic of AHEPA University Hospital due to frequent bloody diarrhoea (>10 times a day). The patient had a pre-existing history of refractory ulcerative colitis and was treated with corticosteroids or immunosuppressants during acute attacks. A full-length colonoscopy was performed and multiple biopsies were obtained for histological examination of inflammation and inclusion bodies and for extraction of DNA for PCR. Computed tomography (CT), general laboratory (WBC, AST, ALT, TKE, CRP) and serological tests were also performed. Using Real-time PCR, biopsy and peripheral blood specimens were tested for the most frequently identified viral pathogens of colitis (CMV, EBV, HSV, VZV).
Results: The abdominal computed tomography (CT) scan did not revealed any abnormalities. The endoscopic and histological findings (severe erosive and edematous mucosa throughout the colon, heavy inflammatory infiltrate with epithelial ulceration) were consistent with UC. No CMV inclusion bodies were detected. The initial laboratory tests were normal except a slightly increased level of CRP. Serological tests for anti-CMV, anti-EBV, anti-HSV and anti-VZV IgG/IgM antibodies excluded primary infection or reactivation. EBV and CMV viral genome was detected only in tissue samples (2.4×10^4 copies/mL, 1.3×10^3 copies/mL, respectively). In blood samples no viral genome was detected. The patient was started on treatment with acyclovir at an oral dosage of 800 mg, five times daily for 5 days, with good resolution of the symptomatology. A further colonoscopy examination showed a clear improvement in the previous picture, with no evidence of inflammation. At that time, PCR on biopsy materials was once more performed, but no EBV or CMV genome was traced. The patient has remained symptom-free since then.
Conclusion: UC patients undergoing immunosuppresive therapy are exposed to an excessive risk of viral infection. We believe that patients with refractory UC should be tested for virus infection before increasing the dose or the number of immunosuppressive drugs. PCR techniques considered to be the most sensitive tool for virus detection and management of infections of the gastrointestinal tract.

R2195 Distinct genotypic distribution of cytomegalovirus (CMV) envelope glycoprotein B in a Cuban cohort of patients with different CMV diseases

V. Kouri, E. González, P. Martínez, V. Capó, R. González, L. Pérez, L. Cardellá, U. Hengge (Havana, CU; Dusseldorf, DE)

Human cytomegalovirus (hCMV) is ubiquitous and seropositivity in the adult population over 40 years of age rates between 60–100% worldwide. The virus is responsible for a wide range of diseases in neonates and immunocompromised patients as well as the most common viral cause of congenital malformations in developed countries.
To investigate the association between human CMV glycoprotein B (gB) genotypes and CMV disease in a Cuban Cohort of Patients with Different CMV Diseases.
We retrospectively analysed 73 biological samples (urine, PBMC, serum CSF and tissues) from 56 Cuban patients with different CMV-related diseases using a multiplex nested PCR for detection of the reported five gB genotypes.
All four main genotypes 1 to 4 were found in the clinical samples while no genotype 5 was detected. Among the individuals analysed genotype gB-2 was the most prevalent (37.5%) followed by gB-1 (30.3%) and mixed infections (16.1%) being mainly detected among immunosuppressed patients (7 out of 9) although there was no association between mixed infections and CMV rejection in transplant recipients. Genotype gB-4 was the least frequent (5 patients), which was almost exclusively detected in mixed infections (4 out of 5, $p < 0.0001$). Genotype gB-1 was more frequently detected in AIDS patients (47.7%) although it was not statistically significant while 66.7% of transplant patients showed mixed infections ($p < 0.05$).
This study represents the first report of human CMV gB genotypes in Cuban patients. This result has allowed us to identify that the main four CMV genotypes are present in the Cuban population with genotypes 2 and 1 being the most frequent strains.

Molecular typing

R2196 Antimicrobial susceptibility and AP-PCR typing of *Acinetobacter* spp. strains isolated from three teaching hospitals, Tehran, Iran

N. Sadeghifard, J. Zaeimi Yazdi, R. Ranjbar, A. Ahmadi, A. Ghasemi, A. Rostamzad (Ilam, Yazd, Tehran, IR)

Objectives: This study was designed to establish antimicrobial susceptibility and AP-PCR typing of Iranian isolates of *Acinetobacter* spp.
Methods: During this study, 67 *Acinetobacter* isolates (including 21 *A. baumannii* and 46 non-*Acinetobacter baumannii* strains, were obtained from three University hospitals in Tehran. The site of these isolates were included the blood, urine, wound, and respiratory tract. Their susceptibility to 17 antibiotics was tested and then all *A. baumannii* isolates were typed by AP-PCR method.
Results: Results showed that all *Acinetobacter baumannii* isolates were resistant to multiple antibiotics including cefoprazone, ceftazidime, ticarcillin/clavulanic acid, aztreonam, meropenem, ceftizoxime, carbenicillin, cefixime, ceftriaxone, ticarcillin, cephotaxime, but were sensitive to colistin. The results of molecular typing by AP-PCR method demonstrated 7 different patterns in the isolates.
Conclusion: Our study as the first report in Iran, established AP-PCR assay as a molecular typing method for multi-drug resistant clinical *Acinetobacter baumannii* strains. Upon our results, because of the presence and spread of multi-drug resistant *Acinetobacter* spp. in the hospitals, more care should be taken for preventing them from producing nosocomial infections in Iran.

Molecular biology, including diagnostics – others

R2197 Detection of three *Candida albicans* virulence genes in a *C. krusei* isolate from Nigeria

F. Okungbowa, R. Chowdhury, K. Pal (Benin City, NG; Kolkata, IN)

Objectives: *Candida* species have become increasingly of medical importance due to the high prevalence in man, of serious health conditions caused by them, especially in immunosuppressed individuals. Moreover, previously uncommon species such as *C. krusei* are now not only commonly isolated but often associated with resistance to antifungal drugs. The expression of three *Candida albicans* virulence genes (Tec1, Tup1 and Rad6) was determined in a *C. krusei* strain with cell morphology resemblance to *C. albicans* isolated from a human male urethra in Benin City, Nigeria, in order to ascertain how the level of virulence in the strain would compare with that of *C. albicans*.
Methods: Chromosomal DNA was isolated from the strain and amplification was done for 40 cycles. Reverse-Transcriptase Polymerase Chain Reaction was also performed.
Results: No amplification of Tec1 and Rad6 genes was detected when chromosomal DNA was used. But the RT-PCR analysis indicated that substantial rRNA synthesis occurred under the growth conditions employed but the virulence genes tup1 and rad6 were not expressed in both the *C. krusei* strain and the clinical *C. albicans* strain that was used concurrently. However, tec1 was detected in the *C. krusei* but not in the *C. albicans*.
Conclusions: An intron (important in *Candida* resistance to antifungal drugs) may be present within the tec1 gene in the *C. krusei* strain. Further investigation is needed to elucidate this. Also, the RT-PCR technique seems to be a more effective method of detecting these genes in *Candida* species.

R2198 Genomic diversity of *Leishmania* parasites isolated by RAPD-PCR

B. Nomanpour, S. Ardehalli, M. Motazedian, N. Sobhani (Kermanshah, Siraz, IR)

Objectives: Cutaneous leishmaniasis (C.L.) is prevalent in different parts of world. Clinical manifestations of cutaneous leishmaniasis vary from a simple to multiple Lesions and chronic forms. Studies have revealed the presence of genetic diversity among leishmania parasites. For better understanding of these diversities characterisation and phylogenic study seem to be necessary.
Methods: In the present study. 82 isolates of cutaneous lesions from different provinces of Iran were cultured on suitable media and characterised by using 8 primers. DNA was extracted for further studies using RAPD-PCR. DNA fingerprints of various isolates were studied by calculating inter and intra-species polymorphisms bands and also genetic distance by Jacquard's distance coefficient.
Results: *Leishmania* major and *L. tropica* were isolated and characterised from Esfahan, Tehran, Pars and Kerman provinces; *L. major* from Khuzestan province; *L. infantum* from Boushehr and Estern Azerbaijan provinces.
Leishmania tropica characterised from Yazd, Khorasan and Golestan provinces where no characterisation of the organisms has previously been reported.
Phylogenic studies demonstrated five main groups within *L. tropica* strains.
A strain isolated from a chronic patient with nine years history of CL in Tehran had 0.35 genetic distances as compared to others and showed some of *L. infantum* polymorphism bands. The possibility of this isolate being a hybrid can not be ruled out.
Leishmania major isolates comprised four main groups by maximum genetic distance of 0.40. A strain of *L. major* isolated from an Afghan refugee had genetic distance of 0.40 which differed from those isolated and studied from Iran.
Three strain of *L. infantum* isolated from Boushehr and Azerbaijan (Meshkin shahr) provinces had genetic distance of 0.37 compared with the standard strain. The inter-train of genetic distance among these organisms were 0.27. The reason for such a difference may be due to different source of standard strain. (Borkinafaso)
Conclusion: Hybridisation seems to be a form of genetic exchange among *Leishmania* species and strains. Geographical proximity, population mobility and the nativity of species and strains may be among the factors involved in possible genetic exchange.

R2199 Plasma is to be preferred above whole blood for automated nucleic acid extraction using the Nuclisens EasyMag system (Biomérieux)

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

Objectives: To evaluate the performance of the NucliSens EasyMag NA extraction system on whole blood and plasma samples compared to Qiagen DNA extraction on whole blood as the reference method.
Methods: All samples were spiked with Phocine Herpes virus (PhHV) to monitor the efficiency of extraction. In a first experiment, 10 EDTA blood samples were extracted in duplo with the Qiagen DNA extraction procedure. Four aliquots of these blood samples were extracted the same day with the NucliSens EasyMag, twice using the generic protocol and twice using the specific protocol A for cell-rich clinical specimens. Input and elution volumes were 200 µL and 110 µL respectively for the 3 extraction methods.
In a second experiment, 28 EDTA blood samples positive for CMV were extracted with Qiagen using an input volume of 200 µL and an elution volume of 75 µL. Plasma was separated from the blood samples and extracted with the NucliSens EasyMag, using the generic protocol with an input volume of 500 µL and an elution volume of 25 µL.
All extracted DNAs were subjected to real-time PCR for PhHV and CMV detection.

Results: The mean Ct value for PhHV detection of the first experiment was 32.81, 34.12 and 37.02 with a standard deviation of 0.25, 0.46 and 1.00 for Qiagen extraction, EasyMag extraction with protocol A and with the generic protocol respectively. Six of the ten blood specimens were repeatedly positive for CMV after Qiagen extraction, of which 4 were repeatedly positive with EasyMag protocol A and 3 with the generic protocol. Mean Ct values were in all cases equal or lower with Qiagen extraction.
In the second experiment, Ct values for CMV were lower in 26/28 plasma samples extracted with EasyMAG compared to the results of whole blood extracted with Qiagen, with a mean Ct difference of 1.19. The mean Ct value for PhHV detection was 30.70 and 32.35 with a standard deviation of 0.14 and 0.38 for EasyMag extraction of plasma and Qiagen extraction of whole blood respectively.
Conclusions: With an input volume of 500 μL and an elution volume of 25 μL, plasma is to be preferred above whole blood for automated nucleic acid extraction using the NucliSens EasyMag system. Moreover, the time needed for plasma isolation and EasyMag extraction for 24 samples is about 1 hour, with a hands-on time of less than 30 min. The method is thus superior in speed and sensitivity compared to Qiagen extraction on whole blood.

R2200 Use of the human albumin gene as an internal control for multiplex real-time PCR in bone marrow transplant patient routine screening

E. McCulloch, K. Harvey-Wood, B. Jones, G. Ramage, C. Williams (Glasgow, UK)

Objectives: Real-time PCR methods to monitor bone marrow transplant patients for viral pathogens are established as part of recognized screening protocols. Real-time PCR for fungal pathogens are being developed. Real-time PCR assays are used because of their high negative predictive value. However to provide confidence in the negative results, the PCR assay must be closely controlled. The use of an albumin gene, found in blood of patients, even those who are profoundly neutropenic, has the advantage over spiking samples with foreign DNA, as it provides a marker that will confirm both success of the extraction process and the PCR reaction. This is particularly important in fungal PCR due to the increased processing of whole blood samples, which require pre-treatment with buffers that remove various components of the blood and disrupt cells prior to automated extraction of DNA.
When using any internal control it is important that this is part of a multiplex reaction rather than separate single amplifications, which provide opportunity for error.
Methods: In our *Aspergillus* real-time PCR assay, DNA is extracted using the EZ1 BioRobot (Qiagen) and a tissue extraction kit, then amplified and detected using an ABI Prism 7000 sequence detector and Taqman probes (Applied Biosystems). Each sample is performed in duplicate using a duplex reaction with which simultaneously amplifies the *Aspergillus* target and albumin gene allowing detection of the internal control and the DNA of interest in a single well.
Results: Our results show that the real-time PCR reaction for albumin and *Aspergillus* can be successfully multiplexed without decreasing the sensitivity of the amplification. This allows *Aspergillus* PCR results to be reported with confidence as true negatives or true positives. Samples where negativity is present with a decreased detection of albumin suggests PCR inhibition or ineffective extraction of the sample. Conversely positive *Aspergillus* with a negative albumin indicates fungal contamination in the assay, in both these cases the samples could be repeated.
Conclusion: The use of human albumin gene in a multiplex real-time PCR limits the margin for error and provides greater confidence in the negative results obtained. This gene could be used in many other real-time PCR assays.

R2201 *Enterobacter aerogenes* strains misidentified as *Klebsiella pneumoniae* and correct identification by sequencing of the QRDR region of the gyrA gene

J. Agüero, M.C. Conejo, A. Sáez, M. de Cueto, L. Martínez-Martínez, A. Pascual (Santander, Seville, ES)

Objective: To verify by genotypic methods the identification of four clinical isolates initially identified as *Klebsiella pneumoniae* showing unusual phenotypic characteristics for this species.
Methods: Four isolates from the same patient were obtained from two blood cultures (isolates 1, 2 and 3, 4) performed within 8 days, during which the patient was treated with imipenem. Initial identification and susceptibility testing was done with the VITEK 2 system. Subsequent identification was performed with the WalkAway System and API 20E. Carbapenem activity was also determined with Etest. The presence of plasmid-mediated AmpC β-lactamases (pACBL) was analysed by multiplex PCR. Sequences from segments of the16S rRNA, rpoB and gyrA (QRDR region) genes were analysed by GenBank database.
Results: The four isolates were identified as *K. pneumoniae* by the VITEK 2. API 20E identified *K. terrigena* (isolates 1, 3, 4) or *Enterobacter aerogenes* (isolate 2). The WalkAway identified isolates 1 and 3 as *K. pneumoniae*, isolate 2 as *E. aerogenes* and isolate 4 as uncommon phenotype. MICs for isolates 1, 2, 3, 4 were: 16, ⩽1, 16, >16 (ceftazidime); 8, ⩽1, 32, >32 (cefotaxime); ⩽1, ⩽1, ⩽1, 8 (cefepime); ⩽1, ⩽1, 8, >8 (imipenem); ⩽1, ⩽1, >4, >4 (ertapenem) and >2, 1, >2, >2 (ciprofloxacin). All were resistant to both amoxicillin-clavulanate and cefoxitin. MICs of oxyimino-cephalosporins did not decrease with clavulanate. pACBL were not detected. The AmpC induction test positive in all 4 isolates. Isolates 1, 3 and 4 presented the same REP-PCR type; isolate 2 showed a different one. Isolates 3 and 4 expressed only an OmpA-like outer memprane protein (OMP). Isolate 1 presented an additional OMP of ca.36 Kda, and isolate 2 two OMPs of ca.35 and 36 Kda. One single β-lactamase band of pI 8.1–8.2 was observed in all 4 isolates. 16S rDNA and rpoB sequences did not differentiate *Klebsiella* sp from *Enterobacter* sp. The gyrA-QRDR sequences of test isolates was highly similar to that of *E. aerogenes* (98%) but not that of *Klebsiella* sp. (⩽95%).
Conclusions: Two strains identified as *K. pneumoniae*/*terrigena* following conventional methods caused bacteraemia in a single patient treated with imipenem. The more resistant strain was later isolated presenting resistance to carbapenems. Additional phenotypic tests and gyrA sequencing demonstrated that the isolates were actually *E. aerogenes* which developed carbapenems resistance after porin loss.

R2202 Touchdown PCR as a tool for improved detection of invasive candidosis

P. Angelov, T. Kantardjiev, V. Levterova, E. Zamfirova, M. Lesseva, R. Vacheva, S. Bobcheva, E. Shopova (Sofia, BG)

Summary: A touchdown conventional PCR based on the internal transcribed spacer sequence (ITS1) was developed for the detection of *Candida albicans* and five other *Candida* species. The assay precisely identifies *C. glabrata*, *C. parapsilosis*, *C. lusitaniae*, *C. guilliermondii*, and *C. krusei*, which are clinically most frequent causative agents of systemic infections. An evaluation of the assay with artificially prepared samples revealed a detection limit of 2–4 genomic copies for *C. albicans* and the other species.
Background: Invasive candidosis poses a major cause of morbidity and mortality in severe immunocompromised and hospitalised patients. Regarding the high mortality rates, there is necessity for rapid and accurate diagnostic methods for rapid diagnostics. The internal transcribed spacer sequences (ITS) are localised between the genes for small subunit r RNA and 5.8S r RNA, and large subunit r RNA, which are conserved among all fungi, but vary among different species, posing suitable target for diagnostic aims.
Aims: To study the ability of touchdown PCR to improve the detection of invasive candidosis and species identification of the pathogen.

Methods: Reaction conditions were optimised, using referent *Candida* strains DNA, and the product was visualised on ethidium bromide-stained agarose gel electrophoresis.
Serum samples from 11 patients with proven invasive candidosis were obtained, and 200 microliters from each sample were subjected to DNA extraction using DNA Purification Kit® (Fermentas®).
Touchdown PCR-assay was developed, using universal fungal primers ITS1 and ITS2 (1), which targets ITS1 region. 40 cycles of the reaction was performed, at annealing temperature ranging 68–58 degrees for the first 10 cycles, and being constant during the next 30 cycles.
Results: The assay reached sensitivity equal to 2–4 *C. albicans* genomes, allowing identification of six *Candida* species.
The touchdown PCR was able to detect fungal DNA in all tested specimens, and *C. albicans*, *C. krusei*, *C. parapsilosis* and *C. glabrata* were identified, proving the identification results, achieved with the blood culture system Bac T/ALERT FA®.
Conclusion: A sensitive and specific touchdown PCR-based assay was developed for the detection of six *Candida* species in serum specimens. Four of the most relevant pathogenic species were successfully identified in serum specimens from patients with sepsis.

Reference(s)

[1] Hibbett D. Ribosomal RNA and fungal systematics. Trans Mycol Soc Jpn. 1992; 33: 533–556.

Diagnostic/laboratory methods (other than molecular)

R2203 Osteoarticular complications of brucellosis (melitensis)

N. Taghavi, M. Haghighi (Tehran, IR)

Objectives: Bone and joint involvement in brucellosis is one of the most common complication that is reported in 20–85% of the cases. This study has been carried out to determine the rate of osteoarticular complications in patients who have been admitted in "Booali Hospital" due to brucellosis in 2003–2005.
Methods: 109 patients had entered this descriptive study. Brucellosis was determined based on clinical picture accompanying the raised titer of specific antibody measured by standard agglutination test (STA). Radiography and bone scans were obtained as needed.
Results: Of 109 patients, 72 (66%) were presented with osteoarticular complication. Spondylitis, sacroiliitis, and knee involvement were the most common complications. 39 (42.2%) patients had experienced the involvement of more than one joint at the same time. 40 (36%) patients were affected with spondylitis. Most of them (75%) were in the range of 55–60 years old. Bone scan was more sensitive for detecting spondylitis.
Conclusion: Spondylitis was the most common osteoarticular complication of brucellosis that was seen more in older patients. Radiography (sensitivity 48.7%) and particularely bone scan (sensitivity 64%) are highly recommended in susceptible cases.

R2204 Panchlamydia PCR for detection and identification of *Chlamydia trachomatis*, *Chlamydophila pneumoniae*, and *Chlamydophilia psittaci* by LightCycler PCR

T. Juretzek, J. Buder, W. Bär (Cottbus, DE)

The aim of this study was to evaluate the performance of a *panchlamydia* real time PCR for screening of the human pathogenic *Chlamydia*, such as *Chlamydia trachomatis*, *Chlamydophila psittaci*, *Chlamydophila abortus* and *Chlamydophila pneumoniae* in clinical specimen. This study included patients with respiratory affects, lung cancer, neonates with respiratory affects with possible participatipon of *Chlamydia trachomatis* or *C. pneumoniae*, patients from the ophthalmology clinic, gynaecology, urology as well as pregnant women were examined. In total3281 Specimens were routinely investigated. 0.96% (16/1662) of adults with respiratory affects were infected by *C. pneumoniae*, 0.3% (5/1662) with *C. psittaci*.
5.8% (34/581) of the pregnants carried *Chlamydia trachomatus* and 0.3% (2/581) *C. abortus*. 2.2% (8/368) of the neonates were infected by *Chlamydia trachomatus* 0.27% (1/368) with *C. pneumoniae*. 2.8% (18/625) of the children carried *C. pneumoniae* in the respiratory tract. 6.6% (3/45) *Chlamydia trachomatus* were detected in swab of eges. The melting curve analysis feature of the Lightcycler allowed identification of *Chlamydia trachomatus* and *C. pneumoniae* directly, whereas *C. psittaci* and *C. abortus* were confirmed by 16 sRNA gene sequencing. The *Panchlamydia* PCR detecteds all 4 above-mentioned *chlamydia* within 3 to 4 h. Due to this *PanChlamydia* PCR it was possile to reduce the cost for PCR and also rare found bacteria like *C. abortus* or *C. psittaci* were detected.

R2205 Prevalence of enteropathogenic and shiga toxin-producing *Escherichia coli* among children with and without diarrhoea in Iran

M.Y. Alikhani, M.M. Aslani, A. Mirsalehian, B. Fatollahzadeh (Hamadan, Tehran, IR)

Objectives: The aim of the study was to determine the rates of detection of enteropathogenic *Escherichia coli* (EPEC) and Shiga toxin-producing *Escherichia coli* (STEC) strains among children in two randomly-selected populations in Iran.
Methods: In total, 1,292 randomly-selected faecal samples from children aged less than 10 years were screened for EPEC and STEC. Of the 1,292 cases participated in the study, 184 had diarrhoea, and 1,108 were healthy/asymptomatic children. The conventional culture method and slide agglutination with 12 different commercial EPEC antisera were used for the detection of EPEC. The colony sweep polymyxin-B extraction method, non-sorbitol fermentation (NSF) phenotype, and slide agglutination with O157:H7 antisera were used for the screening and detection of STEC.
Results: Of EPEC belonging to 11 different serogroups, O111 and O127 were most commonly found in 36.4% of the diarrhoeal cases and 7.2% of the asymptomatic children. A significant association ($p < 0.05$) was found between isolation of EPEC and diarrhoea. 8.7% of the diarrhoeal cases and 2% of children without diarrhoea were infected with STEC, but none of the isolates belonged to the O157:H7 serotype. A significant association ($p < 0.05$) was found between STEC and diarrhoeal cases.
Conclusion: Based on these findings, it can be concluded that different EPEC serogroups may be agents of endemic infantile diarrhoea, and STEC strains are an important enteropathogen among young children.

R2206 Anti-neutrophil cytoplasmic antibodies in severe infection – a neglected association

C. Morath, J. Sis, M. Zeier, V. Schwenger, K. Andrassy (Heidelberg, DE)

Objectives: C-ANCA with target antigen proteinase 3 (PR3-ANCA) and P-ANCA with target antigen myeloperoxidase (MPO-ANCA) are highly sensitive and specific markers for systemic small vessel vasculitides with renal involvement (M. Wegener and microscopic polyangiitis). Although ANCAs have been considered to be highly specific for vasculitides, positive ANCAs have also been observed in patients with severe systemic infections.
Methods and Results: Recently, we observed a female veterinary medical student who was admitted with pneumonia, crescentic glomerulonephritis (S-creatinine 3 mg/dl) and highly elevated ANCA (P-ANCA 1:1260, normal <1:20, MPO 33, normal <2), which turned out to be acute Q-fever infection (coxiella IgG 504 U/L; IgM +++). Renal histology revealed crescentic and necrotic immune complex nephritis (postinfectious glomerulonephritis). Under long-term treatment with doxycycline and levofloxacine, coxiella titers declined slowly from 504 U/L to 90 U/L. Within 6 months renal function improved (S-creatinine) from 3 mg/dl to 1.3 mg/dl and proteinuria decreased from 3.1 g/day to 1.2 g/day. Although crescentic glomerulonephritis is usually treated by

immunosuppression, we avoided this therapy in our patient because of possible harmful effects on the course of Q-fever infection. Whereas Q-fever infection was cured with this regimen, renal function declined progressively, becoming strongly impaired in the follow-up. A second renal biopsy revealed chronic sclerosing glomerulopathy with advanced tubulo-interstitial fibrosis.

Discussion: ANCA with the classical target antigens PR3 or MPO in infectious diseases were shown first by our group in patients with endocarditis, HIV and by other investigators in patients with amoebiasis, leprosy and hepatitis C. ANCA with other target antigens, e.g. elastase and BPI (bacterial permeability inhibitor) could be demonstrated in other bacterial infections (e.g. osteomyelitis, cystic fibrosis).

Conclusion: (1) ANCA are not only markers of small vessel vasculitides, they can also be (false) positive in patients with systemic infections, (2) Determination of target antigens for ANCA are indispensable, (3) Interpretation and therapeutic decisions concerning ANCA associated diseases should only be performed within the clinical context.

R2207 MRSASelect applied on enrichment broths significantly increases the sensitivity of MRSA detection in screening samples

M. Ieven, M. Michiels, H. Jansens, J. Breughelmans, H. Goossens (Edegem, BE)

Background: Early identification of MRSA carriers among hospitalised patients is crucial to prevent its spread.

Our objective was to evaluate the performance of a chromogenic medium, MRSASelect (BioRad) for MRSA detection on surveillance samples both by direct plating and on enrichment broths in comparison with conventional cultures.

Methods: A total of 264 surveillance swabs from 84 patients, previously colonised, were cultured on blood agar (BA), mannitol salt agar (MSA) and MRSASelect (MS) and subsequently into a MRSA enrichment broth containing Brain Heart Infusion, NaCl 7% and oxacillin 4µg/mL. After 24 hrs incubation at 35ºC broths were subcultured onto a secondary MSA and MS plate. All plates were read after 24 and 48 hrs incubation for colony morphology and colour production on MS. Identification and methicillin resistance of pink colonies on MS suspected for MRSA were confirmed by tube coagulase test and oxacillin resistance.

Results: A total of 69/264 (26%) specimens were MRSA positive. After 24 hours incubation, 47/69 (68%) were recovered on the primary MS compared to 61/69 (88%) on routine culture media (RC). Further incubation of the primary plates for 48 hrs resulted in the detection of 53/69 (77%) of MRSA on the MS plate. After enrichment overnight in broth and subculturing for 24hrs on MS agar a total of 60/69 (87%) MRSA were isolated, an increase of 28% compared to the detection on the primary plate. Another 2/69 (3%) were detected after incubation the broth subcultures for another 24 hrs. Compared to the results of culture on BA and MSA, the sensitivity, specificity and negative and positive predictive values of MRSASelect for the detection of MRSA on the primary plate were 67%, 97%, 91% and 87% after 24 hrs, 77%, 97%, 93% and 88.6% after 48 hrs incubation and from broth 85%, 96%, 96% and 87% after 24 hrs and 89%, 96%, 97% and 87% after 48 hrs respectively.

Considering a specimen as a true positive for MRSA if identified and confirmed by any of the culture methods, sensitivity of MS was 68% and 77% on the primary MS plate after 24 and 48 hours, and 87% and 90% after 24 and 48hrs from broth respectively.

Conclusion: Although direct inoculation on MRSASelect results in the detection of only 68% of MRSA after 24hrs, identification is already confirmed after overnight incubation. Broth enrichment subcultured on MRSASelect increases the yield of MRSA detected by 28% compared to direct plating but postpones time to detection by 24hrs.

R2208 Evaluation of two new tests for the detection of *Helicobacter pylori* in stool specimens

S. Blanco, A. Lacoma, M. Forne, C. Prat, I. Latorre, M. Cuesta, J. Viver, V. Ausina, J. Dominguez (Badalona, Terrassa, ES)

Objectives:

1. To evaluate the usefulness of 2 new stool antigen assays, a immunochromatographic tests Letitest *H. pylori* CARD (Laboratorios leti, Madrid, Spain) and an EIA test Immundiagnostik ELISA (Immunodiagnostik AG, Bensheim, Deutschland) in the diagnostic of *Helicobacter pylori* infection and to confirm *H. pylori* eradication after treatment.
2. To compare their accuracy with four stool antigen assays: ImmunoCard STAT! HpSA and Premier Platinum HpSA (Meridian Diagnostics, Cincinnati, OH), Amplified IDEIA Hp StAR and RAPID Hp StAR (DakoCytomation, Cambridge, UK).

Methods: We evaluated stool samples from 80 patients diagnosed with *H. pylori* infection and from 40 patients without infection. To confirm *H. pylori* eradication we evaluated 40 patients who received *H. pylori* treatment, eradication was confirmed with 13C-urea breath test 6 weeks later.

The assays were performed according to the specifications of the manufacturer. Sensitivity, specificity, and positive and negative predictive values were calculated.

Results: The sensitivity, specificity, and positive and negative predictive values of the tests are shown in the table. In the 40 patients evaluated 6 weeks after eradication therapy, the overall agreement between urea breath test and the antigen tests were: Letitest 85%, ImmunoCard STAT! HpSA 82.5%, RAPID Hp StAR 75%, Immundiagnostik ELISA 82.5%, Amplified IDEIA Hp StAR 90% and Premier Platinum HpSA 75%.

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Letitest *H. pylori* CARD	83.8	66.6	91.2	48
Immundiagnostik ELISA	87.3	83.3	95.8	60
RAPID Hp StAR	78.8	55.5	88.7	37
ImmunoCard STAT!	52.5	94.4	97.7	30.9
Premier Platinum HpSA	92.5	72.2	93.7	68.4
Amplified IDEIA Hp StAR	95	66.6	92.7	75

Conclusions: Our results indicate that Letitest and Immundiagnostik ELISA are helpful procedures to determine *H. pylori* infection. Compared to the 13C UBT, both new test shows a comparably good correlation to assess the success of eradication therapy.

R2209 Liaison® VZV IgG and VZV IgM assays: a comparative study

P. Huynen, P. Melin, M. Hayette, J. Mathieu, P. De Mol (Liege, BE)

Varicella-zoster virus (VZV) belongs to the Herpesvirus family. Varicella is the full-blown primary infection and zoster is caused by the reactivation of latent VZV. Varicella and zoster are mainly diagnosed clinically but serology plays an important role, especially when the course of disease is non-typical.

Objectives: VZV serology is currently carried out by microplate analysers in our institute. The aim of this study was to evaluate if the LIAISON® VZV IgG and VZV IgM (DiaSorin, Saluggia, Italy), two fully automated immunoassays, based on chemiluminescence technology (CLIA) could be an alternative method, quick and easy to perform, whose performance meets our current quality requirements. We therefore performed a comparative evaluation and investigated the overall agreement between LIAISON® VZV IgG and IBL VZV IgG ELISA (Immuno Biological Laboratories, Hamburg, Germany) as well

as between LIAISON® VZV IgM and Enzygnost VZV IgM ELISA (Dade Berhing Enzygnost, Marburg, Germany).
Methods: The performances of LIAISON® VZV IgG and IBL VZV IgG ELISA were compared for a total of 165 selected routine serum samples (139 positive, 19 negative, 7 indeterminate). For interpretation of results of the LIAISON® assay, a cut-off value of 150 mIU/mL was used, in order to achieve the highest diagnostic specificity and sensitivity. Discordant results were solved by Euroimmun VZV IgG (Euroimmun AG, Luebeck, Germany) ELISA.
The performances of LIAISON® VZV IgM and Enzygnost VZV IgM ELISA were compared for a total of 160 selected routine serum samples (22 positive, 114 negative, 24 indeterminate). Discordant results were solved by Euroimmun VZV IgM and NovaTec VZV IgM ELISA (NovaTec Immunodiagnostica GmbH, Dietzenbach, Germany).
Results: The comparative study between LIAISON® VZV IgG and IBL VZV IgG ELISA as well as between LIAISON® VZV IgM and Enzygnost VZV IgM ELISA showed overall agreement of 89.7% and 80% respectively. When Euroimmun VZV IgG, Euroimmun VZV IgM and NovaTec VZV IgM tests were used and that a consensus between at least two out of three tests was applied, those values were 96.4% and 97.5% respectively.
Conclusions: The LIAISON® VZV IgG assay is a valid alternative for the quantitative detection of VZV IgG antibodies, since the kit performance is at least equivalent to that of the kits currently available on the market.
With its high specificity, the LIAISON® VZV IgM test, a fully automated method, is also a good alternative for the detection of IgM antibodies.

R2210 Study in changes of CSF in 50 patients with bacterial, viral, and TB meningitis

N. Taghavi, M. Haghighi (Tehran, IR)

Background: Bacterial meningitis is one of the most life threatening condition which without therapy has a high mortality. Differentiation between viral and bacterial meningitis is crucial for appropriate treatment.
Methods: This prospective study was performed on csf changes in 50 patients with bacterial, viral and TB meningitis in infectious department of "Booali Hospital" between year 2002 and 2005. Protein, glucose and cell count of csf samples were analysed.
Results: From 50 patients, 30 cases had bacterial meningitis (proven by culture) 14 cases had viral and 6 cases had TB meningitis. In csf study in bacterial meningitis we had protein >100 mg in 66.6%, csf GLC/serum GLC <20% in 46.6%, leukocyte count in range of 1000–10,000 in 53.3% and PMN dominancy in 93.3% of patients. In patients with viral meningitis we had normal protein in 71.4%, normal GLC in 85.7% and leukocyte count in range of 100–500 in 57.1% with mononeuclear dominancy.patients with TB meningitis had csf protein 45–100 mg in 66.6%, csf GLC/serum GLC <20% in 100%, leukocyte count in range of 100–500 in 100% with mononeuclear dominancy.
Conclusion: concerning the importance of distinguishing of septic and aseptic meningitis, and in the absence of established diagnosis, the csf GLC/serum GLC ratio can be used as a sensitive test to determine the probable septic aetiology. The cutpoint of csf GlC/serum GLC below 25 can be rational reason to start empirical treatment.

R2211 Gram-positive anaerobic bacteria: frequency of isolation and antimicrobial resistance pattern

P. Giannopoulou, A. Kyratsa, I. Graphakos, E. Kontekaki, A. Chondrothanassi, P. Safioleas, K. Zambou, E. Trikka-Graphakos (Athens, GR)

Objectives: To evaluate the incidence of Gram(+) anaerobic bacteria isolated from clinical specimens and their susceptibility pattern.
Methods: During a two-year period (2005–2006) we evaluated Gram(+) anaerobic bacteria isolated from documented infections. For anaerobic culture the following media were used: Brucella Blood agar enriched with vitaminK1 and hemin, Kanamycin-Vancomycin Laked Blood agar, Bacteroides Bile-Esculin agar and Cooked Meat Broth. Preliminary identification was based on Gram stain, colony morphology and susceptibility to high potency discs (colistin10μg, SPS1000 μg, kanamycin1000 μg, vancomycin 5 μg). Species identification was performed by API20A & RapidID32A system. Beta-lactamase production was tested by nitrocephin discs. Antibiotic susceptibility was performed by E-test in the National Reference Center according to CLSI guidelines.
Results: A total of 48 clinical strains were isolated as follow: *PeptoStreptococcus* spp. (23), *Clostridium* spp. (19) and *Eubacterium* spp. (6). 12 strains were isolated from patients suffering from bacteraemia with *C. perfringens* being the most common isolate, 6 from patients with bacterial peritonitis, 7 with pelvic inflammatory disease, 4 with burns, 3 from patients with gas gangrene and finally 19 from wounds and soft tissue infections with *PeptoStreptococcus* spp. as the commonest isolate. Among clostridial infections stands out one case of fatal spontaneous bacterial peritonitis in a cirrhotic patient due to *C. tertium* and 3 cases of gas gangrene due to *C. septicum* and *C. sporogenes*, without underlying malignancy. All strains of *Clostridium* spp. were sensitive to penicillin, while resistance to clindamycin was 12.9% (MICs of 4–>256 μg/mL) & for *PeptoStreptococcus* spp. 12.1% (MICs > 256 μg/mL). Metronidazole, piperacillin/tazobactam and imipenem were highly effective to all isolates. Resistance to ceftriaxone was detected in 4 isolates. All but one isolates were sensitive to tetracycline.
Conclusions: (a) Penicillin remains the drug of choice for clostridial infections, while metronidazole or imipenem are prudent alternatives. (b) *Clostridium* and *PeptoStreptococcus* spp. showed low resistance to clindamycin. (c) Resistance to ceftriaxone and tetracycline require closer surveillance.

R2212 Evaluation of Rubella IgG and IgM assays on the new Vidia® instrument

M.C. Medici, V. Albonetti, M. Martinelli, C. Chezzi, G. Dettori (Parma, IT)

Objective: The aim of the present study was to evaluate the performance of the new VIDIA® Rubella IgG and IgM assays on the fully automated VIDIA instrument, easy to use with a high level of traceability.
Methods: Sensitivity and specificity of the VIDIA RUB IgG and IgM (bioMérieux, France) were evaluated in a retrospective study on 209 serum samples belonging to 195 subjects and in a prospective study on 211 serum samples belonging to 210 subjects, in comparison with VIDAS® RUB IgG and IgM (bioMérieux, France), AxSYM® Rubella IgG and IgM (Abbott, USA) and LIAISON® Rubella IgG and IgM (DiaSorin, Italy) assays.
The samples for which at least one equivocal result have been found whatever the method have been excluded for the analysis, as well as the non resolved samples.
Discrepancies were resolved considering individual clinical data, when available, and/or with repetition of the test as well as with IgG Avidity test for IgM discrepancies.
Results: Sensitivity: For VIDIA RUB IgG, sensitivity was 100% in comparison with 2 out of 3 methods in the retrospective study and with all methods in the prospective one. For VIDIA RUB IgM, in the retrospective study, sensitivity was 100% only in comparison with VIDAS RUB IgM. After resolution of discrepancies, the absolute sensitivity was 100% for all methods except AxSYM Rubella IgM which was 33.33%. In the prospective study the sensitivity was not determinable as no positive serum was obtained.
Specificity: For VIDIA RUB IgG, the specificity has been established at 100% compared to VIDAS RUB IgG and AxSYM Rubella IgG in both retrospective and prospective studies. In comparison with LIAISON Rubella IgG it was 97.29% and 95.24% in the retrospective and prospective studies, respectively. After resolution of discrepancies the absolute sensitivity was found 100%.
The relative specificity of VIDIA RUB IgM compared to VIDAS RUB IgM, AxSYM Rubella IgM and LIAISON Rubella IgM assays

was 99.49%, 98.44 and 99.47, respectively, in the retrospective study. Taking into account clinical information and Avidity results, the absolute specificity was closed to 100%. In the prospective study the specificity of VIDIA RUB IgM was found 100% for all the three methods.
Conclusion: The two evaluated assays, VIDIA RUB IgG and VIDIA RUB IgM, show an excellent sensitivity and specificity.

R2213 Comparison of different culture media and growth conditions for the recognition of *Arcanobacterium haemolyticum*

C. Garcia, A.B. Campo-Esquisabel, F. Unda, C. Ruiz de Alegria, N. Benito, L. Martinez-Martinez (Santander, ES)

Objectives: *A. haemolyticum* (AH) would be easily missed or unrecognized in routine cultures. The aim of this study was to perform qualitative and quantitative comparisons of six culture media and different growth conditions for recognition of AH colonies.
Methods: Forty-seven clinical isolates and the reference strain ATCC 9345 of AH were selected for this study. Identification as AH was accomplished by conventional biochemical tests and sequencing of 16S rDNA. For each strain, a suspension was prepared with a final concentration of approximately 10^3 ufc/mL. All strains were plated onto sets of three plates of the following commercial media: (I) Trypcase Soy Agar with 5% Horse Blood (TSH), Trypcase Soy Agar with 5% Sheep Blood (TSS), Columbia Agar with 5% Sheep Blood (COS), Schaeleder Agar with 5% Sheep Blood (SCS) from bioMérieux. (II) Columbia Agar with 5% Horse Blood (CH) from Beckton Dickinson. (III) Columbia Sheep Blood Agar with colistin/nalidixic (CNA) from Biomedics. Plates were incubated in air, CO_2, and anaerobiosis for 72 h at 37ºC with a first reading after 24 h. Five isolated colonies from each plate were measured with a micrometer of a dissecting microscope, and the average colony size was recorded in millimeters. Analysis of variance (ANOVA) was used to compare colony size on differents media, atmosfera and time of incubation. Quality of media performance was assessed as presence of beta-haemolysis and significance was determined by the Chi-squared test.
Results: After 24 h of incubation the mean colony diameter was 0.2 mm (SD 0.003). The average colony size was significantly greater on TSH, CH and COS (aerobic and CO_2 incubation) and SCS (CO_2 incubation) than under the other media and atmosphere conditions ($p < 0.001$). Haemolysis was visible more often on TSH with CO_2 incubation (61%) than in the other conditions. After 48 h, the mean colony diameter was larger on TSH with aerobic and CO_2 incubation (0.77 mm in both cases) than in the other situations ($p < 0.001$); haemolysis was detected in 100% of strains incubated in TSH. After 72 h of incubation, the average colony size remained greater on TSH – specifically with CO_2 – than on the other media and atmosphere conditions (1.2 mm, SD 0.02).
Conclusions: In TSH larger colony sizes, and earlier appearance of colonies and of beta-haemolysis was observed in comparison with the remaining evaluated media.

R2214 Evaluation of *Helicobacter pylori* stool antigen test for the detection of *H. pylori* infection in paediatric patients

G. Gonzalez Mediero, J.M. Ramos, J.I. García, I. Otero Varela, T. Del Blanco, J.A. Sanchez Conde (Vigo, ES)

Objective: *Helicobacter pylori* plays an important role in the pathogenesis of some several extra or intragastroduodenal diseases. The assessment of *H. pylori* antigen in human stools has been proposed as a valuable non-invasive diagnostic tool. The aim of this study was to evaluate the usefulness of *H. pylori* stool antigen (HpSA Premier Platinum HpSA, Meridiam Diagnostics, Cincinnati, USA) in diagnosis of *H. pylori* infection in children patient.
Methods: 1270 patients have been consulted in the Gastroenterology Paediatric unit as news patients during a period of 4 years (1/01/02–31/12/05). Seventy two patients aged 3–15 years with chronic pain abdominal and suspicion of infection for *H. pylori* were studied in this work; sixty one (4.8% of total patients) were included in this study and eleven discarded for not advice. Stool samples were taken before antibiotic treatment. The patients were evaluated in two stages, the first one for symptoms as pattern of pain abdominal chronic (per-umbilical, epigastria) and study for HpSA and the second one with gastrointestinal gastroscopy for assessment study mucosa for histology, ureasa test and serology (ELISA). Patients were defined as *H. pylori* positive infection if histology was confirmed as gastritis chronic antral and *H. pylori* was showed in the biopsy.
Results: 31 patients were positive for *H. pylori* (50.8%). The media optical densities at 450 nm were 1.088 (range 0.064–3.500) and 0.088 (0.004–0.444) in the patients positive and negative for *H. pylori* antigen respectively. The sensitivity and specificity of method were as follows respectively; ureasa 86.3% and 89.9%, serology 63% and 78% and HpSA was 99% and specificity 40.3%. Predictive value of positive and predictive value of negative test respectively is ureasa 96% and 75%, serology 85% and 67% and HpSA 51% and 98%.
Conclusion: *Helicobacter pylori* antigen test is non-invasive, easy to perform and it can be a test to exclude negative cases when the result is negative, but it can not be considered as a diagnostic test in paediatric group in our area in Vigo (Galicia, Spain). We considered that positive patients who gastrointestinal gastroscopy was negative, must be revised and to evaluate the evolution and prognosis. Until October 2006 none of them were request consultant or revision.
Acknowledgements: All test for *Helicobacter pylori* has been performed by Elena Saavedra as technical personal.

R2215 Routine use of blood culture bottles can reduce detection time in PD peritonitis

V. Cleeve, T. Poole, M. Fitzpatrick, F.K. Gould, T.H.J. Goodship (Newcastle upon Tyne, UK)

Objectives: Peritonitis is a recognized complication of peritoneal dialysis and may result in Tenchkoff catheter removal. Our aim was to assess whether the addition of a blood culture bottle inoculated with PD effluent improved the rate of detection of causative organisms in cases of suspected peritoneal dialysis peritonitis and whether the time to a positive result was more rapid compared to results obtained by direct culture alone.
Methods: A retrospective review was performed of all PD effluents sent for culture from July 2005 – June 2006. Direct samples had been processed using a lysis-centrifugation technique using saponin, inoculated onto a range of agar plates and incubated for 48 hours. Blood culture bottle samples were incubated using a continuous monitoring BacT/Alert system for a maximum of 5 days. Times to positive results were obtained using data from the blood culture computer records and specimen forms. Clinical information was obtained using peritoneal dialysis unit records.
Results: A total of 136 direct samples of peritoneal dialysis effluent were sent for culture and 20 of these were sent with an additional sample in a blood culture bottle. Of the 20, 9 became positive relating to 7 episodes of peritonitis. All 9 bottles grew the same organisms as the corresponding direct sample except in one case where the blood culture bottle sample grew *Streptococcus vestibularis* in addition to the Coagulase negative *Staphylococcus* isolated directly. No direct sample sent with a paired sample in a blood culture bottle was positive without the blood culture sample also being positive.
All 9 samples in blood culture bottles flagged positive within 25 hours. The quickest time was 10 hours. As a number became positive during the night the samples were processed the following morning but a provisional Gram stain report was available by the next day in all 9 cases. Of the corresponding direct samples 5 had provisional culture results the next day but 2 samples required 2 days to obtain a result, 1 sample 3 days and another 4 days.
Conclusion: During this time period the addition of a sample of PD effluent in a blood culture bottle did not improve the detection of causative organisms in cases of suspected peritonitis compared to culture of direct samples but the numbers were small. However, the additional

sample did provide more rapid provisional results for the clinicians without a high increased rate of contaminated samples.

R2216 One-year surveillance study in patients with pulmonary infections caused by anaerobic bacteria

S. Marinkovic, I. Marekovic, M. Jakopovic (Zagreb, HR)

Objectives: Anaerobic bacteria are relatively common pulmonary pathogens. Anaerobic infections are very often polymicrobial, including also microaerophilic and facultative anaerobes. Some studies have shown that patients with underlying carcinoma have high incidence of Gram-negative aerobic pathogens. The purpose of this study was to determine the frequency of different anaerobic bacterial species and their susceptibility to antimicrobial agents. In patients with underlying carcinoma we wanted to determine if Gram-negative aerobic bacteria are frequently present together with anaerobes, which could have implications on empirical treatment of these infections.
Methods: This study was conducted during year 2005 in Department for Microbiology, University Hospital for Lung Diseases "Jordanovac". Anaerobic bacteria were isolated in patients with clinical and radiographic signs of pulmonary infection from specimens obtained mostly at fiber-optic bronchoscopy, either by bronchoscopic aspiration or by bronchoalveolar lavage (BAL). Antimicrobial susceptibility was tested with E-tests in order to determine minimal inhibitory concentrations (MIC) according to NCCLS (CLSI) standards.
Results: Total of 108 anaerobes was isolated in 27 female and 64 male patients. The most frequent isolates were Gram-positive non-spore-forming bacilli (30.56%). Anaerobes alone were detected in 61 (67.03%), and anaerobic and aerobic bacteria together were observed in 30 (32.97%) cases. An average of 1.65 bacterial species per patient was obtained. Underlying carcinoma was present in 24 (26.37%) patients. In 20 (83.3%) of these patients anaerobes were present alone. The level of resistance to metronidazole, ampicillin and clindamycin was 59.3%, 8.3% and 6.5%, respectively. To imipenem and ko-amoxiclav there were no resistant strains.
Conclusion: Anaerobic bacteria are found more often in male patients with pulmonary infection. The most frequent anaerobes in our patients were Gram-positive non-spore-forming bacilli. In most of our patients multiple isolates were found. In contrast to the findings of some studies in patients with underlying carcinoma, anaerobes were more frequently present alone and Gram-negative aerobic bacteria were uncommon. High percent of resistance to metronidazole indicates that clindamycin and β-lactams are better choice when anaerobic infection is suspected.

R2217 Elimination of false negativity of internal amplification control in PCR assay

M. Sayan, Z. Yumuk, S. Çelebi, A. Willke (Kocaeli, TR)

Objectives: Polymerase chain reaction (PCR) is a nucleic acid amplification technique commonly used in routine laboratories as a rapid, sensitive and specific diagnostic tool. However PCR inhibitory components may effect the PCR assay and false negative results may be obtained. To detect false negativity, internal amplification control (IAC) is used in diagnostic laboratories, but various PCR inhibitors (such as heparin, heme, leukocyte DNA etc.) may adversely effect amplification procedure. In this study we evaluated the effectiveness of various pre-PCR processing procedures on IAC in paralel with the serum samples of hepatitis B and hepatitis C patients.
Methods: From January 2005 through October 2006, hepatitis C virus (HCV) RNA (fluorion HCV QNP v2.1 kit-Iontek Ltd. Sti, Istanbul-Turkey) and hepatitis B virus (HBV) DNA (fluorion HBV QNP v2.0 kit-Iontek Ltd. Sti, Istanbul-Turkey) tests were performed by quantitative real-time PCR (iCycler IQ, v 3.0a – BioRad Laboratories, Hercules, CA-USA) to 1440 and 2754 patient samples respectively. We used silica-gel based spin column (SC) and silica-coated magnetic particle (MP) as extraction methods. After then, in the sera which was seen no amplification of IAC, we re-performed PCR tests after using; (1) freeze-thawing, (2) sample dilution, (3) a new sample and (4) a new lot of IAC consecutively.
Results: Totally 211/1440 (14.6%) of HCV RNA PCR assay (SC: 122/905, MP: 89/535) it was seen no amplification in the IAC. Considering HBV DNA PCR results, false negativity was found to be 15/2754 (0.5%) (SC: 6/1724, MP: 9/1030). Comparison of two extraction methods namely SC and MP for both HBV DNA and HCV RNA PCR tests there was no statistically significance related with false negativity. False negativity ratio in IAC has decreased from 14.6% to 6.0% in HCV RNA group and from 0.5% to 0.0% HBV DNA group after applying all four inhibitor elimination methods.
Conclusion: In routine laboratories in PCR assay, inhibitory substances may lead to several problems such as re-test the same sample, higher cost and delayed results. Therefore in this case, the test should be re-performed after freeze-thawing and diluted procedure. If no result obtained, it will be beneficial to re-performed the test with a new sample or new lot of internal control.

R2218 Evaluation of a rapid new stool antigen test for diagnosis of *Helicobacter pylori* infection in adult patients

R. Krausse, G. Müller, M. Doniec (Kiel, DE)

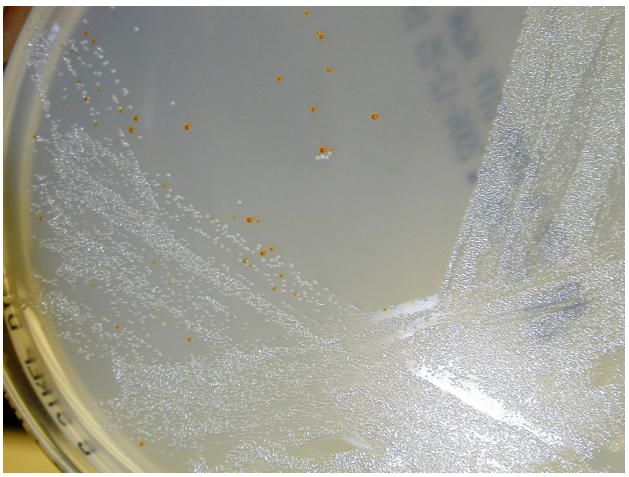
The overall objective of this study was to evaluate the performance of a rapid new stool antigen test (Rapid Hp StAR^{TM}, Dako) for the diagnosis of a *H. pylori* infection in adult patients in our region. The accuracy of the test was defined in relation to the different clinical entities and to the age of the patients. The study included 72 dyspeptic adult patients (37 women, 35 men, mean age 58.4±15 years, range 24–88 years) and was conducted in the years 2002 and 2003. Biopsies were taken for culture, histology and/or rapid urease test. Out of 72 patients, 28 (38.9%) were *H. pylori* positive, 44 (61.1%) were *H. pylori* negative. 18 (64.3%) of the 28 *H. pylori* positive patients had gastritis (n = 11, 39.3%) or gastroduodenal ulcers (GDU) (n = 7, 25.0%) and all other (n = 10, 35.7%) patients had a normal mucosa (NM). Gastritis, GDU and NM were determined in 11 (25.0%), 2 (4.5%) and 31 (70.5%) of the Hp negative patients. Faecal specimens were collected from all the patients and examined for the presence of *H. pylori*. The Rapid Hp StAR^{TM} stool antigen test yielded positive results in 13 and 20 of the 28 *H. pylori*-positive patients (46.4 and 71.4%) after a reading time of 20 and 30 min, respectively. Within the *H. pylori*-negative group of patients (n = 44) the test identified 2 (4.5%), and 7 (15.9%) as *H. pylori* positive after 20 and 30 min, respectively. The sensitivity, specificity, positive and negative predictive value and accuracy were 59.1%, 93.1%, 86.7%, 75.0% and 78.4% after a reading time of 20 min and 76.9%, 82.9%, 74.1%, 85.0% and 80.6% after 30 min. The test achieved the highest sensitivity and specificity among the patients with duodenal ulcers, followed by patients with normal mucosa and gastritis. Moreover, the age (<45 vs > 45 years) and the gender of the patients influenced the efficiency of the test.
Conclusion: This new monoclonal rapid stool antigen test can be used as an alternative non-invasive method to detect *H. pylori* infection. However, a longer incubation time (30 min) is recommended when negative results occur within 20 min; further studies with a greater number of different patient groups are required to confirm its accuracy.

R2219 Comparative study of group B *Streptococcus* differential agar versus LIM broth for detection and identification of *Streptococcus agalactiae* in pregnant women

A. Adler, C. Block, D. Engelstein, D. Hochner-Celnicier, R. Drai-Hasid, A.E. Moses (Jerusalem, IL)

Objectives: Our goal was to compare BD Group B *Streptococcus* Differential Agar (GBSDA, Becton Dickinson), a commercially available modification of Granada medium, with inoculation into LIM broth and subculture onto blood agar plates (LIM-BAP) as a method for GBS screening in pregnant women.

Methods: First, we compared the detection threshold and the ease of use of GBSDA with LIM-BAP by inoculating known numbers of GBS mixed with approximately 10^7 colony-forming units (cfu)/mL of other bacteria (*E. faecalis* ATCC 29212, *S. epidermidis* ATCC 12228, *P. mirabilis* ATCC 7002 and a clinical isolate of *Lactobacillus* sp.). Second, we tested the production of carotenoid pigment in 159 GBS blood culture isolates. Finally, we compared GBSDA, LIM broth with direct GBS antigen detection (LIM-AG) and LIM-BAP as methods for GBS screening in pregnant women presenting in labour to the delivery room. Suspect colonies were identified as GBS by a commercial agglutination test. Identification of non-haemolytic GBS included a positive CAMP test and failure to hydrolyse esculin on bile-esculin agar.
Results: GBS colonies were easily detected on GBSDA (figure) at a threshold of 2000 cfu/mL in a mixed culture. In contrast, GBS was not detected in a mixed culture on BAP subcultured from LIM broth with initial inocula of up to 10^4 cfu/mL. Orange pigment was produced in 149 of 159 GBS blood culture isolates, including one non-haemolytic isolate. Pigment was not produced in 8 non-haemolytic and 2 haemolytic strains. GBS was detected by GBSDA in 49 out of 226 women (21.6%) but in only 42 (18.5%) and 36 (16%, $p < 0.05$) women by using LIM-BAP and LIM-AG, respectively.
Conclusion: GBSDA was faster, easier to use and more sensitive for the detection of GBS from vaginal swabs, compared with LIM-BAP. Although non-haemolytic strains are usually missed by this method, it appears that enrichment in LIM broth offers no advantage for detection of these strains. Therefore, GBSDA may be the culture method of choice for GBS screening in pregnant women.



R2220 Evaluation of a new rapid method for microbial growth analysis and antimicrobial susceptibility test in human biological fluids

A. Tessari, S.G. Parisi, A. Cavallaro (Padua, IT)

Objectives: The Uro-Quick system is an automated rapid method for bacteriuria screening which uses laser nephelometry to detect bacterial growth. We evaluated the Uro4 HB&L system for a rapid micro organisms detection and antimicrobial susceptibility test (AST) directly performed on human biological fluids.
Methods: 233 human biological samples counting respiratory (36%), articular, peritoneal and other drainages (64%), were analysed for culture with Uro-4 system. Gram stain microscopy and plate subculture analysis was performed on every sample analysed with Uro-4 after 6 hours incubation. Sixty-nine samples (57 mono and 12 polimicrobial) detected positive by Uro-4 were then processed for a direct AST (3 hours incubation). Four different antimicrobial panels were designed for Enterobatteriaceae, *Streptococcus*, *Staphylococcus* and non fermenting Gram negatives. Culture and AST performed on Uro-4 were compared with traditional culture methods on agar plates and with VITEK 2 system for AST.
Results: 136 samples were concordant positive. Microbial growth analysis performed on Uro-4 showed 94.4% sensitivity and 93.3% specificity compared with the traditional culture method. Uro-4 test coupled with Gram stain microscopy after incubation gave 100% sensitivity. Positive counted monomicrobial samples (76%), including 69% Gram positive bacteria, 24% Gram negative and 7% yeast, and polimicrobial samples (24%). Direct AST performed on 26 monomicrobial Gram positive cultures resulted in 95% agreement with the reference method (*Streptococcus*/*Enterococcus* panel 100%, *Staphylococcus* 92.4%) meanwhile AST conducted on 31 monomicrobial Gram negative samples revealed a 96% agreement (Enterobacteriaceae 94.9%, non fermenting 97.3%). No very major error (sensible vs. resistant) was detected. Polimicrobial samples gave discordant results by reason of the variable composition of the mixture and the small number of analysis.
Conclusion: Uro-4 is able to perform bacterial growth analysis and a subsequent AST within 7–9 hours, with a great saving of time respect to the traditional cultural and AST methods. The system revealed optimal performance on microbial agents detection when coupled with Gram stain analysis. The Uro-4 AST showed an high level agreement with the reference method when the test was conducted on mono microbial samples. Further investigations are pending for polimicrobial samples.

R2221 Comparison of MacConkey agar with ceftazidime and CAURI agar for isolation of strains with extended-spectrum β-lactamases

I. Štrumbelj, Z. Horvat Šardi, M. Maucec Ivanic, D. Sabotin (Murska Sobota, SI)

Objectives: MacConkey agar with ceftazidime (CMAC) was used in several published studies for isolation of strains with extended-spectrum β-lactamases (ESBL) from faeces. We formulated new agar for the same purpose: CAURI. Objective of the study was to compare both agars.
Methods: Media: CMAC agar was MacConkey agar (Biokar) with ceftazidime (1 mg/L); CAURI agar was Uriselect 4® agar (Biorad) with ceftazidime (1 mg/L) and ampicillin (10 mg/L). Specimens: during an ESBL outbreak we screened 39 specimens from patients for presence of ESBL strains in faeces. Each faeces was inoculated on CMAC and CAURI agar. Isolates were identified by standard methods, presence of ESBL was determined by CLSI method (cefotaxime and ceftazidime disks with and without clavulanic acid). Sensitivity of each agar was determined by dividing number of ESBL isolates on each agar with number of ESBL isolates on any agar. Sensitivities of CMAC and CAURI were compared (chi-square test, statistical significance: $p < 0.05$).
Results: Twenty ESBL isolates were isolated from 14 specimens; two different ESBL species from one specimen were isolated from 6 specimens. Identification of ESBL isolates: 12 *Escherichia coli*, 7 *Klebsiella pneumoniae*, and 1 *Klebsiella oxytoca*. Number of ESBL isolates on CMAC: 17 out of 20 (sensitivity 0.85). Number of ESBL isolates on CAURI: 19 out of 20 (sensitivity 0.95). Difference in sensitivity was not significant ($p = 0.3$).
Conclusion: Compared to CMAC, sensitivity of CAURI was not statistically different, but only twenty isolates in specific epidemiological circumstances were studied. Further studies are necessary to determine possible role of CAURI in detection of ESBL carriers in faeces.

R2222 Serologic diagnosis of human brucellosis using Wright, Rose Bengal, Brucellacapt and Elisa tests in a nonendemic area in Greece

G. Vrioni, S. Kouvardas, A. Pagarliota, N. Charalampaki, A. Tarpatzi, L. Zerva (Athens, GR)

Brucellosis is a widespread zoonosis of great public health importance and economic significance, especially in countries around the Mediterranean basin. Because clinical findings are usually non-specific and blood cultures' sensitivity is low depending on the disease stage, diagnosis is principally based on specific antibodies detection. The

aim of this study was to compare the results of Rose Bengal test (RB), Wright seroagglutination test, an immunocapture-agglutination test (Brucellacapt) and an enzyme-linked immunosorbent assay IgG and IgM against smooth lipopolysaccharide from Brucella melitensis 16M in patients with suspected brucellosis. A total of 606 patients were examined by RB and Wright tests in the General University Hospital "Attikon" (which serves the west area of Attiki with a population of 600,000) from February 2004 to November 2006. Thirty-two of them had positive RB and/or Wright tests (5.3%). Brucellacapt and Elisa tests were performed in 19 out of 32 patients. All patient sera were positive in the Brucellacapt test, while 8 sera had negative either RB or Wright test results. Fourteen patients (11/19, 57.6%) had a Brucellacapt titer >1/640, while only 3 patients had a Wright test titer >1/640 (3/19, 15.8%). No prozone phenomenon was observed in the samples. As regard Elisa test results, 14 patients had positive IgG antibodies and 6 IgM antibodies.
So, in our territory with low endemicity of human brucellosis, RB and Wright tests were still efficient methods for serological diagnosis of the disease, while Brucellacapt test was appeared to be an effective method, increasing the chance of brucellosis diagnosis.

R2223 Comparative study of the instrument robustness of automated blood culture devices: BD BacTec™ versus bioMérieux BacT/Alert™

D. Heyneman, C. Van der Linden (Antwerp, BE)

Instrument robustness of the Becton Dickinson (Sparks, MD) BACTEC™ and the bioMérieux (Durham, NC) BacT/Alert™ automated blood culture devices was assessed by interviewing 278 laboratory managers and senior lab technicians in 5 European countries over a period of less than one month. The main question was "When was the last time you had to call the company for a repair or technical problem (not including normal maintenance)". 101 or 36.3% of the interviewees reported at least one technical intervention since the year of installation of their device. Specific attention was paid to comparability of the test population for both systems. Based on these data it can be concluded that the BacT/Alert™ device requires significantly more interventions than the BD BACTEC™ device. Also the time since last call to the supplier for a technical intervention was significantly shorter for the BacT/Alert™ device than for the BACTEC™ device. When intervention was needed the BacT/Alert™ device was "completely down" just as much times as the BACTEC™ device. The most common problems with automated blood culture devices are hardware related. However, these problems have little impact on patient care because of the availability of back-up and data-retrieval systems.

Methods for antibacterial susceptibility testing

R2224 Are susceptibility testing results for clindamycin from microdilution automated systems reliable for therapeutic decisions?

M. Treviño, L. Moldes, C. Garcia-Riestra, M. Gómez-Rial, M. Jorge, B. Regueiro (Santiago de Compostela, ES)

Objectives: Clindamycin has long been an option for treating both, methicillin-susceptible (MSSA) and methicillin-resistant (MRSA) *S. aureus* infections, particlularly skin and soft tissue infections. Missidentification of inducible MLSB resistance may lead to clinical failure of clindamycin therapy. We studied the prevalence of MLSBi in community- and hospital-associated *S. aureus* isolates, including MRSA and MSSA, at our institution.
Methods: We prospectively collected sequential nonduplicate *S. aureus* isolates exhibiting erythromycin resistance and clidamycin susceptibility, as determined by broth microdilution using an autommated system (VITEK System, boiMérieux, France) from April to November 2006. Testing for MLSBi was accomplished by the agar diffusion (D test) method in accordance with the recommendations of the Clinical and Laboratory Standards Institute (CLSI).
Results: Among 63 *S. aureus* isolates, the overall prevalence of MLSBi was 77.8%, with 75% of MRSA and % 78 of MSSA isolates exhibiting MLSBi. CA-MRSA was not found. Prevalence of MLSBi hospital associated MRSA was 83.3%. CA-MSSA has a lower prevalence of MLSBi than hospital associated MSSA (72.4% versus 83.3%).
Conclusions: Susceptibility results for clindamycin using methods that do not detect induced resistance are not reliable in order to avoid clinical failures in patient who receive clindamycin for *S. aureus* infections with MLSBi.

Public health and community-acquired infections

R2225 Cost analysis on common cold adult patients treated by private general practitioners in Sri Serdang, Malaysia in April 2005

Z. Sekawi, M. Osman, M.H. Juni (Serdang, MY)

Background: Though common cold mortality is not of great concern, its morbidity is enormous in terms of economic loss due to missed days from school and work. $40 billion was spent on its treatment and lost of work hours yearly in the USA. In Malaysia, the quantum cost of common cold is not known.
Objective: To estimate the average cost of its treatment in a locality and eventually, postulate the total cost in Malaysia.
Methods: This was a clinic-based, cross-sectional study. Data was obtained through face-to-face interview at four private general practitioner clinics in Sri Serdang, Selangor in April 2005. The clinics and patients were selected by using convenient sampling method and universal sampling method respectively.
Results: A total number of 222 adult patients were recruited. Majority (48.6%) are in the 20–29 years old age group. Male slightly predominated female (52.3% and 47.7% respectively). The Malay ethnic group accounted for the highest number of respondents (81.5%). Majority had tertiary education (51.0%) and within income range of RM 1,000.00–2,000.00 (US$260–520) (45%). The incidence of adult patients who were treated with common cold at private clinics in Sri Serdang is 4.92%. The average direct cost per patient was estimated to be RM 39.82 (US$10.48). The average total cost per patient was RM 116.39 (US$30.63) per consultation. Based on information from the Ministry of Health Malaysia, the total cost for common cold among adults in Malaysia was estimated about RM 250,921,809.40 (US$66,032,055.10) a year.
Conclusion: This study indicates that the economic impact of common cold in Malaysia is also huge albeit done in a small scale. This study provides a preliminary estimate and hence, future research is needed to obtain a more precise economic burden of common cold in the country.

R2226 Microorganisms prevalence in urethritis in primary attendance

M.A. Orellana, M. Aramendi, G. Galera, M.T. Sanchez, T. Fernandez, L. Bustillo, C. Caballero, M.A. Amerigo (Madrid, ES)

Objective: Urethritis is the most frequent sexually transmitted disease symdrom. The aim of this study is to know the prevalence and tendency of microorganisms producing urethritis in primary attendance.
Methods: Cross-sectional study. It is studied the urethral exudate of 1371 patients, 1248 men and 123 women, for 3 years (January 2003–December 2005). The samples were studied for: GRAM stain, culture in habitual plates, *Chlamydia trachomatis* detection by inmunocromatography method CHLAMY-CHECK-1 (GRIFOLS) and *U. urealyticum* and *M. hominis* by Mycoplasma IST2 method (bioMérieux).
Results: The percentage of positive samples was 36.98%. The isolated microorganisms were: *N. gonorrhoeae* 6.56%, *U. urealyticum* 13.42%, *M. hominis* 1.68%, *Chlamydia trachomatis* 4.45%, *H. parainfluenzae*

1.90%, *H. influenzae* 1.17%, *E. coli* 2.19%, *S. agalactiae* 1.46%, *S. aureus* 0.58%, *Ec. faecalis* 0.29%, *K. pneumoniae* 0.29%, S.pyogenes 0.22%, *Candida* sp 1.75% and other 0.73%. Two or more microorganisms were isolated in 3.36%. In women, the percentage of positive samples was 69.11% and 32.69% in men. In 2003 the percentage of positivity was 28.03% and *N. gonorrhoeae* the most frequent microorganism (5.94%). In 2004 was 40.58% and *U. urealyticum* the most frequent (16.36%) and in 2005 was 41.76% being *U. urealyticum* the most frequent (17.34%).
Conclusions: The isolated microorganisms with more frecuency were: *U. urealyticum*, *N. gonorrhoeae* and *Chlamydia trachomatis*. The most frequent association was *U. urealyticum* + *M. hominis*. Women had more percentage of positive samples than men. In men, the isolated most frequently microorganisms were: *U. urealyticum*, *N. gonorrhoeae*, *Chlamydia trachomatis*, *H. parainfluenzae* and *E. coli*, while in women were: *U. urealyticum*, *M. hominis* and *Candida* sp. The percentage of positive samples were increasing for the years, from 28% in 2003 to 41.76% in 2005.

R2227 *Coxiella burnetii* seroprevalence in the rural part of Bolu, a city located in the western Black Sea region of Turkey

O. Karabay, E. Kocoglu, G. Baysoy, S. Konyalioglu (BOLU, TR)

Objective: The aim of this study is to determine the seroprevalence of *Coxiella burnetii* in the rural part of Bolu located in the western Blacksea region of Turkey.
Materials and Methods: The city is located in the western part of Blacksea region of Turkey with a population of 270.654. It has nine districts in which cattle and sheep breeding and raising is a common activity. The people who were included in the study were chosen by proportionate stratified sampling according to the age and population density of districts of Bolu among the people or their children who breed or raise cattle and sheep. Blood samples were drawn from peoples with the help of local health centres of each district. A questionnaire was filled for each subject enrolled in the study. Blood samples were centrifuged and serum samples were kept at –20ºC until the analysis. *C. burnetii* phase II IgG antibodies were studied by using IFA test with a commercial kit. IgG antibodies were accepted as positive in case of titers equal to or greater than 1/64. Statistical analysis was performed by using Epi Info Ver 6.0. This study was approved by the ethical committee of Abant Izzet Baysal University. Informed consent was obtained from each subject or their parents in case of children.
Results: Until now, 293 samples were obtained in this ongoing study. According to the results, *C. burnetii* phase II Ig G antibody positivity was 20.8%. For those above the age of 18 (n = 248), 59 of them (23.8%) were tested positive whereas this rate was only 4.4% for those below the age of 18 ($P < 0.001$). There was no significant difference regarding to *Coxiella* positivity between male and female ($P > 0.05$).
Conclusion: In this ongoing study, *C. burnetii* seroprevalence was found to be 20.8% in the rural area of a city from the western part of the Blacksea region of Turkey.

- Seroprevalence increase was found to be parallel to the age.
- It was thought that because 50% of the affected population remains asymptomatic, the disease is not widely recognized in this region of Turkey. However *C. burnetii* was found to be an important and common infectious agent for this region.
- Although seroprevalence rates are higher Q fever was underreported in Turkey. For this reason, physicians and local health departments have to remember this important and prevalent infectious agent and Q fever must be taken into consideration in patients with a suitable clinical course.

This study was funded by the AIBU (ABU-BAP-Project No: 2005.08.03.210).

R2228 Study of the incidence of respiratory infectious diseases after the Bam earthquake in 2004

N. Jonaidi jafari, M. Izadi (Tehran, IR)

Objectives: Disasters that result in large areas of standing water potentially lend themselves to increased rates of vector-borne disease. Although vector-borne disease epidemics rarely occur following natural disasters, a theoretical risk exists if flooding provides more breeding sites and if extensive damage to homes allows increased exposure. In this research, we report the incidence of vector-borne disease after the Bam earthquake.
Methods: This is a descriptive follow up study. Considering that, this study was done during a fixed period (in a one month duration a week after the earthquake from 2nd Jan. 2004 to 1st Feb. 2004). All of the patients which refer to the healthcare centres of Bam (either Iranian or other countries) considered and included in our project. For doing this research we go to Bam where was stricken by earthquake and collect data of respiratory infectious diseases such as acute respiratory infection, suspected to measles, and tuberculosis which refer to the healthcare centre after one week of the disaster.
Results: During the study period 6241 cases refer to clinic because of acute respiratory tract infection and incidence of it in one month was 693 in 10000 populations (6.86% of total population). in our study only 11 cases were suspected to tuberculosis. Also there was no any confirmed case of measles and only 10 cases were suspected.
Conclusion: Cold weather, lack of appropriate shelter and heating system and lack of appropriate housing, over crowded population in camps were reasons for increase the incidence of respiratory infectious diseases. There were two major reasons for low incidence of tuberculosis: (1) sufficient supervision of previous tuberculosis patients by the healthcare centres settled in Bam. (2) It is probable that there was no sufficient time to show the clinical manifestation of tuberculosis in the contacted people because of the short study period. Also because of the widespread project of measles vaccination – mass vaccination against measles and rubella (MR vaccination) has been done in Iran before earthquake-cases of suspected measles were rare and there was no any confirmed case of measles.

R2229 Human brucellosis as an emerging disease in Korea

C-S. Lee, B-K. Baek, C-W. Lim (JeonJu, KR)

Objectives: In order to characterise the incidence of humans brucellosis, which is a serious emerging public health and socio-economic problem in Korea.
Methods: Blood samples (n = 87) were collected from 73 individuals with a history of exposure to bovine brucellosis, and from 14 healthy individuals with no history of contact with infected cattle. All samples which were collected from the provinces of Chungnam and Chonbuk, categories as farmers and their family members (n = 34), practicing veterinarians (n = 18), assistants in the Veterinary Service Laboratory (n = 8), Livestock Health Control Association (n = 3), artificial inseminators (n = 3), livestock traders (n = 7) and others (n = 14). Clinical cases included 17 individuals who had been on antibiotic treatment for 6 weeks. The sera were isolated and screened for Brucella reactivity using the ELISA, the Standard Agglutination Test (SAT), 2-Mercaptoethanol Agglutination Test (2-ME), and Western blot (WB).
Result: Based on ELISA (n = 87), 16.09% of the suspected cases were positive (n = 14), 5.74%, equivocal (n = 5) and the remaining 54 samples (OD: 0.2196–0.154, Student's t = 9.8376 <0.0001) were negative (78.16%). Of the individuals on antibiotic treatment (n = 20), 47.05% (n = 8) remained serologically positive, 17.6% (n = 3) were equivocal, and the remaining 35.2% (n = 6) were serologically negative for brucellosis. The highest risk group included farmers and their families, assistants in Veterinary Service Laboratory and practicing veterinarians, they were 29.4%, 25% and 11.1% positive, respectively. All the other serum samples were negative. ELISA results 100% sensitivity with the cut off 1:160 in SAT (n = 22) and 2-ME (n = 22) and specificity 76.65%

and 75.56%, respectively. WB using extracts of Brucella abortus 2308 as the target antigen revealed serum antibodies that recognized several specific bands (16, 26 & 37 kDa) in clinically proven cases only. Serum from individuals that had successful chemotherapy for brucellosis gave negative WB results and was similar to those among healthy uninfected persons.
Conclusion: This is the first time that a comparison of these serological tests has been applied to the diagnosis of brucellosis in Korean persons. Since serology generally indicates evidence of exposure, definitive diagnosis requires isolation of Brucella organisms and/or demonstration of specific Brucella DNA.

R2230 *Chlamydophila pneumoniae* antibodies in Greek patients with neurological disorders

M. Pape, K. Mandraveli, M. Paschalidou, M. Gelagoti, N. Taskos, S. Alexiou-Daniel (Thessaloniki, GR)

Objective: *Chlamydophila pneumoniae* (CP), an intra-cellular parasite, has been implicated in the pathogenesis of a variety of neurological disorders, including atherosclerosis, Alzheimer's disease, optic neuritis (ON), Guillain-Barre (GB) and multiple sclerosis (MS). The correlation of CP with pathogenesis and course of neurological diseases has been studied, but has so far yielded controversial results.
The aim of our study was to investigate a possible relationship between CP infection and neurological disorders in Northern Greek patients.
Methods: Cerebrospinal fluid (CSF) and paired blood samples (S) taken in the acute and the convalescent phase were available from a total of 60 neurological patients (19 males, 31 females). 24 patients had a diagnosis of clinically definite MS, 6 had (ON), 11 had (GB) and 19 had other neurological disorders (OND). Blood specimens and cerebrospinal fluid were tested for the presence of IgG/IgM antibodies against CP using the IFA and the ELISA assay. All the sera were also tested for *Coxiella burnetii*, *Rickettsia typhi*, *Rickettsia conorii*, *Bartonella henselae/quintana*, Cytomegalovirus, Epstein Barr virus, Respiratory syncytial virus, *Mycoplasma pneumoniae* and *Legionella pneumophila* to exclude cross reactions. We also determine the seroprevalence of CP among 60 healthy individuals (control group, 30 men-30 women).
Results: CSF samples were all negative for CP IgG and IgM antibodies. Of the 60 patients, 29 (48%) were seropositive for CP IgG antibodies, having the following diagnoses: MS 15 (25%), ON 3 (5%), GB 7 (12%), OND 3 (5%). Titers 1/64, 1/128, 1/256 were found in 58% (17/29), 28% (8/29), 14% (4/29) respectively. Serological diagnosis of acute CP infection was confirmed by seroconversion only in two GB cases. The seropositivity in the healthy population was 55%.
Conclusions: No significant difference was found in the serology among neurological patients and healthy individuals. CP IgG antibodies were found positive in patients belonging to all groups. This excludes a direct aetiologic relationship between a certain neurological disorder and CP infection. It is possible that a persistent CP infection may play a significant role in the development of the disease by inducing autoimmune reactions.
A surprisingly high rate of CP IgG antibodies was found among patients with ON. Considering that ON may develop to MS, this finding needs to be further investigated.

R2231 Surveillance of bacterial pathogens including *Listeria monocytogenes* associated with acute diarrhoeal disease in the Tyrol

I. Heller, K. Grif, M.P. Dierich, R. Würzner (Innsbruck, AT)

Objectives: An epidemiological survey of acute diarrhoea was conducted in the Tyrol to determine the prevalence of bacterial enteropathogens including *Listeria monocytogenes*.
Methods: From December 2004 to November 2006, more than 1000 stool samples from patients with gastroenteritis were arbitrarily collected and examined for the presence of *Salmonella*, *Shigella*, *Campylobacter*, *Yersinia* and *Listeria* spp.
Results: A bacterial pathogen was detected in 24% of all samples, the most commonly identified agents were *Campylobacter* spp. (12%) and *Salmonella* (11%). In two samples *Yersinia enterocolitica* was detected. Nine *Listeria* isolates were recovered, 3 were *L. monocytogenes*, 5 were *L. innocua* and 1 was *L. grayi*.
Conclusion: *Campylobacter* and *Salmonella* spp. were the most frequently isolated bacterial pathogens, whereas sporadic gastroenteritis due to *L. monocytogenes* appears to be an uncommon illness in the Tyrol.

R2232 Patient demand for antibiotic prescription. An often-neglected cause of antibiotic resistance in the community

A. Alevizos, D. Perimeni, G. Larios, C. Mihas, D. Fanou, M. Papathanasiou, K. Trifynopoulou, K. Stamatiou, K. Kintzoglanakis, A. Mariolis (Athens, GR)

Background: A small, yet important number of patients are inadequately prescribed antibiotic treatment for viral upper respiratory tract infections (VURTIs). General Practitoners (GPs) are one of the main sources of such antibiotic prescriptions. Insistent patient demand has been associated with such antibiotic prescriptions. In the present study we tried to estimate the impact of patients' demand on physicians' decisions in an urban primary healthcare centre in Athens, Greece, a country which currently presents one of the worst antibiotic resistance patterns in Europe.
Methods: The material of our study consisted of 21 GPs of the Health Centre of Vyronas, who were asked to document their rationale for prescribing antibiotics in patients with VURTI, as recorded to the official registry of the Health Centre in a 6-month period, from October 2005 to March 2006, with the typical seasonal peaks in viral respiratory tract infections. The physicians were asked to state the medical reasons as well as the patient demand-related reasons that influenced them the most in making their decisions.
Results: 58 prescriptions of antibiotics in patients with VURTI were found in our registries. 27 out of 58 (46.5%) of prescriptions were documented as necessary by the health status or by other various concomitant medical conditions of the patients (COPD exacerbations, asthma, diabetes, immunosuppression, etc.). According to the physicians' responses, from the remaining 31 prescriptions, 12 (38.7%) were made because of diagnostic uncertainty reasons, while the remaining 19 (61.3%) were actually prescribed because of persistent patient demand. Interestingly, most of the physicians stated that a far greater number of patients with VURTI examined during the same period were not prescribed antibiotic treatment despite their insistent demand.
Conclusion: Patient demand, although not being the most important factor, greatly influences physicians and has a significant effect on antibiotic prescribing for VURTIs. Adequate practice guidelines and intervention strategies from health policy makers aiming to ameliorate antibiotic prescribing patterns in primary healthcare have to take such effect into consideration in order to effectively decrease the expansion of antibiotic resistance in the community.

R2233 Evaluation of continuous ambulatory peritoneal dialysis-related peritonitis attacks in Ankara

R. Ozturk, C. Bulut, G.R. Yilmaz, H. Parpucu, H. Irmak, S. Kinikli, M. Duranay, A.P. Demiroz (Ankara, TR)

Objectives: Peritonitis is a serious and potentially life-threatening complication of continuous ambulatory peritoneal dialysis (CAPD). The aims of this study were to assess demographic aspects, rates of peritonitis, causative organisms, clinical outcomes and treatment approach for continuous ambulatory peritoneal dialysis (CAPD)-related peritonitis cases.
Methods: All peritonitis cases treated in Infectious Diseases and Clinical Microbiology Department between February 2006 and June 2006 were enrolled into this study. The patients with cloudiness of the peritoneal dialysis fluid and/or abdominal pain and with more than 100 white blood cells/mL in their dialysate fluid were accepted as having peritonitis. Gram

stain, and cultures, complete blood count, serum procalcitonin (PCT) and C-reactive protein (CRP) levels were obtained. Conventional methods were used for identification of microorganism. Disc diffusion method was used for antimicrobial susceptibility tests.
Results: Fifty-six episodes of peritonitis occurred in 50 patients. The mean age of the patients was 48.4 years (range: 18–83 years). The overall incidence of peritonitis was 1.6 episodes/patient-year. In 42% of patients, there was only one peritonitis attack; whereas 58% of them had two or more attacks. The most common presenting symptoms of the patients were abdominal pain, cloudiness of the peritoneal dialysate fluid, nausea and vomiting. The median white blood cell count of peritoneal dialysate fluid was 1275/mL (range: 170–7900/mL) in 56 episodes. Cultures were positive in 40 (71.4%) peritonitis episodes; coagulase-negative *Staphylococcus* was the most common organism (20.7%), followed by *Staphylococcus aureus* (4%). In all of the episodes, the empirical therapy administered to the patients was intraperitoneal cefazolin and gentamicin. Antibiotherapies of 18 patients were changed according to antimicrobial susceptibility test results. Two culture negative patients did not respond to initial therapy and the therapy was switched to an empirical therapy with a glycopeptide antibiotic.
Conclusion: Despite all technical improvements during recent decades, peritonitis is still the major reason for CAPD failure. For the accurate treatment of peritonitis attacks, causative organisms and their antimicrobial susceptibilities must be known.

R2234 Clinical features of acute Q fever in a southern area of Spain

J.D. Ruíz, J. De La Torre, J.L. Prada, A. Del Arco, M.D. Martin, L. Merida, I. Escot, J. García-Alegría (Marbella, ES)

Objectives: The aim was to describe the clinical features and outcome of inpatients suffering from acute Q fever (AQF) in an area in the south of Spain.
Methods: Retrospective analysis of clinical and epidemiological data from patients suffering from AQF admitted at Costa del Sol Hospital (Marbella, Malaga), between 1997 and 2005. Diagnosis of AQF was based on detection of antibodies against phase II by indirect inmunofluorescence test (IFA).
Results: 23 cases were admitted. There was a significantly higher sex ratio of males to females (11.5:1). Mean age was 45.6+13.5 years (range 25 to 77 years). Professional risk factors were found in 13% and 21.7% came from rural background. AQF was slight commoner in spring (34.8%). Regarding to clinical features: 91% had only fever (mean duration: 11.3 + 5.5 days), malaise (56.5%), severe headache (34.8%), diarrhoea (34.8%), abdominal pain (21.5%), cough (21.7%), arthralgia/myalgia (19%) and hepatomegaly (43.5%). About lab-data AST/ALT were elevated in 47.8%, an elevated erythrocyte sedimentation rate in 34.7% and trhombocytopenia (17.3%). Chest X-ray were anormal in 17.4%. Clinical features was isolated fever (39.1%), hepatitis (43.5%), pneumonia (8.7%), hepatitis plus pneumonia (4.3%) and pericarditis (4.3%). A patient had a pulmonary thromboembolism related with positive anticardiolipin antibodies. Doxycicline was used in 87%, doxycycline with quinolone in 17.2%. Antibiotherapy mean time was 19.2 + 5.2 days. Two patients had a relapse. All patients were totally recovered within one year. The mean hospital stay was 9.6 + 4.7 days.
Conclusions: AQF was more frequent in male and more cases were diagnosed in spring. In our study AQF clinical presentation was principally only fever and hepatic involvement. The small number of pneumonia cases suggest a *C. burnetii* strains or an infection way different to other geographic areas.

Emerging infectious diseases

R2235 Study of infectivity of Crimean-Congo haemorrhagic fever cases in Sistan va Baluchestan province of Iran, 2003–2004

K. Holakouie Naieni, S. Izadi, M. Salehi, S. Chinikar (Tehran, Zahedan, IR)

Objectives: To determine the infectivity of Crimean-Congo haemorrhagic fever (CCHF) Virus in usual and routine contacts with serologically confirmed cases.
Methods: A cross sectional study was performed during the year 2004 in Zabol and Zahedan districts of Sistan-va-Baluchestan province of Iran. During study serum sample of 57 first or second degree relatives of 8 ELISA confirmed CCHF cases, were tested and all probable routs of transmission (touch, contact with blood, sexual contact, having meal, room mate, home mate with index cases) and also contacts with known risk factors were questioned and recoded during an interview with all participants. All ELISA tests were performed in Pasture Institute of Iran.
Results: Of all 57 people, only one was serologically IgG positive. The descriptive characteristics of the studied people shows high frequency of contact with known risk factors of the disease.
Conclusion: With regard to date of sampling and type of antibody in the serum of the seropositive subject, it is obvious that there is no relationship between infection in this person and the index case in his family. And generally it seems that the infectivity of this virus in usual routine contacts with infected subjects is low.

R2236 First fatal infection due to c-MRSA in Austria

M. Hell, F. Wimmer, R. Lenzhofer, G. Pauser, A. Zimmermann, H. Mittermayer (Salzburg, Schwarzach, Linz, AT)

Objectives: Community aquired MRSA (c-MRSA) infections are registered since the year 2002 in Austria. In our region in the middle of Austria (county of Salzburg) c-MRSA is investigated for its PVL-positivity systematically since 2004. The typing is done in a reference laboratory in Linz.
We report the first fatal infection due to an PVL-positive MRSA in a 34-year old woman in Austria from our region in 2005. Returning from a home holiday from the Philippines she developed high fever being pregnant 2 weeks before delivery. A cesarian section was performed in the local hospital. The patient developed a foudroyant pneumonia and for that reason was transferred to the next teaching hospital.
After 10 days intensive care treating pneumonia was still progredient and therefore she was transferred to the university hospital Salzburg. Antimicrobial therapy was switched from Amoxi/clavulanic acid to Linezolid + Imipenem + Clarythromycin. Pneumonia was accompanied by abscess formation in the lung under this regimen. After another day of treatment she died unfortunatly due to a septic shock and multi-organ failure.
Methods: Routine samples were taken from the tracheal secretions during intensive care treatment. MRSA was found in the first samples and also viral investigations were done. The MRSA isolate was investigated for PVL (Panton-Valentine Leukocidin) and subtyping was performed.
Results: Lung abscesses and necrotising pneumonia were due to a superinfection with a PVL-positive MRSA-ST30-subtype 2 (south westpacific clone). Underlying disease was caused by a influenza A infection. During hospitalisation in all 3 hospitals no transmission of this MRSA was obvious.
Conclusion: This was the first time in our country such a severe infection due to PVL-positive MRSA was seen. Severe community acquired pneumonia has to be redefined in its therapeutic strategies including now also multiresistant organsisms such as c-MRSA.

R2237 Periorbital cellulitis: two-year review of eleven hospitalised patients

S.S. Gundes, S.S. Cetin Akhan (Kocaeli, TR)

Background: Bacterial periorbital cellulitis (BPC) is an uncommon infection associated with severe complications. The aim of this study was to analyse medical outcomes, including risks for complications and mortality, in eleven patients hospitalised for BPC.
Methods: A chart review of all patients admitted to Infectious Diseases Department with the diagnosis of PBC for the period Jan 2005 to Nov 2006 were done.
Results: diagnosis was made clinically with radiological confirmation. The mean age was 39.1 years with female predominance (8/11). Two of the patients hospitalised at least three times for the last year. The infection was documented microbiologically in five (45.4%) cases. *Staphylococcus aureus* (four cases) and *Streptococcus pyogenes* (one cases) were the causative pathogens. None of the blood cultures were positive. The mean CRP volue was 11 mg/dl, sedimantation rate was 54/h. Overall, two patients discharged early ($\leqslant$4 days) and nine patients were hospitalised for more than 4 days. Only one patient died. Four of the patients required surgical drainage. When comparing the two study groups (patients discharged early without complications and patients developed complications or died), low risk group were less likely to have multiple comorbid conditions. Factors associated with bad prognosis were male sex, presence of neutropenia (four cases), chronic leukaemia (three cases), diabetes (three cases), obesity and renal insufficiency. Treatment was either by iv β-lactam/β-lactamases alone or with surgery to drain abscesses.
Conclusion: BPC occurs in any age goup with female predominance. In most of the patients, there is an underlying comorbidity. Prompt diagnosis and treatment can decrease the known significant morbidity rates. Bad prognostic findinds can be usefull in stratifying the admitted patients and deciding the most appropriate means of care including antibacterial treatment, debridement and hospitalisation.

R2238 Seroprevalence of Crimean-Congo haemorrhagic fever virus in humans at risk group in an endemic area

Z. Ozkurt, N. Yilmaz, M. Ertek, A. Ertürk, A. Aydemir, S. Toprak, S. Kara (Erzurum, Ankara, TR)

Objectives: Recently, Crimean-Congo haemorrhagic fever (CCHF) has been seen in some geographic area in Turkey. A seroepidemiological survey was carried out to determine the prevalence of CCHF virus activity in humans at risk group at an endemic area during 2004.
Methods: Sera samples were obtained from a total of 250 people consist of 114 farmers, 80 healthcare workers, 44 slaughterhouse workers and 12 veterinarians. CCHF virus immunoglobulin G (IgG) antibody was investigated by ELISA technique.
Results: Anti-CCHFV IgG was found in 19 of 114 (16.6%) farmers, 16 of 80 (20%) healthcare workers, 6 of 44 (13.6%) slaughterhouse workers, 3 of 12 (25%) veterinarians. In healthcare workers anti-CCHFV antibodies were detected in 7/20 (35.0%) nurses, 5/35 (14.2%) laboratory technicians and 3/25 (12%) physicians.
Conclusion: These data showed that humans may have been inapparent CCHF infection when they exposure the virus. Persons at risk group should be trained, and contact precautions should be strictly applied for prevention of the disease.

Infection control

R2239 Study of urinary tract infection in diabetic and non-diabetic patients and antibiotic sensitivity pattern of isolated organisms

H. Alimohammadi asl, N. Fouladi (Ardebil, IR)

Objectives: The main objective of this study was to determine whether there are any differences in the bacteriological pattern of UTI and the antibiotic sensitivity patterns of the pathogens concerned between diabetic and nondiabetic patients.
Methods: Over a period of one year, a total of 1510 patients suspected to have urinary tract infections (UTI) were studied.
Results: of the test patients 452 of them were diabetics. 102 (22.5%) of 452 diabetic patients had UTIs from which 72 patients (70.5%) were female. In non diabetic group (control group) 120 patients were selected out of 1058 non diabetic patients as having UTI. Regarding age and sex of the patients on diabetic and nondiabetic patients meaningful statistical difference, were found. the infections in both groups were further divided into community acquired and nosocomial acquired UTI in non diabetic patients (73.3%), this organism was isolated in 43.6% of the community acquired UTI in diabetic patients and this finding indicates a meaningful statistical difference in two groups.
The organisms isolated from nosocomial UTI in diabetics showed a significantly greater preponderance of klebsiella spp. (41%) as compared to non diabetics (29%) which statistically is a meaningful difference.
Most of the UTI in diabetics, especially in female, were asymptomatic. In 31 *E. coli* strains isolated from diabetic and non diabetic patients type 1 fimberia also investigated, which in diabetic patients 43.7% of the isolated strains showed this type of filmberia as compared to *E. coli* strains isolated from non diabetic patients (73.3%) indicating a meaningful difference.
Antibiotic sensitivity patterns of the isolated strains were determined using some of the routine antibiotics used in UTI treatment, some of third generation cephalosporins and quinolones, but the results showed no statistical differences. Although when WBC conuts in $1mm^3$ of the infected test urines were compared in diabetic and nondiabetic patients, the sensitivity of the test recorded as 58.3% and 92%, respectively, but the sensitivity of nitrate reduction test in the both groups was not statistically significant.
Conclusions: Most of the UTI in diabetics, especially in female, were asymptomatic. sensitivity and bacteriological patterns between diabetic patients and control group were different. Routine urine culture can be recommended for diabetic women even when there is no urinary symptom.

R2240 Study of epidemiological determinants of occupational exposure to HIV, HBV, HCV in healthcare workers in Iran

A. Hadadi, S. Afhami, M. Karbakhsh, N. Esmailpour (Tehran, IR)

Introduction: Healthcare workers (HCWs) are at substantial risk of acquiring blood borne infections through contact with blood and other products of patients. Our main objectives were to determinant epidemiological characterists of occupational exposure to blood/body fluids and its related risk factors.
Methods: This cross sectional study was conducted on healthcare workers at risk of exposure to blood and other body fluids in three hospitals of Tehran University of Medical Sciences. Using a structured interview, all selected HCWs who were at risk were questioned about the exposure to blood born pathogens in the preceding year (Dec 2003–Dec 2004).
Results: Of the 900 HCWs, 391 (43.4%) had at least one occupational exposure to blood and other infected fluids during the previous year, with the total number of 467 exposures (52.88%) with annual rate of 0.5 exposure per HCW. The highest rate of occupational exposure was found among nurses (26.1%) and housekeeping staff (20.2%) and occurred most commonly in the medical and emergency wards (23.3%, 21% respectively). The rate of exposure in HCWs with less than 5 year experience was 53.8%.
Percutaneous injury was reported in 280 participants (58.8%). History of hepatitis B vaccination was positive in 85.93% of HCW among exposed workers. Sixty one percent had used gloves at exposure time. Hand washing was reported in 91.38% and infectious diseases specialist consultation in 29.38%. There were 72 exposures to HIV, HBV, HCV and exposure to HBV was the most common and in 237 of enrolled cases source was unknown.

Conclusion: Sharp injuries among HCWs are a widespread occupational problem. In this study, job categories, work experience and hospital ward were the most important risks for exposure. An effective and goal oriented education programme targeting HCWS, use of protective barriers, vaccination against hepatitis B are important way to prevent viral transmission among HCWs.

R2241 Preventing healthcare-associated infections: the experience of a 3-year educational programme in the nursing homes of Trentino

I. Caola, P. Dallapè, L. Scartezzini, G. Noro, A. Calì, M. Giordani, E. Negri (Trento, IT)

Objectives: To prevent healthcare-associated infections in nursing homes (NH) of Trento province, Italy, and to reduce the infection risk in the healthcare network.
Methods: In 2004 a three years long programme for education and training of nurses and physicians, co-ordinated by the public institution UPIPA, started. In the first year a residential educational programme was carried out on healthcare workers (HCW) related infections, universal precautions, bacterial antimicrobial resistance, management of microbiological samples. In the second year an inquiry form was sent to all nursing homes to survey data on residents, invasive procedures, required microbiological tests, criteria used for defining infections. On the basis of collected data, the project "Management of urinary catheterisation: procedures auditing for improvement in quality of care" was planned in the third year.
Results: 108 nurses and physicians attended to the residential educational programme in the first year. In 2005, 53.3% of nursing homes returned data on 61.5% of institutionalised population (2486/4040): 2/3 female, 82% over 75 years, 5.8% bedfast. In a prevalence survey, 751 invasive procedures were reported with a 25% of urinary catheterisation (UC). Since UC represents the most frequent invasive procedure with infective risk in NH, an educational project, with residential and "on job" training, was planned for 2006 to improve catheter management. 14 NH (31%) and 56 HCW were involved. The problem of urinary tract infection in the elderly and infection definitions for the surveillance in NH were elucidated in residential educational session. Five multiprofessional working groups developed local practice guidelines for catheter management that incorporated evidence-based advices for preventing infections in clinical activities. After the supervision of the tutors (external infection control nurses) the guidelines were applied. Two prevalence studies on urinary tract infections were carried out (before and after six months from protocol application). Data are on evaluation.
Conclusion: The incidence of preventable healthcare-associated infections is strictly associated with specific education of HCW. The effectiveness of an interventional programme improves when HCW are directly involved in their educational process. The adopted strategy for the development, application and revision of guidelines by HCW promise to be effective in modifying professional behaviours and reducing infection risk.

R2242 Effects of education on the importance of blood cultures by an infection control team: experience in two community hospitals, Kyoto, Japan

T. Saito, Y. Aoki, A. Matsuoka, Y. Iinuma, S. Takakura, N. Fujiwara, F. Kohi, M. Noguchi, S. Ichiyama (Kyoto, Takamatsu, JP)

Objectives: Blood culture is the only method for detecting bloodstream infections. Infection control team at each hospital grasped through ward round that blood culture examinations were not carried out sufficiently. The infection control team of each hospital educated medical staff about the importance of blood culture examinations.
Patients and Methods: This study was conducted from January 2003 to December 2003 at A hospital (179 beds) and from October 2003 to September 2005 at B hospital (500 beds). Each hospital was acute-phase community hospital. The yields of microorganisms and the characteristics of patients with clinical significant bacteraemia and/or fungaemia were compared for pre-education (January to June, 2003 at A hospital and October 2003 to September 2004 at B hospital) and post-education periods (July to December, 2003 at A hospital and October 2004 to September 2005 at B hospital).
Results: During the pre-education period, 193 blood cultures were taken from 111 patients, compared to 467 blood cultures (2.4-fold increase) from 245 patients (2.2-fold increase) during the post-education period at A hospital. Of these, 40 positive cultures were obtained from 30 patients in the pre-intervention period, compared to 65 positive cultures (1.8-fold increase) were obtained from 55 patients (1.7-fold increase) in the post-intervention period. At B hospital, during the pre-education period, 1159 blood cultures were taken from 667 patients, compared to 2175 blood cultures (1.8-fold increase) from 1141 patients (1.7-fold increase) during the post-education period. Among these, 139 positive cultures were obtained from 112 patients in the pre-education period, compared to 213 positive cultures (1.5-fold increase) were obtained from 166 patients (1.4-fold increase) in the post-education period.
Conclusion: The present study has shown that cases of bacteraemia or fungaemia may be overlooked if blood culture examinations are not performed. We consider that the importance of blood cultures must be emphasized and education needs to be continued.

R2243 Occurrence of *Mycoplasma hominis* in amniotic fluid of pregnant women

M. Vydrzalova, P. Lyskova, J. Mazurova (Pardubice, CZ)

Background: Intrauterine infection is a major cause of preterm delivery, perinatal morbidity and mortality of fetus. Intrauterine infection is present in approximately 25% of all preterm births. The most common microorganisms involved in intrauterine infections are *Ureaplasma urealyticum* and *Mycoplasma hominis*. *M. hominis* is commonly isolated microorganism from the female genital tract. This microorganism can be found in 21% to 53% of sexually active women. The transmition from the mother to the developing fetus can happen in uterus during the pregnancy or during the delivery.
The relationship between detection of *Mycoplasma hominis* in mid-trimester amniotic fluid and subsequent pregnancy outcome was investigated. Data collected included indication for amniocentesis, gestational age at amniocentesis, gestational age at delivery, pregnancy outcome and low birth weight of infants.
Method: The amniotic fluids of 184 women who underwent a transabdominal amniocentesis at weeks 15–22 of pregnancy were tested for *M. hominis* by cultivation method on PPLO agar and broth with arginine. The isolates of *M. hominis* were confirmed by polymerase chain reaction.
Results: *M. hominis* were identified in 20 (11%) samples of the amniotic fluid. A total 8 infants had the birth weight below 2500g. Two of them were born to mothers whose amniotic fluid was positive for *M. hominis*. Nine of the women delivered preterm and one of them was positive for *M. hominis*.
Conclusion: The aim of this study was to investigate the occurrence of *M. hominis* in amniotic fluid of pregnant women. The presence of *M. hominis* in amniotic fluid could be associated with low birth weight of infants which was confirmed by other authors. This work was supported by MSM 0021627502

R2244 Group G beta-haemolytic streptococci of human and animal origin

P. Lyskova, M. Vydrzalova, T. Kroupa, J. Mazurova (Pardubice, CZ)

Background: Group G beta-haemolytic streptococci form heterogeneous group of microorganisms. They have been recognized as the cause of various infections, such as skin, urogenital and respiratory infections and otitis externa, in dogs, cats and other animals. In 1935 were group G streptococci first isolated from human material taken from patients

with puerperal sepsis. In humans, they may colonise pharynx, skin, gastrointestinal and female genital tract. In recent years, they have been reported with increasing frequency as a cause of variety of human infections, such as endocarditis, arthritis, meningitis, pharyngitis and sepsis. The aim of the present work is to study group G beta-haemolytic streptococci isolated from various clinical specimens of animal (86 strains) and human (107 strains) origin.

Method: Each collected sample was cultivated on Blood agar. After incubation (37ºC, 24 hours) colonies compatible to group G beta-haemolytic streptococci were subjected to biochemical tests. The susceptibility to antibiotics was determined according to the NCCLS recommendations.

Results: All strains recovered from animals were according to their growth, serological and biochemical properties identified as *S. canis*. Out of total 107 strains of human origin, 101 were identified as *S. dysgalactiae* ssp. *equisimilis* and 6 as *S. canis*. Species differed especially in production of beta-glucuronidase (99% of *S. dysgalactiae* ssp. *equisimilis* strains positive × 17.4% of *S. canis* strains positive), alpha-galactosidase (1% of strains positive × 91.3% of strains positive) and in trehalose fermentation (98% of strains positive× 20.7% of strains positive). All isolated strains were susceptible to penicillin and ampicilin. The least effective antimicrobial agent was found to be tetracycline (20.7% susceptible strains).

Conclusion: Although group G beta-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.

R2245 Elimination of *Staphylococcus aureus* nasal carriage in healthcare workers of a burn centre: effect on incidence of *S. aureus* of burn wound colonisation

A.M.D. Kooistra-Smid, M.K. Nieuwenhuis, E. Zanten van, J. Hiddingh, G.I.J.M. Beerthuizen, W.H.M. Vogels, A. Belkum van, H.A. Verbrugh (Groningen, Rotterdam, NL)

Objectives: *Staphylococcus aureus* colonisation and infection of burn wounds increases morbidity and delays wound healing. *S. aureus* burn wound colonisation may result from nasal and pharyngeal colonisation of patients as well as healthcare workers (HCW). The aim of this study was to evaluate the effect of eradication of nasal *S. aureus* in HCW with mupirocin on the incidence of *S. aureus* burn wound colonisation.

Methods: HCW nasal *S. aureus* was eradicated with one course of mupirocin in July 2004. From July 2003 to June 2006 patients were extensively screened on admission for *S. aureus* carriage and burn wounds were cultured weekly. *S. aureus* burn wound colonisation during the year following mupirocin treatment (July 2004 to July 2005, n = 72) was compared with two control periods, C1 (July 2003 to June 2004, n = 54) and C2 (August 2005 to June 2006, n = 57).

Results: Forty-three (93%) HCW have received the mupirocin course. Nasal eradication was proven successful in 13/15 (87%) of the nasal carriers. HCW nasal carriage rate dropped from 35% to 2%, and gradually increased from 12% after 6 months to 25% after one year. The incidence of burn wound colonisation during admission in the year after the mupirocin course was 27/56 (48%) and for control groups C1 and C2 12/42 (29%) and 14/44 (32%) respectively.

Conclusion: Eradication of *S. aureus* nasal carriage among healthcare workers did not reduce *S. aureus* burn wound colonisation.

R2246 Molecular epidemiology of Gram-negative rods isolated from neonatology ward

K. Kopron, A. Jurczak, J. Jursa-Kulesza, S. Giedrys-Kalemba (Szczecin, PL)

Objectives: Infections in neonatology wards are becoming more and more serious problem, not only clinical and economic but also organisational and legal ones. They are closely related to invasive diagnostic and therapeutic procedures as well as long-term hospitalisation. The aim of this study was to assess the genetic similarity of Gram-negative rods isolated from neonate intensive care unit (NICU) and neonatology ward (NW) for a period of one year.

Methods: The analysis included all neonates (953) born between 1 January 2003 and 30 December 2003 in Clinic of Obstetrics and Perinatology in Szczecin. Infection was found in 55 neonates – 5.77%; 2 neonates dead – 0.20%. Gram-negative rods dominated among aetiological agents. They were isolated from different clinical materials (blood, cerebrospinal fluid, bronchoalveolar lavage, urine, faeces, and swabs from nosopharynx, ear, anus) of infected and healthy newborns: *Klebsiella pneumoniae* (29), *Klebsiella oxytoca* (29), *Enterobacter cloacae* (17), Serratia marcenscens (12) and *E. coli* (7). All strains were typing with using PFGE method and XbaI (*Klebsiella*), SpeI (*Enterobacter*, Serratia), NotI (*E. coli*) restriction enzymes (Bio-Rad).

Results: The occurrence of 4 genotypes and 8 unique patterns of *K. pneumoniae*, 3 genotypes (3 unique) *K. oxytoca*, 5 genotypes *E. cloacae*, 1 genotype (1 unique) S.marcescens, and 1 genotype (5 unique) *E. coli* was confirmed. The particular genotypes grouped from 2–12 isolates. The same genotypes were isolated in NICU and NW, from infected newborns (mainly acquired infections but intrauterine also; the most common clinical forms were systemic infections and pneumonias) and from healthy individuals (colonisation) in different months of the year.

Conclusion: The presence of strains showing apparent genetic similarity suggests that epidemic/endemic strains of Gram-negative rods existed in ward ecosystem and were responsible for clonal character of newborn infections and colonisation of others. This situation provoked that strict sanitary discipline was applied in both wards.

R2247 Adherence to surgical site infection guidelines in Italian heart surgery units

A. Pan, L. Ambrosini, S. Lorenzotti, L. Soavi, L. Signorini on behalf of the GIS-INFCARD investigators

Objective: data regarding adherence to surgical site infection (SSI) prevention guidelines in Italian cardiac surgery units are lacking.

Methods: a multiple choice questionnaire, structured into 8 sections following the Centers for Disease Control 1999 surgical site infection (SSI) guidelines was prepared and sent to 24 units participating to a National study group (GIS-INFCARD) Answers were stratified based upon strength of recommendations: class IA (10 questions), class IB (52 questions), class II (11 questions).

Results: 17 of the 24 units answered to the questionnaire. The responding centres performed in 2005 10249 surgical procedures, that represent about 20% of all heart surgery procedure in Italy. Adherence to class IA recommendations varied between 0 and 100%. The lowest level of adherence was related to hair removal, that is performed systematically in all male patients (0% adherence), contrary to guidelines recommendations. Timing and method of hair removal were adherent to guidelines in 29 and 41% of questionnaires, respectively. While 94% of units had written guidelines on antibiotic prophylaxis, timing of antibiotic administration was adequate in 65% of units only. Adherence to class IA recommendations was 69±34%, with 5 units (29%) showing a ⩾80% adherence. Answers to class IB recommendations were consistent with guidelines in 29 to 100% of questions, with an average of 65±26%; one single unit (6%) showed a ⩾80% adherence. Adherence to class II recommendations was 71±28%.

Conclusions: Adherence to CDC SSI guidelines in Italy is fair. Organisational improvements, especially regarding hair removal and timing of antibiotic prophylaxis, should be implemented in most hospitals. Low level of adherence to timing of antibiotic prophylaxis despite the existence of National guidelines since 2003 indicates that new instruments to improve antibiotic prescribing need to be searched for.

R2248 Syphilis prevalence within a population of intravenous drug users in Lisbon, Portugal

R. Matos, R. Neves, A. Matos, J. Santos, C. Benoliel, A. Moniz (Lisbon, PT)

Syphilis is an infectious disease caused by the spirochete Treponema pallidum subspecies pallidum. The prevalence of cases in the Portuguese population is about 13 per million inhabitants (0.0013%). The main means of transmission is the sexual contact with infected partner. However, transmission is also frequent by contact with contaminated body fluids, such as blood, among intravenous drug users (IDUs). The objective of this work was to evaluate the prevalence of syphilis among intravenous drug users during the past 5 years (2002–2006).

A total of 3118 different individuals were included in this study (mean age 35 years). These individuals were intravenous drug users who attended two mobile drug users support stands in Lisboa (centre). The VDRL assay was used for screening and confirmations were done with TPPA. A VDRL titer $\geqslant$ 32 dils was used to assume a recent infection status.

The main prevalence observed in this population was 8.7% (0.7% recent infections).

Both the positive prevalence rates and the recent infection rates have shown a tendency to decrease during these years (positive infections: 11.1 to 8.3%; recent infections: 0.96 to 0.31%, $p < 0.01$).

The positive cases prevalence has been decreasing since this IDU support programme started (2001), perhaps as a result of several disease control policies, such as syringe exchanges programmes for example.

The recent infections prevalence, albeit it's decrease, is 200 times higher than the estimated for the Portuguese population 0.0013%, as expected in this high risk population.

The control of transmissible diseases can only be overcome with the health control of high risk populations, such as the intravenous drug users. The syphilis situation presented is a good example that the work being done in the IDU's field seems to have good results but is still insufficient for a proper disease control.

R2249 Antimicrobial resistance in strains isolated in 2005 by Romanian National Sentinel System for Nosocomial Infections Surveillance

I. Codita, M. Oprea, A.M. Petrescu, D. Tatu-Chitoiu, V. Ungureanu, M. Ghita, D. Lemeni, R. Oancea, D. Brehar-Cioflec, A. Romaniuc (Bucharest, Timisoara, Iasi, RO)

Objectives: to obtain microorganisms involved in nosocomial infections; to identify them; to test their antimicrobial susceptibility in order to create a local and national data base for better orienting antimicrobial therapy and nosocomial infection prevention; to evaluate hospital laboratories capacities

Methods: Nosocomial infection sentinel system surveillance was done using the methodology recommended by the National Centre for Prevention and Control of Communicable Diseases and involved 17 hospitals, 11 District Public Health Directorates, 4 Public Health Institutes and the National Institute for R&D in Microbiology and Immunology "Cantacuzino".

Results:

1. The National Institute received via Public Health Institutes 438 strains from 10 hospitals: 161 *Pseudomonas aeruginosa* (Ps. ae.), 110 Enterobacteriaceae, 97 *Staphylococcus* spp., 54 non-fermentative Gram negatives, 13 *Enterococcus* strains, 2 fungal strains and 1 anaerobic strain.
2. 10 hospitals sent 4 to 74 bacterial strains. Most laboratories did not send but few from their isolates. Only 4 hospitals sent over 30 strains belonging to the same species; it seems that interest of every hospital was directed towards different, specific microorganisms.
3. 60% of *Staphylococcus aureus* strains (Nr. 84) showed methicilline resistance.
4. 15.5% of Ps. ae strains were resistant to all antimicrobial substances tested using CLSI (formerly NCCLS) M100-S15 standard.
5. ESBL were present in: 11 from 16 *Enterobacter* strains, 44.8% from 29 *Klebsiella* strains, and 21.6% from 37 *Escherichia coli* strains.
6. One *Enterococcus faecalis* strain was penicilline resistant.

Conclusions:

1. Generally, high percentages of all isolated microorganisms sent to the national laboratory were resistant to one or more antimicrobials, confirming EARSS national data.
2. Conclusions should be locally formulated by every participant hospital laboratory for different microorganisms
3. Laboratories isolated few number of enterococci and anaerobic bacteria.
4. Local microbiological data should be integrally collected by the national laboratory in order to elaborate the further study strategy.

R2250 The effectiveness of hand disinfectants in decontaminating *Candida* hand carriage in volunteer healthcare workers

M. Yildirim, I. Sahin, I. Sencan, A. Kucukbayrak, S. Cakir, C. Ozaydin (Duzce, Ankara, TR)

Objectives: The hands of healthcare workers (HCWs) are considered to be important for colonisation and infection of *Candida* spp. Aim of the study is to evaluate the effectiveness of three different types of hand disinfectants in decontaminating candida hand carriage in volunteer healthcare workers.

Materials and Methods: In the controlled, prospective study, eighty (50 female, 30 male) HCWs who had candida hand carriage were included to the study and used the disinfection procedures for four weeks, in Duzce University medical faculty hospital. Mean age of the objects was 29.1 ± 8.09. Study objects were divided into four groups according to used disinfectant agents (alcohol based hand rub, 4% chlorhexidine gluconate, 7.5% povidon-iodine, and control group who were not used any disinfectant). HCWs who did not use the disinfectants properly were excluded from the study. The hands of all participants were tested by culture with the broth wash technique. *Candida* species were identified by the germ-tube test, CHROMAgar *Candida* (CHROMAgar Company, Paris, France), and by API-20C identification kits (bioMérieux-VITEK, France). The chi-squared test was used for statistical evaluation.

Results: In a month of follow up, candida hand carriage were decreased to 50% (13/26) in control group; 78.9% (15/19) in alcohol based disinfectant group; 89.5% (17/19) in 4% chlorhexidine gluconate group and 81.3% (13/16) in 7.5% povidon-iodine group. *Candida* hand carriage was decreased significantly in 4% chlorhexidine gluconate group ($x2=7.70$, $p=0.006$), 7.5% povidon-iodine group ($x2=4.10$, $p=0.0439$) and alcohol based rub group ($x2=3.91$, $p=0.048$) compared to control group. There was not found any side effect (such as contact dermatitis, allergic reactions) that caused to end the hand disinfection procedure in HCWs.

Conclusions: Hygienic hand disinfection (especially with 4% chlorhexidine gluconate) was efficacious in removing candida carriage present on the hands of HCWs. It is recommended that HCWs should perform hand hygiene especially in units (such as intensive care units and neonatal care units) that have high risk for candida infections.

R2251 Comparative evaluation of internists' behaviour regarding hand hygiene in the emergency room and in the wards of a tertiary university hospital in Greece

E. Giannitsioti, S. Athanasia, O. Koulouri, A. Kolias, A. Papadopoulos, H. Fitrou, H. Giamarellou (Athens, GR)

Background: This study was launched in order to compare how hand hygiene policies influence the internists' behaviour in the emergency rooms and in the wards of a Department of Internal Medicine at a tertiary hospital in Athens.

Methods: The study was conducted from May to June 2006. Behaviour of internists in the wards and the emergency room regarding the use of

gloves and the use of alcohol-based hand-rubs during medical procedures was assessed by two independent observers via a standardised registry form. Medical procedures were divided into invasive (i.e blood puncture, catheterisations) and non-invasive (i.e. clinical examination) ones. A bed-railed system of alcohol based hand rubs was installed in every ward. Dispensers of hand rub antiseptic were installed on each table in the emergency room. Statistical analysis was done by Chi-square and Fischer exact test for categorical variables.

Results: There was no difference regarding the use of gloves among internists in the emergency room (22/73, 30.2%) and the Department wards (30/84, 35.7%) while changing gloves between patients reached 95.5% and 93% respectively. However, among internists who did not wear gloves, the adequate use of antiseptic before and after contact with each patient was efficient only in the wards (28/54, 51.8%). Internists in the emergency used antiseptic only before (5/51, 9.8%) or only after (13/51, 24.5%), but never before and after contact with the patient. Rates of invasive procedures did not differ between the emergency rooms and the wards.

Conclusions: Internists were prompt to appropriately use hand rub antiseptic only when they were working in the wards but not in the emergency. This issue may be mainly attributed to the internists' stress during the work in emergency. Moreover, although antiseptic was available in both departments, the bed-rail hand rub dispensers existed only in the wards and presumably enhanced the internists' compliance with hand hygiene rules.

R2252 Evaluation of reported percutaneous/mucocutaneous injuries in a tertiary care hospital

D. Kanyilmaz, A. Erbay, N. Avci, D. Oztas, H. Bodur (Ankara, TR)

Objectives: Healthcare workers (HCW) have the risk of occupational exposure to bloodborne pathogens. This study is planned to examine the characteristics of exposures in HCW.

Methods: Prospective surveillance of percutaneous, mucous membrane, and cutaneous contacts. A structured survey form was administered to HCW by person-to-person interview, who sought medical advice following the injury from The Infection Control Department (ICD).

Results: There were 2938 HCW including housekeeping staff in ANEAH. Thirty-six HCW applied to ICD following the injury from Oct 2005 to Oct 2006 and 34 of them were interviewed. Mean age was 31.8 (range 18–52) and 17 (50%) were female. HCW of the surgical wards were accounted for 35% of the injuries. Most of the injured HCW were working as housekeeping staff (44%). Half of the HCW were working more than five years in their job, no statistically significant difference was obtained between the employment duration of the injured HCW. Almost all of the HCW (88%) described themselves as an experienced staff at their job. Although 94% of the HCW told that they were not tired at the time of the injury, most of the injuries occurred at the last hour of the shift ($p = 0.039$) and most of the injuries occurred on Friday (27%) but no statistically significant difference was found between the days of the week. Needle stick injuries were the most common type. Of the injured HCW, 82.6% were using personal protective equipment. Among HCW surveyed, 82.4% of them had blood screening test for hepatitis B, C and HIV in last 6 months; but only 52.4% had been previously vaccinated against HBV. The main reason for the unvaccinated status was personal neglectfulness (50%). HCW sought medical advice following the injury by attending (71%) and phone call (21%) to ICD. The serologic profiles of the source patients were unknown in 44%, HBsAg was positive in 32%, HCV was positive in 12% and HIV was positive in 3%. Eleven of the HCW injured from a HBsAg positive patient had received postexposure prophylaxis against HBV, including immunoglobulin and vaccination within 72 hours of injury. Also sixteen HCW who were negative screening tests for hepatitis B were enrolled to vaccination programme.

Conclusion: Housekeeping staff injuries ranked first at our facility and most of these injuries caused by devices discarded inappropriately. Hepatitis B immunisation, universal precautions training, barrier protection, and safer disposal systems should be implemented.

R2253 Trends in nosocomial bloodstream infections

L. Galani, V. Sakka, K. Kouvelas, D. Plachouras, H. Kalogeropoulou, A. Gika, H. Fytrou, L. Zerva, H. Giamarellou (Athens, GR)

Purpose: To describe the incidence of nosocomial bloodstream infections (N-BSI), the predominant pathogens, antimicrobial susceptibilities and the trend during a two year period, in a tertiary care teaching hospital in Greece.

Methods: All N-BSIs were identified retrospectively, as a surrogate marker for nosocomial infections, among adult inpatients in three six-month periods between September 2004-February 2005, March 2005–August 2005 and September 2005-February 2006. Comparisons were performed between the first and third period with Fisher's exact test.

Results: During the three study periods, 1524, 1657 and 1769 blood culture sets were received, resulted in 246 (16.1%), 245 (14.7%) and 265 (15%) positive blood cultures, with 76 (31%), 59 (22%) and 64 (24%) to be considered as contaminants respectively. The incidence of N-BSI per 1000 admissions was: N-BSI 11.3 (86 episodes), 8.9 (94 episodes) and 6.6 (90 episodes) respectively ($p < 0.001$). Mean age of patients with N-BSI was 62.7, 66.3 and 66.1 years and median hospitalisation time was 43, 44 and 42 days respectively.

Rates of BSI according to pathogen per 1000 admissions in the three periods were as follows: Gram-positive bacteria 3.4, 2.3 and 3.1 (p NS) [including *Staphylococcus aureus* 1.3, 0.5 and 0.7 (p NS), *Enterococcus* spp. 0.26, 0.6 and 1.03 ($p < 0.001$)], Gram-negative bacteria 8.4, 3.78 and 3.9 ($p < 0.001$) and fungi 1.18, 0.94 and 0.66 ($p < 0.001$). Antimicrobial susceptibility profiles are shown in Table 1.

Table 1

Pathogen		Number (%) susceptible		
		1st period	2nd period	3rd period
MRSA		2/10 (20)	1/6 (17)	4/10 (40)
VRE		0/2 (0)	2/7 (28)	4/14 (29)
Pseudomonas spp.	ImR[a]	1/5 (20)	7/9 (77)	6/14 (43)
Klebsiella spp.	ESBL	14/21 (67)	3/7 (42)	2/6 (33)
	ImR[a]	10/21 (48)	0/7 (0)	0/6 (0)
Acinetobacter spp.	A/S R[b]	11/15 (73)	2/8 (25)	8/13 (62)
	ImR[a]	12/15 (80)	2/8 (25)	11/13 (85)
E. Coli	ESBL	2/4 (50)	1/16 (6)	1/11 (9)

[a]ImR: imipenem resistance; [b]A/S R: ampicillin/sulbactam resistance.

Conclusions: A steady and significant decrease of N-BSI was observed probably due to a strict infection control policy (bed-rail disinfectant solutions, continuous staff interventions). However an increase in enterococcal N-BSI, especially VRE and the high incidence of multidrug resistant Gram(−) bacteria are a cause of concern.

R2254 Implementation of "significant organism precaution" in a teaching hospital: first experience in Iran

A. Nateghian, L. Mehrali, N. Hamdarsi (Tehran, IR)

Introduction: nosocomial infection due to multiple drug resistant organisms is a concerning problem in Iran, particularly in large teaching hospitals. A specific type of contact isolation termed as "significant organism precaution (SOP)" has been proposed to limit the extent of problem, however, no previous experience in terms of such an isolation programme, coordination for it's implementation and executive barriers was available in our country.

Methods and Patients: After holding three workshops for introducing SOP to physicians, nurses and other healthcare workers, we decided to start SOP for all cases with MRSA, VRE & MDR Gram-negative bacilli (defined as resistant by disk diffusion methods to all members of three major groups of antibiotics usually effective in their therapy (i.e.,

aminoglycosides, new quinolones and 3th generation cephalosporins) in Rasul Akram hospital, a 800 beds teaching hospital in Tehran during 2004. For all cases of SOP, data regarding the demographic issues and type of organisms as well as sites of colonisation or infections, availability of required items for SOP and rate of compliance were recorded by an observer throughout their admission.
Results: 183 cases needed SOP during a 6 months period of study and observation.60 cases (33%) were female.mean age of cases was 43.5 (± 16) and most common sites of isolation were urinary tract, ulcers or incisions & blood stream (23%, 19% & 13.5% respectively). MDR Gram-negative bacilli were the most common isolates for which SOP was necessary (86%) followed by MRSA (13%) and VRE (1%). Except for disposable gloves and individual thermometer for each case which were available for most cases, scrub betadine, gowns, masks, personal stethoscopes and isolated rooms were not present or could not provided for most of cases in non intensive care wards and units. For most of these cases, physicians and nurses believed that such interventions and programmes were necessary, however just in 12% of cases they were fully compliant to SOP programme. Rates of availability of required items and compliance were much better for cases admitted to various intensive care units.
Conclusion: SOP programme is a necessary item at least in large teaching hospital in Iran particularly for large number of MDR Gram-negative bacilli but more educational efforts, budget and facilities should be provided if proper implementation of such a programme is to be happen.

Clinical epidemiology of nosocomial infections

R2255 Antimicrobial resistance profiles of *Acinetobacter baumannii* strains isolated in intensive care units

M. Naim, M. Chabani, D. Tiouit (Algiers, DZ)

Objectives: *Acinetobacter baumannii* formely bacterium saprophyte is at present a redoubtable nosocomial pathogen in intensive care units (ICU) because its virulence and of its multidrug resistance. The purpose of this short study is to estimate the profiles resistance of *Acinetobacter baumannii* isolated in military hospital's ICU during 3 months.
Methods: 20 strains isolated in different samples obtained from inpatients hospitalised in ICU over a period going from January till March 2006. The identification of *Acinetobacter baumannii* was performed using classical bacteriological methods (API 10S, API 20NE boiMérieux) associated to the other additional tests (incubation 44ºC, catalase. . .). The antimicrobial susptibility testing were performed using NCCLS methods. Twelve common used antimicrobial agents were tested: ticarcillin (TIC), piperacillin (PIP), ceftazidim (CAZ), imipenem (IMP), gentamicin (GM), amikacin (AN), trimethoprim-sulfamethoxazol (SXT), ticarcillin+clavulanic acid (TCC), ciprofloxacin (CIP), netilmicin (NET), tobramycin (TB), colistin (CS).
Results: The isolates included in our study originated from: wounds (45%), urine (25%), bronchial aspirates (20%), haemocultures (10%). The almost totality of strains presented a simultaneous multidrug resistant to 8 antibiotics with rates from 70–100%: TIC 100%, PIP 100%, AN 100%, GM 100%, CAZ 95%, IMP 80%, TCC 70%, SXT 70%.
The rates of resistance to other antibiotics were included between 45–55% (TOB, NET, CIP)
Colistin were the only antimicrobial agent active to all isolated strains.
Conclusions: These results show distrubing emergence and fast evolution of multidrug resistant in our hospital, that what we have been reported in a poster published in ECCMID 2005.
Is important to say that multidrug resistant concern the major antibiotics as imipenem and amikacin often ending in a therapeutic impasse and a fatal outcome

R2256 *Acinetobacter* infections: a growing threat for critically ill patients

M.E. Falagas, E. Karveli, I. Siempos, K. Vardakas (Athens, GR)

Objective: There has been a concern regarding an increase of *Acinetobacter* infections in critically ill patients in various parts of the world.
Methods: We extracted information regarding the relative frequency of *Acinetobacter* pneumonia and bacteraemia in patients with intensive care unit (ICU) acquired infection from 41 relevant studies (25 prospective, 8 surveillance, 8 retrospective) and regarding the antimicrobial resistance of *Acinetobacter* clinical isolates from 32 in vitro susceptibility studies identified in Pub Med, Cochrane, and Current Contents database searches.
Results: *Acinetobacter* infections most frequently involve the respiratory tract of intubated patients. The available data suggest that *Acinetobacter* pneumonia has been more common in critically ill patients in Asian (ranging from 4% to 44% among patient with ICU acquired infections) and European (0%–35%) hospitals than in USA hospitals (6%–11%). The data also suggest the presence of a gradient in Europe regarding the proportion of ICU acquired pneumonias that are caused by *Acinetobacter* (very low in the countries of Scandinavia, and gradually higher in Germany and the UK, France, Spain and Italy, and finally Greece and Turkey). A higher proportion of *Acinetobacter* isolates from patients in Asian and European than USA ICUs were resistant to various antimicrobial agents, including aminoglycosides and piperacillin/tazobactam.
Conclusions The data suggest that *Acinetobacter* (mainly *Acinetobacter baumannii*) is a growing threat affecting a considerable proportion of critically ill patients in several parts of the world, especially Asia and Europe.

R2257 The relationship between the antibody titre of *Chlamydophila pneumoniae* and clinical presentation, in patients with respiratory tract symptoms

K. Ueda, T. Nakamura, T. Morikawa, A. Moriyama (Yamatokouriyama, JP)

Objectives: Persistent infection by several microorganisms such as *Helicobacter pylori* has been shown to be associated with diseases other than infectious diseases. There have been reports that persistent infection by *Chlamydophila pneumoniae* is a risk factor for cardiovascular diseases but the relation is not clear. We reported at the 2005 Annual Meeting of The Japanese Society of Internal Medicine that when anti-*C. pneumoniae* IgA and IgG in patients with respiratory tract symptoms were measured, the ratio of patients considered to have acute *C. pneumoniae* infection constituted about 10% (12.8%) and patients that were IgG antibody positive constituted about 70% (70.4%). In this study we noticed that there were cases in which IgA or IgG was maintained at strong-positive for half a year or longer. After careful examination, we had an impression that these were serious. Here we studied the difference in clinical presentation between antibody negative and strong-positive to investigate whether infection by *C. pneumoniae* infection is associated with other diseases.
Methods: We investigated IgG in 244 specimens and the clinical presentation of the patients (total 207; 93 males; 114 females; age 16–99; average age 70.4) whose IgG was measured due to their presentation of respiratory tract symptoms at our hospital from 1 August 2004 to 31 August 2006. A specific antibody measuring kit which uses the ELISA method in which the outer membrane complex of *C. pneumoniae* is the antigen (HITAZYME C. Pneumoniae: Hitachi Chemical Co., Ltd.) was used. An index 3 or above was defined as strong-positive and less than 0.9 was defined as negative.
Results: 59 cases (28.5%) were negative and 13 cases (6.3%) were strong-positive. The average age of the negative was 61, and 82 in the positive, indicating that the negative were younger ($P < 0.01$). The ratio of deaths by 31 October 2006 was 15.3% in negative and 61.5% in

strong-positive, indicating a higher ratio in the strong-positive ($P < 0.01$). The cases of cerebrovascular disorder were found in 25.4% of negative and 38.5% in strong-positive ($P = 0.42$).
Conclusion: The strong-positive patients for *C. pneumoniae* antibody were significantly older in age compared to the negative, and significantly more of the strong-positve patients died within 2 years of the measurement as compared to the negative. Although statistically not significant, there were more cases of cerebrovascular disorder in the strong-positive cases.

R2258 Evaluation of nosocomial infections in the hospitalised patients of intensive care unit of a university hospital, Kashan, Iran

A. Khorshidi (Kashan, IR)

Background and Objective: Nosocomial infections is a great problem in hospital that increased cost, duration of hospitalisation and mortality, Regarding to inaccessibility to accurate information of NI and the bacterial agents. antibiotic resistance pattern in Hospital University of Kashan (Iran), this study was done from May 2005 to Apr 2006.
Material and Methods: This descriptive study was conducted by evaluation of all files of admitted patients of ICU in Central Hospital University of Kashan from May 2005 to April 2006, after of finding of infection cases according to symptoms with or without positive culture, The results and demographic characteristic were registered then results of research were presented by descriptive analysis.
Results: Research showed that prevalence of nosocomial infections in ICU was (10.12%) (39 cases of 289 Persons) range of aged in hospitalised infection was 75–45 years.
The most prevalence of NI was: respiratory infections (56.59%), UTI (26.58%), Bacterimia (6.52%), surgical wound infections (4.3%).
The most common Pathogen were determined: *Escherichia coli* (23.3%), *Pseudomonas* and *Enterobacter* (20%), *Klebsiella* (16.60%), *Staphylococcus aureus* (13.3%). *E. coli* and *Pseudomonas* showed the most resistance to ciprofloxacin, cefteriaxon, ceftazidim, amikacin, gentomycin, imipenem.
Conclusion: Considering the high prevalence and increasing of antibiotics resistance particularly in ICU comparing with others studies, it is necessary to consider the predisposing factors of nosocomial infectons in ICU like appropriate use of medical equipment, prevention of Irrational prescription of antibiotics and sedative and regarding improved attention to hygienic principles. Incidentally It is better that Procedure of treatment start on the last information of bacterial agents and antibacterial resistance patterns and suggested to organised an Infection Control Committee in this Center.

R2259 Catheter-related bacteraemia in intensive care unit patients

J. Komarnicka, H. Grezlikowska, E. Naumiuk, A. Sledzinska, A. Samet, M. Wujtewicz (Gdansk, PL)

Objectives: Analysis of aetiology and clinical characteristics of central venous catheter-related bacteraemia (CRB).
Methods: Microbiological results of proven CRB (isolation of the same pathogen from blood culture and from catheter tip) in patients hospitalised in 12-bed Intensive Care Unit in tertiary care hospital were evaluated, in the period from January 2003 to September 2006. Catheter tips – 3 cm, were examined by Maki technique. Blood cultures were performed in BacT/Alert automated system.
Results: From 1th January 2003 to 30rd September 2006, 2442 blood cultures were performed (16.5% positive). 36 of 84 examined catheter tips were positive. 15 patients were considered as proven CRB and two of them had more than one CRB during hospitalisation (total 17 CRB). Aetiological agents were: 8 coagulase negative staphylococci (CNS), 2 *A. baumannii*, 2 *S. maltophilia*, 2 *Candida albicans*, 1 *E. cloacae*, 1 *P. aeruginosa* and 1 *E. faecalis*. In further 10 patients only catheter tip was positive, without a blood culture: 4 CNS, 1 *Candida* sp. 1 *C. albicans*, 1 *E. cloacae*, 1 *E. faecalis*, 1 *A. baumannii* and 1 *P. mirabilis*.
In patients with proven CRB six suffered from sepsis and nine had fever only. All infections were nosocomial.
Conclusion: Most of isolated aetiological agents were multiresistant hospital pathogens (except *E. faecalis*). Because of small number of proven CRB prospective surveillance is essential as well as examining of every catheter tip in situation of removal due to suspicion of infection.

R2260 Epidemiology and antibiotic resistance of bacterial isolates from patients with bacteraemia in an intensive care unit

E. Ikonomopoulou, P. Georgakoloulos, P. Michail, S. Paratiras, G. Stavropoulou, C. Verra, V. Dondou, A. Regli (Patras, GR)

Objectives: The aim of this study was to carry out the prevalence and the antibiotic resistance of bacterial isolates from patients with bacteraemia in the intensive care unit (ICU) of our hospital.
Methods: Blood cultures from ICU patients were performed using the Bactec 9120 (Becton Dickinson) and the Bact – alert (bioMérieux) during 2003 and 2004–2005 respectively. The identification and the antimicrobial resistance of bacterial isolates were carried out by the VITEK system (bioMérieux). MBL production was performed by E-test (Imipenem, Imipenem and EDTA). Colistin sensitivity was performed by E-test (AB–Biodisc).
Results: During a three year period (2003 –2005) 155 patients (99 males/56 females) hospitalised in our ICU and 30 (19.4%) had one or more episodes of bacteraemia. A total of 53 bacterial strains were isolated. The most prevalent pathogen was *P. aeruginosa* 14 (26.4%) followed by *K. pneumoniae* 8 (15%), Coag– staphylococci 8 (15%), *Acinetobacter baumannii* 7 (13.2%), *Candida* spp. 6 (11.3%), *E. faecalis* 3 (5.7%), *Stenotrophomonas maltophilia* 3 (5.7%), *S. aureus* 2 (3.8%) and *E. coli* 2 (3.8%). 5 *P. aeruginosa* strains produced MBLs and 4 *K. pneumoniae* strains produced ESBL. Resistance rates of *P. aeruginosa* and *K. pneumoniae* for commonly used drugs respectively were: amikacin 21.5%/0%, aztreonam 36%/12.5%, cefepime 29%/50%, ceftazidime 21.5%/50%, ciprofloxacin 21.5%/75%, imipenem and meropenem 36%/0%, piperacillin/tazobactam 36%/12.5%. All *Acinetobacter baumannii* strains were multiresistant (resistance rate: 83% to amikacin, cefepime, imipenem and 100% to aztreonam, piperacillin/tazobactam, gentamycin, netimicin, tobramycin and ceftazidime). All *Acinetobacter baumannii* strains were sensitive to Colistin. Among staphylococci all isolates were resistant to oxacillin but they were susceptible to glycopeptides. *Candida* spp. (*C. albicans* 4 strains, 1 strain C. sake and 1 strain *C. glabrata*) were sensitive to Amphotericin B, Fluconazole and Flucytocine.
Conclusions: Bacteraemia happens in 19.4% of ICU patients and multiresistant strains are mainly responsible so we must be careful in antibiotic usage in order not to increase the antibiotic resistance of bacterial isolates.

R2261 Venous access ports. Prospective study to analyse use-related complications

A. Serrera, M. Alonso, J.L. del Pozo, S. Hernaez, A. Martinez, I. Bilbao, A. Aguinaga, A. Ramos, J. Leiva (Pamplona, ES)

Objectives: Venous access ports (VAP) are used in patients receiving long parenteral treatments. The aim of this prospective and observational study was to analyse the complications of VAP use, and to describe the role of different culture techniques for diagnosing VAP-related infection.
Methods: Over a 12-month period all VAPs removed in our hospital were microbiologically examined by the following procedures: culture of the catheter tip (sonication method). Culture of the internal lumen of the port (chamber swabbing and septum sonication). Besides, a macroscopic analysis with search for deposits inside the reservoir was performed. We also analysed the records of all patients (age, sex, clinical diagnosis, underlying conditions, date of VAP insertion and removal, number of days in use, reasons for removal and all significant events during treatment).

Results: From August 2005 to the end of July 2006, 121 VAPs were removed from 121 patients (median age, 48; range, 4 81 years). The mean duration of implantation of the VAPs was 618 days (range, 0 3301 days). The mean VAP uses were 7 (range, 0–45 uses). VAPs were removed at the end of treatment in 99 cases; with 8% of VAP positive cultures. *S. epidermidis* was the most frequent isolated microorganism (66.6%). Eleven VAPs (9.1%) were removed from 11 patients because of suspected VAP-related bloodstream infection. Septum culture was positive in 90.9% of the patients while catheter tip culture was only 60% positive. The isolated microorganisms were as follows: *S. aureus* (3 patients), yeasts (3), Gram-negative bacilli (3), coagulase-negative staphylococci (1), and *A. fumigatus* (1). The overall incidence of VAP-related bloodstream infection was 0.11/1000 VAP-days. Eleven patients had a VAP removed because of mechanical complications; with 50% of positive VAP cultures. *S. epidermidis* was the most common isolated microorganism (87.5%). The overall incidence of VAP-related mechanical complication was 0.11/1000 VAP-days. In 26 patients (21.49%) we found deposits in the internal lumen of the reservoir (84.6% in end-use removal, 7.6% in VAP-infection and 7.6% in VAP-mechanical complication).
Conclusions: Incidence of associated VAP-use complications is low in our hospital. On the basis of data from the present study, it looks that the reservoir is the key piece of the catheter for the diagnosis of VAP-related infections. *S. epidermidis* might play a role in the genesis of some mechanical complications.

R2262 Genotypic structure and susceptibility to antimicrobial agents of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* in Russian burn intensive care units

A. Goncharov, A. Solomenny, B. Aslanov (Saint-Petersburg, Perm, RU)

Objectives: At first we studied the epidemic situation and the genetic structure of hospital population of *Acinetobacter baumannii* developed (during 2002–2004 yrs) in two burns intensive care units in St. Petersburg and Leningrad region. We observed in these hospitals chronic epidemy of an acinetobacter infection linked to contamination by bacteria from mattresses and functional beds on an "air pillow". The aim of this study was to investigate the genotypic structure and susceptibility to antimicrobial agents and bacteriophages of MDR *A. baumannii* and *P. aeruginosa*.
Methods: A total of 56 clinical isolates of *A. baumannii* and 11 isolates of *P. aeruginosa* from 2 burn ICUs (BICU 1 and BICU 2) were characterised with respect to antimicrobial agents and bacteriophages susceptibility. 17 bacteriophages of *A. baumannii* and 12 bacteriophages of *P. aeruginosa* have been isolated from clinical specimens and tested for their antimicrobial activity.
Results: Typing by RAPD-PCR with the M13 primer showed the hospital population of *Acinetobacter* presented as a restricted number of clonal lines, four (13, 10, 5 and 4 isolates) in BICU 1 and two (7 and 6 isolates) in BICU 2, circulating for the prolonged time (>2 yrs.). No identical amplification pattern was indicated in the clone predominating in BICU 1 and BICU 2. Class 1 integron variable segments (2.5 kb in size) were determined in predominating in BICU 1 clonal line. Prevalence of resistance to cefaclor, cefotaxime, ceftriaxone, ceftazidime and gentamicin was significant in BICUs strains. The integron-positive isolates were sensitive to imipenem or polymyxin only. Only one clonal line of *P. aeruginosa* was detected in two burn ICUs using RAPD-PCR. 44.4% of acinetobacteria and 75.0% of *P. aeruginosa* were susceptible to bacteriophages.
Conclusion: The large-scale researches on search and selection of bacteriophages, for their application as a therapeutic agent are necessary.

R2263 Infection control investigations and treatment of discharged patients following exposure to scabies in a tertiary hospital

M. Schousboe, K. Armstrong (Christchurch, NZ)

Objective: Review of an outbreak of scabies originating in an infected patient with Bullous Pemphigus Vulgaris (BPV).
Methods: A 58-year-old woman admitted to a cardiology ward with arrhythmia and cardiac symptoms had a history of BPV, diabetes mellitus and cellulitis of the legs. She stayed 54 days in five wards in two hospitals and mainly admitted into multi-bed rooms with no restriction of her movements. She was treated with methylprednisolone and cyclophosphamide.
"Norwegian" Scabies was diagnosed 16 days before her death. She was isolated with contact precautions and the infection treated topically and given oral ivermectin. However, microscopy of skin scrapings for the scabies mite remained positive until her death.
The infection caused staff and patient exposure, but no transmission was found until a city laboratory reported a further case to the Infection Control Service. A "look back" of patients who could have been exposed to the index case was carried out and information was sent to General Practitioners.
Results: During the subsequent four months, 31 patients were diagnosed with scabies. Age groups were 40–96 years mean age 75. 8 scabies infections were known to have been diagnosed by microscopic identification of the scabies mite or the eggs. The others were a clinical diagnosis.
Contacts were treated with permethrin cream. 6 were known to require several applications. The Infection Control Service (ICS) became the contact for follow-up for 5 patients of which 3 required referral to a specialist for alternative treatment including oral ivermectin.
Several staff consulted the ICS and were treated.
Conclusion: Scabies is difficult to diagnose in immunosuppresed patients or in the presence of dermatological disease. Elderly people have few reactions to the mite. "Norwegian scabies" is a crusted highly contagious form of scabies. It is often overlooked, lacking the itchiness and lesions seen in people with normal immunity.
The outbreak showed spread via formites in hospitals. Scabies is often not considered by medical practitioners. Steroids used as symptomatic treatment will aggravate the infection. Technical skill is required by laboratory staff when recovering and identifying the mite from skin scrapings.

R2264 Epidemiologic characteristics of *Clostridium difficile* infection in a tertiary hospital in Korea

B-M. Shin, H.M. Yoo, E-Y. Kuak, S.J. Yoo (Sanggye, KR)

Introduction: *Clostridium difficile* is one of the most important pathogens responsible for nosocomial diarrhoea. The disease is mediated by two toxin, designated as A and B; therefore, identification of toxin is also important for diagnosis, treatment and infection control of *C. difficile*. The purpose of this study is to provide the basic features of the microbiological, clinical and endoscopic findings in a tertiary hospital in Korea for controlling *C. difficile* infection.
Methods: We evaluated 384 *C. difficile* positive cases that were cultured from Jan. 2004 to June 2005. We tested toxin A enzyme immunoassay using VIDAS CDA II. We amplified toxin A gene in culture positive cases to differentiate toxin A positive, variant and negative strains. Sigmoidoscopic/colonoscopic findings, departments of admission, underlying diseases and histories about antibiotic usages were evaluated.
Results: Of 384 culture positive cases, *C. difficile* were mainly isolated in medical and neuro-surgical parts. According to the age and gender, *C. difficile* were highly positive in elderly patients (>60 years; 67%) and no statistical difference was observed between male and female. Toxin A gene PCR assay revealed that the proportion of positive, variant and negative strains were 31%, 54% and 15%, respectively. Among culture positive cases, only 40% were underwent sigmoido/colonoscopy and 61% of them were diagnosed as pseudomembranous colitis (PMC)/colitis. Toxin A variant strains were highly associated with PMC instead of intact toxin A positive strains. No statistical differences were found in colitis between toxin A positive strains and variant strains. Among antibiotics, cepholosporin and aminoglycoside were the most frequently used agents. The highly associated underlying diseases were cerebrovascular diseases, malignancy, diabetes mellitus and cardiovascular diseases.

Conclusion: We shoud be aware of the *C. difficile* infection in elderly patients with underlying diseases (cerebrovascular diseases, malignancy, diabetes mellitus and cardiovascular diseases) and long-term usage of antimicrobial agents, especially cepholosporin and aminoglycoside. Toxin A variant strain was more important risk factor in PMC compared with toxin A positive strains.

R2265 Hospital-acquired bloodstream infections in a teaching hospital: a five year follow-up

C. Bulut, M.A. Yetkin, F.S. Erdinc, G.R. Yilmaz, M. Yücel, N. Tülek, E.A. Karakoc (Ankara, Samsun, TR)

Objectives: Hospital acquired bloodstream infections are major causes of morbidity and mortality. The aim of this study was to evaluate the clinical and epidemiological features of the patients with hospital acquired bloodstream infections and the risk factors for mortality attributable to that infection in our hospital.
Methods: A prospective active laboratory based surveillance was performed in our hospital between January 2000 to December 2004. Bloodstream infection was diagnosed according to the CDC criteria. Microorganisms isolated from the the blood were identified by using conventional methods. Disc-diffusion method was used for antimicrobial susceptibility test according to NCCLS.
Results: A total number of 234 nosocomial bloodstream infections were detected in 221 patients during the study period. The mean age of the 97 male and 114 female patients were 58.8±15.4 years and 61.2±16.2 years, respectively. The most common causes for hospitalisation were cerebrovascular diseases, malignancy and cardiovascular diseases. Fortysix percent of the patients were hospitalised on the medical wards, whereas 38.2% and 18.1% of the patients were hospitalised on ICU and the surgical wards, respectively. The history of prior antibiotic usage was present on 32 patients. The mean length of stay between admission and occurrence of bloodstream infection was 15.4±13.1 days.
A total of 264 pathogens were isolated (58.3% Gram-positive cocci, 22% Gram-negative rods, 16.3% nonfermenter rods, 3.4% fungi). *Staphylococcus aureus*, *Enterococcus* spp., *Acinetobacter* spp. were the most common isolated microorganisms. Methicillin resistance was detected in 49.2% of *Staphylococcus* isolates. Carbapenems, netilmisin and tobramisin were the most effective antimicrobials against *Acinetobacter* isolates.
The overall infectious mortality was 40.7%. Clinical factors associated with mortality were evaluated and older age (⩾60 years), being an ICU patient, presence of coma and mechanical ventilation were found as independent risk factors in multivariate logistic regression analysis ($p < 0.05$).
Conclusion: Mortality rate attributable to bloodstream infection was high in our study. It is important to know the aetiological microorganism and antimicrobial susceptibility pattern in order to plan the optimal initial empirical antimicrobial therapy.

R2266 Outbreak of *Corynebacterium striatum* infection in an Italian general intensive care unit

C. Iaria, G. Stassi, S.G. Spinella, G.B. Costa, C. Biondo, E. Gerace, F. Ricciardi, A. David, A. Cascio (Messina, IT)

Background: Intensive care units (ICUs) are known to be a focus for the emergence and dissemination of multiresistant bacteria mainly because the most severely-ill patients can be found in the ICU, and almost all of these patients will have been exposed to intense antibiotic pressure and exogenous bacterial colonisation. *Corynebacterium spp.* are widely disseminated in the environment and constitute part of the normal skin and mucous membrane flora. Although both *C. amycolatum* and *C. jeikeium* are currently recognized as important pathogens, the significance and prevalence of *C. striatum* as a causative agent of disease are not well understood. We describe an outbreak occurred in the ICU of our University hospital and try to evaluate the clinical significance of *C. striatum* infection and risk factors associated with it.
Case report: In first months of 2006 13 strains of *Kocuria kristinae* were isolated from 8 patients admitted in ICU. Routine diagnostic cultures of the clinical specimen had been used and identification performed using Biomeieux VITEK 2 system GP card. Antibiotic sensitivity test was performed using the disc diffusion method according to Clinical and Laboratory Standards Institute guidelines for *Staphylococcus*. All the isolates exhibited the same pattern of antibiotic resistance being sensitive only to vancomycin, teicoplanin and linezolid. Considering that in our lab *K. kristinae* had never been isolated before and that using standard biochemical analysis misidentification of coagulase negative *Staphylococcus* as Kocuria had been reported, a genotypic assay (16s rRNA) was performed. Surprisingly, all the strain tested were identified as *C. striatum*.
Overall *C. striatum* was isolated from 7 bronchial aspirates relative to 5 patients, one from a central venous catheter tip and from 5 blood cultures relative to 5 patients. The demographic and clinical data of the patients are reported in table 1. In no one of the patients in which *C. striatum* was isolated from bronchial aspirate a diagnosis of ventilatory associate pneumonia was made. Patients n.4 had the parameters of sepsis and died notwithstanding he was treated with appropriate antibiotic therapy.

Patient[a]	Underlying illness	Clinical specimens	Days in ICU	T (ºC)	Pulse	WBC $\times 10^3$	Therapy	Outcome
1, M/73	Cranial trauma	N.3 bronchial aspirates	7	37	90	12.5	Teicoplanin	Died
2, M/16	Multiple trauma	CVC tip	9	37	117	27.2	Ceftazidime	Recovered
3, M/59	Stroke	bronchial aspirates	19	37	90	10.0	Teicoplanin	Recovered
4, F/16	Multiple trauma	N.3 blood cultures	5	38.2	100	10.3	Teicoplanin Meropenem	Died
5, M/33	Multiple trauma	bronchial aspirate	9	38	78	16.6	Teicoplanin	Recovered
6, F/80	Stroke	N.2 blood cultures	60	37	90	14.4	Piperacillin Teicoplanin	Recovered
7, M/69	Stroke	bronchial aspirate	5	40	103	19.6	Linezolid Levofloxacin	Recovered
8, F/55	Cerebral metastasis	bronchial aspirate	24	38.5	90	17	Teicoplanin	Died

[a]ID, Gender/Age.

Conclusions: Our report highlights the importance of *C. striatum* as a potential human pathogen in ICU patients even if its pathogen role remains to be clarified. Furthermore, we signal the possible misidentification of *C. striatum* as *K. kristinae* using the bioMérieux VITEK 2 GP card.

R2267 Repeated prevalence surveys of surgical site infections in a Turkish university hospital

O. Yildiz, E. Alp, S. Yucel, B. Kiran, B. Tucer, E. Ok, B. Aygen (Kayseri, TR)

Objectives: The aim of this study was to clarify factors influencing the prevalence of surgical site infections (SSI) undergoing general surgery and neurosurgery, as well as to determine the effectiveness of infection control measures for SSI.
Methods: Two prevalence surveys were carried out during the first six months of 2005 and 2006, after implementing infection control measures in an 1136-bedded teaching hospital. A total of 3924 surgical patients (2263 in general surgery and 1661 in neurosurgery wards) were investigated, the number of patients in the two studies were 2026 in 2005 and 1898 in 2006. The patients were assessed for SSI by review of medical records and by discussion with ward nursing and medical staff. The changes in infection control activities during the 18-month period included educational programmes to healthcare workers, increasing the number of infection control nurses, implementing published guidelines on prevention and control of hospital associated infections, postoperative incision and intravascular catheter care.
Results: During the period of observation, 5.5% of 3924 patients had SSI, 16 (0.4%) had superficial incisional SSI, 117 (3%) had deep incisional SSI and 81 (2.1%) had organ space SSI. The infection rate was 4.4% for clean and clean-contaminated surgery, 14.8% for contaminated and dirty surgery; 4.1% for American Society of Anaesthesiologists (ASA) score I, 5.7% for ASA score II, 9.7% for ASA score III, 9.8% for ASA score IV and 33.3% for ASA score V. The prevalence of SSI

decreased significantly from 6.5% in 2005 to 4.4% in 2006 (p = 0.005). In general surgical patients, the SSI rate in 2006 was significantly lower than that of 2005 (5.1% and 7.6%, respectively, p = 0.017). During the same period, the SSI rates were not significantly different for neurosurgical patients (3.4% in 2006 and 4.9% in 2005, p = 0.151).
Conclusion: The risk factors for SSI were higher degree of wound contamination, poor physical status according to ASA classification, prolonged duration and type of operation, inappropriate use of antibiotics for surgical prophylaxis and use of surgical drains.
Implementation of infection control policies can have a significant impact on the prevalence of SSI, and their effectiveness could be measured by repeated prevalence surveys.

R2268 *Enterococcus* bacteraemia: striking high mortality

M. Suppli, R. Aabenhus, Z. Harboe, L. Andersen, M. Tvede, N. Kirkby, J. Jensen (Copenhagen, DK)

Objective: To assess the character and degree of underlying disease in patients with enterococcal bacteraemia compared with *E. coli* bacteraemia. Additionally to determine if such differences can explain variations in mortality between the two bacteria.
Methods: All patients identified with *E. faecium*, *E. faecalis* or *E. coli* bacteraemia at Rigshopitalet, Copenhagen, were included in the study. Demographic, diagnostic and mortality data were compared for patients with *Enterococcus* and *E. coli* bacteraemia. Patients were excluded from mortality analyses if: (a) younger than 16 years, (b) suffering from haematologic cancer or (c) with an unregistered date of death. Statistical analyses were performed using Student's test and Mann–Whitney U-test to compare continuous variables and chi-squared test for equal proportions or Fisher's exact test to compare categorical variables.
Results: 326 patients with *E. faecium* and *E. faecalis* bacteraemia and 225 patients with *E. coli* bacteraemia were included in the study. Mortality at 90 days after the first positive bloodculture was 36.7% among *Enterococcus* bacteraemia patients, and 25.9% among *E. coli* patients (p = 0.03). There was no difference in mortality at 10 and 30 days, (p= 0.57 and 0.45 respectively). No differences in age were observed and median age were 60.5 and 60.0 years in the two groups (p = 0.54). Acute renal failure, respiratory insufficiency, peritonitis, congestive heart failure and endocarditis were more common in patient with *Enterococcus* bacteraemia (P < 0.05), while solid cancer were more common among *E. coli* patients (p = 0.057).

90-days morality

	Dead	Alive	Mortality
Enterococcus	76	131	36.7%
E. coli	45	129	25.9%

P = 0.03 (Fischer). OR(Dead by *Enterococococcus* bacteraemia): 1.66 [1.07–2.59] (Woolf's approximation).

Conclusions: The observed differences in mortality between *Enterococcus* and *E. coli* at 90 days are unrelated to age and haematological malignancies. Also of interest is that solid cancers were more frequent in *E. coli* bacteraemia. Patients with *Enterococcus* bacteraemia had a higher frequency of congestive heart failure and endocarditis. The present findings suggest that Enterococcal bacteraemia is a strong independent predictor of death. Further investigations are needed to explore whether pathogenic processes in the heart are involved in the death of patients with *Enterococcus* bacteraemia, even when endocarditis is not diagnosed.

R2269 Quantitative cultures of endotracheal aspirate in the diagnosis of ventilator-associated pneumonia

F. Ioannidou, A. Kyratsa, I. Graphakos, E. Kontekaki, V. Mamali, A. Chondrothanassi, A. Xaritou, E. Trikka-Graphakos (Athens, GR)

Ventilator associated pneumonia (VAP) is a common complication in patients requiring mechanical ventilation. Invasive and non invasive diagnostic techniques have been used for the diagnosis of VAP. Quantitative culture of endotracheal aspirate is a non invasive procedure, useful and inexpensive.
Objectives: To evaluate the validity of quantitative endotracheal aspirate (QEA) in patients with suspected VAP.
Methods: We investigated 2,119 endotracheal aspirates (EA) of patients undergoing mechanical ventilation for more than 72 hours, in the Intensive Care Unit of our hospital, in a 4 year period (2003–2006). EA were obtained by sterile means using an aspiration catheter, placed in sterile containers and dispatched immediately to the laboratory for quantitative culture. Bacterial pathogens in concentration of $\geqslant 10^6$ cfu/mL, were considered to be the causative agents of VAP. All isolates were identified by standard laboratory methods, while MICS were determined by phoenix automation system (BD).
Results: The most commonly isolated bacteria were *Acinetobacter baumannii* (56%), *Pseudomonas aeruginosa* (28%), *Klebsiella pneumoniae* (8%), *Staphylococcus aureus* (5%) and others (3%). The antimicrobial resistance pattern is presented in the table. A total of 21 out of 43 *Klebsiella pneumoniae* isolates were b-lactamase producers. All *Acinetobacter baumannii* isolates were sensitive to colistin.

Pathogen	Resistance rates[a] (%)							
	AN	FEP	CAZ	CIP	GM	IPM	MP	TZP
A. baumannii	90.4	97.4	98.2	97.4	68.6	87.8	70.2	93.9
P. aeruginosa	55	80	73.3	70	63.3	68.3	70	61.6
K. pneumoniae	5.6	55.6	72.2	77.7	5.6	11.1	11	66.69

[a]AN, Amikacin; FEP, Cefepime; CAZ, Ceftazidime; CIP, Ciprofloxacin; GM, Gentamicin; IMP, Imipenem; MP, Meropenem; TZP, Piperacillin/tazobactam.

Conclusions: (1) *Acinetobacter baumannii* was the predominant isolate with high resistance rates to all tested antimicrobials. (2) QEA is a simple, non invasive and easily repeatable procedure. Early use of QEA is helpful to clinicians in decision making with regard antibiotic use.

R2270 Risk factors of hospital-acquired infections in a neurology-neurosurgery intensive care unit in a tertiary care hospital, Turkey

C. Bulut, G.R. Yilmaz, M.A. Yetkin, F.S. Erdinc, C. Ataman Hatipoglu, E. Yildiz, E.A. Karakoc, A.P. Demiroz (Ankara, TR)

Objectives: Intensive care unit (ICU)-acquired infections are associated with high mortality, excessive length of ICU and hospital stay, and high hospital costs. The risk factors for nosocomial infections may differ according to the type of ICU. The aim of this study was to evaluate the risk factors for ICU-acquired infections in the patients treated in neurology-neurosurgery ICU.
Method: The study was conducted in Ankara Training and Research Hospital, from May 2006 to November 2006. The patients treated for more than 48 hours in 14-bed neurology-neurosurgery ICU were enrolled into the study. The patients were followed until death or three days after discharge by prospective daily surveillance. Nosocomial infections were identified according to CDC criterias. Risk factors for ICU-acquired infections were analysed with a logistic regression model.
Results: Seventy-one ICU-acquired infections occurred in 52 (30.4%) of 171 patients during 1440 patient-days. The overall rate of ICU-acquired infection was 37.4/100 patients and 44.4/1000 patient-days.

The most common site-specific infections were pneumonia (36.0%), urinary tract infections (31.3%), and bloodstream infections (24.0%). Urinary catheter-associated urinary tract infection rate was 15.0/1000 urinary catheter-days; central line-associated bloodstream infection rate was 23.7/1000 central line-days and ventilator-associated pneumonia rate was 40.6/1000 ventilator-days. The utilisation ratios of urinary catheter, central line catheter and ventilator were 0.92, 0.38 and 0.19, respectively. In univariate analysis, age "d60 years, glasgow coma scale score" T10, being a neurology patient, presence of nasogastric tube, central venous catheter, heart failure, and the presence of two or more underlying diseases were determined as significant risk factors for ICU-acquired infections ($p < 0.05$). Multi-variate logistic regression analysis revealed, being a neurology patient ($p < 0.01$), presence of nasogastric tube ($p < 0.05$), and presence of central venous catheter ($p < 0.01$) as independent risk factors.
Conclusion: In that study, ICU-acquired infection rates were found higher when compared with NNIS results. In interpretation of device-associated infection rates, the rates of device-use should be known those high rates made us to take immediate precautions both for decreasing device utilisation and emphasizing the importance of device application and care practices.

R2271 Diarrhoea by *Clostridium difficile* toxin

J.L. Prada, F. Moreno, J. De La Torre, A. Del Arco, J. Aguilar, J.D. Ruíz (Marbella, ES)

Introduction: *Clostridium difficile* (Cd) toxin is a cause of diarrhoea in patients treated with antibiotics. Any antibiotic may produce this problem which start during the antibiotic treatment or even 4 days after its end.
Objectives: to study in our centre the clinical profiles and outcomes of Cd associate colitis.
Method: Retrospective study of all the in-patients suffering from diarrhoea and Cd toxin confirmation in stools between 2000 and 2004. We have used the Microbiology Lab Database and the patient clinical records. Immunocromathographic quickly test in direct stool sample was used to detect toxin A (InmunoCard Stat! Toxin A Meridian)
Results: Cd toxin test was required in 325 samples with positive results in 21 patients (6.8%), one of them with two samples. Average age was 58 years (15–80) and 36.4% were males. 31.8% were diabetics, 22.7% oncology patients without chemotherapy, gastroenterology diseases in 22.7%, bronchial diseases in 22.7%, chronic steroids therapy in 13.7% and chemotherapy in 2 cases (9.1%). Active or previous antibiotic treatment were described in 81.8% ($n = 18$), and there was no references in 3 cases. Betalactamics were the source of the problem in 62.5% (piperacilin/tazobactam was the first cause with the half of the cases) and quinolones in 37.5% (ciprofloxacin and levofloxacin). The previous infection-diagnosis was in the hospital in 72.7%. Outcomes were satisfying in 90.9% (2 death by no-infectious causes) Cd colitis was the main diagnosis in 27.2% of definitive medical reports. In 6 cases (27.3%) there were a hospital admission in the previous 2 months. Colitis treatment was described in 77.3% of medical records, metronidazol was the central therapy with 15 of 17 cases, and vancomycin was used in the other 2. The average of metronidazol-treatment-time was 10 days (5–16) with doses between 250 and 500 mg tid. By departments 45.5% were in General Internal Medicine and 36.4% in the Gastroenterology Unit.
Conclusions: Colitis by Cd is not a common cause of diarrhoea, but must be taken in mind in all in-patients receiving antibiotics, or with previous antibiotics therapy, overall in diabetics.

R2272 Clinical correlates of Mortality Probability Model MPM II and the outcome of a *Burkholderia cepacia* bacteraemia outbreak in an intensive care unit in Guatemala, Central America

I. Cazali, F. Ramirez, C. Mejia, C. Ramirez, M. Silvestre, R. Gordillo (Guatemala City, GT)

Background: The prevalence of multidrug resistance (MDR) Gram-negative bacteria is on the rise, but its effect on patients outcomes is not well established. We assessed the impact of *Burkholderia cepacia* with MPM II on the outcomes of critically ill patients with bacteraemia.
Objectives: To evaluate the outcome and risk factors of *Burkholderia cepacia* bacteraemia according to the MPM II in critically ill patients.
Methods: 17/665 adult critically ill patients with *Burkholderia cepacia* bacteraemia were evaluated retrospectively and compared to a control group with Gram-negative bacteraemia. Demographics, baseline MPM scores, appropriateness of empiric therapy were examined in relation to infection-attributed mortality and clinical cure.
Results: The incidence of bacteraemia was 145.86×1000 patients in eight months. 97/306 (31.7%) blood cultures were positive; 17/97 (17.5%) due to B cepacia in 10 patients. 9 cases were analysed and compared with 9 controls with bacteraemia due to Gram-negative bacteria. There were no significant differences among cases and controls including: age (SD) 44 and 45.8 years, gender, antibiotics use and the MPM II score (49.5% vrs. 46%). 2 (22%) cases vrs. 9 (100%) controls received appropriate antibiotics for bacteraemia. Mortality rate was 37.5% for cases and 66.6% for controls. No *B. cepacia* was found in the intensive care facilities.
Conclusions: In the adult critically ill patients with Gram-negative bacteraemia, *Burkholderia cepacia* was associated with a lower rate of infection-attributed mortality, after adjusting for the severity of illness as compared to other Gram-negatives. Inappropriate empiric therapy or no therapy at all was not associated with mortality. Our results warrant further investigations and confirmation with a prospective study.

Travel medicine, tropical and parasitic diseases

R2273 Prevalence of enteric parasites in people with and without gastrointestinal symptoms in Gonbad, Iran

E. Nazemallhoseini-mojarad, F. Mesgariyan, A. Haghighi, M. Zali (Tehran, IR)

Objective: Geographical conditions and poor nutritional and socioeconomic status contribute to making the Islamic Republic of Iran a favourable area for parasitic infections.
The aim of this study is to investigate the prevalence of intestinal parasitic infection in Gonbad city in Iran.
Method: From February 2005 to July 2006, five hundred and fifty one faecal samples were examined for intestinal parasites. All samples were tested to detection and analysis the parasite by standard method.
Result: Out of 551 faecal samples (81%male and 19%female), 72 isolates (13.1%) including pathogen and non pathogen parasites were positive. The results of this study showed a frequency rate of 48 (8.7), 31 (5.62%), 3 (0.54%), 3 (0.54%), 2 (0.36%), 2 (0.36%), 2 (0.36%) for *Giardia lamblia*, *Endolimax nana*, *Ascaris lumbricuides*, Hookworm, *Enterobius vermicularis*, *Hymenolepis nana* and *Entamoeba histolytica* respectively.
Discussion: In this study *G. lamblia* showed the most prevalent rate in the infections and *E. nana* was higher among the nonpathogenic protozoa. Base on the result of present study, stool examination should be taken for high-risk populations in the low level hygiene to increase knowledge of people about personal and community health and hygiene.

R2274 Current concepts of malaria in Romania

S. Florescu, C. Popescu, M. Cotiga, L. Raduta, C. Voinea, E. Ceausu, P. Calistru (Bucharest, RO)

Objective: Epidemiological, clinical, laboratory and therapeutical aspects of imported malaria in Romania.
Methods: Retrospective study of 22 patients with malaria admitted in the Clinical Hospital of Infectious and Tropical Diseases "Dr. V. Babes" between January 1st, 2004 and October 31st, 2006.
Results: 8 patients were hospitalised in 2004, 7 in 2005 and 7 in 2006. 19 were romanian citizens and 3 were foreigners. Sex ratio: M/F-20/2. 18 patients traveled to Africa (5 to Nigeria, 3 to Guineea, 2 to Ivory Coast,

Camerun and Mozambic, respectively, and 1 to Ciad, Uganda, Mali and Tanzania, respectively); 2 patients traveled to Papua New Guinee, and 1 to India and French Guinee, respectively. Age limits were between 24 and 70 years old. Travel length limits in the endemic zone were 2 weeks and 2 years, with a mean travel duration of 8 months. The interval between arrival in Romania and clinical onset ranged from 3 days to 5 months, and the period from the clinical onset and positive diagnose ranged from 1 day to 2 months. 15 patients had previously malaria. Only 3 patients had malarial chemoprofilaxy. The aetiological distribution was as follows: 13 patients with *P. falciparum*, 5 patients with *P. vivax*, one patient with *P. malariae*; in 3 patients we diagnosed double ethiology (*P. falciparum* + *P. vivax* – 2 patients, *P. falciparum* + *P. malariae* – 1 patient). The thin and thick blood smears were the standard for the positive diagnose in all the patients. The highest value for the parasitaemia was 300‰ (most of the highest values in the *P. falciparum* malaria cases). 15 patients had thrombocytopenia, 6 had anaemia, 8 had hyperbilirubinaemia, 5 had high levels of ALAT, and 3 had high creatinin levels. Ethiological treatment consisted of: Quinine for 11 patients, artemisine derivatives in 10 patients, Atovaquone+Proguanil in 3 patients, Mefloquine in 2 patients, and Artemether+Lumefantrine in 1 patient. 2 patients with *P. falciparum*, and *P. falciparum* + *P. malariae*, respectively, had severe forms of malaria (coma and acute renal failure). 2 patients had high grade resistance (R2) to quinine. All patients survived.

Conclusions: Our clinic admittes a relatively constant number of imported malaria cases. *P. falciparum* malaria is a possible lethal form of the disease, thus the rapid diagnose and treatment is compulsory. The ethiological treatment must take into account the specie of P., the endemic zone, but also the possibility of the quinine resistance.

R2275 Haemophagocytic lymphohistiocytosis associated with visceral leishmaniasis

A. Tapisiz, N. Belet, E. Çiftçi, E. Ince, Ü. Dogru (Ankara, TR)

Visceral leishmaniasis (VL), is a systemic disease caused by the dissemination of protozoan parasite *Leishmania* throughout the reticuloendothelial system. It may mimic or lead to several types of haematological disorders including hemophagocytosis. Infection associated hemophagocytic syndrome implicating *Leishmania* is very rare and often difficult to diagnose. Here, we describe a child with hemophagocytic lymphohistiocytosis (HLH) associated with VL.

A 2-year-old boy presented with a 1 month history of high fever, fatigue, poor feeding, weight loss, pallor and abdominal distension. On physical examination, he was febrile (39.6ºC) and pale. There was severe weight loss and failure to thrive. He had hepatomegaly (4 cm below the right costal margin) and splenomegaly (8 cm below the left costal margin). Neither lymphadenopathy nor bleeding signs were observed. Haematological investigation revealed hemoglobin 5.1 g/dL, white blood cell count 3,300/mm^3 (%16 neutrophils, %76 lymphocytes and %8 monocytes), platelet count 55,000/mm^3. Erythrocyte sedimentation rate was 55 mm/hour, C reactive protein was 9.52 mg/dL and ferritin was 6364 ng/mL. Fibrinogen level was normal. Serum triglycerides were increased to 266 mg/dL with normal cholesterol, and the serum lactate dehydrogenase level was elevated at 2762 U/L. Serological markers for Epstein–Barr virus, cytomegalovirus, parvovirus B19, toxoplasmosis, hepatitis A and B viruses, HIV, salmonella and brucella agglutinins were all negative and blood cultures could not demonstrate any infectious agent.

On bone marrow aspiration hemophagocytosis and leishmania amastigotes were observed. The patient was diagnosed with HLH associated with VL. Liposomal amphotericin B 3 mg/kg/day was administered on 1–5, 14, 21 days. Treatment was well tolerated, no side effects were observed. Symptoms and clinical findings improved gradually. Fever was controlled on the 3rd day of treatment. After 5 days of treatment laboratory testing revealed hemoglobin 8 g/dL, white blood cell count 4,500/mm^3, platelet count 144,000/mm^3. One week later, the hepatosplenomegaly gradually regressed. On discharge, physical examination was normal, with no enlargement of spleen. No relapse was seen in a 6 months follow up period.

VL should be considered for the aetiology of HLH. Awareness of this association and early diagnosis of visceral leishmaniasis may prevent prolonged hospitalisation and potentially harmful investigations and treatments.

Resistance & mechanisms of action of antifungals

R2276 Multicentre study of antifungal susceptibility patterns of yeast isolates and their species distributions in Bulgaria

Z. Ivanova, T. Kantardjiev, A. Kouzmanov, L. Boyanova (Sofia, BG)

Objectives: The National Reference Laboratory of Mycology has started for the first time a survey on fungal infections in Bulgaria to get insight in the species distribution and their antifungal susceptibility pattern.

In the current study is determined the fluconazole and voriconazole susceptibility of yeast strains mainly from genus *Candida* with the use of E-test and micro-dilution kit Micronaut-AM (Merlin). The strains with high minimal inhibitory concentrations (MICs) for fluconazole and voriconazole are additionally confirmed with the referent broth micro-dilution method NCCLS (currently CLSI) M27-A2.

Methods: A total of 235 clinical isolates has been collected in six participating medical centres from January to November 2006. All yeast strains are isolated from diverse body site, of immunocompetent and immunocompromised patients, including HIV infected ones. Etest MICs are determined with Mueller–Hinton agar containing 2% glucose and 0.5 mg of methylene blue per l and were read after incubation for 24 and 48 h at 35ºC.

Results: Sixty-six percent of the isolates are identified as *Candida albicans*, followed by *C. glabrata* (26%), *C. parapsilosis* (6.8%), *C. krusei* (4.7%), *C. tropicalis* (3.4%) and other *C.* non-*albicans* strains (7.2%). Low susceptibility to fluconazole is detected among *C. albicans* strains (3.2%, MIC > 256 mg/L), *C. glabrata* (46%, MIC16–32 mg/L; 23%, MIC > 256 mg/L), *C. krusei* (45.5%, MIC32–64; 36.4%, MIC > 256 mg/L). No resistance to fluconazole and voriconazole is detected in *C. parapsilosis*, *C. tropicalis* and *C. lusitaniae* strains. Resistance to voriconazole is detected only in *C. glabrata* (15.4%, MIC > 32 mg/L) and *C. albicans* (2.6%, MIC > 32 mg/L) strains. Voriconazole showed good activity to most of the tested isolates with MICs in the range of 0.064–0.5 mg/L.

Conclusion: This study confirmed the high percentage of isolated *C.* non-*albicans* strains and showed that species distribution of *Candida* isolates is similar to that in other European countries. An important concern is the low antifungal susceptibility detected in *C. glabrata* and *C. krusei* and their high prevalence.

R2277 Antifungal susceptibility patterns of *Candida* isolates recovered from bloodstream infections: a 3-year study

O. Zarkotou, K. Kopsari, V. Avramioti, G. Chrysos, D. Gianneli (Athens, GR)

Objectives: To determine the prevalence of candidaemia in bloodstream infections (BSIs), to define the species distribution among candida isolates and to evaluate the antifungal susceptibility profiles.

Methods: During a 3-year period (11/03–10/06) a total of 1263 episodes of BSIs were studied. The blood cultures were incubated in BACTEC 9240 Automated Blood Culture System (Becton and Dickinson). The identification of candida isolates to species level was based on Fungichrom kit (International Microbio, France), until April 2006. The isolates recovered after April 2006 were identified to species level by VITEK 2 Compact automated system (bioMérieux). The antifungal susceptibility testing was performed by a colorimetric microdilution method (Sensititre YeastOne Test Panel, UK) which is based on NCCLS M-27-A2 standards and gives MIC results for 6 common antifungal agents: amphotericin B, fluconazole, itraconazole, ketoconazole, flucytosine and voriconazole. The breakpoints for azoles

and flucytosine were those suggested by NCCLS. The strains with MICs >1 for ampotericin B and voriconazole were considered resistant.
Results: During the study period a total of 58 episodes of candidaemia in 56 patients were identified. Candidaemias accounted for 4.6% of BSIs (58/1263) and 5.8% of the patients (56/964) developed BSI due to candida species. The majority of candidaemias were observed in ICU-patients (33/56) followed by surgical patients (14/56). The species distribution study proved a clear predominance of *Candida albicans* (40/58 isolates, 69%). The most prevalent among non-*albicans* species was *Candida parapsilosis* (9/58, 15.5%). All candida isolates were susceptible to amphotericin B. Susceptibility to all antifungals tested was detected in 46.6% of the strains. Resistance to fluconazole and itraconazole was observed in 15.5% and 17.2% of the isolates respectively, while 6.9 and 15.5% of them were susceptible dose-dependent. The resistance rate to voriconazole was 13.8%. Resistance to all azoles tested was detected in 5 *Candida albicans* and one *Candida glabrata* isolate.
Conclusions: *Candida albicans* prevails among fungal isolates in candidaemias in our hospital. The necessity of surveillance for detecting trends in species distribution and antifungal susceptibility patterns is emphasized by the increasing incidence of resistance to antifungals.

R2278 Determination of the antifungal activity of itraconazole, voriconazole, posaconazole, amphotericin B and caspofungin against 45 clinical isolates of zygomycetes using the CLSI M-38A procedure

M. Torres-Narbona, J. Guinea, J. Martínez-Alarcón, T. Peláez, M. Guembe, E. Bouza (Madrid, ES)

Objectives: An increase in the incidence of *Mucor*mycosis has been reported during the last few years in some institutions. However, the "in vitro" antifungal susceptibility profile of clinical zygomycetes has received little attention. The aim of our study was to determine the activity of amphotericin B (AMB), itraconazole (ITC), voriconazole (VC), posaconazole (POS) and caspofungin (CAS) against a collection of clinical isolates of zygomycetes.
Methods: We evaluated the activity of AMB, ITC, VC, POS and CAS against 45 clinical strains of zygomycetes collected during an 18-year period in our hospital. The isolates were identified according to standard methods. The antifungal activity was obtained by means of the CLSI M-38A microdilution procedure. The final concentration of the VC, ITC, POS and AMB in the wells ranged from 0.03 to 16 μg/mL. The final concentration of CAS ranged from 0.25 to 256 μg/mL. The trays were incubated for 24 and 48 hours at 35C degrees. The MIC endpoint was defined as the lowest concentration producing complete inhibition of growth (MIC-0) for all the antifungal drugs studied. The minimal effective concentration (MEC), which was also calculated for CAS, was defined as the lowest concentration at which an abnormal growth was observed.
Results: The distribution of the isolates studied was as follows: *Absidia* (8), *Cunninghamella* (4), *Mucor* (19), *RhizoMucor* (1), *Rhizopus* (11) and *Syncephalastrum* (2). We calculated the MIC_{90}, MIC_{50}, MEC and range for each species and the results are summarised in the table: POS was the most active drug, followed by AMB, ITC/VC and CAS. We did not find significant variations between the MIC_{90} obtained at 24 and 48 hours of incubation, with the exception of the MICs of POS against *Absidia* (1 μg/mL and >16 μg/mL, respectively). *Absidia* and *Mucor* showed the highest MICs for POS. In addition, *Absidia* and *Cunninghamella* were less susceptible to AMB than the other species. VC and CAS were inactive against all the isolates tested.
Conclusions: AMB and POS showed potent in vitro activity against zygomycetes whereas voriconazole and caspofungin presented very poor activity. With the exception of POS and *Absidia*, the antifungal activity of the drugs studied presented a slight variation between 24 hours and 48 hours of incubation.
Jesús Guinea Pharm D, PhD, is contracted by the Fondo de Investigación Sanitaria (FIS), contract number CM05/00171.

Susceptibilities of 45 strains examined.

Anti-fungal	24 h				48 h			
	MIC (mg/L)			MEC	MIC (mg/L)			MEC
	MIC_{50}	MIC_{90}	Range	(mg/L)	MIC_{50}	MIC_{90}	Range	(mg/L)
AMB	1	2	0.125–4		1	4	0.125–>16	
ITC	1	>16	0.25–>16		4	>16	0.5–>16	
VC	16	>16	4–>16		>16	>16	8–>16	
POS	0.5	1	0.125–2		0.5	2	0.25–>16	
CAS	256	>256	64–>256	128	>256	>256	128–>256	128

R2279 Susceptibilities of different clinical isolates of *Candida* spp. to voriconazole, itraconazole and fluconazole

R. Cisterna, G. Ezpeleta, K. Ibarra, A. Morla (Bilbao, ES)

Objectives: There has been an increasing rate of fungal infections during the last two decades that has created a need for standardised methods for determining the in vitro susceptibilities of new and established antifungal agents. The aim of this study is to describe the susceptibility pattern of different *Candida* spp. strains to voriconazole, itraconazole and fluconazole isolated between January 2003 and January 2006.
Methods: 1791 isolates of *Candida* species from different sites were collected for in vitro susceptibility testing to voriconazole using E-test and broth microdilution (BMD) methods. The CLSI (formerly NCCLS) BMD method used RPMI 1640. E-Test MICs were determined with Mueller–Hinton agar containing 2% glucose and 0.5 micro g of methylene blue per ml (MBE agar). All plates were incubated at 35ºC with readings taken after 24 and 48 hours. E-Test MICs of voriconazole read at 24 and 48 hours were compared to BMD MICs read at the same time. MICs were read at the 24 and 48 hours. The interpretative breakpoints described by the CLSI were used to determinate the correlation between the E-test and BMD results.
Results: We observed positive statistical significant ($p < 0.01$) correlations between 93% and 98% between E-Test and the BMD method. The general susceptibility pattern revealed that most of the isolates were sensible to fluconazole (Sensible (S): 87.94% Sensible dose dependant (SDD): 8.23% Resistant (R: 3.83%) but this rate decreased when the isolate considered was not a *C. albicans* (S = 68.84%;SDD= 21.3%; R: 9.86%). Voriconazole had a very good susceptibility pattern with less a 2% of isolates exhibiting a SDD or R MIC to this drug. Itraconazole exhibited a rate of 1.1% of resistant stains and a nearly 4% of isolates with SDD MIC.
Conclusions: These statistical results suggest that E-Test have very good correlation with other methods like BMD to establish the susceptibility of a fungal isolate to different azole drugs. Fluconazole remains to be a good choice in case of *Candida albicans* infection but other azole drugs must be considered when the strain isolated is not a C. Albicans. At this point itraconazole but specially voriconazole could be the drugs of choice for successful treatment of the infection

R2280 Surveillance of azole resistance among *Candida* non-*albicans* clinical isolates from patients with invasive fungal infections

E. Kazakos, E. Moschous, E. Tsiakiri, S. Alexiou-Daniel (Thessaloniki, GR)

Objectives: The aim of the present study was to document the in vitro susceptibilities of *Candida* spp. representing predominantly invasive forms of candidiasis, to currently available triazole agents and ketoconazole in order to assess the rates of resistance per species and identify possible implications for antifungal therapy selection.
Materials and Methods: A total of 68 isolates (4 *C. ciferrii* , 6 *C. dubliniensis*, 3 *C. famata*, 10 *C. glabrata*, 1 *C. haemulonii*, 3 *C. krusei*, 5 *C. kefyr*, 4 *C. lusitaniae*, 24 *C. parapsilosis*, 2 *C. sphaerica*, 6 *C. tropicalis*) were included. The strains originated from blood, respiratory tract specimens, surgical wounds and normally sterile sites and each of them represented a unique infectious episode.

Identification to species level was achieved by the VITEK 2 automated system with greater then 98% probability. Antifungal susceptibility testing to itraconazole, voriconazole, posaconazole, fluconazole and ketoconazole was performed using the Etest assay according to the manufacturers' instructions. MIC data were interpreted by applying the CLSI breakpoints (document M27-A2) and tentative MICs' previously published.
Results: Overall, the rates of resistance recorded among all isolates were 26.4% for itraconazole, 19.6% for posaconazole, 13.2% for voriconazole, 16.1% for fluconazole and 10.1% for ketoconazole. All fluconazole-resistant strains exhibited resistance to posaconazole (MIC⩾8ug/mL) whereas 72.2% of those strains appeared to be less susceptible to voriconazole (MIC⩾2ug/mL). Extended rates of cross-resistance were also documented between fluconazole and itraconazole (100% of fluconazole resistant isolates). Pan-azole resistance was evident in 1 *C. famata*, 5 *C. glabrata* and 1 *C. parapsilosis* isolates.
Conclusions: Although our study is limited by the small number of invasive *Candida* isolates from a single institution, our data provides further evidence regarding the patterns of triazole cross-resistance, previously reported. The degree of resistance recorded highlights the need for introduction of antifungal susceptibility testing in routine practice and raises important clinical issues related to therapeutic options in patients with invasive candidiasis.

Fungal infections

R2281 *Pneumocystis carinii* pneumonia in HIV-negative patients

D. Kofteridis, M. Velegraki, M. Antoniou, G. Papazoglou, T. Anastasopoulos, D. Alegakis, G. Samonis (Heraklion Crete, GR)

Purpose: *Pneumocystis carinii* (renamed jirovecii) pneumonia (PCP), is a life-threatening opportunistic infection occurring in immunocompromised hosts. The aim of this study was to investigate the predisposing factors, clinical features and outcome of PCP in HIV-negative patients.
Materials and Methods: The medical records of 18 hospitalised adult patients with PCP during a 3 year period (2004 to 2006) were retrospectively reviewed using a standardised questionnaire.
Results: Twelve men (67%) and 6 women (33%) were identified suffering of PCP.
Their median age was 65 (range 25–80 years). Eight patients had haematologic malignancies, 5 solid tumours, 2 rheumatoid arthritis, 1 systemic lupus erythematosus and 2 patients had type 2 diabetes, as underlying diseases. Cytotoxic and/or immunosuppressive drugs including steroids were used in 14 (78%). One patient did not have any known immunosuppression. Only ten patients (55%) had absolute lymphopenia at the time of PCP onset, ranging from 100–700 lymphocytes/mm^3 (median 300/mm^3). In 7 (70%) out of 10 patients having a high-resolution computed tomography the ground-glass pattern was a characteristic finding suggestive of PCP. All 18 cases were confirmed microbiologically by direct immunofluorescence technique. On admission only 3 patients (17%) was receiving trimethoprim-sulfamethoxazole prophylactically. Six patients (33%) developed severe symptoms, required mechanical ventilation and were admitted to the intensive care unit. All patients received appropriate therapy consisting of trimethoprim-sulfamethoxazole (sulfamethoxazole 100 mg/kg/day) and corticosteroids. Improvement occurred only in 9 (50%) who survived, whereas the remaining 9 patients died. PCP was the clear cause of death in 5 (26%).
Conclusions: PCP is a life-threatening disease with poor prognosis in patients treated appropriately. The infection occurs mainly in patients treated with immunosuppressives, especially corticosteroids. However it can also occur in patients with no apparent immunosuppression. PCP prophylaxis should be considered in patients treated with corticosteroids and those with malignancies receiving antineoplastic chemotherapy.

R2282 Isolation of *Cryptococcus humicolus* from an immunocompromised HIV-negative patient

S. Baka, S. Antonopoulou, P. Salomidou, S. Meretaki, G. Kaparos, S. Demeridou, A. Velegraki, E. Kouskouni (Athens, GR)

Objectives: Cryptococcosis is a disease caused usually by *Cryptococcus neoformans*, an encapsulated yeast-like organism. The number of species included in the genus *Cryptococcus* has increased over the last years. *Cryptococcus humicolus* is a rare opportunistic yeast which starts to be implicated more often in diseases of severely debilitated hosts. Prevalence data are not available since the reports in the literature are scarce. We report a case of cryptococcaemia by *C. humicolus* in an immunocompromised HIV-negative surgical patient.
Methods: A 39-year old man was referred to our hospital with a history of sigmoidectomy and colostomy 2 months ago because of permeative peritonitis on the ground of acute diverticulitis. Two explorative laparotomies and drainage of the intraabdominal collection were also performed. He presented in a septic condition and 2 sets of blood cultures were sent to the laboratory on admission in the ICU. Identification of the pathogen was performed using the API System (boiMérieux, France) and susceptibility testing was carried out with 2 different methods (NCCLS and E-Test).
Results: Blood cultures became positive after two days of incubation and *C. humicolus* was identified by means of colonies' characteristics, Gram stain morphology and ID 32 API (boiMérieux, France). The isolated strain was sensitive to the antifungal agents studied: NCCLS (amphotericin B, fluconazole, voriconazole) and E-Test (amphotericin B, 5-fluorocytocine, ketoconazole, fluconazole, itraconazole). Two new antifungal agents, caspofungin and posaconazole, were tested and the MIC for caspofungin was 4 in both methods while for posaconazole 0.125 and 0.064, respectively. The patient was immediately put on liposomal amphotericin B, voriconazole and fluconazole. Clinical improvement was seen after 3 weeks of treatment and the patient was discharged from the ICU.
Conclusions: It seems that *C. humicolus* emerges as an important pathogen in HIV-negative patients. Diagnosis must rely on positive cultures followed by identification of the pathogen. Early recognition and proper therapy after appropriate susceptibility testing may improve clinical outcomes.

R2283 Mycotic peritonitis in Saint-Petersburg, Russia

L. Pestova, N. Klimko, T. Bogomolova, I. Vibornova, A. Saturnov, A. Priadko, I. Zuzgin, L. Plahotnyuk, G. Palval (Saint-Petersburg, RU)

Objectives: To determine epidemiology, underlying diseases, clinical symptoms, treatment and outcomes of MP in patients (pts) in Saint-Petersburg.
Methods: We have analysed prospectively demographic, clinical and microbiological data for 29 pts with MP in 6 hospitals, during 1998–2006. Diagnosis of MP was established on EORTC/MSG 2002 criteria.
Results: Male 14, female 15. Age: 3 months–78 years (median 47). Nineteen pts (66%) were hospitalised in surgical ICUs and 10 (34%) in dialysis units. Underlying diseases in surgical pts were pancreatitis (5, 26%), ulcer disease (4, 21%), colon cancer (3, 16%), gallstone disease (2, 11%), abdominal injury (2, 11%), liver transplantation (1, 5%), congenital disease of GI system and kidneys (1, 5%), tuberculosis of kidneys and urinary bladder (1, 5%). Underlying diseases in dialysis patients were chronic glomerulonephritis (4, 40%), diabetes mellitus (3, 30%), cystic disease (1, 10%), methylmalonic aciduria (1, 10%), agnogenic chronic renal insufficiency (1, 10%). Main clinical signs of MP were abdominal pus or unclear dialyzate (100%), fever >38C (93%), septic shock (17%). Fungaemia and/or focal sites were detected in 45% of pts. Forty-one fungal strains were isolated from 29 pts. The most common species were *Candida albicans* 34%, followed by *C. parapsilosis* 25%, *C. glabrata* 18%, *C. krusei* 8%, *C. tropicalis* 5%, *C. zeylanoides* 2%, *C. lusitaniae* 2%, *C. kefyr* 2%, *Trichosporon mucoides* 2%, *T. cutaneum* 2%. Monoinfection was in 62% of pts. The

disease was caused by ⩾2 different fungi in 38% of pts. Susceptible to fluconazole were 52% of strains, SDD 17%, resistant 31%. In 86% of pts abdominal catheters were removed/changed and these patients received antimycotic therapy: fluconazole (84%), amphotericin B (32%), caspofungin (12%), liposomal amB (4%). Therapy with two antimycotic drugs was conducted in 32% of pts. Overall mortality rate was 31%. Mortality rate in patients with removed/changed abdominal catheters who received antimycotic therapy was 20%, 4 untreated patients died (100%) ($p = 0.003$).
Conclusion: Mycotic peritonitis develops in surgical ICUs and dialysis units' pts. Main aetiologic agents are *Candida* spp. (96%), 31% of strains are fluconazole resistant. Effective antifungal treatment, removing of abdominal catheters are important in the management of these infections.

R2284 *Aspergillus* endophthalmitis in an immunocompetent woman: intra-ocular penetration of oral voriconazole

S. Logan, E. Graham, M. Rajan, E. Johnson, J. Klein (London, Bristol, UK)

Aspergillus fumigatus is a recognized cause of endophthalmitis in the immunocompromised. We report a case in an immunocompetent patient. There are only three previous reports in the literature of endogenous spread of *Aspergillus* species to the eye in apparently immunocompetent individuals.
Voriconazole penetration into the vitreous has not been assessed in the inflamed eye.
Initial management with oral voriconazole produced good results. We were able to demonstrate equivalent voriconazole concentrations in serum and vitreous samples by means of a bioassay.
Despite negative fungal culture of the vitreous fluid after treatment with voriconazole the appearance of the eye worsened and she required enucleation.
This case report serves to highlight the potential for endogenous *Aspergillus* endophthalmitis to occur in immunocompetent individuals. Voriconazole appears to penetrate the inflamed eye well, the prognosis, however, remains poor.

R2285 Comparison of antifungal susceptibility test methods for yeasts using broth microdilution, EtestTM and Fungitest

O. Perovic, C. Rosmarin, J. Wadula, S. Gould, K. McCarthy, D. Orton, D. Antelme, L. Bhagoobhai, A. Duse (Johannesburg, ZA)

Introduction: Antifungal drug resistance is likely to be a result of the increased use of antifungal agents as treatment and prophylaxis and is associated with high morbidity and mortality. Appropriate initial therapy for invasive fungal infections may require the introduction of susceptibility testing for selected isolates. Reference guidelines are based on a broth microdilution method. Agar dilution, EtestTM, disk testing, and modified broth microdilution technique, have been deemed to be easier to perform, time efficient and less laborious. An evaluation of three test methods was proposed in our hospitals to select for the most optimal test.
Objectives of our study were to determine the susceptibility of mucosal and invasive isolates of *Candida* species using the broth microdilution technique and to compare the EtestTM and Fungitest® test methods to the reference method.
Methods: Test evaluations were performed at the Johannesburg, Chris Hani Baragwanath hospitals and Mycology Reference Unit during 2006. Eighty eight invasive clinical isolates of *Candida* species including *albicans, glabrata, parapsilosis, tropicalis, krusei, dublinsiensis, guillermondii*, were analysed.
The CLSI broth microdilution method M27-A2, EtestTM and Fungitest were performed.
Antifungal susceptibility profiles were compared amongst fluconazole, and amphotericin B. All assays were carried simultaneously and results read at 24 h as well as 48 h of incubation. The tests were performed according to manufacturer instruction.
Results: MIC 90 and 50 for amphotericin B and fluconazole for all our isolates are presented in the Table. For amphotericin B all three test methods had a high concordance. For fluconazole there was poor agreement between Fungitest® and broth microdilution test and a high number of major errors were identified.

Susceptibility of *Candida* spp. to antifungal agents determined by CLSI M27-A2 broth dilution MIC reference method

Species	No. tested	Antifungal agent	MIC (μg/mL)	
			MIC_{50}	MIC_{90}
Candida albicans	46	Amphotericin B	0.125	0.25
		Fluconazole	2	8
Candida dubliniensis	1	Amphotericin B	0.125	0.25
		Fluconazole	32	64
Candida glabrata	13	Amphotericin B	0.25	32
		Fluconazole	0.5	64
Candida guilliermondii	1	Amphotericin B	0.5	1
		Fluconazole	32	64
Candida krusei	9	Amphotericin B	0.25	0.5
		Fluconazole	32	64
Candida parapsilosis	5	Amphotericin B	0.25	0.5
		Fluconazole	8	16
Candida tropicalis	4	Amphotericin B	0.25	0.5
		Fluconazole	16	32

The best agreement was observed with the EtestTM 48hours for *Candida* species where correlation was 88% at 48 h compare to 58.7% at 24 hours. Fungitest® showed 6.25% and 46.87% at 24 h and 48 h respectively.
Conclusion: We founded that the EtestTM for fluconazole performed better in predicting resistance than the Fungitest®. Also, results of EtestTM at 24 hours were equivalent to 48 hour readings, thus allowing for a more rapid result, a feature not found for the Fungitest®. Therefore the EtestTM would be more suitable and efficient in a routine academic microbiology laboratory.

R2286 The frequency of isolation and the susceptibility to antifungals of *Candida* species in a large academic hospital: three-year surveillance

M.C. Ghezzi, G. Raponi, C. Mancini (Rome, IT)

Objective: The pattern of *Candida* species commonly isolated in various clinical settings is continuously changing. Purpose of this study was to analyse the frequency of isolation of different species of *Candida* and the variation of their susceptibility to antifungals, during a three years surveillance study.
Methods: *Candida* species were isolated from clinical samples of patients hospitalised at the "Azienda Policlinico Umberto I" Academic Hospital of Rome, Italy during three years (2003–2005). Strains were identified by germ tube test and by API 32C®. The susceptibility testing was performed by Sensitre Yeast One® test on clinically based request, and the results were interpreted according to the manufacturer's and CLSI guidelines.
Results: A total of 3022 strains of *Candida* were identified during the study period. The specie most frequently isolated was *C. albicans* (83.9%) followed by *C. glabrata* (7.8%), *C. tropicalis* (5.1%) and other *Candida* species (4.2%). The frequency of isolation of *C. albicans* steadily decreased during the study period, starting from 90% in 2003 to 80% during 2004 and 2005. Instead, the isolation of *C. glabrata* and *C. tropicalis* doubled in the same period from 4.5% to 9.5% and from 2.2% to 4.8% respectively. Antifungal tests were performed on 110 strains isolated from blood stream (50.9%), lower respiratory tract (26.3%), wounds (16.4%) and urinary tract (6.4%). *C. albicans* showed very low frequency of resistant isolates, with 3 strains in 2004 and one in

2005 resistant to at least one antifungal. Conversely, *C. glabrata* showed low susceptibility to itraconazole (18%), to fluconazole (59%), and, to a lower extent, to amphotericin B (88.3%) and to voriconazole (94%). Furthermore, the number of *C. glabrata* strains resistant to at least one antifungal steadily increased from 0 in 2003 to 10 strains in 2005.
Conclusions: The results of this study indicated that the pattern of *Candida* species isolated during the years varied in our clinical setting, showing an increasing incidence of non-*albicans* species, particularly *C. glabrata* and *C. tropicalis*. The susceptibility data evidenced that strains of *C. albicans* were highly susceptible to the test drugs, together with an increasing rate of *C. glabrata* strains resistant to antifungals. The usage of azoles in the prophylaxis of fungal infections in our hospital probably influenced the diffusion of non-*albicans* species, some of which are frequently resistant to these drugs.

R2287 Antifungal utilisation in a haematology unit in Turkey: a part of the FUNGOBASE Study

A. Azap, Ö. Kumbasar, H. Akan (Ankara, TR)

Objective: To evaluate antifungal (AF) usage in haematology unit of a tertiary care hospital.
Methods: The study was conducted in 60 bed haematology unit of Ankara University Medical School. The unit has 20 beds for stem cell transplantation. AF prescriptions in 2004 and 2005 were analysed. Data were collected retrospectively by the help of an AF prescription surveillance programme (FUNGOBASE). Renal toxicity was described as $\geqslant$50% increase in the basal serum creatinin level.
Results: AFs were used in 215 febrile episodes not responding to broad spectrum antibiotics in a two years period. Amphotericin B deoxycholate (AmB-d) was the initial therapy in 192 (89%) episodes. In 109 (56%) of 192 episodes no other AF was used during the course of neutropenia, and the median duration of AmB-d therapy was 12 days (4–46 days). In 32 (17%), 38 (20%), and 13 (7%) of 192 episodes AmB-d was stopped due to infusion related toxicities, nephrotoxicity, and treatment failure, respectively. The most common AF choice in patients who did not tolerate AmB-d (nephrotoxicity and infusion related toxicities) was lipid formulations of amphotericin B (AmB) (74%) and caspofungin (11%). Lipid formulations were used for a median duration of 15 days (3–76 days). Combination AF therapy was used as initial treatment in 2 patients and for salvage in 13 patients. Combination therapy succeded in 7 (46%) patients.
Conclusion: AmB is a broad spectrum antifungal agent. The reasons limiting AmB-d use are nephrotoxicity and infusion related toxic effects. Its main advantage on other AF agents is low cost. In our setting AmB-d was well-tolerated by 56% of the patients. Despite its disadvantages AmB-d is still the main drug in the treatment of invasive fungal infections especially in countries with limited sources.

R2288 Mycological and pathological findings of sinus material from patients with chronic sinusitis

F. Can, M. Demirbilek, L. Ozluoglu, N. Haberal, B. Akkuzu, E. Aydin, H. Arslan (Ankara, TR)

Background: Fungi are important aetiologic agents of sinusitis but incidence and fungal types have not been systematically studied. This study was designed to evaluate the incidence of fungi and dispersal of species in chronic sinusitis another aim of the study was to saw the correlation among microbiological and pathological findings.
Methods: Specimens of mucin, sinus secretions, and/or tissue were obtained intraoperatively from 60 cases of chronic sinusitis (without diabetes mellitus and/or immunosupression) and sent to the mycology and pathology laboratory. For microbiological findings; specimens were treated with Sputolysin and cultured on Sabouraud's dextrose agar, Mycosel agar, and Brain-Heart Infusion agar plates; and incubated at 30ºC (and 37ºC) for up to 6 weeks. Identifications were made according to standard procedures. For pathological findings; conventional H&E and Gomorie's methamine silver (GMS) stain were employed in all cases.
Results: The fungal species were demonstrated in 21 of 60 specimens. *Aspergillus* spp. was the commonest isolate (48%) and it was followed by dematiaceous fungi which found in 7 patients. *Candida albicans* were isolated from 2 patients and *Curvularia lunata*, *Paecilomyces lilacinus*, *Chrysosporium* spp. were isolated from one patient each.
Although allergic fungal sinusitis was detected in 17 of 21 patients (81%) that fungi were isolated by culture, it was found to be 51% (20 of 39) in fungal culture negative patients in histopathological examination ($p < 0.5$). According to the histopathological findings one patient had chronic invasive fungal sinusitis and *Chrysosporium* spp. was isolated from that patient.
Conclusion: Our data showed that allergic sinusitis was the most common part of chronic sinusitis (37 of 60) and fungi was isolated from 46% of them. *Aspergillus* spp. and dematiaceous fungi are most relevant species. These data would be important for clinicians while choosing the empiric drug therapies for chronic sinusitis.

R2289 Epidemiology of candidaemia and antifungal susceptibility patterns in a Turkish university hospital

S. Serin Senger, M. Demirbilek, O. Kurt Azap, F. Timurkaynak, H. Arslan (Ankara, TR)

Objective: *Candida* species are the fourth most common cause of hospital-acquired bloodstream infection. Our objective was to evaluate the *Candida* species and antifungal drug resistance.
Methods: The study was conducted retrospectively from Feb. 2005 to Oct. 2006. Antifungal susceptibility test was performed according to CLSI M27-A protocol and MIC values were also determined by using E-test and RPMI 1640 agar with 2% glucose.

In vitro susceptibilities of bloodstream *Candida* spp. isolates.

Antifungal agent	MIC (mg/L)		
	MIC_{50}	MIC_{90}[a]	Range
***C. albicans* (n = 25)**			
Fluconazole	0.25	1	0.06–16
Itraconazole	0.02	0.09	0.008–0.25
Voriconazole	0.012	0.064	0.002–0.64
Caspofungin	0.03	0.06	0.012–0.25
Amphotericin B	0.047	0.12	0.002–0.125
Non-*albicans* *Candida* spp. (n = 7)			
Fluconazole	0.75	ND	0.12–>256
Itraconazole	0.03	ND	0.016–0.5
Voriconazole	0.016	ND	0.003–0.19
Caspofungin	0.03	ND	0.002–>32
Amphotericin B	0.094	ND	0.003–0.5
Total (n = 32)			
Fluconazole	0.25	1	0.06–>256
Itraconazole	0.02	0.09	0.008–0.5
Voriconazole	0.016	0.064	0.002–0.64
Caspofungin	0.03	0.06	0.002–>32
Amphotericin B	0.047	0.12	0.002–0.5

[a]ND, not determined.

Results: A total of 32 candidaemia episodes were encountered in 31 patients. Overall, 25 (78.1%) episodes were due to *C. albicans*, followed by 2 (6.3%) of *C. tropicalis*, 2 of *C. famata*, and 1 (3.1%) of *C. parapsilosis*, *C. glabrata*, and *C. guillermondii* each. The invitro activities of all tested agents are outlined in the table. Only one *C. albicans* strain (4%) was susceptible dose-dependently (SDD) to fluconazole, all others were susceptible. The same strain was also SDD to itraconazole, but the voriconazole MIC value was <1 mg/L. MIC values of non-*albicans* strains were higher than *C. albicans* strains. Resistance to fluconazole was detected in the *C. parapsilosis* strain and this

strain was SDD to itraconazole. *C. guillermondii* strain was resistant to caspofungin (MIC > 32 mg/L). None of the other strains of *Candida* spp. had MIC value greater than 1 mg/L to caspofungin, amphotericin B and voriconazole. Twenty four (77.4%) of the patients were in ICU, 6 (19.4%) of whom had burns. CVC were in place in 29 (93.5%) patients. Prior antibiotic usage was present in 30 (96.8%) patients. Fifteen (48.4%) patients were subject to total parenteral nutrition. As an underlying condition, 8 (25.8%) had prior abdominal surgery, 10 (32.3%) had malignancy, and 10 (32.3%) had chronic renal failure. Prior fluconazole usage within a month of the first positive blood culture for *Candida* spp. was present for 22 (71%) patients, two of whom had fluconazole-resistant or -SDD candidaemia. 11 episodes were treated with fluconazole and 18 with caspofungin. The overall mortality rate was 45.2%.

Conclusion: *C. albicans* was found as the most common cause of candidaemia. Our study suggests that fluconazole resistance has not emerged among bloodstream isolates of *C. albicans*, although isolated from patients at high risk. Although number of non-*albicans Candida* strains is too low, fluconazole resistance was detected in one strain (14.3%). Present study shows that even for the patients at high risk, fluconazole still remains the drug of choice for *C. albicans* strains.

R2290 A four-year epidemiological study of aetiological agents of yeast infections and antifungal susceptibility profiles in the centre of Portugal

C. Paulo, J. Marques, C. Mourão, A. Meliço-Silvestre, A. Florindo, T. Gonçalves (Coimbra, PT)

Objectives: The objective of this study was to characterise the aetiological agents of yeast infections in the Portuguese population, together with the antifungal susceptibility profiles.

Methods: Yeast isolates were collected from October 2002 to October 2006, from patients attending or hospitalised at the Centro Hospitalar de Coimbra. Species identification was primarily obtained using the commercial methods Bichro-latex Albicans Fumouze (Fumouze Diagnostics, France) and Api ID 32C (BioMérieux, Portugal), and antifungal susceptibilities using the ATB Fungus 2 INT (BioMérieux, Portugal) and the Fungitest (Sanofi Pasteur). Then, all the confirmed aetiological agents were subjected to re-identification by RFLP of the 5.8 S-ITS and/or by sequencing the contiguous D1/D2 fragment from the rRNA 26 S gene. All the isolates were deposited in a pathogenic yeast collection, the PYM collection. The following patient data was collected and registered in a database: age, gender, underlying disease, specimen collected.

Results: A total of 727 yeast isolates were obtained, and confirmed as aetiological agents of yeast infections, during the four-year surveillance study. Half of the samples studied belong to patients older than 60 years. The most represented specimens in this survey were isolated from respiratory system infections (292), followed by urine (152), blood (48), catheters (49), gynaecological (47), intra-abdominal (20) and skin lesions (54). Among these, only 2% were not correctly identified using the commercial methods described above, when confirmed by the molecular biology techniques. As expected, the predominant pathogen is *Candida albicans* (60%), followed by *C. tropicalis*, *C. parapsilosis* and *C. glabrata*. Moreover, other species belonging to the *Candida* genus were also found as aetiological agents, together with *Saccharomyces cerevisiae*, *Geotrichum capitatum*, *Cryptococcus neoformans*. The isolated pathogens proved, in vitro, to be susceptible to the tested antifungals, included in the methods used. Infrequently, some isolates exhibited resistance to itraconazole (21 isolates).

Conclusion: The data obtained during this study represents the first broad epidemiological study on yeast infections in the Portuguese population. As expected from similar studies in different countries the predominant pathogenic yeast is *C. albicans*. Albeit, these results showed that there are some regional specificities in what regards yeast infections aetiology.

AIDS and HIV infection

R2291 HIV infection, HAART, and gynecomastia. Epidemiological, clinical, and potential pathogenetic correlates

R. Manfredi, L. Calza (Bologna, IT)

Introduction: Gynecomastia (G) is an emerging untoward event in patients treated with HAART.

Patients and Methods: Through a cross-sectional study performed on around 1,000 HIV-infected patients (p) treated with antiretrovirals at our reference centre in Bologna (Italy), we identified all cases of G related to the administration of at least 12 consecutive months of HAART, to assess possible correlations of G with a spectrum of clinical, laboratory, and therapeutic variables (and including all adverse effects of HAART itself). All p with true G (as distinguished from lipomastia by an ultrasonography assay) were considered evaluable, while p with other predisposing conditions (endocrine disease, alcohol abuse, liver cirrhosis, and use of drug possibly predisposing to G), were carefully ruled out.

Results: Twenty-one out of 616 evaluable HIV-infected male p (3.4% of our p population), developed a true G when aged 12–58 years. Seven p out of 21 never received protease inhibitor (PI)-containing therapies, while efavirenz-based regimens apparently prompted G in 7 p who were naïve for PI, and worsened this disturbance in three further p who abandoned PI for efavirenz. Considering nucleoside analogues (NA), two p developed G during treatment conducted with dual isolated NA. Comparing the different administered NA, stavudine seemed to be the most commonly used compound, also taken for the longest time ($p < 0.01$). A complete hormonal workup did not detect significant abnormalities, save in one p, who had slight serum FHS, LH, and testosteron abnormalities (with normal prolactin levels). When considering the eventual correlation with the most common HAART-induced disturbances, some forms of lipodystrophy was concurrent in all the 21 p with G, while hypertriglyceridaemia, hypercholesterolaemia, and hyperglycaemia were found in 15, 9, and 3 p, respectively. During the subsequent 12–36-month follow-up, a spontaneous ameliorement of G was never observed, notwithstanding eventual HAART modifications. Due to local hypersthesia, tenderness, and discomfort, two p resorted to surgery.

Conclusions: G is probably an underestimated problem in the setting of HAART. The frequent association of G with other HAART-related dysmetabolism suggests a possible common pathogenetic causes.

R2292 Aetiology of withdrawal lopinavir/ritonavir in a group of HIV-infected patients and evolution of subjects changed to other treatments in a simplification strategy

V. Moreno, E. Valencia, I. Jiménez-Nácher, J. González Lahoz (Madrid, ES)

Objectives: (1) To analyse the aetiology of withdrawal Lopinavir/ritonavir (LOP/r) in a group of HIV infected patients with prolonged viral suppression and (2) to analyse the evolution of subjects changed to other treatments in a simplification strategy.

Methods: We reviewed the clinical history of 101 HIV+ patients treated with LOP/r who were switched off to other treatment when they were with complete viral suppression. A data base was developed and the epidemiological, clinical, serological and laboratory characteristics were studied. All the simplification cases were separately analysed and the subjects with treatment failure were reviewed. Statistical study was done by SPSS 13.0.

Results: Causes of LOP/r withdrawal were: toxicity in 7 cases, treatment interrupttion in 3 and simplification in 61 (65%). Two patients died, 10 were lost and in 8 was no possible to find the aetiology. Mean age in 61 patients who were simplified was 42 years (r: 27–59) and 52 (85.2%) were men. They had been receiving LOP/r for at least 12 months, 73.8% were heavily pre-treated and mean CD4+ cell was $613 \pm 343\ mm^3$ (r: 108–1800). Twelve patients (19.7%) had AIDS and 24

(39.3%) had hepatitis C virus (HCV) co-infection. Twenty five patients were simplified to receive a protease inhibitor (PI) free schedule (13 TrizivirR, 10 efavirenz and 2 nevirapine) and 36 received another PI: 25 atazanavir/ritonavir (ATZ/r), 8 ATZ and 3 fosamprenavir/rito-navir (FOS/r). One year, after simplification, virological failure developed in 11 cases (18%) and mean time was 6 months. Forty seven subjects (77%) continued with complete viral suppression, 1 patient withdrew recommended treatment and 2 were lost. All failures took place in pre-treated patients and the most frequent were in TrizivirR (46%) and efavirenz (30%) group. Patients simplified to nevirapine and FOS/r continued with undetectable viral load. Only two of 33 subjects receiving ATZ (one without ritonavir) developed virological failure (6%). The results did not depend on HCV co-infection or having AIDS but previous treatments and the presence of resistance mutations influenced the evolution. After LOP/r withdrawal, there were statistical differences in metabolic and hepatic parameters without clinical significance.
Conclusions: Simplification was the main cause of withdrawal LOP/r in patients with suppressed viral load. This strategy should not be considered in pretreated persons overall if PIs-free schedule is going to be used.

R2293 Prevalence of genotypic resistance to nucleoside analogues, non-nucleoside analogues and protease inhibitors in HIV-infected persons in Athens, Greece

G. Panos, G. Charatsis, V. Paparizos, M. Kazantzi, M. Falagas (Athens, GR)

Objective: To study the prevalence of genotypic resistance to nucleoside analogues (NRTIs), non-nucleoside analogues (NNRTIs) and protease inhibitors among HIV-1 infected persons in Athens, Greece.
Methods: Patients followed at 2 HIV Units were examined for emergence of antiretroviral resistance mutations (ARMs) in this observational study where complete therapy history was available. HIV-1 genotypic resistance was performed by using the HIV-1 TRUGENE® Genotyping kit and ViroSeq™ HIV-1 Genotyping system. All mutations were recorded according to the October/November 2005 IAS-USA Drug Resistance Mutations Figures.
Results: Our data are from 234 patients that underwent genotypic testing out of 2069 followed during 1987–2004. The most frequently observed ARM of each drug category were to NRTIs at codons M184V [present in 149 tests (63.6%)], M41L [79 tests (33.8%)], K70R [66 (28.2%)], M184VI [58 (24.8%)], T215YF [53 (22.7%)], D67N [82 (35.0%)], T215Y [72 (30.8%)], K219Q [47 (20.1%)], K219E/Q [54 (23.1%)], and L210W [49 (20.9%)], respectively. The most prevalent mutations related to NNRTIs were K103N [present in 59 tests (25.2%)], G190A [50 (21.4%)], and Y181C [48 (20.5%]. Mutations in the protease gene showed that the ARM at residue L63P was the most prevalent present in 119 samples (50.9%). Other protease mutations commonly found were V82A/F/T/S [85 (36.3%)], I54L/M/V [93 (39.7%)], L90M [62 (26.5%)], M46I/L [67 (28.6%)], I84V [27 (11.5%)], L33F [16 (6.8%)], and D30N [12 (5.1%)].
Conclusion: L90M (26.5%) was among the most frequently observed key protease mutations in our series, probably attributed to the steady increase in prevalence of non-B subtypes in Greece (mainly subtype A).

R2294 *Helicobacter pylori* infection in symptomatic HIV-seropositive and seronegative patients: a case-control study

G. Panos, E. Xirouchakis, V. Tzias, G. Charatsis, I. Bliziotis, V. Doulgeroglou, N. Margetis, M. Falagas (Athens, GR)

Objective: We conducted this study to evaluate the prevalence and morbidity of *Helicobacter pylori* infection in HIV seropositive patients.
Methods: Case-control study of HIV seropositive and seronegative patients in a tertiary care hospital in Greece.
Results: HIV seropositive patients were infected by *H. pylori* less often than HIV seronegative controls [12/58 (20.7%) versus 38/58 (65.5%), $p < 0.001$]. The mean CD4 count was lower for *H. pylori* negative than *H. pylori* positive HIV infected patients ($p < 0.007$). Also, among HIV patients, prior use of antibiotics or proton pump inhibitors was more common in those without *H. pylori* infection, a result that almost reached statistical significance ($p = 0.06$). The grading of the density of *H. pylori* infection and the grading of the histomorphological findings according to Sydney classification were similar between HIV seropositive and seronegative patients with *H. pylori* infection.
Conclusion: Our data suggest that HIV seropositive patients are infected by *H. pylori* less often than the general population. In addition, among patients with HIV infection, these with decreased CD4 cells are less likely to be co-infected with *H. pylori*.

R2295 Study of the genotypic-resistant pattern in plasma of a group of HIV-infected Cameroonian pregnant women and children

O. Turriziani, G. Russo, M. Lichtner, M. Bucci, A. Stano, I. Sauzullo, F. Mengoni, A. Ialungo, V. Vullo, G. Antonelli (Rome, IT)

Objective: To study the genotypic resistance pattern in plasma-HIV RNA derived from HIV-1 infected Cameroonians pregnant women and children.
Patients The samples used in this study derived from a cohort of HIV infected mothers and their children who participated in a PMTCT programme (Prevention mother to child transmission) based on HIV-NET protocol. 49 Cameroonians HIV-1 infected pregnant women and 7 HIV-1 infected children.
Methods: RNA was extracted from 140 μL of plasma using a QIAmp Viral RNA kit (Qiagen, Milan, Italy) following the manufacturer's instructions. Sequencing was undertaken using CLIP, a DNA sequencing technique for direct sequencing of small quantities of amplified template (TruGene HIV-1, Bayer Diagnostics).
Results: Eight out of 49 pregnant women examined and 4 out of 7 children who underwent the treatment develop resistance to NVP. Three of the 4 children harbour a resistant virus different from that of the mothers who indeed harbour a wild variant of the virus. This indicates that the virus has not been transmitted by the mothers but they develop resistance after the treatment received after birth. Since the mothers and children have been treated once and with a single dose of nevirapine, the data also show that NNRTI resistant variants persist also in absence of the drugs.
The sequence analysis of the protease region also revealed the presence of minor PI mutations in 44 of 49 mother's samples studied (median 5 mutations; range 0–7). Interestingly the PR sequence from children revealed the same PI substitutions in 4 cases, while in 3 children the mutations detected were different from those revealed in mother's samples.
Conclusions: These data confirm that one dose of NVP is sufficient to select NNRTI resistant variant which can persist for long time also in the absence of the selective drug. Furthermore our results show that HIV-1 from Cameroonian patients seems to display a peculiar pattern of minor substitution associated to PI resistance. Further studies are needed to verify whether the use of PIs in Cameroon may result in the rapid emergence of PI resistant strain.

R2296 Antiretroviral drug resistance among drug-naive HIV-infected patients in Korea

S-H. Lee, E-K. Rheu, D-H. Cah, S-J. Lee, H-S. Rheu, H-H. Kim, G-J. Cho (Busan, KR)

Objectives: The prevalence of HIV drug resistance mutations in durg-naive patients has been shown to differ with geographic origin. The purpose of this study was to assess the prevalence of transmitted antiretroviral drug resistance mutations in drug naive patients in Korea.
Methods: Genotypic resistance was determined by using ViroseqTM Genotyping System in 51 antiretroviral treatment naive HIV-infected patients between March 2005 and September 2006. Transmitted drug resistance was estimated according to the IAS-USA 2006 definition,

taking into account only major mutations in protease and all mutations in reverse transcriptase including revertant mutaions at codon 215.
Results: The median age was 42 years and 46 (90%) were male. Median CD4 cell count was 131/mm^3 and mean plasma RNA level was 4.98 log copies/mL. Among 51 patients studied, 46 (90%) were newly diagnosed patients. None of them were recent seroconverters. 46 (90%) were infected with subtype B and 5 (10%) with the non-B subtype strains (3 as CRF01_AE; 1 as CRF02_AG; 1 as subtype A). Of all 51 subjects tested, we did not find any primary mutations of NRTI, NNRTI as well as PI regions, resulting in an estimated prevalence of 0% in this study. Although no primary mutations were found, V118I was found in 2 patients and T69S in 1 patient for NRTI, V179D/E in 3 patients and A98G in 1 patient for NNRTI, and 50 of 51 (98%) patients carried one or more secondary PI mutations/polymorphisms.
Conclusion: Prevalence of transmitted antiretroviral drug resistance in drug-naive patients was still low in Korea.

R2297 Pharmacokinetics of atazanavir in HIV-HCV co-infected patients with or without cirrhosis

F. Gatti, A. Loregian, P. Nasta, S. Pagni, K. Prestini, A. Matti, S.G. Parisi, L. Biasi, M. Puoti, C. Boldrin, G. Palù, G. Carosi (Brescia, Padua, IT)

Objectives: To evaluate the influence of liver cirrhosis on atazanavir (ATV) pharmacokinetics (PK) in HIV/HCV co-infected patients (pts) treated with atazanavir ± ritonavir (ATV 400 mg QD or ATV/r 300/100 mg QD).
Methods: 14 HIV/HCV co-infected pts receiving ATV/r 400/100 mg QD and 4 pts receiving ATV 400 mg QD were included. According to liver stiffness (LS) value obtained by fibroscan® at the moment of PK determination or histological diagnosis patients were classified in 2 groups:
- NC, no cirrhosis (10 pts); LS < 12 kPa or Knodell fibrosis score (Kfs) 1–3;
- C, cirrhosis (8 pts); LS ⩾12 kPa or Kfs 4.

ATV plasma levels were determined by High Performance Liquid Chromatography.
Samples for the 24 hour PK curve were collected at t = 0–1.5–3.5–8 h at the steady state. C trough (Ct) was determined before the daily dose. PK analysis of plasma ATV concentration–time data were conducted using non-compartmental methods with WinNonlinProfessional software, version 4.1. The area under the concentration–time curve during a dosing interval (AUC_{0-24}) was calculated using the log linear trapezoidal method. Results are expressed as median and interquartiles.
Parametric and non parametric tests have been used for comparison of continuous variables between groups when appropriate and linear regression analysis to test correlation between variables ($p < 0.05$ was considered as significant).
Results: All but one pts had HIV-RNA <50 copies/mL and 14/18 pts were on TDF+3TC/FTC. Median (range) LS was 6 (5–9) kPa for NC and 19 (13–21.5) for C pts ($p = 0.0005$). Median (range) ATV Ct level was 390 (180–475) ng/mL in C and 525 (170–620) ng/mL in NC pts (p = NS). Median ATV AUC was 32,833 (22,480–39,707) ng·h/mL in C and 25,156 (16,929–50,194) ng·h/mL in NC (p = NS); no statistically significant correlation was found between AUC and LS ($p = 0.12$).
The analysis restricted to the pts taking ATV/r showed no statistically significant difference of Ct or AUC between groups, but a significant inverse correlation was found between AUC and LS ($p = 0.03$) and a trend toward a significant correlation was found between Ct and LS ($p = 0.06$).
Conclusions: The slight reduction in Ct observed in cirrhotic patients with mild disease could be related to the reduction and/or diversion of the liver blood flow related to initial portal hypertension associated to increased liver stiffness and would probably become significant in case of severe cirrhosis; this could also explain the inverse correlation found between AUC/Ct and LS.

R2298 Oral side effects of antiretroviral therapy in HIV-infected patients

M. Nechifor, I. Gradinaru, V. Luca (Iasi, RO)

Objective: To assess the incidence of oral side and adverse effects of some associations of antiretroviral chemotherapic drugs associations in HIV-infected patients (both children and adults).
Method: The study was performed on 72 children (age between 3 and 18), and 64 adults HIV-infected of both ganders, hospitalised in the Infectious Diseases Hospital "Sf. Parascheva" Ia^{o}i between 2000 and 2005. All the included patients had CD4 lower than 400/mm^3 and before hospitalisation had not received any other antiretroviral therapy. The study included only patients that were clinically surveyed over 90 days. Patients (both children and adults) received the following chemotherapeutic protocols: Zidovudine + Indinavir + Lamivudine (23 children and 18 adults); Efavirenz + Indinavir + Zalcitabine (29 children and 24 adults). Oral cavity was daily examined.
Results: Main side effects noticed were: erosions on gingival mucosa and inferior lip (6.2% of adults and 5.5% of children); ulceration of jugal mucosa (8.3% of adults and 3.1% of children), oral mucosa hyperpigmentation (3.1% of adults and 4.1% of children), xerostomia (11% of adults and 10% of children), necrotic stomatitis (2.7% of adults and 3.1% of children). In some patients there were more than one side effect. The presence of saburral tongue and oral candidosis was considered as disease complication (though it might be induced also by antiretroviral therapy). The incidence of oral side effects was about 27–28% in children and about 22–23% in adults. There were not observed in this study major difference regarding oral side effects between children and adults. Oral side effects observed did not impose discontinuation of antiretroviral therapy.
Conclusions: Oral side effects of antiretroviral therapy represent a certain fact which should be considered in the clinical practice.

R2299 The knowledge level of high school students in Antakya city, Turkey about AIDS

Y. Onlen, N. Savas, C. Ozer, L. Savas, F. Incecik, A. Kanatli (Antakya, Adana, TR)

Aim: The aim of the current study was to investigate the knowledge levels of junior High school students, sources of their knowledge, the socio-economical facts acting on their kowledge on AIDS
Methods: This is a cross sectional, descriptive study including a total of 2838 students (1368 females and 1470 males) from 16 high schools in 4 different types (State, Anatolian, Trade and Private high schools). A questionaire composed of 5 different sections was used to analyse the knowledge level seperately; how AIDS is an ilness, how AIDS can spread, risk factors for AIDS, ways of protection of AIDS, and the source of their knowledge on AIDS in sections from 1 to 5 respectively. In the first 4 sections the right answers were graded with 2 score, wrong answers with 0 and with 1 when the student does not have an idea. The maximum score for the first 4 sections of the questionnaire were 12, 38, 14, 14 respectively. In the 5th section the probable sources of knowledge such as school, friends, family news papers, books and, TV were questioned.
Results: The mean score received from the questionnaire were 49.50±6.50 (minimum:27, maximum:69). The mean score for the females was 49.69±6.46, it was 49.32±6.66 for the males ($p > 0.05$). The mean score for first 4 section were 8.2±2.15, 24.7±4.16, 9.70±2.30, 6.90±1.79, respectively. No statistically significant difference was observed between the 15–16, 17–18 and 19–20 age groups on knowledge about risk factors for AIDS ($p > 0.05$), while there were meaningful differences, how AIDS is an ilness, how AIDS can spread, ways of protection of AIDS ($p < 0.05$). Trade school students received the lowest score from the questionnaire concerning in the total of the questionnaire, how AIDS is an ilness, how AIDS can spread, risk factors for AIDS, ways of protection of AIDS. While the highest score about risk factors were received by Anatolian high school students, the highest

score how AIDS is an ilness, how AIDS can spread, ways of protection of AIDS were received by private high school students.
Conclusion: High school students in this region do not have enough knowledge about AIDS. They need education especially on ways of protection and contamination.

Hepatitis

R2300 Frequency of haematogenic contagious infections to blood donors in a general hospital of thoracic diseases in Athens, Greece, 2001–2005

D.K. Kalikatzaros, M.S. Stagas, N.T. Tsagarakis, N.K. Kendroy, I.K. Kamatsos, E.A. Anastasakoy, S.M. Mayrea, E.H. Hristaki (Athens, GR)

Objectives: The purpose of our study is to see the frequency of haematogenic contagious infections of hepatitis B, hepatitis C, Human Immunodeficiency Virus (HIV) and HTLV among blood donors of our hospital during the period 1/1/2001 and 1/12/2005.
Materials: Positive results of laboratory examination of 18658 blood donors for hepatitis B, hepatitis C, HIV and HTLV was recorded. The recording was made by sorting the results per year. All blood donors came to our hospital to donate blood during that period of time and were Greeks and non-native.
Methods: The detection of HBsAg, anti-HCV, and HIV was made with the microsomic immunoenzymic assay method MEIA-AxSYM of ABBOTT and the detection of HTLV antibodies was made with the photometric method MUREX of ABBOTT.
In the case of positive HCV confirmation was made with the RIBA method at the Hygienist School of Athens, while positive HIV samples were reexamined with Western Blot.
Results: From the total of the blood donors tested we had the results shown in the table. Regarding the HIV after re-examining with the Western Blot method only one sample was found positive. Of all blood donors, positive for an infection 76.9% were Greeks and 23.1% were foreigners. 0.005% was positive for HIV (after re-examination), 0.34% for hepatitisB, 0.2% for hepatitis C and 0.15% for HTLV.

Blood donors	Positive	HIV	HbsAg	HCV	HTLV
2001	14 (0.37%)	4	3	2	5
2002	49 (1.3%)	2	24	11	12
2003	31 (0.87%)	1	10	9	11
2004	24 (0.63%)	3	13	8	0
2005	27 (0.72%)	4	14	9	0

Conclusions: The frequency of those infectious diseases among the blood donors is at a low and satisfactory level. Nevertheless we must be very strict and thorough in choosing blood donors regardless of their nationality.

R2301 Genotype of hepatitis C virus in Libya

M. Dow, A. Alamari, A. Elahwel, A. Abdulla, S. Albagar, A. Ranganathan (Sirte, LY)

Objective: Information about the incidence of genotypes in hepatitis C virus (HCV) infected patients in Libya is limited. Aim of the study to investigate the incidence of hepatitis C virus (HCV) genotypes in Sirte, Libya.
Method: Sera were obtained from 78 (48 males and 30 females) Libyan patients who were anti-HCV positive by ELISA (Enzyme-Linked Immunosorbent Assay). The studied patients ranged in age from 20 to 80 years old. HCV RNA was genotyped by PCR (Cobas Amplicor Roche, Switzerland).
Results: In this study, half of the 78 patients (54%) were infected with genotype 4. Genotype 1 was the second most dominant genotype with a prevalence of 11.5%. Genotypes 2 and 3–6 were found in 6.4 and 7.6% of the patients, respectively.
Conclusion: in this study, most of the Libyan isolates were genotype 4. The higher presence of genotype 4 in the Libya may indicate a larger immigration from neighbouring country (Egypt) and Central African countries, where genotype 4 is predominant. Few of the Libyan isolates were closely related to the European ones where the majority of patients are infected with genotype 1, 2 or 3.

R2302 Hepatitis C virus genotypes distribution in infected patients

A. Suarez, M. Alvarez, C. Sanchez, J. Picazo (Madrid, ES)

Objectives: Hepatitis C Virus (HCV) is a frequent cause of chronic liver disease, and clinical features of HCV infection as well as responsiveness to treatment appear to vary with different genotypes, so the aim of this study was to evaluate the genotype distribution and identify its frequency when a Human Immunodeficiency Virus HIV infection is associated.
Methods: A total of 2176 patients infected by HCV were tested for genotype, 503 of them were co-infected with HIV.
HCV genotyping was carried out in all patients using a reverse transcription-nested polymerase chain reaction (PCR) technique, and a reverse hybridisation system of the amplified product (Inno-Lipa. Innogenetics) The protocol was performed and interpreted according with the recommendations of the manufacturer.
Clinical diagnoses were confirmed by histopatological analysis.
Results: The patients had a mean age of 53.4±10.6 years (range 25–82) and 65% were males, (co-infected patients: mean age of 36.3±6.1 years, and 84% males). HCV type 1 was detected in 1612 patients, in particular 1127 cases showed subtype 1b, type 2 in 42 cases, type 3 in 311 cases, type 4 in 176, type 5 in 8 cases, only one patient with type 6 and eight patients were infected with more than one genotype. Among the 503 HIV-co-infected patients (23.1%), the distribution of HCV genotypes was as follows: type 1 in 292 (19.3% of genotype 1b), 25.3% genotype 3, 14.9% genotype 4, 0.2% genotype 2 and none genotype 5 or 6. Five patients had mixed genotypes.
All patients suffering from hepatocellular carcinoma were infected by genotype 1b and 73% of the alcoholic cirrhosis patients. Genotype 1b was higher in non-co-infected patients (61.6% versus 19.3%), while genotypes 1a, 3 and 4 were more frequently in HIV-co-infected patients (all differences were statistically significant $p < 0.001$). However there were not significant differences between genders or among risk factors in the distribution of HCV genotypes among the HIV co-infected patients.
Conclusion: A high prevalence of HIV and HCV infection has been found in our results.
A strong association between genotype 1b and the development of a severe liver disease were detected.
The genotypes vary distribution depends on the co-infection with HIV, and that knowledge would be useful to the prognosis and treatment of the HCV infection.

R2303 Detection of total anti-hepatitis A antibodies by the Immulite® systems compared to Abbott HAVAB® EIA

P. Sen, F. Bronstein, C. Cervantes, D. Sustarsic (Los Angeles, US)

Objective: Hepatitis A virus (HAV) infection is diagnosed by the detection of antibodies to HAV in serum or plasma. This study compares the performance of IMMULITE® 2000 and IMMULITE® 2500 to ABBOTT HAVAB® EIA in detecting total anti-HAV (IgM and IgG).
Methods: IMMULITE® 2000 and IMMULITE® 2500 total anti-HAV assays are solid phase sequential competitive chemiluminescent immunometric assay. In the first cycle, the patient sample is incubated with HAV antigen reagent and bead coated with polyclonal anti-HAV antibody for 30 minutes. After a spin-wash step, alkaline phosphatase-labeled polyclonal anti-HAV antibody reagent is added to the patient

sample and incubated for an additional 30 minutes. After another spin-wash step, a chemiluminescent substrate is added, and the signal is generated in proportion to the bound enzyme.
Results: Approximately 257 samples were assayed on IMMULITE® 2000 and IMMULITE® 2500. On IMMULITE® 2000, 170 of the 257 were reactive for total anti-HAV antibodies, which correlated to ABBOTT HAVAB® EIA results; 70 were non-reactive on both methods. Of 15 discordant samples, 7 were resolved by ABBOTT AxSYM. Overall agreement, relative sensitivity and specificity after resolution were 96.1%, 100% and 89.7%, respectively. On IMMULITE® 2500, 170 of the 257 were reactive for total anti-HAV antibodies, which agreed with ABBOTT HAVAB® EIA results; 65 were non-reactive on both methods. Of 22 discordant samples, 7 were resolved by ABBOTT AxSYM. Overall agreement, relative sensitivity and specificity after resolution were 94.2%, 100% and 85.5%, respectively. Precision studies were also performed on 3 lots of IMMULITE® 2000 and 1 lot of IMMULITE® 2500 to assess lot-to-lot consistency. Nine samples, including controls, ranging from non-reactive to reactive were assayed for a 20-day period following the CLSI [NCCLS] document EP5-A, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guidelines. Overall total precision ranged from 6.3% to 14.7% on IMMULITE® 2000 and 4.1% to 5.7% on IMMULITE® 2500.
Conclusion: Results of this study show that the IMMULITE® systems of total anti-HAV assays are comparable to ABBOTT HAVAB® EIA/AxSYM assay for the serological detection of total antibodies to hepatitis A virus.

R2304 Detection of anti-hepatitis A IgM antibodies by the Immulite® systems compared to Abbott HAVAB-M® EIA

P. Sen, F. Bronstein, C. Cervantes, D. Sustarsic (Los Angeles, US)

Objectives: The presence of anti-HAV IgM in serum or plasma indicates an acute or early convalescent stage of infection to Hepatitis A virus (HAV). This study compares the performance of IMMULITE® 2000 and IMMULITE® 2500 to ABBOTT HAVAB®-M EIA in detecting IgM antibodies to HAV.
Methods: IMMULITE® 2000 and IMMULITE® 2500 anti-HAV IgM assays are solid phase 2-site sequential chemiluminescent immunometric assay. In the first cycle, the pre-diluted patient sample is incubated with protein buffer and bead coated with monoclonal murine anti-human IgM antibody for 30 minutes. After a spin-wash step, HAV antigen reagent and alkaline phosphatase-labeled polyclonal anti-human IgM antibody reagent is added to the patient sample and incubated for another 30 minutes. After another spin-wash step, a chemiluminescent substrate is added, and the signal is generated in proportion to the bound enzyme.
Results: Approximately 257 samples were assayed on both IMMULITE® 2000 and IMMULITE® 2500. On IMMULITE® 2000, 37 of the 257 were reactive for anti-HAV IgM antibodies, which agreed with ABBOTT HAVAB®-M EIA results; 207 were non-reactive on both systems. Of 13 discordant samples, 8 were resolved by ABBOTT AxSYM. Overall agreement, relative sensitivity and specificity after resolution were 98.1%, 97.3% and 98.6%, respectively. On IMMULITE® 2500, 36 of the 257 were reactive for anti-HAV IgM antibodies, which correlated to ABBOTT HAVAB®-M EIA results; 208 were non-reactive on both systems. Of 13 discordant samples, 8 were resolved by ABBOTT AxSYM system. Overall agreement, relative sensitivity and specificity after resolution were 98.8%, 97.3% and 99.1%, respectively. Precision studies were also performed on 3 lots of IMMULITE® 2000 and 1 lot of IMMULITE® 2500 to assess lot-to-lot consistency. Ten samples, including controls, ranging from non-reactive to reactive were assayed for a 20-day period following the CLSI [NCCLS] document EP5-A, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guidelines. The overall total precision ranged from 7.8% to 14.7% on IMMULITE® 2000 and from 5.6% to 9.5% on IMMULITE® 2500.
Conclusion: Results of this study show that the IMMULITE® systems of anti-HAV IgM assays are comparable to ABBOTT HAVAB®-M EIA/AxSYM assay for the serological detection of IgM antibodies to hepatitis A virus.

R2305 Phylogenetic, clinical and virological features of hepatitis B virus among Iranian HBsAg carriers

G. Bahramali, M. Sadeghi-Zadeh, S. Amini-Bavil-Olyaee, S. Alavian, A. Behzad-Behbahani, B. Rabbani, E. Sabouri, F. Barkhordari, R. Bagheri, F. Mahboudi (Tehran, Shiraz, IR)

Objective: Hepatitis B virus (HBV) infection is one of the major epidemiological problems around the world. Our previous reports indicated that genotype D of HBV was dominant one in Iranian HBV carriers. Herein, we have investigated a large number of cases in different stages of the disease to show phylogenetic, virological and clinical features of HBV in Iranian infected patients.
Methods: One hundred and twenty HBsAg carriers were selected from patients attending the hepatitis clinic centres. Liver function, clinical and serological status, viral load and HBV genome variability were analysed for each patient. The patients were categorised into four groups;18.8% inactive carrier patients, 45.4% chronic hepatitis B infected cases, 25.4% patients with cirrhosis and 10.9% with hepatocellular carcinoma (HCC).HBV genotype (HBsAg), BCP/Core and YMDD motif sequences were successfully amplified for Sixty two samples and sequenced in both direction.
Results: Phylogenetic analysis revealed genotype D and sub-genotype D1 with a high bootstrap value. Subtypes ayw2 and ayw3 were detected in 98% and 2% of samples, respectively. All patients with HCC and 64.5% of total patients were shown to be HBeAg-negative. The highest ALT rate was detected in patients with cirrhosis and HCC. There was no significant correlation between HBeAg negativity and ALT value in the studied cases. The prevalence of the precore mutation was 78% (70% in chronic infection patients, 20% in HCC patients and 10% in cirrhosis patients). The BCP double mutation was detected in 48% of the patients as well. Twenty-five percent of the patients who had lamivudine therapy showed mutation in the YMDD motif (50% among HCC patients, 33% in hepatitis B chronic carriers and 16.6% in cirrhotic subjects). No HBsAg escape mutant was detected in studied cases.
Conclusion: This work confirmed the earlier studies that the genotype D of HBV was the predominant one in Iranian patients. The rate of precore and BCP mutation was high among the studied cases and closely associated with HCC. The FLLA mutation was also found in patients who suffered from HCC. Nevertheless, no association was observed between ALT and HBeAg status. Analysis of these mutants may prove useful for clinical evaluation and choice of therapy.

R2306 Epidemiological and clinical features of a hepatitis A outbreak among adults in Albania

A. Harxhi, K. Pano, D. Kraja, S. Bino, E. Kakarriqi, D. Ulqinaku (Tirana, AL)

Objectives: To present the epidemiological and clinical features of acute hepatitis A outbreak among adults in a inner city in Albania.
Methods: During July and August 2006 an outbreak of acute hepatitis A occurred in the city of Tirana, Albania. Overall there were 420 cases of acute hepatitis A reported to Public Health Authorities in the city of Tirana. More than 65% of reported cases were above 15 years of age. 126 adult cases hospitalised and diagnosed with acute viral hepatitis A based on WHO case definition were included in the study.
Results: Around 90% of hospitalised cases (113) were between 15 to 25 years of age. There was an equal number of male and female cases. All the cases were inner city inhabitants, living in two to three close neighbourhoods. These young people belonged to families with a high socio0economic level. Epidemiological investigation revealed only drinking water as a common-source of the outbreak. Regarding clinical features, main prodromal symptoms included fatigue, malaise, anorexia, nausea and vomiting. Fever was present in 28% of the cases while arthralgias in 32% of the cases. Splenomegali was present in 42%

of patients. Jaundice was not present in 6 cases (5%). Mean serum bilirubin level was 6 mg/dl, ranging from 0.3 to 19 mg/dl. Peak AST and ALT levels were 2500 and 3500 UI respectively. No fulminant form of acute hepatitis was observed. The serological diagnosis was based on the detection of IgM anti HAV in 49 cases (39%).
Conclusion: The pattern of this common-source water-associated outbreak indicates that Albania is still considered a country of moderate/intermediate endemicity of HAV infection.

R2307 Significant reduce in hepatitis B prevalence among blood donors admitted to an Ahwaz blood transfusion service due to educational and vaccination programmes

M. Jalali Far, J. Torabi Zadeh Maatoghi, S.M. Sajadi, M. Paridar, A. Ghasem Zadeh, A. Nasimian (Ahwaz, Tehran, IR)

Although blood transfusions save the life of many patients, they carry a risk of blood-borne diseases such as viral hepatitis. Hepatitis B is one of the major diseases of mankind and is a serious global public health problem. Of the 2 billion people who have been infected with the hepatitis B virus (HBV), more than 350 million have chronic (lifelong) infections. These chronically infected persons are at high risk of death from cirrhosis of the liver and liver cancer, diseases that kill about one million persons each year. Because of the great number of blood dependent patients such as thalassaemia and other haemoglobinopathies patients, determination of HBV prevalence helps us to recognize the risk factors and increase the blood safety.
In this cross-sectional descriptive survey, we studied 39,032 blood donors admitted to Ahwaz blood transfusion service and related blood centre between 17 Apr 2005 and 19 Feb 2006 by using non-random simple sampling. 36,252 (92.9%) male and 2,780 (7.1%) female; 8,817 (22.6%) single and 30,215 (77.4%) married; 2,351 (6%) was illiterate and has the lowest rate and blood donors with diploma and below the diploma consisted the majority of blood donors: 28,948 (74.2%). Age was 17–65 years, mean: 33.03, the largest age group was 15,792 (40.5%) donors under 29 years old and the smallest age group was donors over 49 years with 4.9%]. For all of them we did HBs-Ag based on Enzyme immuno assay (Dade Behring kit).
We found 0.8% (324/39,022) of blood donors were HBs-Ag reactive. We found significant difference between HBV infection and job, marital status, age group, educational level and blood group, but gender was not significant. The HBV prevalence was higher in blood donors who were over 49 years groups, farmer, illiterate and married.
Our study showed a significant reduction in hepatitis B infection among blood donors in comparison with previous studies in this region and other regions in Iran. This is because of the wide spreading educational and vaccination programmes and the rigid rules in donor selection in Ahwaz. Further educational programmes should focus especially on married and illiterate blood donors. HBc-Ab screening for detection all previous and window period infection are recommended.

R2308 Prevalence of hepatitis C virus genotypes in chronic infected patients of haemodialysis department

M. Kozachenko, A. Kachanka, B. Samoliuk, I. Karpov (Minsk, BY)

Objectives: At hemodialysis centres patients are well known to be infected with virus hepatitis not only because of haemotransfusion but also because of asepsis rules violation while haemodialysis. This category of patients faces a greater risk of being infected with years. Hepatitis C virus (HCV) genotypes have been associated with different treatment response.
The aim of this study was to determine the prevalence of HCV genotypes, serum HBV-DNA and HCV-RNA levels and severity of liver disease in chronic infected patients.
Methods: 126 patients, who were diagnosed with chronic kidney deficiency and assigned to programmed haemodialysis, were examined with the help of ELISA-method. If parenteral hepatitis markers were defined, molecular-biological methods were employed in order to continue the examination of the patients, in particular a real time mode PCR method was adhered to.
Results: 89 patients (70.6%) got positive results for HBV- and HCV-infection. On grouping aetiologic indicators we registered the following: HBV-infection – 42 patients (47.2%), HCV-infection – 27 patients (30.3%), mixed infection HBV+HCV – 20 patients (22.5%). PCR DNA was positive in 47 cases, PCR RNA was positive in 33 cases. After the classification of genes of the patients infected with HCV-infection 13 patients were registered to have 1b genotype, 6 patients 3a, 4 patients 2a, 1 patient 1a, and 1 patient 1b/3a.
Conclusion: Thus, hemodialysis department patients take a high risk of being infected with various kinds of parenteral virus hepatitis. In order to prevent the infection it is necessary not only to observe the rules of aseptic procedures and hemodialysis very carefully but also to conduct a preliminary vaccination of the patients who need a programmed hemodialysis against hepatitis B. Further working out and development of monitoring and specific treatment protocol seems to be very promising.

Virology (non-HIV/non-hepatitis)

R2309 Bladder carcinogenesis via viral infection

H. Badawi, H. Ahmed, A. Ismail, N. El-Khafif, A. Helmy, A. Badawy, S. Mansy, M. Saber (Imbaba, EG)

Objectives: To investigate the role of human papillomaviruses (HPV16, 18), cytomegalovirus (CMV), Epstein-Barr virus (EBV), and herpes simplex virus (HSV-2) in the etiopathogenesis of cancer bladder, using polymerase chain reaction (PCR) and Electron Microscopic Studies (EMS). It is also a trial to highlight the possible correlation of such infections with the apoptosis of buffy coat cells and serologic responses using Enzyme Linked Immunosorbent Assay (ELISA).
Methods: This study was conducted on 80 patients, Urosurgery Department, Theodor Bilharz Research Institute (TBRI), who were identified as three groups, cancer bladder (group I, 20), cystitis (group II, 20), cancer bladder with cystitis (group III, 20), and a fourth group of 20 normal healthy subjects as controls. They were all subjected to the following: Detection of viral genomic sequences by PCR and EMS on bladder tissue biopsies, buffy coat cells, serum and urine samples, serological detection by ELISA of HPV IgG using Baculovirus recombinant HPV virus-like particles and IgG & IgM of EBV, HSV-2 and CMV and detection of CMV antigen (pp65) in PMNL by monoclonal antibody.
Results: Among all Patients 56.6% were virally infected with different viruses under the study. HPV16 was detected in 53% cases, HPV18 in 24%, CMV in 48%, EBV in 41% and HSV-2 in 48%. Infection with multiple viruses (74%) was significantly associated with either cancer or cystitis than single infection (26%) ($P < 0.01$). EMS of the examined cases showed remarkable apoptotic changes in lymphocytes and neutrophils of 75% of either cystitis or cancer cases and were absolutely associated with virally infected cases. IgG antibodies to HPV16 VLPs were detected in 66.7% and 11.4% of HPV16 DNA positive and negative patients respectively and in none of healthy controls.
Conclusion: Our study confirms significant association of mixed viral infection with bladder cancer in Egyptian patients which suggests the interesting hypothesis of a viral synergistic action in bladder carcinogenesis. The sensitivity and accuracy of PCR could be increased by adding EMS. PCR on serum or urine samples proved to be non-sensitive. ELISA for detection of anti-HPV16 VLPs could be used in conjunction with HPV DNA detection techniques for accurate clinical diagnosis and epidemiological studies. Detailed investigations on the different apoptotic pathways in general and on the viral manipulations of these pathways are necessary so as to open up new strategies for therapy.

Mycobacterial infections

R2310 Determination of the cut-off value of pleural fluid CA-125 in patients with pleural effusion, to identify the underlying cause (TB or malignancy)

S. Shokouhi, M. Samanabadi, L. Gachkar (Tehran, IR)

Background: Primary and secondary infections and malignancies are inflammatory causes of fluid accumulation in pleural space. TB is one of infective causes of pleural effusion and is similar to malignancies because of its subacute and chronic process, although their management is extremely different.
CA-125 is a glycoprotein tumour marker with molecular weight of 200 kD, which is found on the surface of ovarian and some normal and inflammatory cells. In both malignancy and tuberculosis, this tumour marker increases in serum and consequently it increases in pleural fluid of these cases.
Objective: to evaluate and compare CA-125 tumour marker in pleural effusion resulted from malignancies and tuberculosis.
Study design: 27 patients affected by tuberculosis (18 men & 9 women), with the mean (±SD) age of 37/3 13/9 and 23 patients affected by malignant tumour (16 men & 7 women) with the mean (SD) age of 57/9 17/7 were evaluated during 2004–2005; In malignant cases, diagnosis was done through pathologic inspection of biopsy samples and cytology of pleural fluid. For recognition of tuberculosis, culture & smear of sputum or gastric lavage, biopsy of pleura and pleural fluid PCR methods were used. Pleural fluid sample were gathered and by CLIA method, the amount of their CA-125 were measured. The cut-off value of CA-125 was obtained from a ROC curve.
Results: The mean (±SD) level of CA-125 in pleural fluid was 159.1±214, and 2149.2±4513.6 U/mL in tuberculosis and malignancies, respectively; which showed a statistically significant difference between these two groups ($p < 0.01$).
Conclusion: we may use CA-125 marker level in pleural effusion as a diagnostic index for differentiation of TB and malignancy induced pleural effusions.

R2311 Prevalence of tuberculin reactivity among healthcare workers from an Iranian hospital

M. Zangeneh, S. Soleimany Amiry, M. Jamshidi Makani, S. Davar Siadat, S. Haghighi, M. Yarjanli (Tehran, Bandar-Abas, IR)

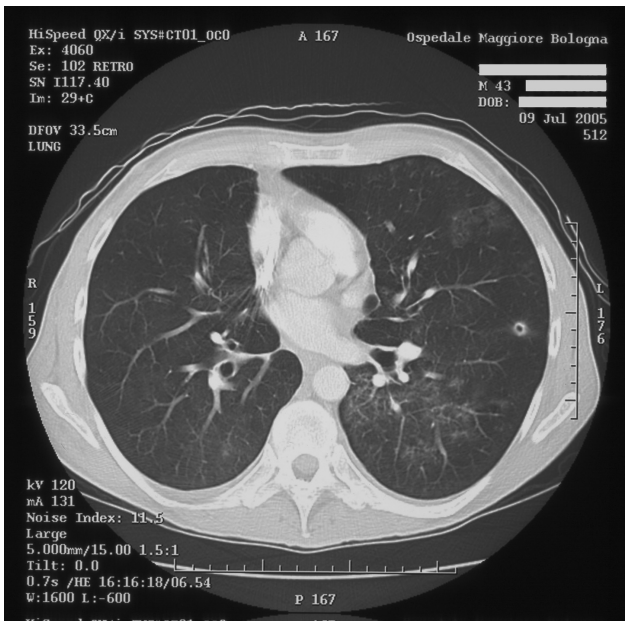
Objective: According to WHO, the prevalence of tuberculosis (TB) is increasing worldwide and healthcare workers are at special risk for tuberculosis. The prevalence of infection is directly proportional to the duration of employment in the hospital. Lifetime risk of developing active tuberculosis is about 10%. Tuberculin test (PPD) is a reliable method for monitoring the exposure of HCWs to TB. According to CDC reports HCWs need to have pre-employment health screening, a two-step PPD test and at least annually. The two-step PPD test conform a negative result. The prevalence of tuberculosis infection in HCWs is unknown in Iran. In this study we wanted to determine the prevalence of tuberculin reactivity among HCWs in our hospital.
Method: To evaluate the frequency of tuberculosis infection in our hospital, we performed a prevalence study of tuberculin reactivity among 170asymptomatic HCWs in the hospital. Trained nurses inoculated purified protein derivative (PPD) using the mantoux technique in tow step. All HCWs tested completed a questionnaire about demographic data, previous TB infection, previous contact with active tuberculosis patients, BCG vaccine scar, and immunosuppressive conditions.
Results: 150 (86/5%) returned for test reading including 82 female, 68 male with mean age of 32/3 years, 69 nurses, 37 service workers, 5 office workers, 2 lab workers, 24 operation room workers, 1 physician, 12 secretaries. Overall, in first step 41 (27/3%) of 150 HCWs was PPD positive, in second step from 50 HCWs 15 (10%) were PPD positive. The rate of PPD reactivity among person with a history of BCG vaccination was the same (P = 0/309). The rate of reactivity was related to hospital setting (P = 0/045). Induration among reactors was 93 (62%) 0–5mm, 32 (21/3%) 6–10mm, 9 (6%) 11–14mm, 15 (10%) >15 mm.. In this study PPD reactivity was same as general population.
Conclutions: The low prevalence of tuberculin reactivity in this study may reflect low rate of mycobacterium tuberculosis infection & disease in the community. In addition, in this hospital most of HCWs are young with low history of work in the hospital.

Infection in the immunocompromised host & transplant recipients

R2312 Nocardiosis as an unexpected complication of end-stage, untreated HIV disease

R. Manfredi (Bologna, IT)

Introduction: AIDS presenters are increasing worldwide, due to HIV infection lasting undiagnosed-neglected for many years.
Case report: A 43-year-old drug-alcohol abuser was recently diagnosed with HBV-alcohol-related liver cirrhosis, and an advanced, untreated HIV infection with a CD4+ count of 14 cells/μL, and an initial AIDS-dementia complex. Hospitalised owing to >15% weight loss, fever, cough with blood emission, and multiple pulmonary infiltrates, when undergoing a HRCT a consolidated 4–5 diameter lesion involving the apical-dorsal left upper lung lobe was accompanied by multiple subpleuric lesion, with excavations at right lower lobe. Either tubercular, bacterial, other opportunistic infections, or a malignancy, were suspected. While waiting for microscopy, cytology, and culture examinations of respiratory secretions-BAL, based on blood cultures which yelded a multi-sensitive *S. epidermidis* strain, a broad-spectrum therapy including cefazolin, and later cefriaxone and fluconazole was attempted. After the microscopic-culture isolation of Nocardia asteroides (testing susceptible in vitro to co-amoxiclav, chloraphenicol, cotrimoxazole and gentamycin), treatment was adjusted to include cotrimoxazole, and a triple HAART was conducted for 12 days, until an overwhelming anaemia-leukopenia needed RBC transfusion and G-CSF administration, followed by a modified antimicrobial therapy (imipenen-amikacin), in the suspect of cotrimoxazole intolerance. A slowly progressive clinical ameliorement occurred, as confirmed by repeated X-ray-HRCT controls, associated with a partial immune recovery obtained thanks to HAART.



Discussion: In patients with recently diagnosed HIV disease and a deep immunodeficiency, the differential diagnosis of multiple pulmonary infiltrates with tendency to excavation includes tuberculosis-atypical

mycobacteriosis, but also bacterial infection and malignancies. In our case, the diagnostic difficulties were complicated by the emerging of cotrimoxazole intolerance which prompted to a severe anaemia-leukopenia, so that a second-line therapy for nocardiosis was performed favourably. Notwithstanding Nocardia spp. infects immunosuppressed hosts, however nocardiosis remains very infrequent in advanced HIV disease, when only sparse pulmonary, cerebral and skin localisations were anecdotally reported. Our case underlines that opportunism may go beyond the most usual disorders, and its treatment may be conducted effectively with second-choice agents.

R2313 Alert pathogens in blood of haematological patients from 2004 through 2005

H. Marchel, M. Wróblewska, R. Kamola, A. Majewska, M. Luczak (Warsaw, PL)

Methods: Analysis comprised the following alert pathogens: MRSA, VRE, ESBL-positive and carbapenem-resistant Gram negative rods. Only non-repetitive strains were included in the study. Blood cultures were tested using a BacT/Alert system (bioMérieux). Identification of the isolates was done with API or VITEK tests (bioMérieux). Antimicrobial susceptibility testing was assessed with a disk-diffusion technique (according to the NCCLS recommendations) or VITEK system (bioMérieux), as well as E-tests (AB Biodisk). ESBL production was evaluated using a double-disk technique, according to Jarlier. Resistance to vancomycin was detected on BHI agar plates with vancomycin 6 μg/mL (Emapol).
Results: In the analysed period there were 6863 patients hospitalised in 2004 and 7331 in 2005. In total, 6099 culture bottles were examined in 2004, yielding 647 (10.61%) positive and 5452 (89.39%) negative results. In 2005, 6350 bottles were cultured, with 638 (10.05%) positive and 5712 (89.95%) negative results. In 2004, 17 strains of *S. aureus* were cultured (including 4 MR), while in 2005 – 12 strains of *S. aureus* (including 2 MR). In 2004, a number of strains of *Enterococcus* spp. amounted to 15 (including 1 VRE), while in 2005 – 20 (including 4 VRE). In 2004 there were 2/7 ESBL-positive *K. pneumoniae* strains isolated, while in 2005 it increased to 10/28. In the analysed period no ESBL production was detected in cultured *E. coli* strains. In 2004 among carbapenem-resistant strains predominated non-fermenting rods (8 strains of *S. maltophilia*, 4/8 strains of *P. aeruginosa*, and 1/7 strains of *K. pneumoniae*). In 2005, all carbapenem-resistant strains represented non-fermenters (7 strains of *S. maltophilia*, 7/15 strains of *P. aeruginosa*, and 5/10 strains of *A. baumannii*).
Conclusion:

1. In the analysed period there was a decrease in the number of isolated *S. aureus* strains and an increase in isolation of *Enterococcus* spp., enteric rods of the Enterobacteriaceae family and non-fermenting rods.
2. A decrease in percentage of MRSA strains was recorded, with simultaneous increase in frequency of isolation of VRE and carbapenem-resistant Gram-negative rods.
3. Frequency of ESBL-positive phenotype among the Enterobacteriaceae family isolates was variable, with highest rates detected in *K. pneumoniae* strains.
4. In this study emergence of carbapenem resistance among enteric rods of the Enterobacteriaceae family was recorded.

R2314 Disseminated infection with *Fusarium solani* in an infant with congenital acute lymphoblastic leukaemia

A. Charissiadou, A. Stathi, A. Velegraki, K. Chryssaki, M. Moschovi, A. Sergounioti, K. Samantas, A. Pangalis (Athens, GR)

Fusarium species are emerging pathogens, with significant morbidity and mortality, in patients with haematologic malignancies. They invade through blood, vessel walls, cause tissue infection and necrosis and disseminate widely.
Case report: A 2-month-old female infant was admitted to the hospital with severe anaemia (Hgb: 5g/dL), high WBC and low platelets, (counts 546,000 and 60,000/mm^3 respectively), hepatosplenomegaly, biochemical picture of liver obstruction and abnormal high level of serum uric acid. Bone marrow aspiration revealed a 99% infiltration with blasts and she was diagnosed as having Acute Lymphoblastic Leukaemia, pre-b (immunophenotype CD34:85%, DR:98%, CD19:98%, CD10:0%, CD20:1%) with central nervous system involvement. She was immediately started induction chemotherapy (New York II protocol). During the neutropenia phase she developed FUO, received Ceftazidime, Amikacin, Vancomycin, Amphotericin B, Fluconazole, Imipenem, entered the ICU and finally she recovered from this infection. She remained neutropenic and two months after her admission, while on chemotherapy and antibiotic prophylaxis, she developed a second febrile episode, accompanied with signs of low respiratory system infection. Meanwhile, diffuse skin lesions appeared, presented as macules with necrotic centre and an ecthyema gangrenosum-like appearance. Rhinitis, stomatitis, high level of CRP and PCT (up to 260 mg/L and 2 ng/mL respectively) were also present. Skin biopsy and cultures from blood, Broncho Alveolar Lavage and skin lesions, revealed a rapid growing fungus with branched hyaline hyphae which was identified as *Fusarium solani*, with conventional and molecular methods. *F. solani* was also found directly in samples from blood, CSF and BAL by multiplex PCR. Galactomannan antigen was detected in serum by ELISA method. The initial antifungal treatment with Amphotericin B and Fluconazole changed. Fluconazole was stopped and Voriconazole, the only antifungal agent in wich the fungus was sensitive (MIC: 0.5 μg/mL, measured by Broth Microdilution method) was added. *F. solani* was never recovered from clinical samples. Remission of the ALL was never achieved although she was administered a second course of chemotherapy. She died four months after her initial admission to the hospital, from her disease and the complications of the treatment.
Conclusion: *Fusarium* an opportunistic fungal agent was recovered from the blood, skin lesions and BAL from an infant with congenital ALL.

Community-acquired infections

R2315 Comparison of macrolides, quinolones, and amoxicillin/clavulanic acid for the treatment of patients with acute bacterial exacerbations of chronic bronchitis: a meta-analysis of randomised controlled trials

M. Falagas, I. Siempos, I. Korbila, K. Manta, G. Dimopoulos (Athens, GR)

Objective: We evaluated the comparative effectiveness and safety of macrolides, quinolones, and amoxicillin/clavulanic acid (A/C) for the treatment of patients with acute bacterial exacerbation of chronic bronchitis (ABECB) by performing a meta-analysis of randomised controlled trials (RCTs).
Methods: PubMed, Current Contents, and the Cochrane Central Register of Controlled Trials were searched to identify relevant RCTs.
Results: 20 RCTs (21 comparisons) that studied 7,730 patients and compared macrolides with quinolones (8 RCTs), A/C with quinolones (4 RCTs), or A/C with macrolides (9 RCTs) were included in our meta-analysis. There was no difference regarding treatment success in intention to treat (ITT) and clinically evaluable (CE) patients between macrolides and quinolones [ITT: odds ratio (OR) = 1.01, 95% confidence intervals (CI) 0.81–1.27; CE: OR= 0.94, 95% CI 0.73–1.21], A/C and

quinolones (CE: OR= 0.86, 95% CI 0.60–1.24), or A/C and macrolides (ITT: OR= 0.99, 95% CI 0.49–2.04; CE: OR= 1.44, 95% CI 0.71–2.94). The treatment success in microbiologically evaluable patients was lower for macrolides compared with quinolones (OR= 0.46, 95% CI 0.32–0.66), but it was similar between A/C and quinolones (OR= 0.83, 95% CI 0.49–1.40), and A/C and macrolides (OR= 1.31, 95% CI 0.57–2.98). There was no difference regarding mortality between macrolides and quinolones. Less quinolone-recipients experienced a recurrence of ABECB after resolution of the initial episode compared with macrolide-recipients during the 26-week period after therapy (data from 2 RCTs). Adverse effects in general were similar between macrolides and quinolones (OR= 1.12, 95% CI 0.96–1.16); however, administration of A/C was associated with more adverse effects (mainly diarrhoea) than quinolones (OR= 1.37, 95% CI 1.04–1.79) or macrolides (OR= 1.72, 95% CI 1.22–2.42).

Conclusion: Overall, macrolides, quinolones, and A/C may be considered equivalent for the treatment of patients with ABECB in terms of short-term effectiveness. However, quinolones are associated with better microbiological success and fewer recurrence of ABECB than macrolides, while A/C with more adverse effects than both comparators.

Antimicrobial clinical trials

R2316 Comparison of cefuroxime and amoxicillin/clavulanate in the treatment of acute sinusitis in a sample of Iranian population

A. Rastegar lari, F. Alinejad, M. Rahmani, H. Mohamadi, H. Mostafavi, G. Farzandy, R. Alaghehbandan (Tehran, IR; St. John's, CA)

Objectives: Acute sinusitis is a common upper respiratory tract infection worldwide, which can be severely complicated if inappropriate treatment is applied. The aim of this study was to assess and compare efficacy of cefuroxime and amoxicillin/clavulanate in the treatment of acute sinusitis in an Iranian sample population.

Methods: A randomised clinical trial study, comparing the efficacy of two oral antibiotics, cefuroxime (Exiroxime®) and amoxicillin/clavulanate in the treatment of acute sinusitis, was conducted in 2005. A total of 99 patients were enrolled in the study. The clinical diagnosis of acute sinusitis was based on association of suborbital pain, purulent rhinorrea and purulent discharge on the middle nasal meatus. All patients were also radiographically examined and their diagnoses were confirmed. Patients were randomly assigned to either receive 10 days of treatment with cefuroxime 250 mg twice daily (n = 57) or receive amoxicillin/clavulanate 500/125 mg three times daily (n = 42). Patients' responses to treatment were assessed during and at the end of the treatment.

Results: A satisfactory clinical outcome (cure or improvement of symptoms) was found in 86% (49/57) and 71.4% (30/42) of the clinically evaluable patients treated with cefuroxime or amoxicillin/clavulanate, respectively ($p > 0.05$).

Conclusion: Findings of this study suggest that cefuroxime (twice daily) is more effective than amoxicillin/clavulanate (three times a day) in the treatment of patients with acute sinusitis. A larger study is needed to elaborate on the findings of this study.

R2317 Doxycycline and streptomycin versus doxycycline alone in the treatment of human brucellosis

E. Martínez Alfaro, P. Geijo, F. Marcos, F. Mateos, J. Blanch, I. Tárraga, C. Rosa, J. Solera (Albacete, Cuenca, Talavera de la Reina, ES)

Background: Tetracyclines are at the present time the antibiotics of choice in human brucellosis. Brucellae is exquisitely susceptible to tetracyclines with MICs (minimum inhibitory concentration) <1 mg/L. Prolongued treatment reduces the rate of relapse.

Objective: We conducted a pilot study comparing treatment with doxycicline 45 days plus streptomycin 14 days versus doxycycline alone 60 days.

Material and Methods: 20 patients were included, all of them >18 years old. Diagnostic criteria were: (1) Isolation of Brucella species in blood cultures, or (2) the finding of a titer >1/160 of standard tube agglutination antibodies. Patients with spondylitis, endocarditis or neurobrucellosis were excluded.

Results: 10 patients were included in the doxycycline-streptomycin group and 10 int the doxycycline alone group. 90% of the patients were male and the mean age was 37 ± 11. 8 patients (44%) had positive blood cultures. Focal forms were arthritis (4 patients), orchitis (3 patients), sacroiliitis (1) and hepatitis (1). The 10 patients in the doxycycline-streptomycin group cured without sequelae. 4 (40%) patients in the doxycycline alone group had relapses of the disease.

Conclusions: The high percentage of relapses in the treatment with doxycycline alone does not allow to indicate this treatment for human brucellosis.

Paediatric infections

R2318 Pyomyositis in children

G. Manoudis, G. Mitsionis, V. Kontogeorgakos, N. Kordalis, I. Sionti, T. Xenakis (Ioannina, GR)

Introduction: Pyomyositis is an acute pyogenic infection of the skeletal muscle. Pyomyositis is common in the tropics but rarely reported in temperate climates.

High fever and painful swelling of the affected limb are the commonest symptoms. Leucocytosis is present with elevated erythrocytes sedimentation rate (ESR) and C-reactive protein (CRP). The most common causative organisms are *Staphylococcus aureus* and *Streptococcus pyogenes*. Magnetic Resonance Imaging (MRI) is the image modality of choice.

Purpose: Diagnosis, clinical course and treatment outcome of children with pyomyositis.

Material and Methods: Between 2002 and 2004 5 children, 2 boys and 3 girls were diagnosed and treated for pyomyositis. The mean age of patients was 7.2 years, ranging from 3 to 14 years. The locations of involvement were gluteus muscles in 3 cases, quadriceps in one case thus in one case there was bifocal involvement of gastrocnemius and biceps muscle. The causative organisms, cultured from blood, were *St. aureus* in 3 cases, *Str. pyogenes* in one case though in one patient no organism could be identified. 4 children had fever (>38.5ºC), elevated ESR and CRP (ESR: 73–110, CRP: 142–410) and raised WBC count (14580–19900). One patient had normal temperature and increased ESR (<55). A history of prior trauma at lower limb was obtained in all patients. MRI plays a significant role in the early recognition and diagnosis of pyomyositis, identifying muscle inflammation and detecting bone involvement. Intravenous antibiotics were administered and were followed by oral agents for an additional period of time. The duration of therapy ranged from 6 to 8 weeks. No surgical intervention was needed. MRI was used in order to evaluate response to the therapy.

Conclusion: Although pyomyositis is a rare disease, it should be considered in the differential diagnosis of acute onset of musculoskeletal pain in children. Early diagnosis and antibiotic treatment are important as abscess formation, which require surgical drainage, sepsis and other major systemic complications can be avoided.

R2319 Invasive infections due to *Haemophilus influenzae* in paediatrics

N. Rivas, G. Yerino, A. Gaiano, A. Medina, A. Mónaco, M. Vázquez, A. Procopio, E.L. López (Buenos Aires, AR)

The invasive infections by *Haemophilus influenzae* (Hi) b has decreased after vaccination. From January 1998 to December 2005 we carried out our study at Hospital de Niños.

Objective: to describe the characteristics of Hi infections in our population. Data were collected from clinical files. Positive cultures for Haemophilus influenzae from sterile places were included. Invasive infections were 78 in 77 patients. Hi type b 20 (26%) and Hi not b 58 (74%). Age:18m (medium) range 8d-216m. Males 53/78 (69%).

Variables	*Haemophilus influenzae*		
	not B, n = 58	B, n = 20	
Complete vaccination	56 (96.5%)	2 (10%)	
Seasonal period			
Atumn–winter	37 (63.7%)	14 (70%)	
Spring–summer	21 (36.2%)	6 (30%)	
Predisposing disease	52 (89.6%)	6 (30%)	p:<0.001
Chronic pneumopathy	21 (36.2%)	4 (20%)	
Immune deficiency	12 (20.6%)	–	
Malnutrition	8 (13.7%)	1 (5%)	
Nephropathies	6 (10.3%)	–	
Cardiopathies	5 (8.6%)	1 (5)	
Clinical forms			
Pneumonia without effusion	33 (56.8%)	10 (50%)	
Pneumonia with effusion	6 (10.3%)	2 (10%)	
Peritonitus	5 (8.6%)	–	
Meningitis	4 (6.8%)	5 (25%)	p = 0.043
Bacteraemic without focus	4 (6.8%)	–	
Cellulitis	3 (5.1%)	1 (5%)	
Septic arthritis	1 (1.7%)	2 (10%)	
Pioventricullitis	1 (1.7%)	–	
Endophthalmitis	1 (1.7%)	–	
R to ampicillina	11 (19%)	12 (60%)	p = 0.001
Sepsis	9 (11.5%)	1 (5%)	p = 0.43
Hospitalisation ⩾14 days	38 (48%)	10 (12.8%)	p = 0.16
Mortality	4 (6.8%)	–	

Conclusion: The infection by Hi type b was observed mainly in children without completed immunisation. Hi b were significantly more resistant to ampicilin than Hi not b. Predisposal conditions, complication and mortality were more prevalent in Hi not b infections.

R2320 Features of clinical course of measles in outbreak

M. Javakhadze, K.H. Baquzanashvili (Tbilisi, GE)

Objectives: Description of the peculiarities of the clinical course of measles during the measles outbreak at the beginning of the XXI century.
Methods: Open type observational-descriptive study.
Results: In 2004, at the beginning of the XXI century, measles outbreak was reported in Georgia. 392 patients with the measles diagnosis were hospitalised during one year whereas there were total 6847 cases of measles registered in Georgia. The cases of the disease were registered during the whole year and reached its peak in the months of May and June. It was adults (aged 15–30) who got mainly infected, and the most vulnerable age group turned out t be the citizens of 20–29. The hospitalised patients with the measles diagnosis had the following disease symptoms: prolonged prodromal period with severe course of disease and with complications. The main reason for hospitalisation of the patients was the severe course of the disease and complications. Most (50%) of the patients had bronchopneumonia, 2% of the patients had bronchopneumonia with encephalitis and 1% had meningoencephalitis. 3% of the patients had gingivitis and 6% had ulcerous damage of oral cavity. There was one fatal case due to measles of the patient infected with tuberculosis registered.
Conclusion: The reason for the measles epidemics in Georgia was the violation of anti-measles vaccination and absence of re-vaccination (the vaccination schedule did not envisage any re-vaccination up to 2000).
The severe course of the disease, according to statistically confident factor analysis, was simultaneously influenced by the following factors: age, vaccination status, duration of prodromal period, duration of temperature, duration and intensity of rush.

R2321 Herpes simplex encephalitis

S. Rafiee Tabatabaei, A. Karimi, M. Hadipour Jahromy (Tehran, IR)

Objective: To evaluate clinical presentation, radiological and cerebrospinal fluid findings and outcome of children with Herpes Simplex encephalitis (HSE).
Methods: The chart of all the patients (n = 18), who were admitted to the Mofid Children Hospital from 2001–2005, were retrospectively reviewed. All of them were included in this study.
The variables were identified (including demographic data, signs and symptoms at presentation and laboratory investigations such as analysis including, PCR and neuroimaging). Diagnosis of HSE was classified into definite, probable and possible.
Results: Clinical findings included fever, loss of consciousness, seizure, confusion, focal neurologic signs, vomiting, headache and behavioral changes. CSF, analysed in all patients, was abnormal in fifteen patients. From eleven persons underwent CT, nine patients had abnormal changes and from 8 patients who had MRI, 4 of them had hyper intensity in temporal lobe of their brain. Definite HSE was diagnosed in seven; Probable HSE in ten, and one patient had Possible HSE.
Conclusion: HSE remains a serious illness with high mortality and morbidity despite appropriate antiviral therapy. Adequate antiviral therapy should be started as soon as possible. CSF examination, Computerised tomography and magnetic resonance imaging may provide some clues of HSE, but negative findings should not deter or delay treatment in suspicious cases.

R2322 *Chryseobacterium meningosepticum* septicaemia in a N.I.C.U.

M. Simou, T. Lazaraki, G. Liosis, I. Zorou, G. Saroglou, H. Kada (Athens, GR)

Chryseobacterium meningosepticum is a Gram negative rod widely distributed in nature. It is highly pathogenic for newborn infants usually causing nosocomial infections, with high mortality rate and serious neurological sequelae in surviving patients.
C. meningosepticum is waterborn, ubiquitously found in the hospital enviroment and has been associated with infection via contaminated water. This organism is usually resistant to antibiotics commonly used for empirical treatment.
In January 2006, a two day old fullterm neonate was transferred from the postnatal ward to Neonatal Intention Care Unit of our Hospital with early onset neonatal sepsis.
Blood culture performed in Bact/Alert 3D-60 system (boiMérieux) was positive. The microorganism grew on blood agar and chocolate agar but not on MacConkey agar. The identification was performed in VITEK automated system (bioMérieux). *Chryseobacterium meningosepticum* was identified. The strain was sensitive only to Ciprofloxacine, Trimethoprim/Sulfa and Vancomycin and resistant to all other classes of antibiotics.
Surface areas, sinks and taps in the nursery were sampled and cultured with no detection of *C. meningosepticum*.
Despite proper antibiotic administration the neonate expired within 48 hours.
Two weeks later the same strain of *C. meningosepticum* was isolated from a sample from a sink tap.
Control of infection in the N.I.C.U. requires an understanding of the reservoirs and modes of transmission of nosocomial microorganisms. Samples for bacteriological examination should be taken periodically to determine the degree of contamination in the environment and equipment.
The possible role of hospital staff, new borne babies and their mothers in the transmission of *Chryseobacterium meningosepticum* should be considered together with the measures taken to reduce environmental contamination.

R2323 Serological investigation of bacterial and viral pathogens in children

I. Varzakakos, K. Papavasileiou, T. Panagea, G. Oikonomopoulos, H. Papavasileiou, A. Makri, A. Voyatzi (Athens, GR)

Purpose: The aim of this study was to investigate the incidence of community acquired lower respiratory tract ilness (LRTi), caused by *M. pneumoniae*, *C. pneumoniae*, Influenza A and B in hospitalised children, seasonal distribution and association with age and sex.
Material and Methods: A total of 150 patients from 2 months to14 years old with symptoms and signs compatible with CAP were enrolled during a 2 year period (2004–2006). Thorax radiography and paired sera were obtained from each patient and the course of illness was monitored uniformly. Specific IgG and IgM antibodies for *C. pneumoniae*, *M. pneumoniae*, influenza A and B were determined by indirect immunofluorescence (Vircell, Spain) and Immunocard based IgM EIA (Meridian France) for the detection of antimycoplasma IgM antibodies. Diagnosis was established by the presence of specific IgM antibodies or by detection of fourfold increase of IgG antibody titers.
Results: Specific IgG and IgM positive antibodies titer was found for *Mycoplasma pneumoniae* in 28 patients (18.6%), *C. pneumoniae* in 3 (2%), influenza A in 9 (6%) and influenza B in 12 cases (8%). In one case coexistence of *M. pneumoniae* and *C. pneumoniae* was observed (0.7%) and in 2 cases coinfection of *M. pneumoniae* and influenza B was demonstrated (1.4%). No significant difference between sexes was found. The average age for *M. pneumoniae* infections was 7–12 years and for *C. pneumoniae* was 2–5 years. Significantly higher frequency of *M. pneumoniae* infections was recorded during the two last winter months and the springtime while *C. pneumoniae* infection mostly occurs in autumn.
Conclusion: The results show the high incidence of *M. pneumoniae* in the aetiology of lower respiratory tract infections and the relatively low frequency of *C. pneumoniae* infections in Greek hospitalised children, especially in children older than 7 years. The knowledge of the prevalence of bacterial or viral respiratory pathogens or coexistence of them helps to choose the appropriate antibiotic treatment in children with CAP. Special antibiotic treatment with macrolides is advisable to be induced for better treatment of *C. pneumoniae* infection.

R2324 Risk factors in the development of latent forms of intrauterine infections in neonates with perinatal pathologies

N. Gorovenko, Z. Rossokha, S. Podolskaya (Kiev, UA)

Objectives: The prevalence of intrauterine infections in neonates is mainly studied in case of evident clinical presentations. But latent forms of these infections are also dangerous because of reducing the resistance of neonates. There is a risk of intrauterine infections manifest forms in neonates with severe perinatal pathologies. The purpose of the research was studied the prevalence of intrauterine infections latent forms in neonates and past histories analysis.
Methods: The prevalence of causative agents of intrauterine infections was investigated in neonates with severe perinatal pathologies and in neonates of the comparative group (clinical healthy neonates). Also was analysed retrospectively risk factors. The neonate's blood analysis with the purpose of detecting DNA of *Toxoplasma gondii*, *Chlamydia trachomatis*, *Mycoplasma hominis*, Herpes virus I/II types, Cytomegalovirus was tested by means of polymerase chain reaction (PCR). Differences in these groups were assessed by the Pearson chi-square analyses.
Results: There were detected latent forms of intrauterine infections in 74 of 117 (63.24%) neonates with perinatal pathologies as compared to the comparative group the neonates where the frequency of latent forms accounted for 9 of 70 neonates (12.85%). When analysing perinatal risk factors it has been detected a significantly increase (16.68, $P < 0.001$) in the rate of complications in obstetric and gynecologic past history of infected patient's mothers (56.75%; 42 of 74 mothers) as compared to uninfected patient's mothers (16.27%; 7 of 43 mothers). Thus, the presence of such complications in the past history in the patient's mothers is a risk factor for the development causing intrauterine infections latent forms.
Conclusion: The neonates with perinatal pathologies whose mothers had complications in the past history are to be studied with the purpose of detecting latent forms and preventing clinical presentations.

R2325 A child with endocarditis due to *Enterobacter cloacae* responded to long-term antibiotic treatment without cardiac surgery

R.R. Klont, J.G. Noordzij, P. Bot, T. Schülin-Casonato, A. Warris (Nijmegen, NL)

Background: Infective native valve endocarditis due to Gram-negative bacilli is rare. *Enterobacter* endocarditis occurs primarily in patients with foreign bodies, mechanical and prosthetic valves, intravenous drug abuse and cardiac surgery.
Case report: A seven-year-old boy with insulin dependent diabetes mellitus presented with fever. One year earlier the central venous catheter (CVC) which was required for the administration of insulin, was replaced because of a catheter related infection. His medical history was otherwise uneventful. Blood cultures taken at admission were positive for *E. cloacae*. Treatment was started with trimethoprim-sulphamethoxazole (SXT; 30/6 mg/kg/d) intravenously. Ciprofloxacin (CIP; 16 mg/kg/d) and cefotaxime (100 mg/kg/d) were added after 24 h when repeated blood cultures were again positive for *E. cloacae* and the patient began to deteriorate. The next day the CVC was removed without improvement of the patient's condition. Culture of the tip of the CVC was negative. Due to persistent positive blood cultures antibiotic treatment was switched to CIP and tobramycin (7 mg/kg/d) on day 6. The patient developed a septic shock and was transferred to the intensive care. On day 11, antibiotic treatment was changed to meropenem (MEM; 60 mg/kg/d). New blood cultures were positive for *E. cloacae* although susceptible in vitro for SXT, CIP, MEM, and ceftriaxone with a minimal inhibitory concentration (MIC) of $\leqslant 0.5$ mg/L, $\leqslant 0.125$ mg/L, $\leqslant 1$ mg/L, and $\leqslant 1$ mg/L respectively. Gentamicin (7 mg/kg/d) was added 4 days later because of persistent positive blood cultures and high fever. Ultrasound of the abdomen and left subclavian vein, cerebral CT scan, and PET scan showed no abnormalities. CT scan of the abdomen showed an enlarged spleen and lymphadenopathy but no abscess. Transoesophageal echocardiography revealed an abnormal structure in the right atrium (not detected by earlier transthoracic echocardiography), consistent with endocarditis. The dosage of MEM was increased (120 mg/kg/d) and the patient became afebrile 5 days later. The last positive blood culture was obtained 7 days after initiation of this antibiotic regimen and 27 days after his first presentation.
Conclusion: *E. cloacae* endocarditis is a rare complication of catheter usage. The optimal treatment of *E. cloacae* endocarditis is not known. Our patient recovered from this serious infection by antibiotic therapy and is currently doing well being on antibiotics without the need for cardiac surgery.

R2326 Brodies abscess: a diagnostic dilemma

A. Pillai, S. Thomas, N. Walker (Glasgow, Manchester, UK)

Introduction: Brodie's abscess is a subacute osteomyelitis which presents a diagnostic difficulty, as characteristic signs and symptoms of the acute form of the disease are absent. It has an insidious onset, mild symptoms and lacks a systemic reaction. Unlike other abscesses, markers of infection are rarely raised making diagnosis difficult. In children and young adults Brodie's abscess can mimic various benign and malignant conditions including Ewing's and osteogenic sarcoma.
We report a case of Brodie's Abscess in a 16 year old where aspiration to rule out infection was delayed due to possible pitfalls of seeding an undiagnosed neoplasm. Urgent and appropriate imaging was necessary. We highlight a diagnostic dilemma for the microbiologist where input comes only upon definitive tissue diagnosis following which aggressive

treatment with prolonged antimicrobial therapy and close microbiology support is needed.
Case report: A previously healthy 16 year old presented to the orthopaedic clinic with a 4 week history of upper left leg pain. There was associated night pain but no weight loss, systemic symptoms, history of trauma or raised infection markers. On examination there was localised tenderness to the upper third of the tibia. Initial plain X-Ray demonstrated a lytic abnormality suggestive of chronic infective process, but a neoplasm couldn't be ruled out. An urgent CT demonstrated a single lesion of the tibia with bony destruction and either free fluid or solid tissue with sclerotic margins. It was unclear whether an area of bone within the lower margin represented a sequestrum or bony nidus. At this point exclusion of malignancy was essential prior to aspiration of the lesion.
The patient was referred to an orthopaedic oncologist and an MRI was arranged. The MRI report was consistent with infection. The patient was taken to theatre for urgent deroofing, curatage and cavity washout. 50ml of thick purulent material was evacuated from the cavity under pressure which grew *S. aureus* sensitive to Flucloxacillin. Bone histology confirmed sclerotic, inflamed bone consistent with Brodie's abscess.
Discussion: In children, differentiation of Brodie's abscess from possible neoplasm requires clinical suspicion and careful radiological imaging. Once diagnosed, immediate input and ongoing discussion with microbiology is essential. Effective treatment requires prolonged, monitored antimicrobial therapy. A multidisciplinary team approach is essential.

R2327 Urinary tract infection in asymptomatic jaundiced newborns

Z. Mosayebi, A. Movahhedian (Kashan, IR)

Objectives: The incidence of neonatal urinary tract infection (UTI) varies from 0.1% to 1% of all infants. Previous studies have noted that jaundice may be one of the first signs of bacterial infection in infants. The aim of this study was to evaluate the incidence of UTI in asymptomatic jaundiced newborns admitted for evaluation and treatment of indirect hyperbilirubinaemia in Beheshti Hospital (Kashan-Iran) during 15 months period.
Methods: We prospectively investigated 377 asymptomatic jaundiced newborns during the study period.A bilirubin work up as well as urinanalysis and urine culture by bag were performed for all patients. If the urine culture was positive, a suprapubic aspiration were done. Newborns with positive urine culture by suprapubic aspiration results were accepted as having UTI.
Results: Of 377 newborns with indirect hyperbilirubinaemia UTI was diagnosed in 13 cases (3.4%). Isolated organisms included *Escherichia coli* (70%), *Klebsiella pneumoniae* (23%) and *Staphylococcus epidermidis* (7%). UTI was more common in male and term newborns.
The mean serum bilirubin level of neonates with UTI was 19.4 mg/dl (SD = 3.2). Eight newborns with UTI had bilirubin level greater than 20 mg/dl.
Patient with the onset of jaundice after 7 days of age had a higher incidence of UTI.
Conclusion: UTI can be associated with neonatal indirect hyperbilirubinaemia. We recommend that urine culture be included as part of the evaluation in asymptomatic jaundiced newborns.

R2328 Isolation of group B *Streptococcus* from blood cultures of newborns at a large Brazilian hospital

G. Duboc-almeida, M. Buratinni, R. Teberges, F. Rossi (Sao Paulo, BR)

Background: Group B *Streptococcus* (GBS) remains an important cause of serious neonatal infection. The majority of infections in newborns occur within the first week of life (early-onset disease). Approximately, 10–30% of pregnant are asymptomatic GBS carriers representing a risk to newborns. In 2002, the CDC published new guidelines for prevention of GBS perinatal disease which advocates universal screening of all pregnant women between 35–37 weeks of gestation. Despite that, no official programme was available in our hospital until recently.
Objective: To analyse the occurrence of GBS in blood cultures collected from patients from 2000 to 2005 at Hospital das Clínicas, a 2000 bed tertiary hospital, particularly in newborns.
Methods: Blood cultures were processed in a Bactec 9240 and GBS were identified according to conventional methods and using a latex agglutination test.
Results: During the study period, 93 patients with positive blood culture for GBS were identified. Of these, 27 (29%) were children, 22 were newborns. The median age of the newborns at index blood culture was 2.5 ± 8.7 days. 6 patients were male. In 72% of the cases, the episode was classified as early-onset sepsis.
Conclusions: These data demonstrated the occurrence of GBS as a cause of neonatal sepsis in our hospital. The high percentage of early onset sepsis emphasizes the need to implement the CDC recommendations for the prevention of perinatal group B streptococcal disease.

Vaccines

R2329 Prevenar use in children older than five years

G. Kuli-Lito, G. Sulcebe, Z. Ylli, E. Kallfa (Tirana, AL)

Prevenar (PCV7) is used successfully in infants and children up to 24 months. After its wide use and inclusion in immunisation schedule, a substantial decrease in incidence of bacteraemia is noticed during last five years in countries where the vaccine is routinely applied.
Objectives: To demonstrate the seroconversion rate after the use of Prevenar in children older than 5 years. To evaluate the clinical benefits and vaccine safety in this age group.
Material and Methods: We vaccinated with Prevenar 35 children aged 5–11 years who meet the criteria of high or moderate risk of invasive pneumococcal infection. Antibody titers were determined by the method of ELISA at the day 0 and 4–6 weeks after vaccination. 20 unvaccinated children, with no significant difference in age and gender, were considered as control group. Paired pneumococcal antibody titers were obtained from them at the same time interval. Solicited local and systemic reactions were recorded in subjects up to 2 weeks after vaccine administration. The follow up period was one year.
Results: A satisfying seroconversion is registered in children 4–6 weeks after vaccination. The geometric mean of pneumococcal antibody values at day 0 and 4–6 weeks after, were respectively 46.78 (R: 11.8–260) and 150.16 (R: 36.4–451). The difference between two values resulted statistically significant ($p < 0.002$). The same calculations were done for pneumococcal antibodies values even for the patients of control group. The mean geometric values at first and second measurement were respectively 20.67 (R: 14.6–168) and 29.81 (R: 16.8–183), $p > 0.05$. The patients had a clinical improvement after vaccine application. No important respiratory tract diseases or invasive infections were seen during the follow up period. No vaccine related serious adverse events were reported for vaccine group. The overall rate of systemic reactions (high fever) was 2.86%.
Conclusions: PCV7 is immunogenic even when administrated to children older than 5 years old. No important side effects are reported after vaccine use.

R2330 A serological survey of measles among a group of adults receiving measles, rubella booster mass vaccination in Iran, 2003. A preliminary report

A. Emami Naeini, M. Rostami (Isfahan, IR)

Objective: Measles immunisation in Iran is an expanded programme. Since 1973 to December 2003, all infants received live attenuated measles vaccine (A.I.K strain, Razi institute, Iran) at 9th and 15th months of life. Before 2003, we had confronted outbreaks of measles in previously vaccinated adults from all over the country. Epidemiological serologic studies revealed a low (40.7%) positive antibody titer in some

vaccinated individuals, implying that the majority of vaccinated subjects were susceptible to measles. In December 2003 Ministry of health of Iran performed one of the biggest vaccination campaign against measles and rubella for all 5 –25 year old persons. Around one-half of the Iranian population received the measles (Edmonston strain) and Rubella (RA 27/3 strain) vaccines at the same time. The aim of this preliminary study is to find out seroprevalence of measles antibody titer in a group of subjects who received the infancy and booster dose of MR vaccine.
Methods: We conducted this cross-sectional survey on subjects referring to a university clinic for marriage consultation. The subjects100 (50 male and 50 female) aged 19to28. All had received their routine vaccines against measles during their infancy, had no physician diagnosed measles and revaccinated in December 2003. We considered Immune Status Ratio (ISR) of $\leqslant 0.90$ as negative, 0.91–1.09 as intermediate, $\geqslant 1.10$ as positive. The IgG was measured by using ELISA method. Data were analysed by Chi-Square test, and one –way analysis of variance using SSPS software version12.
Results: ninety-six (96%), three (3%) and one (1%) of the subjects had positive, negative and intermediate antibody titers, respectively. The mean serum titer in female was 1.92IU/μL and in male 1.95.
Conclusion: In our study 96 (96%) of subjects (49 male, 47 female) had positive antibody titer, meaning that there has been a good vaccine induced protection for the majority of vaccinated subjects and it shows our mass vaccination dramatically increased the number of seropositive persons and there is a significant differences between pre and post vaccination ($P < 0.0001$). No statistically significant correlation was found between antibody titer and age ($P = 0.06$). The correlation between antibody titer and sex also did not turn to be statistically significant ($P = 0.05$). After 2003 our measles vaccination schedule changed to MMR (Measles, Mumps, Rubella) vaccine at 15 months of age and a second booster dose at 4–6 years old children.

Internet and electronic resources

R2331 Choleracafe: an e-learning instrument for professionals involved in international health promotion in the fields of communicable disease prevention, surveillance and treatment

F. Riccardo, L. Pacifici, E. Scaroni, A. De Rosa, L. Nardi, G. Russo, G. Ceccarelli, V. Vullo (Rome, IT)

Objective: The response of the international community to a disaster pivots on the need for cooperation among different countries, organisations and professional figures. Epidemics are a likely consequence of the poor hygienic conditions in IDP (internally displaced people) settlements in particular during late and post emergency phases.
Our objective is to construct a website with a double aim. On one hand to provide updated news, manuals and links for humanitarian operators on the field, promoting information sharing to achieve common quality standards for assistance worldwide. On the other hand, through the use of a forum, to provide a space of discussion and alert from the field in matters related to novel outbreaks and evolving humanitarian crisis.
Methods: Professional figures such as psychologists and international law and geo-policy experts have worked together with hygienists and infectious disease physicians with the aim of providing a comprehensive and colloquial approach to the issues raised in epidemics and humanitarian crisis in particular in developing countries. The effort has produced a website with a phpnews data insertion, a multiple page forum and several specific thematic areas.
Results: The website, www.choleracafe.com, is a pilot programme approved of by the University of Rome "La Sapienza" department of Infectious and Tropical Diseases. It is composed of sections dedicated to: Infectious diseases, vaccination and prophylaxis, minimum standards, epidemiological surveillance, psychological support, legal issues and human rights, aero transport of highly contagious diseases and provides useful links regarding country information and international organisations. Moreover a manual called "Basic International emergency setting field manual" has been compiled by the working group and is freely downloadable from the website. This instrument acts as a digest of the current standards in topics such as disaster management, psychological support, water and sanitation, reporting, evaluation, media and communication.
Conclusions: E-learning has developed in recent years to become a major source of information that is easily accessible, specific and universally accepted. Cholera Cafe' is a website, forum and news resource for Professionals involved in International Health Promotion in the field of Communicable disease prevention, surveillance and treatment that has moved beyond organisation-specific websites.