Abstracts accepted for publication only

Pathogenesis

R2243 Temporal and geographical analysis of clones involved in the increase in serogroup 1 pneumococci

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Introduction: The national reference centre receives all pneumococci isolated from invasive infections from more than 100 of 182 laboratories in Belgium. A marked increase in serogroup 1 (SG1) isolates was observed from 2003 onwards. Overall prevalence of SG1 increased from 8.2% (1998–2002) to 13.6% (2003–2006). In 2006, 58.7% of blood isolates in the age group 5–10 years belonged to SG1 and 34.3% of the isolates in age group 10–19 years. Since 2003 macrolide and tetracycline resistance has appeared in SG1 isolates.
Methods: For the period 1998–2002 and 2003–2006, we received 6622 and 7021 isolates respectively. Of these, 200 randomly chosen serogroup 1 isolates were analysed via multi-locus sequence typing (MLST) as described by Enright & Spratt (Microbiol. 1998; 144: 3049). The MLST data were also compared to available data on strain characteristics (antibiogram) and patient characteristics (geography, age, clinical data on diagnosis and treatment) to investigate trends in clonal selection and spread.
Results: The increase in SG1 was primarily linked to the increase in two sequence types (ST350 and ST304). A clear temporal-geographical pattern of spread could be established for these sequence types. Resistance development was limited to ST350 and was not observed in the other sequence types. A temporal-geographical pattern could also be established for resistance development and spread.
Conclusion: The increase in SG1 and the increased resistance in SG1 are linked to the spread of specific sequence types throughout Belgium. The difference in resistance development between the two dominant sequence types cannot be explained by the available data and needs to be further examined.

R2244 Prevalence of virulence genes (ctxA, tcpA, zot and ace) among *Vibrio cholerae* isolated from outbreaks during 2005 in Iran by multiplex PCR method

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Objective: The virulence of *V. cholerae* is related on presence of ctxAB and tcpA genes. These genes are alleged to be exclusively associated with clinical strains of O1 and O139 serogroups. In the present study, we examined the presence of virulence genes ctxA, tcpA, zot and ace among El Tor variants.
Methods: Thirty nine El Tor variants were obtained from outbreaks during 2005 in Iran. After detection of isolates by biochemical methods, and serotyping, chromosomal DNA was extracted by standard phenol/chloroform method. The oligonucleotide primers for each of the selected virulence-associated factors were designed based on available GenBank sequences for *V. cholerae* O1 E1 Tor for all genes. A multiplex PCR assay was performed and the PCR product was run and visualised in 1.5% agarose gels stained with ethidium bromide.
Results: The PCR analysis of the strains revealed that 100, 100, 82.1 and 82.1% carried the ctxA, zot, tcpA and ace genes, respectively. Isolates with tcpA gene negative had ace gene positive and isolates with ace gene negative had tcpA positive, so there was not any strain with tcpA gene negative and ace gene negative and vice versa.
Conclusion: the results of present stydy reveald that 100% strains carry ctxA and zot genes, but just 82.1% strains had tcpA and ace genes.

R2245 Comparison of Austrian, Hungarian and Macedonian meticillin-resistant and meticillin-sensitive *Staphylococcus aureus* strains in relation to accessory gene regulator type

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Objectives: The purpose of this study was to examine the presence of genes responsible for different accessory gene regulator (agr) types in *Staphylococcus aureus* strains isolated from invasive clinical samples, and compare according to country origin and meticillin resistance.
Methods: Classical microbiological methods were used for the phenotypical identification of the strains. By detecting the genes encoding thermostabile endonuclease (nucA) and 23S rRNA the genetic confirmation of the species of the strains was done. According to the presence of the mecA gene 48 Austrian (AT) meticillin-resistant *S. aureus* (MRSA) and 128 meticillin-sensitive *S. aureus* (MSSA), 110 Hungarian (HU)MRSA and 94 MSSA, 73 Macedonian (MK) MRSA and 29 MSSA strains were studied. The genes responsible for four different agr types were detected by multiplex polymerase chain reaction. The pulsed-field gel electrophoresis of the strains was also done to confirm the heterogenic feature of the bacterial groups.
Results: The agr1 gene was detected in 46%, 47% and 93% in AT, HU and MK MSSA strains, while in 40%, 20% and 97% in AT, HU and MK MRSA ones, respectively. Both the AT and HU MSSA strains harboured the agr2 gene in 34%, while the MK MSSA isolates carried this gene only in 7%. The agr2 gene carriage of the AT, HU and MK MRSA strains was 58%, 74% and 3%, respectively. The agr3 gene could be detected in AT, HU MSSA and MRSA strains in 15%, 13% and in 2%, 6%, respectively. The agr4 gene was harboured by only AT and HU MSSA strains in 5% and 6%.
Conclusion: The presence of the agr1 gene was significantly characteristic for the MK MSSA and MRSA strains compared to the AT and HU ones ($p < 0.001$). The frequency of the agr2 gene characterised significantly the HU MRSA strains ($p < 0.01$). Comparing all the MSSA strains with MRSA ones the prevalence of agr2 gene was significant for the MRSA isolates ($p < 0.001$). Our results may indicate alterations in the regulation of virulence factor genes.
Supported by ÖAD and TéT grant No.: A-19/02 and the Hungarian National Scientific Research Fund (OTKA) T046186.

R2246 Correlation of adherence and haemagglutination abilities of *Staphylococcus saprophyticus* strains

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Objectives: *Staphylococcus saprophyticus* is an important pathogen in human urinary tract infections, especially in young, sexually active women. Bacterial adherence to the urinary mucosa is commonly regarded as the crucial initial step in the pathogenesis of urinary tract infections. The aim of this study was to compare the adherence ability of *S. saprophyticus* strains to the Buffalo green monkey kidney (BGMK) cell line with the hemagglutination (HA) ability of those strains, isolated from the middle clean-catch urine samples of 60 young women in Zagreb Institute of Public Health.
Methods: The strains were divided in two groups: thirty studied strains were isolated in great number ($\geqslant 10^4$ CFU/ml) and pure culture from women with urinary tract infection, and another thirty strains were isolated in small number ($\leqslant 10^3$ CFU/ml) and mixed culture from urine of women without urinary tract infection. Examination of the HA activities with sheep erythrocytes was performed in microtiter plates, and for

adhesion assay the continuous epithelioid BGMK cell line was used. Proportions were compared by the chi^2-test and for correlation of the hemagglutination and adherence abilities the Spearman's coefficient of correlation was used.
Results: The results of the study showed that the strains isolated in great number and pure culture from the urine of women with urinary tract infections mostly expressed hemagglutinin (73% of the strains), had statistically significant ($p < 0.01$) greater HA capability and adhered good or excellent to the BGMK cell line in comparison with the strains isolated in small number and mixed culture which mostly did not express the hemagglutinin (only 36% of the strains), had statistically significant ($p < 0.01$) smaller HA capability, and did not adhered or adhered poorly to the used cell line. In both studied groups of strains the statistically significant correlation (rs=0.556, $p = 0.001$ and rs=0.543, $p = 0.002$, respectively) was observed between the adherence and the HA ability of strains, as well as the greater adherence capability of strains with higher HA titers.
Conclusions: This in vitro study showed statistically significant difference in virulence and pathogenicity between the two studied groups of strains. As the HA ability correlated with the adherence ability of strains, the simple and easy to perform HA test could be used in clinical laboratory for estimation of adherence ability and virulence potential of *S. saprophyticus* strains.

R2247 In vitro investigation of the intercellular cross-talk between opportunistic bacteria and eukaryotic cells

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Objective: to investigate the potential functional responses of eukariotic mammalian cells to the exposure to *Pseudomonas* (*P.*) *aeruginosa* and *Staphylococcus* (*S.*) *aureus*.
Material and Methods: HeLa cells were grown in 6 multiwell plates in DMEM supplemented with 2% foetal calf serum and 24 h later standardised microbial suspensions were added over the monolayer in an interior filter well. The logarithmic phase microbial cultures supernatants (MLCA) as such/MLCA inactivated at 100 C degrees for 20 minutes/ MLCA diluted 1:2 in fresh culture medium were added directly over the HeLa cells monolayer. After 24 h of incubation, cells from the monolayer were harvested, stained with annexin V and propidium iodide and analysed by flow cytometry. The extra-cellular level of IL-1, IL-2, II-4, IL-6, IL-8, IL-10, IL-12, TNF-alpha and IFN-gamma produced by HeLa cells in the presence of bacterial cultures supernatants was assessed by ELISA.
Results: Our studies have indicated that HeLa cells respond to bacterial cultures and their respective supernatants in specific ways. The necrosis rate of HeLa cells at 24 h was significantly increased in the presence of *P. aeruginosa* culture supernatants, comparatively with the whole bacterial culture, demonstrating that the bacterial soluble factors could accelerate the killing of host cells, probably by inducing a strong pro-inflammatory effect demonstrated by the stimulation of IL-6 production correlated with the inhibition of IL-10. The killing activity was preserved after thermic inactivation. In exchange, in the case of *S. aureus*, the death rate of HeLa cells was much more increased in the presence of whole bacterial cultures, comparatively with the respective supernatants. The decreased necrosis rate is sustained also by the anti-inflammatory effect produced by the soluble staphylococcal antigens and demonstrated by the increased level of IL-10. However, in both bacterial infection models, the HeLa cells necrosis rate was significantly reduced after decreasing the soluble mediators concentrations by adding fresh medium.
Conclusion: Our results demonstrate that eukaryotic cells are able to detect bacterial chemical signals and to respond appropriately. The understanding of the mechanism of crosstalk between the bacterial products and host cells may contribute to the elaboration of an efficient strategy for controlling the severity of tissue damages and autoimmune diseases, associated or consecutive to bacterial infections.

R2248 Relationship between *Helicobacter pylori* babA and babB status with other virulence factors and their correlation with disease outcome in Iran

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Objectives: *Helicobacter pylori* (Hp) is an important human pathogen associated with gastrointestinal diseases such as gastritis, peptic ulcer disease (PUD) and gastric cancer. A number of pathogenic factors have been described for this bacterium, and some of them have been proposed as predictive markers of the clinical outcomes. However, with the exception of the cag and vacA status, there is no global consensus regarding the role of the other introduced virulence factors. Therefore, the aim of this study was to investigate the status of Hp strains regarding the Hp babA and babB (blood group antigen binding) adhesins and assess any existing association between the status of these genes and clinical outcomes in Iranian patients.
Methods: Hp virulence genes were amplified by means of gene-specific polymerase chain reaction in clinical isolates of Hp from 72 Iranian patients (16 GC, 12 PUD, 44 NUD patients).
Results: Eleven categories of genotypes were identified with the following frequencies: Group 1: A+B+/cagA+/s1 (38.0%); Group 2: A+B+/cag+/s2 (2.8%); Group 3: A+B+/cag–/s1 (1.4%); Group 4: A+B–/cag+/s1 (7%); Group 5: A+B–/cag+/s2 (2.8%); Group 6: A–B+/ cag+/s1 (22.5%); Group 7: A–B+/cag+/s2 (14.1%); Group 8: A–B+/ cag–/s1 (1.4%); Group 9: A–B+/cag–/s2(4.2%); Group 10: A–B–/ cag+/s 1(2.8%); Group 11: A–B–/cag+/s2 (2.8%). Frequency of Group 1 in GC, NUD and PUD patients is 75%, 27.3% and 27.3% respectively and Group 7 is prevalent in 36.4% of PUD patients. babA prevalence in GC, NUD and PUD patients was 75%, 47.7% and 33.3% and babB prevalence was 93.8%, 77.3% and 91.7% respectively. 75% of Hp isolates were babA/babB double positive in GC patients.
Conclusion: Frequency of babB is higher among Iranian GC and PUD cases but babA frequency is limited to GC cases. Infecting strains from Groups 1 and 7 were found to be associated with GC and PUD respectively. Co-presence of cagA, s1vacA, babA and babB may work synergistically in exacerbating the resulting inflammation which may create grounds for development of intestinal metaplasia and eventually GC, whereas co-existence of cagA, s2vacA, babB may cause susceptibility to PUD development. In GC cases presence of babA/babB double positive are significantly prevalent ($p = 0.044$). Application of this analysis on additional samples will allow for a more concrete conclusion.

R2249 Association of *Helicobacter pylori* and chronic idiopathic urticaria

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Objective: The aim of this work was determined the relation of the infection with *H. pylori* and the dermatological disease Chronic Idiopathic Urticaria (CIU).
Methods: We studied 60 patients with CIU diagnosis at baseline and 6 weeks after therapeutic intervention to eradicate *H. pylori* in the infected patients. *H. pylori* status was established by UBT-C14 and we performed Elisa's to determinate the antibodies response to CagA (Cytotoxin-associated gene product) and *H. pylori* Whole-Cell (WC) antigens.
Results: 44 patients were UBT-positive and 16 UBT-negative; no statistically significant differences were founded in the mean age, gender, duration of disease, and symptoms between both groups. No correlation between *H. pylori* density (UBT DPM mean value) and symptoms severity was found. Serology correlated in 88.6% of UBT-positive patients and 5 (11.4%) of them were false negative for *H. pylori* WC ELISA but 4 (80%) of those patients were positive for CagA ELISA. In contrast, only 5 (31.3%) of UBT-negative patients shows concordance in the serology (WC & CagA negative); with 4 (25%) false positive for

H. pylori WC and CagA serology and 9 (43.7%) were false positive for CagA. A decrease in WC OD net values was find in the UBT-positive patients with clinical improvement after *H. pylori* successful eradication (100%) compared with UBT-negative patients ($P < 0.05$), but not for CagA OD net values. Severity of symptoms showed a correlation with increasing OD values of WC and CagA in the UBT-negative group ($P > 0.05$).
Conclusion: *H. pylori* eradication is associated with clinical improvement of CIU. In *H. pylori*-positive patients, CagA serology is more reliable than WC serology. Successful eradication of *H. pylori* correlated with clinical improvement, but not with the severity of the disease. UBT-negative group showed increase in the WC and CagA OD values in relation to severity of the disease. All these findings strengthen the role of *H. pylori* in the maintenance of CIU disease.
Grants: FONACIT S1–2000000408, G-2005000371. LOCTI – IBA-02–2007 /IBA-03–2007. ALUMNWARE – INALUM.

R2250 Investigation of imipenem efficacy against carbapenem-susceptible but heteroresistant *Acinetobacter baumannii* clinical isolates by experimental pneumonia

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Objectives: The clinical consequences of the recently described carbapenem heteroresistance of *Acinetobacter baumannii* remain unknown. The aim of this study was to investigate the efficacy of imipenem treatment against apparently carbapenem-susceptible, heteroresistant *Acinetobacter baumannii* clinical isolates by experimental infection model.
Materials: The study included four previously characterised *A. baumannii* clinical isolates: one apparently carbapenem-susceptible *A. baumannii* exhibiting unstable carbapenem heteroresistance, one with stable heteroresistance, one carbapenem-susceptible without heterogeneity and one carbapenem-resistant. Approximately 107 CFUs of the isolates were inoculated intratracheally to cause experimental pneumonia in Wistar rats. The animals were treated with 30 mg/kg body weight imipenem/cilastatin intraperitoneally q. 6 h for 24 h, sacrificed, the lungs excised and homogenised and cultured with serial dilutions, to estimate the bacterial counts yielded. Equal numbers of animals remained untreated as controls of the experimental infection.
Results: No bacterial growth was observed from the animals that were infected by the two carbapenem-heteroresistant isolates and the susceptible *A. baumannii* and treated with imipenem. The carbapenem-resistant isolate yielded approximately 100 CFU/ml of the lung homogenate after imipenem treatment. All isolates were grown from the lungs of the untreated animals, in numbers ranging from 13 x 103 to 125 x 103 CFUs/ml, assuring the success of the experimental infection.
Conclusions: The results of the present study indicate that the heteroresistant *A. baumannii* isolates tested may be treated with equal success as susceptible ones, with the administration of adequate imipenem dosing regimens.

R2251 *Veillonella parvula* LPS stimulates cytokine production in a TLR4-dependent manner

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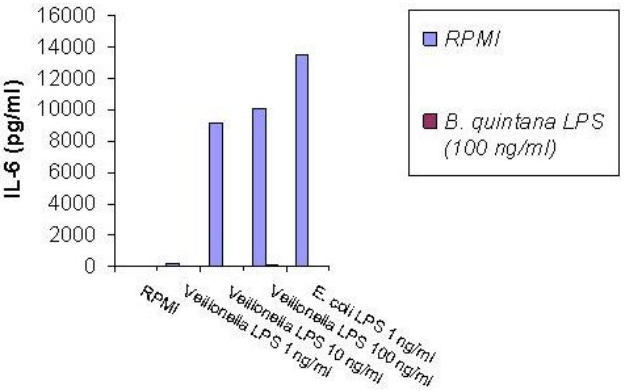
Objectives: *Veillonella parvula* is a Gram-negative coccus present in animal and human mouth and gastrointestinal tract. Oral *V. parvula* is involved in the development of early periodontal disease. Very little is known about cellular and molecular mechanisms responsible for innate immune response following microbial population change inside dental plaque leading to severe periodontitis. Moreover, the interaction between oral *V. parvula* LPS and TLR has not been studied yet. The aim of this study was to investigate the role of TLR4 for the recognition of *Veillonella parvula* LPS in human peripheral blood mononuclear cells.
Methods: Experiments were carried out in peripheral blood mononuclear cells isolated from healthy volunteers. PBMCs were stimulated with increasing concentrations of *V. parvula* LPS, with or without addition of specific TLR4 antagonist. IL-6 concentrations were determined in supernatants by ELISA. Stimulation of PBMCs with *E. coli* LPS was used as control for TLR4 engagement.
Results: *V. parvula* LPS stimulated cytokine release in human PBMC in a dose-dependent manner. Pretreatment of cells with a TLR4 antagonist significantly reduced IL-6 production in PBMC stimulated both with *Veillonella* and *E. coli* LPS. However *V. parvula* LPS was 10 to 100 fold less active than *E. coli* LPS for cytokine induction.



Conclusions: *V. parvula* LPS is an active stimulus of cytokine production, although significantly less active than enterobacteriaceae LPS. Cytokine induction stimulated by *V. parvula* LPS is mediated by TLR4 engagement.

R2252 Inhibitory effect of hyperimmune LPS antiserum in attachment of *E. coli* O157:H7 to HEP-2 cell and murine model

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Objective: Enteroheamorragic *E. coli*(EHEC) strain of serotype O157:H7 is associated with humans disease. The organism have also been associated with a spectrum of sporadic and endemic human illnesses ranging from nonbloody diarrhoea to the haemolytic uremic syndrome. This work was carried out to study of inhibitory effect of hyperimmune LPS antiserum in attachment of bacteria to HEP-2 cell and murine model.
Methods: Bacterial strain was isolated from human. Hyperimmune LPS antiserum was prepared by injecting to rabbit. Different dilution of antiserum prepared. These dilution added to the suspension of bacteria(2×10^8 cfu/ml) for 30 minutes then added to the HEP-2 cell and after 3–6 hours the cells examined with staining and light microscope. For murine model these mixture gave to mice with gavage and the inhibitory attachment of these dilution attained with staining and electron microscope of intestinal tract of mice.
Results: The result showed that 1/1280 dilution can prevent the attachment of the bacterium to HEP-2 cell. For murine model the result showed 1/640 dilution of antiserum was inhibitory.
Discussion: Our results suggest that the prepared antiserum can use to prevention of bacterial attachment and may be useful for control of the disease that cause with this bacteria.

R2253 Risk factors for hospital-acquired *Acinetobacter baumannii* bacteraemia

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Objectives: Acinetobacters are increasingly detected as aetiological agents of hospital acquired infections in seriously ill patients, in recent years. The aim of this study was to evaluate risk factors for *Acinetobacter baumannii* bacteraemia.

Methods: Prospective laboratory-based active surveillance has been performed in our hospital. Hospital acquired *A. baumannii* infections detected between January 2005 and December 2006 were enrolled to the study. The definitions of hospital acquired infections were established by using CDC criteria. Conventional methods were used to identificate *Acinetobacter* isolates. Disc diffusion method was used for antimicrobial susceptibility testing of these microorganisms.
Results: Total of 117 *A. baumannii* infections were included in the study. These infections were detected in 58 male and 59 female patients. The mean age of the patients was 59±19 years. *A. baumannii* was isolated most commonly from the neurosurgery intensive care unit (ICU) (30%), followed by neurology ICU (22%) and internal medicine wards (12%). Of the isolates, 28.5% were isolated from pneumonia, 22% from primary bacteraemia, 21.5% from surgical site infections, 20.4% from urinary tract infections and 7.6% from other infections. Predisposing factors detected in the patients with *A. baumannii* infection were urinary catheter (81.3%), central venous catheter (41%), mechanical ventilation (30%) and tracheotomy (15%). The rate of the patients who had coma was 46%. The mean duration of time between hospitalisation and occurrence of infection was 18±15 (3–108) days. In seventy patients (57%) *A. baumannii* isolates were multi-drug resistant (MDR).
A. baumannii bacteraemia was detected in 51 episodes. Bacteraemia and other infections due to *A. baumannii* were compared. Male gender, insertion of urinary catheter, presence of central venous catheter, presence of peripheric arterial catheter, mechanical ventilation, total parenteral nutrition and being an ICU patient were found as risk factors for *A. baumannii* bacteraemia ($p < 0.05$). On multivariate analyses only mechanical ventilation was found as a risk factor ($p < 0.05$).
Conclusions: In ICUs, patients with mechanical ventilation, urinary catheter, central venous catheter, total parenteral nutrition are at risk for *A. baumannii* bacteraemia. High mortality rate and antibiotic resistance of these microorganisms are still important problems.

Animal models including experimental treatment

R2254 Comparison of *Klebsiella pneumoniae* virulence in mammalian and non-mammalian animal models

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K. pneumoniae is an important cause of nosocomial infections occurring at almost all body sites, but with highest incidence in the urinary and the respiratory tracts.
The main populations at risk are neonates and predisposed and immunocompromised hosts. *K. pneumoniae* typically has both smooth LPS (O antigen) and capsule (K antigen) on its cell surface, and both contribute to the pathogenicity of this species. The aim of this study was to compare using different *K. pneumoniae* well defined mutants lacking only the capsule, the O-antigen LPS, several parts of the LPS core (outer and inner) with or without capsule, several mammalian and non mammalian animal models.
In *K. pneumoniae*, the O-antigen LPS is critical for serum resistance, changes in surface hydrophobicity, adhesion to UEC cells and urinary tract infection and colonisation in rats. The capsule is essential in the *K. pneumoniae* experimental model of pneumonia, while the colonisation of the urinary tract by *K. pneumoniae* requires a complete LPS with O-antigen. The *K. pneumoniae* virulence tested as LD50 in mice inoculated intraperitoneally seems to be dependent on the capsule and the complete LPS (probably full core LPS and O-antigen molecules). The two non mammalian experimental models used, unicellular Dictyostelium ameobae and the insect Drosophila melanogaster, renders similar results to the ones obtained in the experimental model of pneumonia about the important role of the capsule.

R2255 Efficacy of tigecyclin vs. vancomycin in ampicillin- and gentamicin-resistant *E. faecium* experimental endocarditis

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Objectives: Tigecyclin is the first available glycycycline. It is a new drug exhibiting broad-spectrum activity against aerobic and anaerobic bacteria including multidrug resistant Gram-positive and Gram-negative pathogens.
The aim of this study was to evaluate the efficacy of tigecyclin compared to vancomycin in an animal model of experimental endocarditis due to an ampicillin (AMP) & gentamicin (GEN) resistant and tigecyclin glycopeptides susceptible clinical isolate of *Enterococcus faecium*.
Methods: We used a rabbit model of left sided experimental endocarditis. One day after the insertion and fixation of a polyethylene catheter into the left ventricle, animals were inoculated with *E. faecium*. Thirty-six hours later they were randomly assigned to a control group (C) and groups receiving intravenous tigecycline 7 mg/kg/q12 h x 5 days (T) or intravenous vancomycin 50 mg/kg/q12 h x 5 days (V). At the end of treatment all animals were sacrificed. Therapy response was determined by blood cultures and quantitative (log10 cfu/gr of tissue) cultures of aortic valve vegetations, liver, spleen, kidney and brain.
Results: The results obtained of the different therapeutics groups are expressed in the Table.

Groups	Vegetation[a,b]	Liver[a,b]	Kidney[a,b]	Spleen[a,b]	Brain[a,b]
C	7.86±0.91 (0/10)	5±2.42 (0/10)	5.8±2.54 (0/9)	5.23±2.67 (0/9)	4.63±1.85 (0/9)
V	5.64±0.25 (0/8)	3.97±0.18 (0/5)	40.3±0.77 (0/5)	3.63±0.4 (0/5)	3.77±0.56 (0/5)
T	5.34±0.32 (0/8)	4.38±1.32 (2/8)	4.33±1.23 (2/8)	4.48±1.29 (2/8)	3.42±0.97 (2/8)
Mann-Whitney 2-tailed test	C vs V $p < 0.0001$ C vs T $p < 0.0001$ V vs T p = ns	C vs V $p = 0.0007$ C vs T $p = 0.0117$ V vs T p = ns	C vs V $p = 0.012$ C vs T $p = 0.0206$ V vs T p = ns	C vs V $p = 0.004$ V vs T p = ns	C vs V $p = 0.029$ C vs T $p = 0.0111$ V vs T p = ns

[a] Mean±SD $\log_{10}$ CFU/g of tissue.
[b] No. of sterile vegetations/No. of treated animals.
All the blood cultures of the V and T group at the end of treatment were negative.

Conclusions: Although both regiments reduce significantly the bacterial count per gram of vegetations, treatment with V was found to be more potent than QD. Also animals treated with V had lower bacterial counts per gram of liver, brain and spleen tissue.

Biofilms

R2256 Biofilm production by clinical strains of *Acinetobacter baumannii* isolated from patients hospitalised in two tertiary care hospitals

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Objectives: Biofilm poduction by microorganisms is at present increasingly recognised as virulence factor, particularly relevant to opportunistic pathogens, such as *Staphylococcus epidermidis*, *Pseudomonas aeruginosa* and *Candida* spp. However, scarse reports could be found in literature on biofilm formation by clinical strains of *Acinetobacter baumannii* isolated from hospitalised patients. The aim of the study was to evaluate whether factors such as bacterial genotype, site of isolation, resistance to carbapenems and duration of hospitalisation are related to biofilm formation by clinical strains of *A. baumannii*.
Methods: In total 34 randomly selected, clinical strains of *Acinetobacter baumannii*, isolated from patients hospitalised in two tertiary care hospitals (24 strains from hospital A and 10 strains from hospital B), were examined for biofilm formation. They originated from various clinical specimens (blood, catheter tip, urine, wound swabs, bronchial aspirates). Bacteria were cultured according to standard bacteriological techniques. Susceptibility of the isolates to antibacterial agents was tested by a disk-diffusion method (according to CLSI recommendations) and E-tests. The strains were typed retrospectively by RAPD and PFGE

analysis. Bacterial biomass in biofilms was determined quantitatively using a spectrophotometer (O.D. 600 nm).
Results: There was a great variability in the ability of the tested strains to produce a biofilm. Analysis of biofilm formation by the studied clinical strains of *A. baumannii* revealed three groups of strains, regarding their ability to produce a biofilm. However, no relation could be found between the ability of biofilm production and molecular type, carbapenem resistance or site of isolation of the clinical strains of *A. baumannii*. Interestingly, in two cases an increase in biofilm formation could be detected in *A. baumannii* isolates cultured from the same patient upon prolonged hospitalisation.
Conclusion: A better understanding of biofilm formation by *Acinetobacter* and genetic basis for control of this process are required to develop novel strategies for dealing with infections caused by these opportunistic and often multi-drug resistant nosocomial pathogens.

R2257 Antimicrobial efficacy of commercial dentifrices with triclosan or stannous fluoride

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Dentifrices with antimicrobials augment mechanical control of bacteria in oral biofilms and provide important clinical benefits such as reductions in gingivitis.
Objectives: This study assessed antimicrobial effects of commercial dentifrices with triclosan/copolymer (Colgate Total; TCN/copolymer), stannous fluoride (Crest Pro-Health; SnF2) or fluoride (Crest Cavity Protection; F). Antimicrobial effects were determined on a battery of 17 oral and non-oral bacterial strains representative of species in the normal oral cavity, in dental caries, in periodontal disease, and in halitosis. An ex-vivo test examined the effects of dentifrices on supragingival plaque from 10 adults as representative of the effects on bacteria in naturally-occurring biofilms.
Methods: Antimicrobial effects on 17 bacterial strains were determined by broth-based procedures for minimal inhibitory concentrations (MIC's). The lowest concentration of dentifrice inhibiting microbial growth after incubation is reported. For the ex-vivo study, dental plaque was collected from all teeth of the 10 subjects. Plaque samples were dispersed and serial dilutions plated on solid media containing different concentrations of each dentifrice and on control media without dentifrice. Bacterial colonies were enumerated following incubation.
Results: The TCN/copolymer dentifrice demonstrated the lowest MIC's. Substantial inhibition of oral Gram-negative bacteria (such as *Veillonella* sp. and periodontal pathogens including *Aggregatibacter actinomycetemcomitans*, *Eikenella corrodens*, and *Fusobacterium nucleatum*) and Gram-positive bacteria (*Streptococcus* sp.) was observed by the TCN/copolymer dentifrice. Analyses of the ex-vivo studies by 2-way ANOVA with subjects and dentifrice in the model demonstrated significant effects by the dentifrices ($p < 0.0005$). Post-hoc Tukey multiple comparison tests indicate significantly higher inhibition by TCN (>80%) than by either of the other dentifrices ($p < 0.00005$). No significant differences were observed between the SnF2 and F toothpastes ($p = 0.99$).
Conclusions: Results demonstrate significantly higher antimicrobial effects from the TCN/copolymer dentifrice against a panel of bacteria compared to the SnF2 and F dentifrices. TCN/copolymer dentifrice resulted in significantly higher ex-vivo inhibition of dental plaque bacteria in naturally-occurring biofilms compared to the other dentifrices ($p < 0.0005$).

R2258 Antibiofilm activity of bismuth ethandithiol, bismuth dimercaprol, silver nitrate and EDTA on biofilm producing *Pseudomonas aeruginosa*

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Objectives: The purpose of this investigation was to study the effect of four different biocides against biofilm producing *Pseudomonas aeruginosa*.
Methods: Initially, MICs of EDTA, BisEDT (bismuth ethandithiol), BisBAL (bismuth dimercaprol) and silver nitrate on 2 biofilm producing strains of *P. aeruginosa* (strain 214 isolated from clinical specimen and standard strain 8821M) were determined by microdilution method according to CLSI standard. Subsequently, biofilm formation of these strains in the presence of biocides was determined by modified microtitre plate method.
Results: Results showed that in the presence of these biocides, biofilm formation is inhibited on the average up to 80% in MIC concentration. Our finding also showed that BisBAL and BisEDT are more effective in preventing biofilm formation.
We also investigated bactericidal activity of these biocides. The results showed differences in antibiotic susceptibility of planktonic and biofilm cell populations. Complete eradication of Planktonic cells were observed using with 16–32 fold MIC of EDTA, 16 fold MIC of silver nitrate, 64 fold MIC of BisBAL and 16 fold MIC of BisEDT, While biofilm was completely eradicated by treatment with 32–64 fold MIC of EDTA, 512–1024 fold MIC of silver nitrate, 1024–2048 fold MIC of BisBAL, and 2048 fold MIC of BisEDT.
Conclusion: Our finding showed that these biocides were effective in reducing biofilm formation and can be used for inhibiting of biofilm formation in industry by cleaning of surfaces and application of catheter impregnating with biocides in medicine.

R2259 Antibiotic susceptibility of *Pseudomonas aeruginosa* biofilm to ulifloxacin and other fluoroquinolones

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Objectives: *Pseudomonas aeruginosa* is one of the most medically relevant biofilm forming bacterium and an important causative agent of many human infections. Since ulifloxacin, the active form of the prodrug prulifloxacin, is a quinolone with a potent antipseudomonal activity, this study intended to determine in vitro the activity of ulifloxacin compared to other fluoroquinolones (FQs) on *P. aeruginosa* PAO1 biofilm.
Methods: the activity of ulifloxacin, ciprofloxacin, levofloxacin and moxifloxacin on *P. aeruginosa* PAO1 was studied both using a polystyrene 96-wells plate biofilm susceptibility assay (MBEC™; Innovotech) and the standard microdilution method (CLSI). Biofilm was formed on the pegs of a modified microtiter lid culturing *P. aeruginosa* PAO1 in Trypticase Soy Broth for 7–9 h to obtain a bacterial biofilm population from 3×10^6 to 5×10^7 colonies forming units (CFU) /peg. Activity of FQs on biofilm was determined after 24 h treatment with drugs (concentrations range from 1 to 512 mg/L) evaluating the bactericidal activity on biofilm disrupted by sonication and measuring bacterial regrowth both in fresh broth medium and on agar plates by CFU determination.
Results: MICs were obtained following the standard microdilution method using as initial inoculum the broth culture which originated biofilm. MICs expressed in mg/L were 0.25 for ulifloxacin, 0.5 for ciprofloxacin, 2 for levofloxacin and 4 for moxifloxacin.
A complete eradication of sessile cells and the minimum biofilm eradication concentration (MBEC) on 7.5 h biofilm (3×10^6 CFU/peg) was obtained with ulifloxacin at 2 mg/L and at higher concentrations with the other tested FQs.
High killing effect was obtained on older biofilm (9 h and 5×10^7 CFU/peg) at concentrations of about 4 times MIC values for all FQ. In particular a 5 log reduction was obtained at the following concentrations: 1–2 mg/L of ulifloxacin, 2 mg/L of ciprofloxacin, 4–8 mg/L of levofloxacin and 8–16 mg/L of moxifloxacin.
Conclusions: FQ antibiotics exerted a remarkable bactericidal activity against *P. aeruginosa* PAO1 biofilm. In particular ulifloxacin showed the highest activity at concentrations equivalent to those found in plasma and tissues after oral treatment.

R2260 Correlation between antifungal drug susceptibility and morphological changes of *Candida albicans* and *Candida parapsilosis* biofilms

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Objective: While the effects of older antifungals on *Candida* biofilms (BF) are well-documented, less is known about the effects of newer triazoles and echinocandins against *Candida* BF. Our objective was to determine the activities of voriconazole (VRC), posaconazole (PSC), caspofungin (CAS) and anidulafungin (ANID) against *Candida albicans* (CA) and *Candida parapsilosis* (CP) BF, to compare these activities with their planktonic (PL) counterparts and to correlate the susceptibility with the corresponding morphological changes.
Methods: Two CA and 1 CP clinical BF-producing strains were used for susceptibility testing. A Green Fluorescent Protein-tagged CA strain was used for microscopy. BF were grown on silicone elastomer disks in 12-well or 96-well plates at 37ºC under constant shaking for 48–72 h. Mature BF were incubated in RPMI-1640 containing no drug (controls), VRC, PSC, CAS or ANID at two-fold dilutions for 24 h. BF formation and antifungal activities were assessed by XTT assay as changes in BF metabolic activity. BF MICs were determined as minimum antifungal concentrations causing $\geqslant$50% reduction in the BF metabolic activity compared to controls. PL MICs were determined by CLSI M27-A2 method. Confocal laser scanning microscopy (CLSM) was used to evaluate the effects of VRC and ANID on BF morphology. ANOVA with post-test analysis was employed.
Results: PL MICs for CA-M61, CA-GDH2346 and CP-PA/71 were of VRC 0.01, 4, 0.03, of PSC $\leqslant$0.001, $\leqslant$4;0.001, 0.01, of CAS 0.06, 0.03, 0.06 and of ANID 0.003, 0.003, 0.125 mg/l, respectively. By comparison, the BF MICs of VRC were >256, >256, 256, of PSC >64, >64, >256, of CAS 0.06, 0.03, 1 and of ANID 0.5, 0.12, 2. The maximum inhibitory effect of CAS against CA BF was noted for both strains at 2 mg/l (37$\pm$9% vs. control $p < 0.01$) and of ANID at 0.5 or 8 mg/l (48.5$\pm$3.4% or 40.3$\pm$4.1% vs. control, $p < 0.001$) depending on the strain. The corresponding values of both CAS and ANID for CP BF were 16 mg/l (41.2$\pm$9%, $p < 0.001$; 47.6$\pm$7%, $p = 0.01$). CLSM showed that VRC-treated CA BF were morphologically similar to untreated controls; whereas, ANID-treated CA BF were greatly distorted with shorter hyphae, looser network and vacuoles on the cell wall. However, complete sterility of the silicone substrate did not occur.
Conclusions: Echinocandins exhibit relatively low MICs for *Candida* BF; whereas, MICs of triazoles are very high. These findings correlate with the morphological changes observed in drug-treated BF and provide insight into mechanisms underlying BF resistance.

R2261 Biofilm-production is strongly correlated with the presence of accumulation associated protein

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Objectives: The implication of biofilm in chronic bacterial infection in many species has triggered an increasing interest in the characterisation of proteins involved in biofilm formation. The purpose of this study was to investigate the role of polysaccharide intercellular adhesin (PIA), accumulation associated protein (Aap) and biofilm-associated protein (Bap) in biofilm formation among *Staphylococcus hominis*.
Methods: A total of 50 genetically unrelated *Staphylococcus hominis*, recovered from various orthopaedic clinical specimens were tested for biofilm-production by Christensen, and for the production of PIA, AAP and BAP. Staphylococcal cells were suspended in 100 mM PBS containing 5 mM EDTA and 1 mM PMSF. Cells were centrifuged, and suspended in 1 ml digestion buffer [50 mM Tris/HCl pH 7.5, 20 mM MgCl2 and 30% raffinose (Sigma)]. To each sample protease inhibitors (Complete cocktail; Roche), and lysostaphin (Sigma) were added, and the suspension was incubated in a 37ºC water bath for 30 min. After centrifugation at 6000 g, the supernatant fraction, which contained the wall-associated proteins, was analysed by SDS-PAGE (4–20% separation gel). PIA was detected with the PIA-specific antiserum (1:5,000), AAP was detected with AAP-antiserum (1:2,500 dilution) and BAP was detected by anti-Bap serum (1:2,000 dilution). Bound antibodies were visualised by anti-rabbit immunglobulin G-alkaline phosphatase conjugate (1:5000; Sigma).
Results: Eighteen out of fifty *S. hominis* tested (36%) were found to be biofilm-producers. Among them, 88.8%, 50% and 38.8%, respectively were found to produce AAP, PIA and BAP. Strong production was detected in six strains (33.3%) that simultaneously expressed AAP, PIA and BAP. A week biofilm production was associated with the combination of AAP and BAP, or AAP and PIA in two independent strains, while detection alone of PIA was found in two strains.
Conclusions: These results clearly demonstrate that PIA is not the only protein responsible for biofilm-production, but other proteins, such as AAP, also play a crucial role.

R2262 Effect of sitC deletion on planktonic and sessile growth of *Staphylococcus epidermidis*

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Objectives: To investigate the role of the SitABC transporter in bacterial growth and metal cation uptake during in vitro planktonic and sessile growth and in an in vivo rat model for foreign body infections.
Methods: A biofilm forming *S. epidermidis* strain 10b, a PIA negative strain Tü3298 and an existing *S. epidermidis* sitC deletion mutant (Tü3298 sitC::erm) were used. For in vitro studies bacteria were grown overnight in RPMI without Fe (fRPMI) or in iron depleted BHI (fBHI) and re-incubated in different media with iron (25µM FeCl3) and/or manganese (25µM MnCl2). Siderophore activity was measured with a Chrome Azurol S Liquid Assay. For in vivo studies a subcutaneous rat model was used. Catheter fragments, inoculated with a low inoculum of *S. epidermidis*, were implanted subcutaneously in rats as described (Vandecasteele et al. BBRC. 2002; 291: 528). Gene expression was quantified by real-time PCR.
Results: *S. epidermidis* Tü3298 sitC::erm was not able to grow in a Fe-limited environment, in contrast to its wild type strain. Siderophore production in fBHI and certainly in fBHI plus free Fe demonstrated the bacterial need for siderophores for growth. For the sitC deletion mutant, a strong beta-haemolytic activity was observed. All strains tested were able to adhere to the catheter surface and to form a biofilm in our in vivo model. SitC was higher expressed in *S. epidermidis* Tü3298 during initial attachment, whereas in strain 10b expression was highest during the maturation and persistence phase. One week post-implantation the number of biofilm adherent bacteria was transiently higher in the sitC deletion mutant. High expression of fur and a low expression of sirR were measured in the mutant strain in contrast to their expression levels in the wild-type strain with lower number of sessile bacteria.
Conclusion: In vitro experiments suggest that the SitABC transporter favours Fe uptake over Mn2+ uptake. The more pronounced haemolytic activity of the mutant strain, indicated a switch to Fe uptake from haem in the mutant strain and the presence of another siderophore-dependent Fe uptake system(s) for bound Fe. A deletion of sitC had, directly or indirectly, a negative effect on expression of sirR and a positive influence on expression of fur.

R2263 Evaluation of linezolid, vancomycin, gentamicin, and ciprofloxacin activity against *Staphylococcus aureus* biofilms using XTT and Resazurin

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Objective: *S. aureus* (SA) biofilms (BF) show reduced susceptibility to antimicrobials. The activity of linezolid (LNZ), vancomycin (VAN), gentamicin (GEN), and ciprofloxacin (CIP) against SA BF was evaluated using the tetrazolium salt XTT, and the redox indicator resazurin (RZ).

Methods: 10 meticillin and quinolone resistant (MRSA) and 7 meticillin susceptible (MSSA) SA strains isolated from patients with catheter-related bacteraemia were studied. BF were obtained on flat bottom microtiter plates inoculated with 10^6 cfu/mL in Trypcase Soy Broth (TSB). Growth control (GC) and sterility control (SC) were included. Plates were incubated (24 h, 37ºC), and then washed and filled with Mueller-Hinton broth containing 2-fold dilutions of LNZ, VAN, CIP, and GEN (2048–0.125 mg/L). After incubation (37ºC, 24 h) plates were washed, and TSB containing 0.2 mg/L XTT + 0.1 microM menadione or RZ 0.0015% as appropriate was added. After 2.45 h of incubation at 37ºC, the absorbance of XTT (492 nm) or the fluorescence of RZ (530/590 nm excitation/emission) was read. SC values were subtracted to those obtained from the BF wells. The % of metabolic activity (MA) of BF exposed to antibiotics respect to their respective GC was calculated. Concentrations (mg/L) that inhibited >75 and >90% (CI75, CI90) of MA were considered; ANOVA was used to compare log10 of CI75 and 90. Studies were run in triplicate.
Results: XTT and RZ rendered similar results. For MSSA, the geometric mean concentration that resulted in CI90 was 4.9 mg/L for VAN, 9 mg/L for GEN, 2.3 mg/L for CIP and 50 mg/L for LNZ. For MRSA, the geometric mean that achieved the CI90 was of 8 mg/L for VAN, 157.6 for GEN and 1024 for LNZ. Only CIP resulted in 100% of MA at 2048 mg/L. For MSSA the log10 CI90 for VAN, GEN and CIP were significant lower with respect to that of LNZ ($p < 0.05$). SA. For MRSA, the log10 CI90 of VAN was significant lower than that for GEN ($p < 0.01$) and both VAN and GEN showed significant lower log10 CI90 respect to LNZ ($p < 0.021$).
Conclusions: XTT and RZ performed equally for detection of the activity of LNZ, VAN, GEN, and CIP activity against SA BF. For both categories of SA LNZ showed significant higher log10 CI90 with respect to the other drugs. 100% inhibition of MA was only seen in BF of CIP-susceptible SA exposed to high concentrations of CIP.

R2264 Comparison between proliferation intensity of *S. aureus* on unmodified and new C60-modified polymers

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Objectives: The number of new biomaterials in medicine is growing and it is important to know the ease by which they are colonised by microorganisms. The aim of the present study was to assess the intensity of *Staphylococcus aureus* proliferation on the surface of some new composite materials modified with C60 fullerene compared to materials of unmodified polymers.
Methods: The conventional test pellets were solid blocks (diameter = 10 mm, h = 1 mm) of different materials: polymethylmethacrylate (PMMA), PMMA-fullerene composites (PMMA-F), poly(2-hydroxymethylmethacrylate) (PHEMA) and PHEMA-fullerene composites (PHEMA-F), which were sterilised using UV. The samples were immersed in a fresh suspension of *S. aureus* ATCC 25923 in nutrient broth (with a defined microbial concentration) and incubated at 37ºC for 24 h. Afterwards, the pellets were washed with sterile saline solution (to remove the nonadherent cells) and dried at 37ºC. Two methods were used in order to evaluate the microbial proliferation intensity on the different materials. The first consisted in performing imprints by pressing the surface of the pellets against the surface of three Mannitol Salt Agar (MSA) plates and counting the bacterial CFUs after 24–48 h incubation at 37ºC. The second one referred to transfer (using an automate pipette) defined aliquots of staphylococcal suspension (obtained by vortex shaking the pellets in a nutrient broth) into a sterile microplate. After 20 h incubation at 37ºC, the optical densities (OD) measurements of the staphylococcal cultures were read at 450nm by a microplate reader (BIO-TEK, Powerwave AS), and the values could be compared.
Results: In case of the first method, no bacterial growth was observed on MSA at 24 h. The results at 48 h were for: PMMA-F: 3 CFUs, PMMA: less than 200 CFUs, PHEMA-F: more than 200 CFUs, and PHEMA: uncountable CFUs. The OD values were in accordance with the results obtained by the first method, regarding the microbial adherence and proliferation degree on the tested materials.
Conclusion: *S. aureus* presented higher tendency for proliferation on PHEMA. Composite materials modified with C60 fullerene seem to be less favourable for microbial proliferation than the unmodified polymers, but further studies are warranted.

R2265 Pioneer coloniser bacteria in biofilm formation on galvanised steel in a model cooling tower system

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Objectives: Cooling tower water systems provide an ideal environment for microbial biofilm. Biofilm in cooling towers is one of the most common problem that include opportunist pathogens such as *Legionella pneumophila* and corrosion agent sulphate reducing bacteria (SRB). Some bacteria have a higher tendency to produce biofilm than others. Especially, *Pseudomonas* and *Aeromonas* strains are acknowledged to be the pioneer colonizer and predominant in biofilm formation. But we have not observed these bacteria in the first month in our previous study related microbial biofilm. The aims of this study were to examine biofilm formation and to observe the first attachment/ detachment of *Pseudomonas* spp., *Pseudomonas aeruginosa*, *Aeromonas* spp., SRB and filamentous fungi on galvanised surfaces during one month in a model cooling tower water system.
Methods: Galvanized steel was used as test material. Biofilm was removed from coupon surfaces and resuspended in steril tap water. For enumeration of heterotrophic bacteria, *Pseudomonas* spp. *Pseudomonas aeruginosa* and *Aeromonas* spp., biofilm suspensions were spread plate respectively onto R2A Agar, *Pseudomonas* Agar Base with CFC supplement, *Pseudomonas* Agar Base with CN supplement, *Aeromonas* Medium Base with Ampicilin. Postgate medium B and Sabouraud Dextrose Agar were used for isolation of SRB and filamentous fungi, respectively. Glass was used as control.
Results: Colonisation of the galvanised steel and glass by microorganisms occurred very fast from the beginning of the experiment. The values of heterotrophic bacteria on galvanised steel and glass surfaces rose during 30 days. *Pseudomonas* spp., *Pseudomonas aeruginosa* and *Aeromonas* spp. were not determined in the biofilm on both of the surfaces after 19 days. SRB were determined in the biofilm on both of the surfaces during the experiment. Filamentous fungi were firstly seen on surfaces of galvanised steel and glass at 30 min.
Conclusion: Our results revealed that biofilm was formed on galvanised steel in spite of its toxic effect on microorganisms. In addition we established that although *Pseudomonas* spp., *Pseudomonas aeruginosa* and *Aeromonas* spp. were the pioneer colonizer, they were not surprisingly determined in biofilm on both of the surfaces after 19 days. The presence of filamentous fungi on both of the surfaces showed variable. Also we saw that SRB took part in the biofilm formation from the beginning of its.

R2266 The prevention of microbial biofilm formation on net prosthetic appliance for hernioplasty

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Objectives: To evaluate the ability of net prosthetic appliance to prevent the microbial biofilm formation.
Methods: Net prosthetic appliance is made of lavsan polyfilaments. Antimicrobial effect was attained in vitro when the composition of mixed high dispersed silver with high-molecular weight polyvinylpyrrolidine (PVP) was placed on polymeric net. Cell Wells™ 6-Well with Lid were used. Control – net without mixed composition. The piece of net was placed on the plate bottom with nutrient medium, then infect with *S.aureus* ATCC 6538 was added. The plates were incubated at 37ºC, 1 week, 2 weeks or 3 weeks. The CFU/ml as a testing parameter was evaluated during the experiment. In vivo the net prosthetic appliances were placed subcutaneous on guinea-pigs in conditions of microbial

contamination. Experimental animals were disabled on the 1st, 3d and 5th day of experiment. Then the pieces of net prosthetic appliances were placed on Petri dishes and incubated at 37ºC, 24 h; after what the CFU/cm^2 of each example were evaluated. The tissue histological examination was performed in surgical zone in 10–15 days.
Results: In vitro control plates showed solid infect growth after 24 h and the increasing level of CFU during 3 weeks up to 10^9 CFU/ml. Experimental examples showed no CFU in 1 and 2 weeks, during the 3d week 10^2 CFU/ml were obtained. In vivo net prosthetic appliance with tested composition helped to reduce the content of *S.aureus* in wound. Control nets possessed 10^3 CFU/cm^2 on the 1st day; 10^6 CFU/cm^2 on the 3d day and 10^9 CFU/cm^2 on the 5th day. The examples of silver-net tested after 1st, 3d and 5th day were sterile and possessed antimicrobial effect against *S.aureus* by agar diffusion method. Their prolonged antimicrobial effect was obtained after more than 5 days as well, this is necessary for optimisation of wound reparative processing. No pus process was in each experimental wound where silver-nets were placed. Control animals possessed the signs of sub-clinical festering.
Conclusions: High-molecular weight polyvinylpyrrolidine mixed with silver penetrates to lavsan polyfilaments pores leading to pseudo-monofiber formation. Tested composition PVP and silver is biodegradable in 1 week after net application. The net pores cleared that way are ready for conjunctive tissue growth. Net prosthetic appliance with antimicrobial composition was successfully applied in different clinics in Russia.

Antimicrobial pharmacokinetics, pharmacodynamics and general pharmacology

R2267 Heterogeneity of the mutant selection window: selection of resistant *Staphylococcus aureus* at ciprofloxacin constant concentrations simulated close to the MIC or the mutant prevention concentration

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Objective: Expected enrichment of resistant mutants at antibiotic concentrations that fall into mutant selection window (MSW) might depend not only on the time inside the MSW but also on the position of the pharmacokinetic profile in the MSW. To test this hypothesis, the selection of ciprofloxacin (CIP)-resistant *S. aureus* was studied at CIP constant concentrations (CCs) simulated closer to the bottom of the MSW (slightly above the MIC – "lower case") and to the top of the MSW (slightly below the mutant prevention concentration (MPC) – "upper case").
Methods: Two meticillin-resistant strains of *S. aureus*, one with a wide MSW (ATCC 6538) and another with a narrow MSW (ATCC 43300) [respective MPC/MIC ratios are 16 and 4], were exposed to constant-rate infusion of CIP for 3 consecutive days. With *S. aureus* ATCC 6538 and ATCC 43300, simulated CCs were 1.3–2 times greater than the MICs ("lower case") and 1.5 times smaller than the MPCs ("upper case"). Bacterial growth on agar plates containing 2×–16×MIC of CIP was tested daily. Time courses of mutants grown on CIP-containing plates were expressed by areas under the bacterial mutant kinetic curves (AUBMCs) measured from the start to the end of the infusion.
Results: Susceptible sub-populations of *S. aureus* ATCC 43300 were completely replaced by mutants resistant to 2× and 4×MIC of CIP by the end of the infusion (both lower and upper cases – CC 0.75 and 1.5 mg/L, respectively). Similar replacement occurred with *S. aureus* ATCC 6538, but only in the lower case (CC 0.5 mg/L). In the upper case (CC 2.7 mg/L), CIP-resistant mutants of *S. aureus* ATCC 6538 were enriched later and to a lesser extent than in the lower case: for mutants resistant to 2–16×MIC of CIP, estimated AUBMCs were 2–3-fold smaller in the upper case than in the lower case. There were no "position-associated" differences in the enrichment of resistant *S. aureus* ATCC 43300, probably due to comparable CCs at both the lower and upper positions within the MSW.
Conclusion: A more rapid and pronounced enrichment of CIP-resistant staphylococci occurs with organisms exhibiting a wide MSW. The possible heterogeneity of the MSW should be considered to better predict the enrichment of staphylococcal resistance.

R2268 Comparison of vancomycin and teicoplanin trough serum levels in patients treated for orthopaedic device infections

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Introduction: no study has compared the pharmacokinetic performances of the glycopeptide agents vancomycin (V) and teicoplanin (T) for the treatment of pts with orthopaedic device infections (ODI).
Objectives: to compare trough serum concentrations of V and T in patients (pts) treated for with both loading doses and high daily dosages for ODI.
Methods: medical charts for pts treated with either V or T for ODI and for which the results of at least 2 trough levels were available were reviewed. Patients with V or T therapy were compared on the following parameters: age, weight, renal clearance, protides, fever, white blood cells, CRP level, and trough serum levels of V or T.
Results: 52 patients hospitalised from May 2000 to December 2005 were eligible for the study. Intravenous loading doses were administered in 26 pts treated with V (0.5–1g infused during 1 hour) and in 26 pts treated with T (10 mg/kg bid for 5 injections). Following the loading dose, one-daily10–12 mg/kg T infusion and continuous V infusion of 30 mg/kg were administered. No significant differences in the patients' demographic parameters were observed between T and V groups.
Trough levels performed at day at day 2±1 and at day 5 ±1 were significantly higher in T than in V patients (26.1 vs 16 mg/L, $p = 0.01$ and 27.8 vs 19.9 mg/L, $p = 0.01$, respectively). When considering an expected trough value of >25 mg/L, this target was reached at day 2+/1 in 1/26 V pts vs 10/26 T pts ($p = 0.002$), and at day 5 ±1 in 6/26 V pts vs 13/26 T pts ($p = 0.04$).
Conclusion: teicoplanin trough serum concentrations obtained during the first week of treatment are significantly higher than those of vancomycin in patients treated with both loading doses and high daily dosages for ODI.

Mechanisms of action and resistance

R2269 Molecular characterisation of antimicrobial resistance mechanisms and genotype of Irish Enterobacteriacae

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Objectives: There is a dearth of information on the development and spread of antibiotic resistance in Gram-negative hospital-acquired infections in Ireland. This study aimed to identify the molecular mechanisms of resistance and the genotypes among Irish Enterobacteriacae.
Methods: Antibiotic susceptibilities were determined by breakpoint agar dilution method. The resistant isolates were screened for the presence of β-lactamase and ESBL genes, plasmid mediated ampC genes, qnr genes, aac(6′)Ib-cr and aminoglycoside genes. The gyr and par genes of fluoroquinolone resistant isolates were amplified by PCR and sequenced. The genetic relatedness of all isolates was performed by PFGE and analysed using BioNumerics software.
Results: Number of isolates positive for genes/genetic mutations:
Klebsiella pneumoniae: 13 acc-1, 2 dha-1, 1 Shv, 1 CTX-M group 1, 1 aac(6′)-Ib-cr, 2 gyrA and 1 parC mutation.
Enterobacter spp.: 3 dha-1, 5 Shv, 4 Tem, 1 Oxa, 6 CTX-M group 1, 1 group 9, 2 group 26, 3 qnrA, 2 aac(6′)-Ib-cr, 2 aac(6′)-IIc, 9 gyrA and 5 parC mutation.
Escherichia coli: 1 Shv, 7 Tem, 2 Oxa, 4 CTX-M group 1, 2 group 8, 5 group 9, 1 group 26, 1 qnrA, 1 qnrB, 16 gyrA, 1 parC and 1 parE mutation.
One of the *Enterobacter* species isolates, which tested positive for the aac(6′)-Ib-cr gene, was an *Enterobacter aerogenes*. This gene has never been identified in this genera prior to this study.

This is the first detection of isolates with blaOXA genes (in Enterobacteriacae), CTX-M groups 8 and 26, the Qnr genes, aac(6′)-Ib-cr gene, aac(6′)-IIc gene and mutations within the DNA gyrase and DNA topoisomerase genes in Ireland. A novel amino acid mutation, L445H, was identified in the parE gene of an *E. coli*.
The PFGE patterns analysed by BioNumerics did not suggest the predominance of a particular clone within any of the bacterial species. The resistant *E. coli* isolates 14815 and 28265 appeared to be very closely related, with only 1 band difference. Isolate 14090 was identical to 28265. Isolate 14090 was susceptible to fluoroquinolones and cephalosporins.
Conclusions: These findings indicate that the transferable antibiotic resistance genes, which have been associated with outbreaks in other European countries, are present in Ireland. The PFGE results suggest that resistance is spreading due to horizontal transfer of resistance genes rather than vertical transfer of resistant isolates.

R2270 First report of carbapenem-resistant *Bacteroides fragilis* strain isolated from an intensive care unit in Italy

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Objectives: A carbapenem-resistant *Bacteroides fragilis* strain isolated in August 2005 from a patient admitted to the intensive care unit of University Hospital in Verona (Italy) was investigated for carbapenemase activity and presence of cfiA genes and activating insertion sequence (IS) elements.
Methods: The imipenem-resistant *B. fragilis*, designated BF417, was identified with API Rapid ID32A. Metallo-β-lactamase (MBL) production was investigated with E-test MBL strips and spectrofotometric assay, following the imipenem hydrolysis of crude cell extracts. PCR techniques were used to amplify the cfiA gene and the insertion sequence (IS) elements. Sequence was performed by an ABI capillary sequencing equipment.
Results: The strain BF417 showed high-level of resistance to imipenem and meropenem (MIC > 32 mg/L) and other β-lactam antibiotics: piperacillin (MIC > 256 mg/L); cefoxitin (MIC > 256 mg/L); amoxicillin/clavulanate (MIC > 256 mg/L).
The MBL E-test showed the presence of a functional metallo-β-lactamase. The hydrolysis assays showed that the crude cell extracts from strain BF417 hydrolysed the imipenem at the rate of 4.5×10^9 moles/min/mg protein.
Sequence analysis of PCR amplicons revealed a cfiA gene with 99% of identities to previously described *B. fragilis* cfiA-1 and an insertion sequence (IS) element with 88% of identities to IS614B element.
Conclusions: To our best knowledge this is the first carbapenem-resistant *B. fragilis* isolated in Italy, bearing a cfiA gene coding a MBL. The cfiA gene ia activated by a new IS element which possesses an overall identity of 88% to IS614B element.

R2271 Prevalence and efflux mechanism of macrolide resistance in *Campylobacter jejuni* and *Campylobacter coli* from different sources

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Objectives: The prevalence and efflux mechanism of resistance against erythromycin, azithromycin and clarithromycin in *Campylobacter jejuni* and *Campylobacter coli* isolated from humans, poultry, farm chickens and water in Zenica-Doboj Canton, Bosnia and Herzegovina during 2001–2006 was investigated.
Methods: Antibiotic resistance phenotypes of *Campylobacter jejuni*/coli isolated from humans (n = 222), poultry meat (n = 50), farm chickens (n = 15) and water (n = 17) were determined by disc diffusion and Etest methods, and with reference agar dilution and broth microdilution method with CellTiter-Blue® reagent and automated fluorescence signal detection. The involvement of efflux pumps was evaluated as well by broth microdilution measurements of MICs with/without the efflux pump inhibitors phenylalanine-arginine beta-naphthylamide (PAB′N, 20 mg/ml) and naphtyl-methyl-piperazine (NMP, 80 mg/ml) in 25 selected isolates.
Results: The high proportion of *C. coli* from humans (119/222), poultry meat (29/50), farm chickens (8/15) and water (15/17) was found, 56.5%, 56.9%, 53.3% and 88.2%, respectively. Erythromycin resistance in human, poultry, farm chicken and water isolates was 21.3%, 30.6%, 38.5% and 57.1%, respectively, and it was in the range 17.1%-58.3% and 20.2%-50% in *C. jejuni* and *C. coli* isolates. Prevalence of azithromycin and clarithromycin resistance was similar. The PAB′N and NMP restored the MICs of two low-level resistant isolates (MICs 8–16 μg/mL, n = 2) to the level of susceptible strains, and reduced the MICs of all susceptible strains (n = 14). In high-level resistant isolates (MICs over 32 μg/mL, n = 9) the use of PAB′N increased the susceptibility by 4–16-fold, while NMP restored the susceptibility of two HLR strains. In the case of azithromycin and clarithromycin, PAB′N increased susceptibility up to 6- and 128-fold, respectively.
Conclusion: Unusually high prevalence of *C. coli* and the resistance to macrolides in both *C. jejuni* and *C. coli* isolated from humans, poultry, farm chickens, and water was noted. Efflux activity was confirmed as involved mechanism at erythromycin, azithromycin and clarithromycin resistance. However, the results confirm that PAB′N and NMP efflux pump inhibitors are moderately active in reversing macrolide resistance in *Campylobacter* isolates. Further studies of mechanisms involved in macrolide resistance of *Campylobacter* are required, as well as monitoring of macrolide resistance of human, animal and environmental isolates.

R2272 Study on SHV-type extended-spectrum-β-lactamase in Hunan province

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Objective: To invesigate the prevalence of SHV-produing Enterobacteriaceae isolates in Hunan province. To identify the subtype of SHV enconding gene and study the epidemiological aspect of SHV-producing isolates.
Methods: 171 clinical strains of muti-resistant Enterobacteriaceae isolates were collected from three affiliated hospital of Central South University in the region from October 2004 to July 2005.ESBLs producing isolates were confirmed by means of phenotypic confirmatory tests as recommended by the CLSI/NCCLS. PCR and DNA sequencing were used to determine the genotype of SHV enzymes. The homology of SHV-producing strains were detected by RAPD.
Results: 26 of 142 ESBLs-producing Enterobacteriaceae isolates were confirmed to produce blaSHV genes, there are 19 *K. pneumoniae*, 5 *E. cloacae*, 1 K.ozaenae and 1 *C. freundii*. 5 subtypes of SHV-type β-lactamases were determined, including 9 strains of SHV-28, 7 strains of SHV-12, 7 strains of SHV-1, 2 strains of SHV-11 and 1 strain of SHV-5. There were 6 RAPD types in 19 isolates of SHV-producing *K. pneumoniae*, 5 RAPD types in 5 isolates of SHV-producing *E. cloacae*.
Conclusions: SHV-12 is the predominant genotype of SHV-type β-lactamases producing Enterobacteriaceae in Hunan province, and clone spread has played a certain role in SHV-type β-lactamase producing *K. pneumoniae*. SHV ESBLs are not found in *Escherichia coli*.

R2273 Is *Bartonella bacilliformis*, endemic pathogen of the Andean areas, intrinsically resistant to quinolones?

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Objectives: To characterise the resistance to quinolones and the sequence of the gyrA and parC gene in *Bartonella bacilliformis*, responsible of the Carrion's disease in the Andean countries.

Methods: Three *B. bacilliformis* were included in the study. Two of them were isolated before of 1957 (CIP 57.17 and CIP 57.18, Institute Pasteur), previously to the introduction of the quinolones in the clinical practice, while the third was recovered in 1996. The specie was confirmed by amplification and sequence of the rRNA16S gene. The QRDR sequence of the gyrA and parC genes was characterised by PCR amplification and posterior DNA sequence. The susceptibility to nalidixic acid (Nal) and ciprofloxacin (Cip) was established both by disk diffusion and by the E-test method. In all cases the plates were incubated at 28ºC in a 5% CO2 atmosphere and controlled once to day in order to avoid contaminations.
Results: The three strains exhibit resistance to Nal by the two tested methodologies, possessing a MIC > 256 mg/L by the E-test method. While, the two ancient isolates presents a MIC of 0.25 mg/L to Cip, and the remaining isolate has a MIC > 32 mg/L to Cip.
In accordance with that is reported in the Gene Bank, the three strains presents an Ala in the position 85 of the parC gene, equivalent to the 80 of the QRDR of the parC gene of *Escherichia coli*. Meanwhile, the two isolates pre-quinolone age presents a Ala in the position 91 of the gyrA gene, as is described in Gene Bank, possition equivalent to the 83 of the gyrA gene of *E. coli* while the remaining presents a Val.
Conclusions: Despite its isolation previous to the introduction of the quinolones in the clinical practice, the studied strains, presents a constitutive resistance to quinolones, which may be related with the presence of Ala in the position 91 and 85 of the QRDR of the quinolone-targets (GyrA and ParC). To our knowledge this is the first report of a clinical isolate of *B. bacilliformis* presenting an amino acid substitution in the QRDR of GyrA. These results dissapointed the current clinical guidelines that recommended the use of ciprofloxacin to treat bartonellosis in some countries of this area.

R2274 Molecular characterisation of penicillin non-susceptible *Streptococcus pneumoniae* isolated in Turkey

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Objectives: The mechanism of penicillin resistance in pneumococci is alteration in the structural genes of penicillin-binding proteins (PBPs). In this study, the diversity of PBPs 2b, 2x, and 1a of Turkish penicillin non-susceptible strains of *Streptococcus pneumoniae* (PNSP) was investigated.
Methods: *S. pneumoniae* clinical isolates were collected at five centres in different cities of Turkey from children and adults. Susceptibilities of all isolates were determined by CLSI broth microdilution testing of penicillin, ceftriaxone, levofloxacin, erythromycin and clindamycin. Serotypes were determined by sequential multiplex-PCR and quellung reaction with specific antisera for *S. pneumoniae*. PNSP isolates were examined for the PBP genes pbp2b, pbp2x, pbp1a by PCR and restriction fragment lenght polymorphism (RFLP). All PCR-RFLP fingerprints were analysed and compared using Bionumerics software and assigned to specific profiles.
Results: Of the 297 nonduplicated pneumococcal isolates, 128 (43.1%) showed reduced susceptibility to penicillin (MICs ⩾0.12g/L). Of these, 22 were invasive and 106 were non-invasive. Twenty five different serotypes were recognised, while four strains were nontypeable. The most frequent serotype was 14 (17.9%). Antimicrobial susceptibilities of PNSP isolates are shown in Table 1. Intermediate resistance (MIC 0.12–1g/L) to penicillin were found in 54 (42.2%) penicillin-nonsusceptible isolates and high-level resistance (MIC ⩾2 g/L) in 74 (57.8%). Thirty four (26.6%) of PNSP isolates showed a multi-drug resistance pattern. PBP profile clusters comprised 56 groups. The largest group included 43 strains of seven different serotypes: 14, 9V, 6A, 23F, 19F, 13F, 22F; all strains had penicillin MICs ⩾1g/L. Strains belonging to this predominant profile were found in all 5 centres. The second profile included eight serotype19A strains.
Conclusions: Penicillin nonsusceptibility among strains from Turkey was relatively high with a rate of 43.1%. The major serotype associated with resistance was serotype 14 which accounted for 17.9% of these isolates. Analysis of PBP 2b, 2x and 1a profiles showed a relatively heterogenous population with 56 different profiles determined. However, one specific profile associated with MICs of ⩾1g/L was associated with a variety of serotypes in all 5 centres.

Table 1. Antimicrobial susceptibilities of PNSP isolates

	MIC_{50}	MIC_{90}	Range	Susceptible, n (%)	Intermediate, n (%)	Resistant, n (%)
Penicillin	2	>2	0.12–>2	0	54 (42.2)	74 (57.8)
Ceftriaxone	1	2	⩽0.25–4	78 (60.9)	48 (37.5)	2 (1.6)
Levofloxacin	1	2	0.5–>8	118 (92.2)	7 (5.5)	3 (2.3)
Erythromycin	⩽0.25	>8	⩽0.25–>8	70 (54.7)	2 (5.5)	56 (43.7)
Clindamycin	⩽0.25	>8	⩽0.25–>8	84 (65.6)	0	44 (34.4)

R2275 Detection of KPC-2 in a clinical isolate of *Proteus mirabilis*: first reported description of carbapenemase resistance in this species caused by a KPC β-lactamase

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Background: Carbapenem resistance due to the blaKPC carbapenem hydrolysing β-lactamase is a major healthcare concern in the U.S. Since its discovery in 2001 blaKPC has been shown to be present in a variety of Enterobacteriaceae including *Enterobacter*, *Citrobacter*, *Salmonella*, and *Serratia* species as well as *Pseudomonas aeruginosa*. In August 2006 we instituted a prospective study to determine the prevalence of KPC-producing organisms in our hospital. During this time we identified an isolate of *Proteus mirabilis* that was resistant to ertapenem, meropenem, and imipenem. PCR analysis using blaKPC-specific primers produced an amplicon of the expected size. Herein we describe the first reported *Proteus* species possessing a plasmid with a functional blaKPC-2 gene.
Methods: blaKPC-specific polymerase chain reaction was performed on total DNA and plasmid DNA. Plasmid isolated from *P. mirabilis* was amplified and sequenced using KPC-specific primers and BigDye Terminator sequence technology.
Results: blaKPC PCR assays were positive from both total and plasmid DNA from a carbapenem resistant isolate of *P. mirabilis* recovered from blood cultures from a single patient. Zone diameters for ertapenem, impenem, and meropenem were 10, 6, and 6 respectively. The imipenem MIC for the isolate was >16 ug/ml. Sequence analysis of PCR generated DNA indicated 99.5% homology to previously the published KPC-2 gene sequence.
Conclusions: These results suggest that blaKPC is transferable to *Proteus mirabilis*. While this organism is not generally considered a highly virulent pathogen, it does highlight that fact that blaKPC can be harboured in normal and/or opportunistic bacteria, which could in turn provide an opportunity for dissemination of this resistance factor to other compatible organisms within the intestinal milieu. Further, this data suggest that PCR analysis should be done on all carbapenem resistant organisms to screen for the presence of the blaKPC gene.

Resistance surveillance

R2276 Easy accessible surveillance tool for clinical decision-making of empirical antibiotic therapy

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Objectives: In patients with severe infections like septicaemia, early and appropriate empiric antibiotic therapy is of utmost importance, resulting in decreased mortality of patients or shorter length of stay. However, the always changing pattern of susceptibility of pathogens and geographic differences in epidemiology makes it necessary to start empirical therapy based on local data. Often microbiological laboratories produce only once or twice a year statistical data with regard to epidemiology and susceptibility. Normally, the clinician himself has no opportunity to access the laboratory information system for statistical analyses.

Methods: We developed a new surveillance tool to support analysis of microbiological data by clinicians. It is based on a business programme produced by Oracle and accessible via internet explorer.
Results: By means of this tool, the clinician is able extract information including graphs from the past until the present day – Hospital as a whole, department and single ward; group of pathogens or a single pathogen; activity of an antibiotic against different pathogens or a single pathogen; percentage of susceptibility; development of susceptibility over different periods of time, e.g. years, quarter of a year, month; detection of pathogens with predefined resistance mechanisms like MRSA, VRE or ESBL-producing bacteria; pre-defined warnings, e.g. MRSA > 20% or decrease of susceptibility >3% of a given combination of antibiotic and pathogen over different periods of time; detection of pathogens with a unique pattern of resistance within a hospital. The number of inquiries is not limited and can be easily tailored to local needs.
Conclusion: Regular analysis of microbiological data by means of this easy to handle surveillance tool enables clinicians to optimize empirical antibiotic therapy based on recent local data and also to detect emergence of resistant strains in a very early phase.

R2277 Susceptibility of CTX-M producing *Escherichia coli* against non-β-lactamase agents

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Objectives: To investigate the activity of different non-β-lactam agents on CTX-M producing clinical isolates of *Escherichia coli* in the County of Östergötland, Sweden
Methods: From 2002 until August 2007 145 clinical isolates of CTX-M producing *E. coli* were further analysed. Ninetyfive isolates belonged to CTX-M group 1, 50 isolates to CTX-M group 9. Minimal inhibitory concentrations (MICs) for nitrofurantoin, colistin, fosfomycin, ciprofloxacin, tigecycline, trimethoprim, trimethoprim-sulfamethoxazole (TMP-SMX) and gentamicin were determined with Etest (AB Biodisk). The susceptibility was determined according to the breakpoints of the Swedish Reference Group for Antibiotics. For fosfomycin there were no available breakpoints and susceptibility was assessed according to the wild-type distribution from EUCAST. MIC50 and MIC90 were calculated. Genetic relatedness between isolates was not investigated.
Results: Susceptibility of *E. coli* belonging to CTX-M group 1 and 9 are shown in the table.

Agent	MIC_{50}		MIC_{90}		Susceptibility (%)	
	group 1	group 9	group 1	group 9	group 1	group 9
Nitrofurantoin	16	8	64	32	68	80
Colistin	0.25	0.25	0.5	0.5	99	98
Fosfomycin	1	1	4	4	94	94
Ciprofloxacin	32	1	32	32	28	46
Tigecycline	0.25	0.25	0.5	0.5	99	100
Trimethoprim	32	32	32	32	31	26
TMP-SMX	>128	>128	>128	>128	37	28
Gentamicin	2	1	64	64	51	76

Conclusions: The frequencies of susceptibile isolates were low for ciprofloxacin, trimetoprim, TMP-SMX, gentamicin and nitrofurantoin for isolates belonging to CTX-M group 1 and CTX-M group 9 However, there was a tendency towards higher susceptibility to nitrofurantoin and ciprofloxacin among isolates belonging to CTX-M group 9. Isolates in both groups showed high susceptibility for fosfomycin, colistin and tigecycline and these agents may offer therapeutically options

R2278 Multidrug-resistant bacteria in German intensive care units, 2001–2007

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Objectives: The proportions of meticillin-resistant *S. aureus* (MRSA), vancomycin-resistant *E. faecium* and extended spectrum β-lactamase (ESBL) producing enterobacteriaceae have increased worldwide in the recent years. We analysed the resistance situation for these pathogens in German ICUs.
Methods: From 2001 to 6/2007, 47 ICUs participating in SARI (Surveillance of Antibiotic Use and Bacterial Resistance in Intensive Care Units) were included in the analysis. Among other things, ICUs collected prospective, unit-based data on patient days and resistance proportions of MRSA, VRE faecium, 3rd generation cephalosporin-resistant *E. coli*, *K. pneumoniae* and imipenem resistant *P. aeruginosa*. Isolates were nonduplicate. Trends over time were tested using the Cochran-Armitage trend test.
Results: MRSA resistance rates decreased from 26.3% in 2001 and 17% in 2007 ($p < 0.001$). Imipenem resistant *P. aeruginosa* fluctuated without a clear trend between (24.2% in 2001 and in 27.6 2006, $p = 0.381$). The same was true for 3rd generation cephalosporin resistant *K. pneumoniae* (3.9% in 2001 and 8.2 in 2007, $p = 0.642$). In contrast, 3rd generation cephalosporin resistant *E. coli* increased continuously from 1.1% in 2001 to 10.1% in 2007 ($p < 0.001$). Vancomycin resistant *E. faecium* showed a sharp peak in resistance in 2005 of 5.6%, but decreased again to 2.7% in 2007 ($p < 0.109$).
Conclusion: The continous increase of 3rd generation cephalosporin-resistant *E. coli* (an indicator for ESBL production) is alarming and requires attention and immediate action with respect to antibiotic policy and infection control.

R2279 Susceptibility to tigecycline by Etest against extended-spectrum β-lactamase-producing and AmpC-hyperproducing isolates of *Escherichia coli* and *Klebsiella* spp.

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Objectives: Third generation cephalosporines resistance (TGCR)in enterobacteria is increasing not only because of harbouring extended-spectrum β-lactamases (ESBL) but the AmpC hyperproduction (AmpC). 123 ESBL enterobacteria isolates (EI) (108 *Escherichia coli* and 15 *Klebsiella* spp.), 23 AmpC EI (19 *E. coli* and 4 *Klebsiella* spp.) and 16 *E. coli* isolates without TGCR isolated from patients of our clinical area, were investigated for in vitro activity of tigecycline.
Methods: Rutine identification and determination of susceptibilities of antimicrobial agents were tested by using MicroScan or Phoenix systems. ESBL production was confirmed according to CLSI guidelines. Tigecycline E-Test was performed on freshly Mueller-Hinton agar and according to manufacter instructions.
Results: According CLSI interpretative criteria, 3 AmpC EI, 3 ESBL EI and 1 isolate without TGCR were intermediate. No resistant strains were found. 95.6% of EI were susceptible to tigecycline. According EUCAST interpretative criteria, 5 AmpC EI, 3 ESBL EI and 1 isolate without TGCR were resistant and 1 isolate without TGCR was intermediate. 93.8% of EI were susceptible. *Klebsiella* spp. and AmpC EI showed major MIC50 and MIC90 than *E. coli* and ESBL EI.
Conclusions: Even though there are no comparable number of isolates between the different groups of EI tested, tigecycline seems to be more active against *E. coli* and ESBL EI than against *Klebsiella* spp. and AmpC EI. Nevertheless a good activity of tigecycline against EI was found out. Comparing clinical results with tigecycline treatments in knowing isolates producing infections would be interesting.

R2280 Antimicrobial resistance of Spanish *Pseudomonas aeruginosa* from blood according to the European Antimicrobial Resistance Surveillance System (EARSS) experience (2006–2007)

J. Oteo, O. Cuevas, J. Campos on behalf of the Spanish members of The European Antimicrobial Resistance Surveillance System

Objectives: Antibiotic resistance in invasive *Pseudomonas aeruginosa* (PAE) has been monitored in Europe since 2005 by the European Antimicrobial Resistance Surveillance System (EARSS). The goal of this study is to analyse the antimicrobial susceptibility data of invasive PAE collected by 36 Spanish hospitals during 2006–2007 in comparison with data from other European countries.
Methods: Each laboratory identified strains and tested their susceptibility using its own methods. To assess the comparability of susceptibility test results, a quality assurance exercise was performed.
Results: We report data on 691 invasive PAE isolated from blood from the same number of patients; 477 (69%) were males and 214 (31%) females. Twenty-seven patients were <15 years old, 307 between 15–65 years, and 357 >65 years. Resistance figures were as follows: ciprofloxacin (CI), 23.7%; gentamicin (G), 14%; tobramycin (T), 13.3%; imipenem (I), 13.3%; ceftazidime (CE), 8.5%; piperacillin/tazobactam (P/T), 6.3%; and amikacin (A), 2.5%. Sixteen isolates (2.3%) were simultaneously resistant to CI-G-I-CE-P/T, and 68 (9.8%) were resistant to three or more of these antibiotics. Resistance to piperacillin/tazobactam, imipenem and ceftazidime was higher in ICUs (12.8%, 22.2% and 14.9%, respectively) than in Medicine departments (4.8%, 9.4% and 6.9%, respectively) ($p < 0.002$). Resistance rates in isolates from children <15 years was lower than in >14 years for all antibiotics ($p < 0.05$). No statisitical differences were observed in the other age groups. In comparison with other European countries participating in EARSS in 2006, resistance to carbepenems and fluorquinolones was lower in Spain than in Greece (46.6% and 45.5%, respectively), Italy (21.3% and 36.1%), Portugal (20.7% and 20.7%), Czech Republic (32.6% and 46.5%) and Turkey (32.7% and 30.1%), but higher than in the United Kingdom (6.2% and 7.6%, respectively) and The Netherlands (2.1% and 8.7%). Similar resistance figures than in Spain were observed in Austria and France.
Conclusions: In contrast with other pathogens, mainly community-acquired pathogens, antibiotic resistance in PAE in Spain is not amongst the highest in Europe. However, emergence of co-resistance to multiple antibiotics can generate important therapeutic problems.

R2281 Prevalence of antimicrobial resistance among uropathogens causing acute uncomplicated cystitis in female outpatients in South Korea: a multicentre study in 2006

Y.H. Cho for the Korean Association of Urogenital Tract Infection and Inflammation

Objective: The Korean Association of Urogenital Tract Infection and Inflammation (KAUTII) conducted a survey of the antimicrobial susceptibility patterns of uropathogens responsible for female acute uncomplicated cystitis in South Korea in 2006. KAUTII has already reported similar data in 2002, which are compared with the results of the present study. The present study is now the second report of an ongoing programme that provides nationwide and regional references needed to select empirical therapy.
Methods: This study was carried out with the participation of 22 hospitals in South Korea. A total of 301 isolates were obtained from female outpatients with acute uncomplicated cystitis. The antimicrobial susceptibilities to ampicillin, ampicillin/sulbactam, gatifloxacin, ciprofloxacin, gentamicin, trimethoprim/sulfamethoxazole (TMP/SMX) and tobramycin were determined by VITEK 2 antimicrobial susceptibility test systems. The antimicrobial susceptibilities to commonly prescribed drugs were determined.
Results: The most prevalent causative organism was *Escherichia coli* (71.1%), followed by enterococci (13.0%), coagulase-negative staphylococci (5.3%) and other species of Enterobacteriaceae (10.6%). Among all Enterobacteriaceae isolates, 31.4% were susceptible to ampicillin, 52.3% to ampicillin/sulbactam, 97.6% to piperacillin/tazobactam, 78.9% to ciprofloxacin, 80.3% to gatifloxacin, 86.8% to cefazolin, 99.6% to amikacin, 80.5% to gentamicin, 81.1% to tobramycin and 73.9% to trimethoprim/sulphamethoxazole (TMP/SMX). The resistance rates of *E. coli* to ciprofloxacin and gatifloxacin were 23.4% and 21.8%, respectively, and 12 (11.8%) of 102 strains were confirmed of producing extended-spectrum β-lactamase (ESBL). All the ESBL-producing strains were also resistant to fluoroquinolones. Enterobacteriaceae were highly susceptible to piperacillin/tazobactam and amikacin (>97%). There was a small increase in susceptibility to TMP/SMX (73.9%) compared with the same study in 2002 (62.1%).

Susceptibility of urinary Enterobacteriaceae isolates from female outpatients with acute uncomplicated cystitis to various antimicrobial agents

Antimicrobial agent	Percentage of susceptible strains in 2006/2002		
	E. coli (n = 214/291)	Other Enterobacteriaceae (n = 32/20)	Total (n = 246/311)
Ampicillin	35.2/37.2	6.3/15.0	31.4/35.5
Ampicillin/Sulbactam	52.4/44.5	51.6/50.0	52.3/45.0
Piperacillin/Tazobactam	98.6/97.4	90.6/95.0	97.6/97.2
Ciprofloxacin	76.6/84.8	93.8/95.0	78.9/85.7
Gatifloxacin	78.2/N.A.	93.8/N.A.	80.3/N.A.
Cefazolin	92.4/92.2	50.0/60.0	86.8/89.1
Amikacin	99.5/99.0	100/100	99.6/99.1
Gentamicin	77.6/81.7	100/80	80.5/81.5
Tobramycin	78.2/85.9	100/80	81.1/85.3
TMP/SMX	70.6/61.3	96.8/70	73.9/62.1

TMP/SMX, trimethoprim/sulphamethoxazole. N.A., not available.

Conclusions: Similar to 2002, the high prevalence of resistance to ampicillin, ampicillin/sulbactam and TMP/SMX still exists. In acute uncomplicated cystitis, fluoroquinolones and cephalosporins show adequate rates of susceptibility for empirical use. The increasing number of ESBL-producing or fluoroquinolones-resistant strains remains a serious clinical problem in South Korea.

R2282 Ventilator-associated pneumonia in a tertiary hospital's polyvalent intensive care unit: a sixteen-month study

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Objectives: ICU is an area of elevated nosocomial infection rates. In our polyvalent 10-bed Unit of a tertiary care hospital Ventilator-associated pneumonia (VAP) is the second more frequent infection, complicating a high percentage of mechanically ventilated patients. Our aim was to study the incidence and outcome of VAP as well as the trends of the infective flora.
Methods: We studied all patients who were hospitalised above 72 hours in our ICU during the last 16 months. Protected bronchial samples of all patients with suspected VAP were taken and cultured and standard diagnostic criteria were followed.
Results: 341 patients were hospitalised from 1st August 2006 until 1st December 2007 in our ICU. 118 patients were excluded due to length of stay (LOS) less than 72 hours. 223 patients were studied and 42 were proved to suffer of VAP. Mean age was 55.09±18.57 years, male/female ratio was 33/9, mean APACHE II score was 23.8±5.63, and average LOS was 25.7±10.84 days. 44 episodes of VAP were recorded, two of them being of double pathogen cause and 7 had concomitant bacteraemia. The mean VAP onset day was 12.25±6.6 days. The most prevalent pathogens were Gram-negative bacteria: *Acinetobacter baumannii* 19 (42.2%), *Pseudomonas aeruginosa* 18 (40%), *Klebsiella pneumoniae* 4 (8.8%), *Stenotrophomonas maltophilia* 2 (4.4%) and of Gram-positive cocci only *Staphylococcus aureus* 2 (4.4%). The incidence of late VAP due to MDR Gram-negative pathogens was 28 (63.6%) and 6 of them (13.6%) were sensitive only to colimycin. The sensitivities are presented

in the table. Positive outcome was found in 26 patients (61.9%) and was reversely related to APACHE II score, LOS and MODS.

	aminog	carbap	cephalo	cipro	pip/tazo
A. baumannii	31.5%	21%	5.2%	0	5.2%
P. aeruginosa	50%	27.7%	16.6%	44.4%	88.8%
K. pneumoniae	75%	50%	25%	25%	25%

Conclusion: Even though the percentage of MDR pathogens was high, the survival rate was fairly good. Colimycin is still 100% efficient on MDR Gram-negative pathogens, given both intravenously and aerosolised for the treatment of VAP, and remains our only constant ally.

R2283 Antimicrobial resistance and co-resistance in *Escherichia coli* isolates from blood cultures. Data from the Regional Surveillance System of Emilia-Romagna, Italy

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Objectives: To describe the trends of antimicrobial resistance and the patterns of co-resistance of *Escherichia coli* isolates from blood cultures in Emilia-Romagna region, Italy.
Methods: Data were collected through the regional system for surveillance of antimicrobial resistance which was started in Emilia-Romagna (a northern Italy region of about four million inhabitants) since 2003. The number of laboratories participating to the network increased from 11 in 2003 to 19 in 2006. Each laboratory was requested to provide data on all bacterial cultures performed, according to a specified electronic format; files were centrally transferred through secured protocols. Standard codes were used.

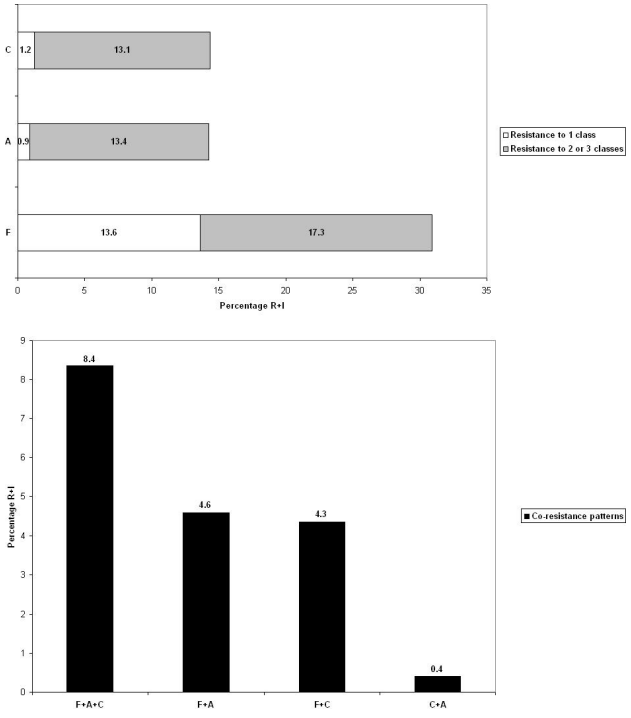

Figure 1. *Escherichia coli* isolates from blood cultures: co-resistance to fluoroquinolones (F), aminoglycosides (A) and third-generation cephalosporins (C), Emilia-Romagna region 2006.

Results: The prevalence of antimicrobial resistance of *E. coli* had a rapid rise during the surveillance period (2003–2006). Resistance to third generation cephalosporins of isolates from blood increased from 4.8% in 2003 to 14.3% in 2006, due to a fast spread of extended-spectrum β-lactamases phenotypes. Similar trends of resistance were observed for aminoglycosides (from 9.6% in 2003 to 14.3% in 2006) and fluoroquinolones (from 25.1% in 2003 to 30.9% in 2006). A high prevalence of co-resistance was also observed among the considered antibiotic classes (third generation cephalosporins, aminoglycosides and fluoroquinolones): most isolates resistant to one of these three classes were resistant to at least one of the remaining two (Figure 1). High prevalences of resistance and co-resistance were also observed for *E. coli* cultured from urine and other sites.
Conclusion: The surveillance data from Emilia-Romagna show a high prevalence of resistance for *E. coli* which is likely to further increase due to the promoting effect of co-resistance toward third generation cephalosporins, aminoglycosides and fluoroquinolones. The burden of resistance and the speed of its spread are impressive and raise concerns for the future availability of an effective therapy for infections caused by this organism. Therefore urgent control measures are needed to counteract this phenomenon in Emilia-Romagna region.

R2284 Real-time PCR for the detection of qnrA gene in Enterobacteriaceae: the incidence of plasmid-mediated quinolone resistance in a London teaching hospital

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Objectives: Mechanisms of resistance to quinolones are classically chromosomally mediated. However, the plasmid encoded gene, qnrA, encodes a protein that binds to the subunit of topoisomerase IV, preventing quinolone binding and conferring resistance. The plasmid encoded nature of the gene may facilitate spread in a hospital environment. The aim of this study was to determin the prevalence of strains harbouring the qnrA gene in our hospital.
Method: A previously described PCR for the detection of qnrA was modified to a real-time PCR format and used retrospectively to screen 45 extended-spectrum-β-lactamase enterobacterial isolates collected at the Royal Free Hospital between January 2005 and December 2007. In addition, 59 isolates of quinolone resistant Enterobacteriaceae were screened prospectively between October and December 2007.
Results: Of the isolates from the retrospective and prospective study, 4.4% and 1.7% carried the qnrA gene, respectively.
Conclusion: Enterobacteriaceae exhibiting quinolone resistance encoded by the plasmid-medicated qnrA gene were isolated from patients in our hospital but the incidence was low.

R2285 Genetic diversity of metallo-β-lactamases produced by carbapenem-resistant *Pseudomonas aeruginosa*

N. Rizk, S. Zaki (Alexandria, EG)

Objectives: To investigate the prevalence of metallo-β-lactamases (MBLs) among clinical isolates of carbapenem resistant (CR) *Pseudomonas aeruginosa* (*P. aeruginosa*), and to characterize their molecular types.
Methods: A total of 134 non-repetitive clinical isolates of *P. aeruginosa* were collected and tested for carbapenem resistance by disk diffusion method with imipenem and meropenem. Minimum inhibitory concentrations (MICs) were determined by the microdilution technique according to CLSI guidelines. CR isolates were screened for the presence of MBLs by imipenem EDTA double disk synergy test. Isolates with screen positive results were confirmed by EDTA inhibition of imipenem hydrolysis with their crude cell extracts, and by PCR using primers for detection of bla IMP-1, bla IMP-2, bla VIM-1, bla VIM-2, bla SPM-1, and intI1 genes.
Results: Of the total 134 *P. aeruginosa* clinical isolates included in this study, 75(56%) isolates were CR, among them, MBL phenotype was

detected in 30(40%) isolates by the double disk synergy test. EDTA sensitive hydrolysis of imipenem by crude cell extracts confirmed 14 (46.7%) isolates as MBL producers. PCR detected MBL genes among 15 (50%) isolates; 11(733%) isolates were positive for bla VIM-2, and 4(26.7%) isolates were positive for bla IMP-1. The integrase gene (intI1) was detected in all of the 15 genotypically MBL producing isolates. No isolate harbouring bla VIM-1, bla IMP-2, or bla SPM-1 was detected.
Conclusion: Continued screening for MBLs production among CR *P. aeruginosa* isolates will be essential to control dissemination by this important resistant mechanism, ensure optimal patient care, and the timely introduction of appropriate infection control procedures. PCR is an important step, since EDTA based screening tests can give false positive results.

R2286 MYSTIC 2007: European results

P. Turner (Macclesfield, UK)

Objectives: MYSTIC (Meropenem Yearly Susceptibility Test Information Collection) is a longitudinal global surveillance study that examines the activity of meropenem and other broad spectrum agents against clinically significant non-copy isolates. The data presented here cover the European 2007 results.
Methods: Isolates were collected from centres in Belgium, Croatia, Czech Republic, Finland, Germany, Poland, Spain, Sweden and the UK and tested by CLSI reference methodology (agar dilution or broth microdilution) and interpreted by associated criteria.
Results: See the table.

Organism	n	Percentage susceptibility*						
		MEM	IPM	CAZ	CPM	P+T	CIP	GM
S. aureus (MS)	391	100	100	34	98.8	97.4	88.5	92.9
CNS	131	89.3	91.6	48.1	84.2	89.3	70.2	84.6
Enterobacteriaceae	2008	99.4	98.1	84.3	94.6	83.3	82.6	84.9
K. pneumoniae	345	99.7	99.7	80.6	86.8	76.2	83.2	68.3
E. cloacae	276	100	98.9	72.5	97	81.5	93.5	88.6
P. aeruginosa	591	77	58.7	65.8	55.4	78.3	67.9	52.6
Acinetobacter spp.	129	73.6	80.6	65.8	55.4	78.3	67.9	52.6

*CLSI criteria.
MS: meticillin-susceptible; CNS: coagulase-negative staphylococci;
MEM: meropenem; IPM: imipenem; CAZ: ceftazidime; CPM: cefepime;
P+T: piperacillin + tazobactam; CIP: ciprofloxacin; GM: gentamicin.

Conclusions: Overall meropenem proved to have the broadest spectrum of activity followed by imipenem. Carbapenems are less active against *Pseudomonas aeruginosa* and *Acinetobacter* spp. than against Enterobacteraiceaea. The importance of surveillance programmes such as MYSTIC in tracking the occurrence of these types of strains is emphasised.

R2287 Trends in susceptibility over last five years for European isolates of *Pseudomonas aeruginosa* from the MYSTIC programme

P. Turner (Macclesfield, UK)

Objectives: The importance of longitudinal surveillance programmes such as MYSTIC (Meropenem Yearly Susceptibility Test Information Collection) is that they allow trends over a number of years to be examined and the information provided can aid in rational choice of antibiotics. The data presented here examines the percentage susceptibility of European isolates of *Pseudomonas aeruginosa* over the last five years.
Methods: Since 2003 nearly 5.500 European isolates of *P. aeruginosa* have been examined as part of the MYSTIC Surveillance Programme. The Minimum Inhibitory Concentrations (MICs) of a number of anti-pseudomonal compounds: meropenem, imipenem, ceftazidime, piperacillin+tazobactam, ciprofloxacin and gentamicin have been determined by CLSI methodology and their percentage susceptibilities at associated breakpoints determined yearly.
Results: See the table.

Year	n	Percentage susceptibility*					
		MEM	IPM	CAZ	P+T	CIP	GM
2003	1195	78.1	70.2	74.5	78.8	66.2	71.2
2004	1112	73.4	66.9	73.1	77.8	66.4	63.2
2005	1118	75.5	66	72.4	78.9	67	63.2
2006	1052	77.4	66.8	74	84.2	66.4	67.1
2007	591	77	58.7	65.8	78.3	67.9	52.6

*CLS1 criteria.
MEM: meropenem; IPM: impenem; CAZ: ceftazidime;
P+T: piperacillin + tazobactam, CIP: ciprofloxacin, GM: gentamicin.

Conclusions: With the possible exceptions of imipenem and gentamicin percent susceptibility for the compounds has been remarkably consistent over the last five years. Piperacillin+tazobactam although appearing to be the most effective compound has elevated results due to the very high susceptibility breakpoint (<64 mg/L) granted to it by CLSI for *P. aeruginosa*.

R2288 Antimicrobial susceptibility of isolates from various body sites in Europe: the T.E.S.T. Program

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

Objectives: Bacterial resistance patterns vary over time and geography. Surveillance studies help to identify those patterns to help guide therapy. The Tigecycline Evaluation Surveillance Trial (TEST) is an ongoing global surveillance study that serves to recognize current trends in resistance. This report evaluates differences in susceptibilities of strains from different body sites, collected in Europe 2004–2007.
Methods: 21,381 strains isolated from 8 body sites were collected and identified from 2004–2007 at 79 hospitals in 22 countries in Europe. MICs for each strain were determined per EUCAST guidelines at each facility using broth microdilution. MIC50/90 was analysed to identify any significant differences in antibiograms from different sources.
Results: Tigecycline (TIG) MIC50 values for almost all organism/specimen pairings were +/− 2 log2 dilutions of each other, with no single source giving a higher MIC50 than the others. The same was seen for TIG MIC90 values, which were also almost always within 1–2 log2 dilutions of the MIC50, except for *S. pneumoniae*, whose TIG MIC90 were 3–4 fold higher than the MIC50 for all specimen sources. Comparator drugs also generally showed no variability in susceptibility of isolates from various body sites. A notable exception was *E. faecium* vs. vancomycin, for which the MIC90 of blood isolates was significantly higher than other sources.
Conclusions: Bacteria isolated from more than 8 different body sites had generally similar antibiograms, with no isolates from any single source showing significantly different sensitivity patterns. TIG's broad spectrum of activity and consistently low MIC90/MIC50 ratios, including strains resistant to other drugs, may make it an excellent therapeutic option when treating infections often caused by strains refractory to treatment with other agents.

R2289 In vitro activity of tigecycline against pathogens isolated from lower respiratory infections – The Global T.E.S.T. Program 2007

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

Objectives: 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 Tigecycline Evaluation Surveillance Trial (TEST), a global longitudinal surveillance study, determined the in vitro activity of TIG and 13 comparators against Gram-positive and Gram-negative species isolated from lower respiratory infections.

Methods: 6,266 lower respiratory pathogens from 42 countries 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 guidelines.

Results: TIG activity against pathogens isolated from lower respiratory infections is shown in the table.

Organisms (n = 331)	%Sus	Tigecycline MIC (μg/ml)		
		MIC_{50}	MIC_{90}	Range
Acinetobacter spp. (n = 795)	na	0.5	1	⩽0.008–8
P. aeruginosa (n = 1,224)	na	8	>16	⩽0.008–>16
Enterobacter spp. (n = 879)	93.7	0.5	2	0.06–8
Enterococcus spp. (n = 82)	100	0.12	0.25	0.015–0.25
E. coli (n = 406)	100	0.12	0.25	0.03–2
Klebsiella spp. (n = 924)	94.9	0.5	2	0.03–16
H. influenzae (n = 951)	na	0.12	0.25	0.12–0.25
S. aureus (n = 663)	99.8	0.12	0.25	0.03–1
MRSA (n = 293)	99.7	0.12	0.25	0.03–1
S. pneumoniae (n = 680)	na	0.03	0.12	⩽0.008–0.25
S. marcescens (n = 548)	96.5	1	2	0.12–>16
S. agalactiae (n = 54)	100	0.03	0.12	⩽0.008–0.25

*na = breakpoints not available.

Conclusions: Tigecycline showed excellent inhibitory activity against all pathogens from lower respiratory infections in this study, with the exception of *P. aeruginosa*. Tigecycline demonstrated MIC90 values of ⩽0.5 μg/ml against Gram-positive pathogens (including resistant phenotypes) and MIC50 values of ⩽1 μg/ml against the Enterobacteriaceae and *Acinetobacter* spp., validating the potent inhibitory activity of TIG against these pathogens.

R2290 In vitro activity of tigecycline and comparators against vancomycin-resistant enterococci from western Europe

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

Objectives: Tigecycline (TIG), a member of a new class of antimicrobials (glycylcyclines), has been shown to have potent expanded broad spectrum activity against most commonly encountered species responsible for community- and hospital-acquired infections. The T.E.S.T. Program determined the in vitro activity of tigecycline compared to amoxicillin-clavulanic acid, piperacillin-tazobactam, levofloxacin, ceftriaxone, linezolid (LZD), minocycline (MIN), vancomycin (VAN), ampicillin (AM), penicillin (PEN), and imipenem against vancomycin-resistant enterococci (VRE) collected from 94 hospitals in 22 Western European countries from 2004 through 2007.

Methods: 76 VRE (15 *Enterococcus faecalis*, 61 *E. faecium*) clinical isolates were identified to the species level at each participating site and confirmed by the central laboratory. Minimum Inhibitory Concentrations (MICs) were determined by the local laboratory using supplied broth microdilution panels and interpreted according to EUCAST guidelines (tigecycline susceptible <0.25 mg/L for enterococci).

Results: %S of all VRE to TIG, LZD, and MIN were 100, 100, and 71.7, respectively. For *E. faecalis* strains, the most active drugs were TIG (100%), LZD (100%), PEN (93.4%) and AM (93.3%). For *E. faecium*, the three most active drugs were TIG (100%), LZD (100%), and MIN (78.7%).

Conclusions: TIG exhibited outstanding activity against VRE, inhibiting 100% of strains at ⩽0.25 mg/L with an MIC90 of 0.12 mg/L, which was 32-fold lower than the LZD MIC90 of 4 ml/L. The exceptionally broad spectrum of TIG, which includes many other multi-resistant Gram-positive and -negative bacteria in addition to VRE, should make it a very important addition to hospital formularies.

R2291 Antibiotic resistance of *Salmonella enterica* serovars in a paediatric hospital in Greece

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Objective: was to investigate the frequency, the distribution of various *Salmonella* serotypes and to determine their antimicrobial resistance profiles in a Paediatric Hospital.

Material and Methods: During 2004–2007 a total of 644 *Salmonella enterica* isolated from hospitalised children with gastroenteritis. The age balanced from 1 month to 14 years. The identification of *Salmonella* spp. was performed by classical methods and susceptibility to antibiotics was tested by a disk diffusion method. MICs were determined by automatic system VITEK 2 (bioMerieux – France). The serotypes were confirmed from the National Reference Centre for *Salmonella*.

Results: Out of 6762 stool specimen, the 1202 (17.8%) were found positive for enteropathogens.644 (53.5%) *Salmonella* spp. and 473(39%) *Campylobacter* were recovered.17 different serotypes were identified being: *S. enteritidis* the most predominant (74.7%) followed by S. Typhimurium (13.1%), S. Oranienburg (2.1%), S. Abony (1.5%) and the remaining strains(8.6%) belonged to a wide variety of serovars. All serotypes were susceptible to fluoroquinolones. The majority of *S. enteritidis* isolates were found susceptible to the following antibiotics: streptomycin (S), spectinomycin (Sp), chloramphenicol (C). Additional,increased resistance was observed to nalidixic acid 11% (MIC50,90 4, ⩾32) and less to ampicillin (A) 9.8% (MIC50,90 ⩽2, 16), co-amoxiclav (AMC) 3.5% (MIC50,90 ⩽2, 4), cefotaxime (CTX) 1% (MIC50,90 ⩽1) and trimethoprim-sulfamethoxazole (SXT) 2.6% (MIC50,90 ⩽1/19). In contrast high level resistant exhibited serotype *S. typhimurium* to tetracycline (Te) 10% (MIC50,90 ⩽2, 8), A 44% (MIC50,90 ⩽2, ⩾32), AMC 16% (MIC50,90 ⩽2, 16), CTX 4% (MIC50,90 ⩽1), SXT 4% (MIC50,90 ⩽1/19), Sp 8.7%, S 13% and C 8.7%.Multidrug resistance phenotype among *S. typhimurium* strains probably related with the appearance and spread of the phage type DT104, recently established in Greece.

Conclusions: All strains were susceptible to fluoroquinolones. Resistance to nalidixic acid presents an indicator for the detection of low-level resistance to ciprofloxacin. The high incidence of resistance to A in *S. enteritidis* recorded in past years, seems to reduce. *S. typhimurium* phage type DT104 presented the multidrug resistance phenotype(ACSSpSuTe). Presence of ESBLs was not observed. The emergence of resistance to CTX should be followed by an efficient surveillance for antimicrobial drug sensitivity.

R2292 High co-trimoxazole resistance by bacteria associated with oral lesions in a population of Ugandan HIV-infected and AIDS patients

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Objective: Opportunistic infections continue to cause a significant amount of morbidity and mortality in sub-Sahara African patients infected with HIV. This study was designed to assess the effect of prolonged routine cotrimoxazole prophylaxis on bacteria isolated from oral lesions in a population of HIV/AIDS patients in South Western Uganda.

Methods: Exactly 605 swabs (469 from females and 136 from males), were randomly collected from oral lesions of The AIDS Support Organization (TASO) HIV/AIDS patients in 4 Districts (Masaka-142, Rukungiri-152, Bushenyi with Mbarara-305) of Uganda. Sample processing for isolation, identification and antibiogram of bacteria was

done aseptically using standard Microbiological techniques. Randomized Block Design (RBD; alpha = 0.05) was used to compare both the prevalence and resistance of each bacterium between districts and bacteria within districts.
Results: Overall bacteria prevalence was highest in Mbarara/Bushenyi (50.4%) followed by Rukungiri (20.8%) and Masaka (20.3%). Between Districts, *Streptococcus mutans* (30.9%) from Mbarara/Bushenyi were the most predominant bacteria followed by *Proteus mirabilis* (16.4%) from Rukungiri. Within Districts, *Streptococcus mutans* (31.7%) from Masaka were the most prevalent followed by: *S. aureus* (11.3%). In Rukungiri, *Streptococcus mutans* (22.4%) was the most prevalent followed by *Proteus mirabilis* (16.4%). In Mbarara/Bushenyi, *S. mutans* (31.5%) was the most prevalent followed by: *S. aureus* (11.5%). Within districts, all bacteria isolates from Rukungiri were resistant (100%) to cotrimoxazole except *Branhamella catarrhalis* which showed (77.8%) resistance. In Mbarara/Bushenyi, the most resistant strains (100%) were *S. aureus*, *B. catarrhalis* and Non-haemolytic *Streptococcus*, followed by *Klebsiella pneumoniae* (84.6%). Absolute bacterial resistance (100%) in Masaka, were observed in 4 strains: *B. cerius*, *S. aureus*, *E. coli* and *B. subtilis*. There was a significant difference ($p < 0.05$) when the prevalence of different bacteria strains in the oral mucosa were compared within districts and between districts. There existed significant difference ($p < 0.05$) in the effect of the antibiotics when compared between different bacteria and within each bacterium
Conclusion: Prolonged use of cotrimoxazole prophylaxis could be associated with observed cotrimoxazole resistance by our bacteria isolates and could explain observed rebound of oral lesions with bacteria aetiology in the studied population.

R2293 Countrywide dissemination of CTX-M-3 type extended-spectrum β-lactamase in Turkey

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Objectives: To investigate the prevalance, of CTX-M type β-lactamases and properties of the genetic elements responsible for the spread of these among ESBL positive Enterobacteriaceae family which were isolated from 7 different hospitals between November 2002-July 2003. Clonal relationship between CTX-M producers was also evaluated.
Methods and Results: In this study blaCTX-M, blasSHV and blaTEM presence was sought by PCR in 70 (*E. coli* (n = 32), *K. pneumoniae* (n = 24), K. ornitholytica (n = 3), *E. cloacae* (n = 4), *E. aerogenes* (n = 4), *P. mirabilis* (n = 2), *S. sonnei* (n = 1)) isolates which were chosen randomly as 10 isolates per centre. blaCTX-M, blaTEM and blaSHV production rates were 75.7%, 81.4%and 91.4%, respectively. CTX-M prevalence varied between 50% (Uludag University School of Medicine) to 100% (Hacettepe University School of Medicine) among centres. Sequence analysis has shown that all of the CTX-M positive isolates included in this study produced CTX-M-3, ERIC-PCR results revealed no clonal relationship between the isolates from different centres although clusters of up to 4 isolates were detected within individual centres. Conjugation experiments revealed that plasmids with molecular sizes of 46–85 kb were responsible from the transfer of blaCTX-M.
Conclusion: As a result, the widespread appearance of CTX-M-3 is noteworthy in our country. According to ERIC-PCR results however clonal dissemination of CTX-M β-lactamase producers between different centres is not considered

R2294 Sensitivity profile to some new antibiotics of most frequently encountered bacterial species in infections in a hospital in Romania – Comparison 2003 vs. 2006

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Objective: To evaluate the evolution of bacterial resistance to some highly active, new antibiotics of the most frequently encountered bacterial species in the Emergency University Hospital, Iasi, Romania, in 2006 compared to 2003.
Methods: Retrospective evaluation of charts and records of hospital's Microbiology Laboratory (from January 1 to December 31, 2003 and from January 1 to December 31, 2006). Bacterial sensitivity to antibiotics was performed using disk diffusion method. For each antibiotic, results were expressed as: sensitive, intermediate or resistant, according to CLSI Guidelines. Comparison between resistance profile in 2003 and 2006 was performed using chi-squared test.
Results: In the investigated period, there were 1917 biological samples from hospitalised patients sent for identification and antibiogram. The bacterial species was identified in 76.00% of cases (1457 samples). The three most frequently encountered identified species were: *Staphylococcus aureus* (512 samples, 35.14% of identified species, 26.71% of total samples), *Acinetobacter baumannii* (178 samples, 12.22% of identified species, 9.29% of total samples) and *Pseudomonas aeruginosa* (162 samples, 11.12% of identified species, 8.45% of total samples). Comparative evaluation of resistance profile for *Staphylococcus aureus* revealed un-modified sensitivity to vancomycin (0 in 2003 vs. 2.21% in 2006) and linezolid (2.49% in 2003 vs. 3.32% in 2006) and increasing sensitivity to teicoplanin (from 36.1% in 2003 to 49.08% in 2006, $p < 0.01$) and cefpirom (from 26.14% in 2003 to 33.95% in 2006, $p < 0.05$). Percentage of meticillin-resistant strains increased from 51.03% in 2003 to 70.18% in 2006 ($p < 0.01$). *Acinetobacter baumannii* developed significant resistance to imipenem (from 18.29% in 2003 to 31.76% in 2006, $p < 0.01$). In case of *Pseudomonas aeruginosa*, we also noticed increasing resistance to imipenem (from 25% in 2003 to 33.78% in 2006, $p < 0.05$), while sensitivity to cefpirom remained un-modified (45.45% in 2003 and 45.94% in 2006).
Conclusions: *Staphylococcus aureus* isolated strains keep satisfactory sensitivity to vancomycine and linezolid (about 97%). Surprisingly, *Staphylococcus aureus* sensitivity to teicoplanin and cefpirom increased. Increasing resistance to imipenem of *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, as well as increasing percentage of MRSA strains are to be considered. Rational use of carbapenems in infections should be implemented.

R2295 European survey of antibacterial activity against *S. pneumoniae* from 2006–2007: focus on fluoroquinolones

D.J. Farrell, C. Couturier (London, UK; Paris, FR)

Objectives: To document the activity of a range of antibacterial agents, including levofloxacin, against strains of *Streptococcus pneumoniae* from patients with community-acquired respiratory tract infections (RTIs).
Methods: Isolates were collected between January 2006 and April 2007 in 17 European countries (156 centres) from patients with community-acquired RTIs either in the community or those hospitalised for less than 48 hours. In vitro antibacterial activities and susceptibilities of *S. pneumoniae* isolates were determined at a central laboratory using Clinical and Laboratory Standards Institute methodology and interpretive criteria.
Results: Overall, 10.3% (range: 0–33% across countries) of the 3026 *S. pneumoniae* isolates collected were resistant to penicillin, while 10.5% exhibited intermediate penicillin susceptibility. Rates of penicillin resistance varied widely across Europe, being over 10% in 7 countries (Eire, Finland, France, Hungary, Poland, Slovakia and Spain). Macrolide (erythromycin) resistance was observed in 26.0% of isolates overall, ranging from 3.8% in the Netherlands to 52.2% in Italy; 10/17 countries had over 20% of resistance. The overall susceptibility of *S. pneumoniae* isolates to the fluoroquinolones levofloxacin and moxifloxacin was high at 98.4% and 98.6%, respectively (range: 93.3–100% for both agents). The fluoroquinolones also demonstrated activity against isolates resistant to other classes of antibacterials: of the 312 isolates resistant to penicillin, 97.4% and 97.8% remained susceptible to levofloxacin and moxifloxacin, respectively, while the equivalent susceptibility rates against the 734 multidrug-resistant isolates (those resistant to $\geqslant 2$ antibacterial classes) were 95.4% and 95.6%.
Conclusions: This surveillance study demonstrates high levels of resistance to macrolides and β-lactams among *S. pneumoniae* isolates collected across Europe. In contrast, the fluoroquinolones remain

active against these isolates, including those resistant to several different antibacterial agents. Overall, susceptibility of *S. pneumoniae* to levofloxacin and moxifloxacin was high and comparable in all countries studied.

R2296 Pan-resistance to commonly used antibiotics and nonsusceptible to tigecycline *Acinetobacter baumannii* clinical isolates

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Objectives: To describe five panresistant to commonly used antibiotics and intermediately susceptible or resistant to tigecycline *Acinetobacter baumannii* clinical isolates.
Methods: We examined five *A. baumannii* isolates originated from equal numbered patients treated in different wards of our hospital. Four out of five patients were hospitalised in two different ICUs and the fifth in the neurosurgical ward. The sources of the examined isolates were blood (n = 1), vein catheter (n = 1) and bronchoalveolar excretions (n = 3). The identification of the isolates and the susceptibility testing were performed using the automated VITEK 2 system (Biomerieux, France). The resistance to colistin was confirmed by E-test method (AB Biodisk, Solna, Sweden) according to CLSI guidelines. The tigecycline MIC levels were also determined using E-test strips in accordance to the manufacturer's recommendations. Isolates having MIC values between 2 to <8 mg/L were considered as intermediately susceptible to tigecycline and MIC values ⩾8 mg/L as resistant to tigecycline according to breakpoints recommended by the British Society of Antimicrobial Chemotherapy [1].
Results: The examined five *A. baumannii* isolates were resistant to all aminoglycosides, b-lactams, carbapenems, monobactames, quinolones and colistin. The obtained tigecycline MIC values were 3 mg/L (blood and vein catheter samples, n = 2), 4 mg/L (bronchoalveolar excretions samples, n = 2) and 8 mg/L (bronchoalveolar excretions sample, n = 1).
Conclusion: The nonsusceptible to tigecycline and panresistant to the commonly used antibiotics *A. baumannii* isolates rapidly nullify the whole of our therapeutic armamentarium leading to therapeutic failure. It is very challenging to reach the knowledge of the tigecycline-nonsusceptible mechanisms of this bacterium and to discover newer antimicrobials to reduce the abilities of this dangerous pathogen.

Reference(s)

[1] R. Hope et al.: Determination of disc breakpoints and evaluation for Etests for tigecycline susceptibility testing by the BSAC method. Journal of Antimicrobial Chemotherapy (2007) 60, 770–774.

R2297 Biocide and antibiotic resistance in staphylococcal isolates from household pets

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Objectives: Biocides are synthetic compounds found in a wide range of clinical, veterinary and domestic cleaning products. Their widespread use is raising concerns regarding their impact on antibiotic resistance. The aim of the study was to compare biocide/antibiotic resistance in coagulase-positive staphylococci isolated from household pets and to investigate the possibility of any linkage between biocide/antibiotic resistance amongst the test strains.
Methods: A total of 136 strains of meticillin-resistant *Staphylococcus aureus* (MRSA), 48 strains of meticllin-sensitive *Staphylococcus aureus* (MSSA) and 96 strains of *Staphylococcus intermedius* were obtained from household pets in a U.K. veterinary diagnostic laboratory. Antibiotic susceptibility testing was performed on all isolates using the VITEK® (1) automated instrument (bioMérieux, France). Strains resistant to oxacilin, >2 mg/L were confirmed using the PBP2a latex kit (Oxoid, Basingstoke) to detect the gene product mecA. All isolates were screened for biocide resistance on Iso-sensitest (Oxoid, Basingstoke) media containing one of the following biocides at pre-determined concentration; benzalkonium chloride 4 mg/mL, cetrimide 5.5 mg/mL, chlorhexidine 4 mg/mL and triclosan 0.5 mg/mL.
Results: A generally higher level of resistance to antibiotics was seen amongst the MRSA isolates compared to the MSSA isolates. Surprisingly resistance to biocides (especially triclosan) was found to be more prevalent in MSSA than in MRSA. The chi-squared test was used to compare differences in antimicrobial susceptibility amongst the test strains. Statistical analysis using the kappa coefficient was used to determine whether a relationship exists between resistance to the antibiotics and biocides tested. No significant linkage was found between antibiotic and biocide resistance.
Conclusion: This study provided no evidence to link biocide/antibiotic resistance in coagulase-positive staphylococci isolated from household pets.

R2298 *Campylobacter* infections in a central hospital, West-Tallinn, Estonia in 2000–2005

I. Zolotuhhina, R. Voiko, K. Kirs (Tallinn, EE)

Objectives: *Campylobacter* species are the most common cause of bacterial gastroenteritis in many developed countries. In the last years there has been a considerable increase in the incidence of *Campylobacter* infections diagnosed in West-Tallinn Central Hospital. The purpose of the present study was to show the significance of *Campylobacter* in the aetiology of bacterial diarrhoea and to give some strain characteristics of the isolated bacteria.
Methods: During the study period all patients admitted to the hospital with the symptoms of gastroenteritis were routinely examined for the presence of *Campylobacter* spp. in stools. The isolatoin and identification procedures included conventional bacteriological methods. *Campylobacter jejuni* strains were differentiated on the basis of the hippurate hydrolysis tested with sodium hippurate tablets (Rosco Diagnostics). Susceptibility to erythromycin (ERY), ciprofloxacin (CIP) and tetracyclin (TE) was determined by disc diffusion and Etest methods perfomed on Mueller-Hinton agar with 5% sheep blood incubated at 42ºC for 24 hours in microaerobic atmosphere.
Results: During the period of 2000–2005 the aetiological role of *Campylobacter* spp. as a cause of bacterial gastroenteritis was increasingly growing from 14.7% in 2000 to 35.4% in 2005 of all bacteriologically confirmed cases. In 2003 and 2004 campylobacteriosis was diagnosed more frequently than salmonellosis (33.5% versus 24% in 2003 and 30.5% versus 16% in 2004). *C. jejuni* was isolated in 93% to 100% of all cases in different years. Among the patients children under 5 years old constitued 44% to 56%. Another big group was young people from 20 to30 years old (10–16% of all cases). 276 strains were tested for antimicrobial susceptibility. The results showed that most of the isolates were susceptible to TE and ERY. Reduced susceptibility to CIP increased from 3.1% in 2000 to 19.3% in 2005. Co-resistance to 2 drugs was also detected.
Conclusions: *Campylobacter* is one of the most important intestinal pathogens specially among children of early age. Resistance to commonly used antibiotics is increasing. ERY remains relatively effective for the treatment of campylobacteriosis. The number of *Campylobacter* strains with reduced susceptibility to quinolones is rapidly growing.

R2299 Tigecycline in vitro activity against vancomycin-resistant enterococci in the U.S. analysed by CDC Regions

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Background: The percentage rates of vancomycin-resistant *Enterococcus* spp. (VRE) vary by country and region. Tigecycline, a new glycylcycline, has been shown to have potent activity against commonly encountered species, including those with resistant phenotypes. The purpose of this study was to determine regional variations, if any, and the current activity of tigecycline (TIG) against VRE in the United States.

Methods: 622 clinically relevant isolates of vancomycin-resistant *Enterococcus faecalis* and vancomycin-resistant *Enterococcus faecium* were collected from patients in 193 hospitals across the United States from 2004 to 2007. MIC's were determined by broth microdilution and interpreted following CLSI guidelines. Regions are defined by the CDC.
Results: Results are summarised in the table.

Region	n	Tigecycline MIC_{90} (μg/ml)	% Susceptibility
All Regions	622	0.12	100
East North Central	133	0.12	100
East South Central	34	0.12	100
Middle Atlantic	196	0.12	100
Mountain	16	0.06	100
New England	19	0.12	100
Pacific	35	0.12	100
South Atlantic	115	0.12	100
West North Central	31	0.12	100
West South Central	43	0.6	100

Conclusions: Tigecycline demonstrated consistant potent activity against VRE in the United States, regardless of region of isolation, with an overall MIC90 of 0.12 μg/ml. The exceptionally broad spectrum of tigecycline, which includes many other multi-resistant Gram-positive and Gram-negative bacteria in addition to VRE, will make it a very attractive addition to hospital formularies.

R2300 Susceptibility patterns of tigecycline and comparators from clinical isolates in Spain and Portugal 2004–2007

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Background: Development of bacterial resistance continues to cause concern world wide, but the availability of newer agents offers clinicians options for therapy. Tigecycline 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 Iberia from 2004 to 2007 were evaluated for susceptibility to several commonly used antimicrobials.
Methods: 1710 strains were collected and identified at 9 sites in Spain and Portugal. MICs were determined at each site using EUCAST guidelines on microdilution panels.
Results: The tables summarise results.
Conclusions: Tigecycline's in vitro activity was comparable to or greater than commonly prescribed antibiotics against both Gram-negative and Gram-positive pathogens, including resistant phenotypes. Tigecycline's MIC90 of 1 μg/ml was the lowest of all comparator agents in this study.

Drug	Enterobacteriaceae (n = 736)		*Acinetobacter* spp. (n = 116)	
	%Sus	MIC_{90}	%Sus	MIC_{90}
Tigecycline	97.8	1	na	1
Amikacin	98.8	4	49.1	>64
Cefepime	88.9	2	31	>32
Ceftazidime	0	16	25.9	>32
Imipenem	99.7	1	56.9	>16
Levofloxacin	86.4	4	20.7	>8

Drug	*S. aureus* (n = 216)		*Enterococcus* spp. (n = 132)	
	%Sus	MIC_{90}	%Sus	MIC_{90}
Tigecycline	100	0.25	100	0.12
Levofloxacin	78.7	8	62.1	>32
Linezolid	100	2	100	2
Minocycline	100	0.5	53	>8
Vancomycin	100	1	99.2	2

R2301 Effectiveness of an active intervention to improve notification in an antimicrobial resistance surveillance system in a developing country

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Background: Surveillance is the first step for antimicrobial resistance contention. Adherence to periodic notification is an important problem in surveillance systems, our work describe the impact of an active intervention to improve this notification in the Antimicrobial Resistance Surveillance System (ARSS) in Bogota-Colombia.
Methods: Created in 2004, all institutions of high and intermediate complexity with an institutional laboratory of microbiology in the city were potential eligible. Training in software Whonet and quality control of the laboratories was realised. Initially in 2005 we designed a passive system of digital capture of information, across Internet. Follow-up of notification was done with especial indicators. In 2006 there we designed an active intervention composed by retraining in Whonet, incorporation of variables of nosocomial infections, double sending of information (laboratory and infections committee), calls and mails, continuous assessment of the notification and visits to institutions problem. Change in notification was evaluated with a Wilcoxon test.
Results: 52 institutions trained since 2004 initiated notification in 2005, proportion of gross notification was 68.6% and timely notification was 58.3%. In 2006 after the intervention, statistical difference in notification percentages ($p < 0.002$), were observed 96.1 and 95.1% respectively (Figure 1). ARSS has generated trends in bacterial resistance for hospital system in Bogota, alert system, technical advices and bulletins to improve technical choices.
Conclusions: Our Active interventions to improve the notification had a significant impact in the system. The ARSS has showed a more exact panorama of the antimicrobial resistance in our city.

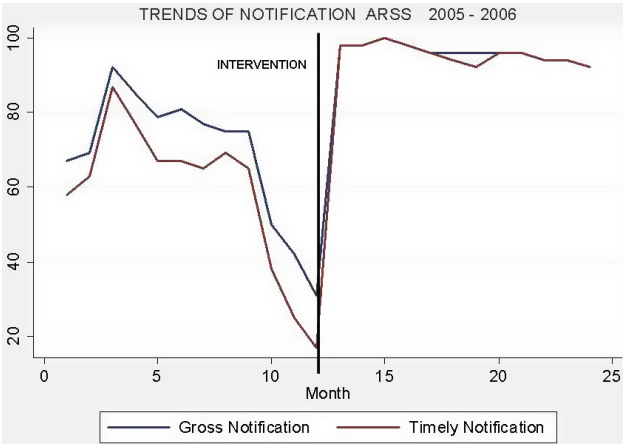

Figure 1

R2302 Wide variation in rates of ESBL production among *Escherichia coli* causing bacteraemia in the Asia-Pacific region: A SENTRY Program Report

J.M. Bell, J.D. Turnidge, R.N. Jones (Adelaide, AU; North Liberty, US)

Objective: Extended-spectrum β-lactamase (ESBL) producing *E. coli* have been reported with increasing frequency in both hospital- and community-acquired infections worldwide. We examined blood culture isolates of *E. coli* from our APAC region to determine the prevelance of ESBL and multidrug-resistant (MDR) strains.
Methods: Blood culture isolates were consecutively collected from 42 medical centres in nine countries in the APAC region. All isolates were tested against a wide range of antimicrobial agents using CLSI methods and broth microdilution panels (TREK Diagnostics). MDR was defined as resistant (R) to greater than three drug classes apart from cephalosporins. ESBL phenotypes were defined as those strains demonstrating elevated MICs (>1 μg/ml) to ceftazidime or ceftriaxone or aztreonam (CLSI).
Results: A total of 1,621 isolates were received. *E. coli* was the commonest bacteremic species overall and accounted for 26% of isolates (range of 39.1% Taiwan to 7.5% Thailand). ESBL percentages ranged from 0 to 75. In India and mainland China MDR was detected in the great majority of strains, while in other countries, MDR was present in approximately 50%. All strains were susceptible to carbapenems and polymyxins.

Country (no. tested)	Centres	Rank[a]	EBSL, N (%)	MDR, N (%)
Australia (60)	5	1	1 (1.7)	3 (5.0)
Hong Kong (12)	1	1	0	1 (8.3)
India (67)	10	2	50 (74.6)	43 (64.2)
Korea (39)	3	1	5 (12.8)	11 (28.2)
Mainland China (181)	10	1	109 (60.2)	116 (64.1)
Philippines (22)	1	1	3 (13.6)	6 (27.3)
Taiwan (36)	2	1	3 (8.3)	7 (19.4)
Thailand (3)	2	4	2	2

[a]Rank of frequency of *E. coli* from blood cultures.

Conclusions: ESBL-producing *E. coli* are very prevalent among bacteraemia isolates from India and mainland China, but still uncommon in Australia. Many of these isolates were also R to multiple other drug classes limiting therapeutic options.

R2303 Occurrence and resistance in pathogens from hospitalised patients with pneumonia in the Asia-Pacific region: a SENTRY Program Report (2006)

J.M. Bell, J.D. Turnidge, R.N. Jones (Adelaide, AU; North Liberty, US)

Objective: As part of the SENTRY Antimicrobial Surveillance Program we examined clinical isolates from the Asia-Pacific region to determine the occurrence and resistance (R) rates of pathogens from hospitalised patients with pneumonia.
Methods: Up to 50 consecutive isolates were collected from each of 42 medical centres in nine countries in the Asia-Pacific region centre during 2006. All isolates were tested at a central monitoring laboratory against a wide range of antimicrobial agents using CLSI methods and broth microdilution panels (TREK Diagnostics). ESBL phenotypes were defined by elevated MICs (>1 μg/ml) to ceftazidime or ceftriaxone or aztreonam.
Results: A total of 1,642 isolates were collected. Gram-negative pathogens (GN) were dominant in Indonesia (99%), Thailand (97%), India (94%), Philippines (88%), Hong Kong (86%), mainland China (84%), and Singapore (80%). *Acinetobacter* spp. was the most frequent isolate in these countries, accounting for 63% and 75% of all isolates in Singapore and Thailand, respectively. Only Korea (70%), Taiwan (65%) and Australia (51%) had <70% GN. ESBL phenotypes were present in 70, 50 and 20% of *E. coli*, *Klebsiella* spp. and *P. mirabilis*, respectively. In Australia, *S. pneumoniae* and *S. aureus* accounted for 45% of strains. Carbapenem-resistant (CP-R) *Acinetobacter* spp. (class D/B) was >60% in Hong Kong, Korea, Singapore, Taiwan and Thailand; and also common in India (52%) and China (25%). CP-R *Pseudomonas* spp. was also common with >60% of strains R from Taiwan, Korea, Singapore resistant. One *E. cloacae* from India was imipenem-resistant. No CP-R isolates were detected in Australia. All staphylococci were susceptible to vancomycin, linezolid and quinupristin/dalfopristin. No vancomycin-R enterococci were detected.
Conclusions: There are very high rates of R in pathogens causing pneumonia in hospitalised patients in most countries in the Asia-Pacific region.

Molecular epidemiology of resistance genes, strains & serotypes

R2304 Molecular detection of ESBL-producing Enterobacteriacee in northeastern Italy

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Objectives: The prevalence of extended spectrum β-lactamases (ESBL), especially the CTX-M type, increased dramatically during the past 10 years. Moreover, a molecular epidemiology shift was observed in Europe from TEM and SHV types to the predominant CTX-M enzyme. Among ESBL producers, *E. coli* emerged as a cause of urinary tract infections either in hospital or community settings.
Aim of this study was to assess by molecular methods the prevalence of different enzymes among ESBL-producing Enterobacteriacee in Trieste, North-Eastern Italy.
Methods: During 2007 we analysed 80 ESBL-producing bacteria (70 *E. coli*, 5 *K. pneumoniae* and 5 *K. oxytoca*) isolated from urinary tract (n= 60), respiratory tract (n = 6), blood (n = 5) or various other sites (n = 13). 57 strains were isolated from hospitalised patients and 23 from outpatients.
E. coli was recovered in 65 cases (92.8%) from adult patients, 43 hospitalised and 22 outpatients, while the majority of *Klebsiella* spp. (7 out of 9) were isolated in paediatric patients.
Identification and screening for ESBL was performed with VITEK 2 ID-GN and AST-N041cards. All isolates tested positive were confirmed by ESBL Etest strips with cefotaxime/ cefotaxime+clavulanate and ceftazidime/ ceftazidime+clavulanate. The confirmed isolates were examined by PCR for the presence of ESBL-encoding genes blaTEM, blaSHV and blaCTX-M, according to Mugnaioli et al (Antimicrob Agents Chemother, 2006).
Results: Of the 70 ESBL-producing *E. coli*, 61 (87.1%) were positive for blaCTX-M, alone (30, corresponding to 42.8%) or in combination with blaTEM (31, 44.3%). Only 8 isolates showed other resistance genes (1 blaTEM alone, 1 blaSHV and 7 with associated blaTEM and blaSHV). In *K. pneumoniae*, bla SHV was present in all 5 isolates, alone (n = 1) or associated with blaTEM (n = 2) or blaTEM and CTX-M (n = 2). In *K. oxytoca*, 3 strains carried blaSHV and 1 blaTEM, while bla CTX-M was absent; only one strain resulted negative for all the ESBL tested.

	n.	TEM	SHV	CTX-M	TEM+ SHV	TEM+ CTX-M	TEM+ SHV+ CTX-M	Negative
E. coli	70	1	1	30	7	31	0	0
K. pneumoniae	5	0	1	0	2	0	2	0
K. oxytoca	5	1	3	0	0	0	0	1
Total	80 (100%)	2 (2.5%)	5 (6.3%)	30 (37.5%)	9 (11.3%)	31 (38.8%)	2 (2.5%)	1 (1.2%)

Conclusion: In our area, ESBL-producing *Klebsiella* spp. are rarely detected, while *E. coli* is becoming the predominant ESBL-producing

species, as reported in other Italian areas and other European countries. Among ESBL-positive Enterobacteriacee, CTX-M type is most frequently encountered, involving about 87% of *E. coli* strains. Finally, ESBL resistance is not exclusively found in hospital settings, but it is spreading even in the community, involving especially elderly patients with urinary tract infection.

R2305 Evaluation of resistance mechanisms and epidemiology of *Acinetobacter* isolates from intensive care units in a Turkish university hospital

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Objectives: Multiresistant *Acinetobacter* spp. (MR-A) are responsible for the increasing number of nosocomial infections. Local epidemiolgy has outmost importance for determining therapeutic options in patients infected with MR-A.
Methods: We examined one-hundred-fifty *Acinetobacter baumannii* strains isolated from blood cultures between 1997 and 2007 at the Hacettepe University Medical School. All of the strains were isolated from nosocomial infections. In vitro activity of netilmicin, amikacin, ciprofloxacin, imipenem, piperacillin, cefoperazone/sulbactam, tetracycline and colistine, were evaluated by the minimal inhibitor concentration (MIC) technique using broth microdilution method according to CLSI criteria. Presence of PER-1 and other β-lactamase profiles (IMP-1, VIM-1, SPM and OXA-58) of the strains were determined using PCR. DNA fingerprinting by PFGE was used for epidemiological analysis of the strains.
Results: Resistance rates are given in the Table. Sixty (40%) of 150 isolates were found positive for PER-1 gene. No isolates with IMP-1, VIM-1 and SPM genes were determined. OXA-58 was detected in 75 (50.0%) of 150 isolates. Eight distinct patterns were detected with genomic fingerprinting by macrorestriction analysis with PFGE.

Table. In vitro susceptibility of *Acinetobacter* isolates

	MIC_{50}	MIC_{90}	Range	Susceptibility Results (n = 150)					
				Sensitive		Intermediate		Resistant	
				n	%	n	%	n	%
Amikacin	128	256	0.50–>256	39	26.0	4	2.7	107	71.3
Netilmicine	2	8	<0.125–256	137	91.3	9	6.0	4	2.7
Ciprofloxacin	32	128	<0.125–>256	30	20.0	3	2.0	117	78.0
Imipenem	32	>256	<0.125–>256	46	30.7	18	12.0	86	57.3
Piperacillin	>256	>256	1–>256	13	8.7	15	10.0	122	81.3
Cefoperazone/sulbactam	32	128	<0.125–>256	60	40.0	28	18.7	62	41.3
Tetracycline	8	256	<0.125–>256	63	42.0	42	28.0	45	30.0
Colistine	0.25	0.50	<0.125–1	150	100.0	–	–	–	–

Conclusions: In a multiresistant population of *Acinetobacter* isolates, PER-1 and OXA-58 were frequently detected. Along with the other possible mechanisms, these two ESBL-type of enzymes seem to contribute to multiresistance pattern of our *Acinetobacter* isolates.

R2306 Antimicrobial susceptibility and macrolide-resistance mechanisms among clinical isolates of *Streptococcus pneumoniae* in Bulgaria, 2006–2007

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In total, 186 clinical isolates of *S. pneumoniae* collected from four University Hospitals in Bulgaria were analysed to determine their susceptibility to 13 antimicrobials by the means of MIC testing and their mechanisms of macrolide resistance. Of these pneumococci, 46.8% were penicillin-nonsusceptible (28.5% penicillin-intermediate and 18.3% penicillin resistant strains). Erythromycin resistance was found in 30.1% of the isolates and 20.4% were clindamicin resistant. The susceptibilities to other antimicrobial agents were: amoxicillin (95.7%), amoxicillin-clavulanic acid (95.7%), cefuroxime sodium (73.1%), cefotaxime (93%; all the rest 7% were intermediately resistant), chloramphenicol (89.2%), tetracycline (67.2%), ciprofloxacin (93%), trimethoprim-sulfamethoxazole (45.7%), rifampin (100%), and vancomycin (100%). Among 55 erythromycin-resistant *S. pneumoniae* isolates, strains harbouring erm(B) genes (n = 33; 60%) were found to be predominant over strains with mef(A)/mef(E) genes (n = 17; 30.9%). Five isolates (9.1%) carried both erm(B) and mef(A)/mef(E) genes. We observed the prevalence of erythromycin ribosome methylase macrolide resistance mechanism than macrolide efflux compared to isolates collected in 2001–2005. Serotyping of *S. pneumoniae* isolates suggested that serotype (or serogroup) 14, 6 and 19 were predominant (%) among erythromycin-resistant strains. Among mef(A)/mef(E) positive isolates serotype 14 was predominant, among erm(B) positive isolates serogroups 6 and 19 were the most prevalent.

R2307 Detection of ambler class A, B, and D in multidrug-resistant *P. aeruginosa* from central regions of Korea

S.H. Koo (Daejeon, KR)

Objectives: Outbreaks of multidrug-resistant *P. aeruginosa* give rise to significant therapeutic challenges in treating nosocomial infections. In order to acquire data useful in preventing the extension of multidrug-resistant *P. aeruginosa*, we identified genetic types and frequencies of ESBL of Ambler class A and D, and carbapenemase of class B and D, from a medical centre in central area of Korea.
Methods: A total of 37 selected strains of multidrug-resistant *P. aeruginosa* isolated from March 2006 to May 2007 were analysed. For the detection of multi-resistance, broth dilution methods were carried out according to the CLSI guidelines. The strains showed resistance to imipenem/meropenem, piperacillin, and ciprofloxacin. MBL-producing strains were selected by the inhibitor-potentiated disc diffusion (IPD) method. Genotype analysis of β-lactamase was carried out using PCR and DNA sequencing. Clonality was tested using enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC PCR) in 19 OXA-10 producing strains.
Results: Twenty-nine of 37 isolates harboured blaOXA-10 (56.8%), blaOXA-2 (18.9%), and blaOXA-1 (5.4%), respectively. IMP-1 producing strain was the only one case harbouring blaOXA-1. The strains haboring ambler class A β-lactamase or class D carbapenemase were not detected. One strain revealed a combination of IMP and blaOXA-1, while the other had OXA-2 and OXA-10. The strains-producing Ambler class D β-lactamase showed significantly higher resistances in aminoglycoside, compared to non-producers. The ERIC PCR pattern of the 19 OXA-10 producing strains indicated that the isolates were closely related in terms of clonality.
Conclusion: OXA type β-lactamases of multidrug-resistant *P. aeruginosa* are widespread, but their extended-spectrum derivatives and carbapenemases are rare in central regions of Korea. The strains harbouring OXA β-lactamase were defined to have an effect on cross-resistances by their significantly higher resistance against aminoglycoside. To prevent further spreading of the multidrug-resistant *P. aeruginosa*, consequent monitoring and effective clinical policies are required.

R2308 Characterisation of extended-spectrum β-lactamases in clinical *Escherichia coli* isolates in a Spanish hospital

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Objective: To characterize the genes encoding ESBLs, their genetic environments and the phylogenetic groups of broad-spectrum cephalosporin-resistant clinical *Escherichia coli* isolates recovered in a Spanish hospital.
Methods: Seventy-seven broad-spectrum cephalosporin-resistant *E. coli* isolates recovered in the Hospital San Pedro (Logroño, Spain) during 2004–2007, presented a positive ESBL-screening test and were included in this study. Genes encoding CTX-M, SHV and TEM type β-lactamases were analysed by specific PCR-RFLP and/or PCR-sequencing. The

genetic environment of blaCTX-M genes were characterised by PCR-mapping and the phylogenetic groups of ESBL-positive isolates were determined by PCR (chuA, tspE4.C2, and yjaA genes).
Results: The origin of the 77 ESBL-positive isolates were: urine (77%), blood (5%), wounds (5%), others (13%). The bla genes encoding the following ESBLs were detected in our 77 *E. coli* (number of isolates): blaCTX-M-14 (46), blaCTX-M-14 plus blaSHV-12 (3), blaCTX-M-67 (1), blaCTX-M-15 (2), blaCTX-M-9 (7), blaCTX-M-9 plus blaSHV-12 (1), blaSHV-12 (10), and unknown mechanisms (7). Thirty-seven of the 70 CTX-M and SHV-positive ESBL-isolates harboured a TEM β-lactamase. The surrounding regions of blaCTX-M genes detected in our isolates were as follows (number of isolates): ISEcp1-blaCTX-M-14-IS903 (38), blaCTX-M-14-IS903 (4), ISEcp1-blaCTX-M-14 (1), ISCR1-blaCTX-M-9-orf1005 (7), blaCTX-M-9-orf1005 (1), ISEcp1-blaCTX-M-67-IS903 (1), and blaCTX-M-15-orf477 (2 isolates). Forty-eight per cent of the ESBLs-positive isolates were classified into the A or B1 phylogenetic groups, and 52% into the B2 or D phylogenetic groups.
Conclusions: The ESBLs of the CTX-M-type are predominant in this hospital (80%), mainly of the CTX-M-9 group (77%), associated to SHV-12 (10%). Different genetic environments associated to ISEcp1 and ISCR1 were detected among our blaCTX-M-positive isolates.

R2309 Staphylococcal cassette chromosome mec profiles of *Staphylococcus aureus* isolated in 13 Korean hospitals

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Background: There has been a nationwide increase in infections caused by *Staphylococcus aureus* resistant to multiple antimicrobial agents in Korea. The prevalence of MRSA in *S. aureus* in tertiary-care hospital is nearly 70% since the mid-1990s. We assessed the staphylococcal cassette chromosome mec (SCCmec) profiles and Panton-Valentine leukocidin (PVL) on Korean nationwide collection of *S. aureus* strains from tertiary-care hospitals in Korea.
Methods: We collected a total of 250 clinical isolates of *S. aureus* from 13 clinical laboratories over the country during 2 months in 2007. Meticillin-resistant *S. aureus* (MRSA) isolates were confirmed by oxacillin susceptibility test and PCR detection of mecA gene. SCCmec typing was performed by multiplex PCR. Panton-Valentine leukocidin (PVL) gene was also detected by PCR.
Results: Among 250 isolates, 175 strains (70.0%) were MRSA. Of the 175 MRSA isolates, 0 (0%), 129 (73.7%), 31 (17.7%), and 15 (8.6%) isolates belonged to SCCmec types I, II, III, and IV, respectively. The most prevalent subtype was SCCmec type IIb (n = 109, 62.3%). SCCmec type IVa strains known as community-associated MRSA in Korea were 10 (5.7%) isolates. None of the MRSA isolates were PVL-positive.
Conclusions: SCCmec type II strains are the most frequent SCCmec type in Korea, followed by SCCmec types III and IV. SCCmec subtype IIb strains were detected in 12 hospitals throughout the country and have increased in Korea, compared to previous study (62.3% in 2007 vs. 33.7% in 2002). SCCmec type I strains or PVL-positive *S. aureus* strains were not detected, though they are often isolated in Europe.

R2310 A new association of known genetic elements in a Tn916-family transposon carrying tet(M), erm(B), and mef(E)

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Objectives: In *Streptococcus pneumoniae*, macrolide resistance can be conferred by two distinct genes, erm(B) and mef. Pneumococcal isolates, carrying both erm(B) and mef, have been increasingly observed in South Africa, in Asian countries, and in the United States. Dual-gene isolates are rarely found in European countries, although one such strain was described in Hungary. The genetic elements carrying macrolide resistance genes in this Hungarian pneumococcal isolate were characterised.
Methods: Antimicrobial susceptibility to penicillin, erythromycin, clindamycin, and tetracycline were determined by Etest. PCR assays were performed: to detect the presence of the resistant determinants mef(E), erm(B), and tet(M), to define the genetic organisation of the elements and a possible association among them, and to explore the chromosomal location.
Results: In the multiresistant pneumococcal isolate from Hungary belonging to serotype 19F, the resistance determinants mef(E), erm(B), and tet(M) were identified. Analysis of the genetic elements carrying the resistance genes performed by PCR mapping and by comparing the amplicons obtained with those obtained with control strains, showed that mef(E) was found included in the mega element, erm(B) in a Tn917-like transposon, and tet(M) in a Tn916-like transposon. Since descriptions in literature were reported where Tn2009 harboured mega in orf6 of Tn916, and Tn3872 harboured Tn917 in orf9 of Tn916, PCRs targeting these two ORFs were performed. The results obtained confirmed the insertion of mega and Tn917 in orf6 and orf9, respectively. The linkages among the three elements, confirmed by PCR, indicated the presence of a novel composite transposon. The chromosomal location of this novel composite transposon was identical to that previously found in other Tn916-family transposons.
Conclusions: The multiresistant *S. pneumoniae* isolate from Hungary harbours a novel Tn916-family transposon carrying mef(E), erm(B), and tet(M). This transposon corresponds to a new modular combination of three well-known genetic elements: mega, Tn917 and Tn916.

R2311 Panton-Valentine leukocidin positive, t008 spa-type meticillin-resistant *Staphylococcus aureus* in a Romanian western county hospital

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Objectives: To preliminary evaluate the possible hospital isolation of the USA300 genotype community-associated meticillin resistant *Staphylococcus aureus* strain in a Romanian Western county hospital.
Methods: *Staphylococcus aureus* strains were identified using conventional, API and automate methods (VITEK 2). Meticillin-resistance was screened by the cefoxitin disc method and confirmed by mecA PCR. MecA gene was detected by a triplex PCR variant optimised in our laboratory, detecting concomitantly the nuc gene coding for the thermonuclease and the lukS/F genes coding for the Panton Valentine leucocidin (PVL). PFGE was performed according to the HARMONY protocol. Spa-typing was done by DNA sequencing analysis of the protein A (spa) gene hypervariable region according to the SeqEUNet.org recommended protocols.
Results: 91 *S. aureus* strains have been analysed at the National Nosocomial Infections and Antimicrobial Resistance Laboratory, National Institute of Research-Development for Microbiology and Immunology "Cantacuzino", Bucharest, including the SeqEUNet.org 2006/2007 6 months study and all ICU strains received during the January 2006–June 2007 interval. Among these, 4 strains received from a Romanian Western county hospital isolated in January/February 2007 harboured the t008 spa-type, similar with the spa-type of the USA300 community associated genotype, which has been recently discovered in Germany (Witte et al. – JAC Advance Access published online on October 13, 2007). Two of the 4 strains were MRSA. These MRSA strains proved lukS/F positive. The two strains were isolated from 2 patients, 3 months and 14 years old, respectively, receiving healthcare in the ICU of the Western county Romanian hospital. Both t008, PVL positive MRSA strains harboured the same pulsotype. The Romanian strains pulsotype looks very similar with the USA300 pulsotype (Johnson et al – Em. Inf. Dis., 13, 8, 2007).
Conclusion: The t008 spa-type MRSA strains received from a Romanian Western county hospital could be related with the USA300 genotype strain based on the presence of lukS/F genes, spa type and PFGE profile. Further molecular studies, including SCCmec typing and searching for the arginine catabolic mobile element (ACME) determinant will help us to clearly establish the clonal relatedness of these 2 identical Romanian strains with the USA300 genotype community associated strain.

R2312 Characterisation of carbapenem-resistant *Acinetobacter baumannii* carrying different OXA-type carbapenemases from two hospital outbreaks in Berlin, Germany

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Methods: Non-duplicate *A. baumannii* isolates were collected between June, 2005 and November, 2006 from clinical specimens from patients hospitalised on different wards in hospital 1 and between March and June, 2007 from patients hospitalised in hospital 2, respectively. Species identification was confirmed by gyrB multiplex PCR and ARDRA. Antimicrobial susceptibility was determined by VITEK 2 and agar dilution methods. Detection of IMP-, VIM- and OXA-type carbapenemases was performed by PCR. Epidemiological typing of isolates was performed by RAPD and PFGE.
Results: Forty-nine and fourteen *A. baumannii* isolates were collected from hospitals 1 and 2, respectively. Data on the CR isolates is summarised in the table. Molecular typing revealed that isolates from hospital 1 had 6 PFGE patterns (A-D, G, H), of which pattern A was the most prevalent. Patterns A1-A4 (5 isolates) were closely related to pattern A. Isolates with patterns A2, A3, B and C were isolated once each and were carbapenem-sensitive. Hospital 2 yielded two PFGE patterns, I and J, of which I was the predominant strain. All but the 4 isolates from hospital 1 mentioned above were resistant to all penicillins, cephalosporins, ciprofloxacin, gentamicin, tobramycin, amikacin, imipenem, and meropenem, and remained susceptible only to colistin, i.e. isolates were pandrug-resistant. All *A. baumannii* isolates carried the intrinsic OXA-51-like gene. With the exception of PFGE type H, all CRAb isolates had either an OXA-23-like, OXA-58-like or OXA-24-like gene. One isolate with PFGE pattern G carried an OXA-58-like and an OXA-24-like gene. PFGE type H was negative for IMP- or VIM-type metallo carbapenemases.
Conclusion: Carbapenem resistance was associated with the spread of distinct clonal strains that carried an OXA-type carbapenemase. In hospital 1 the predominant strain had closely-related carbapenem-sensitive isolates. Resistance was associated with this strain acquiring an OXA-23-like or OXA-58-like gene while one isolate has acquired both of these genes. Molecular typing of all the CRAb isolates reveals that there was no inter-hospital spread of CRAb, but rather it is the dissemination in two different locations of strains that have acquired an OXA-carbapenemase.

	Hospital 1						Hospital 2	
PFGE-pattern	A	A1	A4	D	G	H	I	J
No. of isolates	30	2	1	8	2	2	13	1
with OXA-23	30	2						
with OXA-58			1	8	1		13	
with OXA-24					2			1
Imipenem MIC (μg/ml)	16–64	32–64	32	16–32	⩾256	8	16–32	64

R2313 Mechanism of resistance to antimicrobial agents in *Shigella* strains isolated from stools among children under 5 years of age in southern Mozambique

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Objective: To characterise the molecular mechanisms of antimicrobial resistance of *Shigella* spp. strains isolated from stools among children under 5 years of age in Southern Mozambique
Methods: *Antimicrobial susceptibility testing*: *Shigella* strains isolated from children <5 years of age complaint with diarrhoea in Manhiça District Hospital (MDH), Southern Mozambique were tested for antimicrobial susceptibility to ampicillin (Amp), chloramphenicol (Chl), cotrimoxazole (SXT), tetracycline (Tet), nalidixic acid (Nal), Ceftriaxone (Cro) by disk diffusion method.
Detection of the mechanism of resistance: The presence of the cmlA and floR genes associated to Chl resistance; tetA, tetB, and tetG genes associated to Tet resistance; genes encoding β-lactamases (OXA-1, OXA-2, TEM-like, SHV-like) associated to Amp resistance, and dihydrofolate reductase genes were carried out by PCR and electrophoresis in 2% agarose gel.
Results: The molecular mechanisms of antimicrobial agents resistance in *Shigella* strains [Ampicillin (Amp), Tetracycline (Tet), Chloramphenicol (Chl) and Trimetoprim-Sulfametoxazole (SXT)] were analysed. At total 111 isolates were analysed. The most frequently detected encoding β-lactamases genes were oxa-like, from which oxa-1 (55 isolates) was the most frequent followed by carb (7 isolates), while tem (4 isolates) and oxa-2 (3 isolates) were recovered in low prevalence.
Resistance to Tet were mainly related to tetB (47 isolates), tetG (18 isolates), and tetA (9 isolates), genes. Nor floR neither cmlA genes were found to be related to Chl resistance; however CAT is not researched yet. Several genes encoding dihydrofolate-reductases (dfrA1, dfrA5, dfr14, dfr7) were responsible for SXT resistance strains, being dfrA1-like (52 isolates), the most frequent followed drf14 (24 isolates) and dfr7 (3 isolates only).
Conclusions: Despite the fact that the isolates were recovered in a specific geographic area, an high heterogeneity in the molecular mechanisms of antimicrobial resistance has been detected.

R2314 Detection of clindamycin resistance in *Staphylococcus aureus*: a comparison of agar diffusion tests, VITEK 2 and real-time PCR

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Objectives: The study of antibiotic resistance mechanisms in *S.aureus* has never been more relevant with an exponential rise in public concern over nosocomial MRSA infection. Community acquired MRSA is a further problem, especially with the recognition that Panton-Valentine Leucocidin is most commonly present in community MRSA and related strains of MSSA.
Clindamycin is favoured for treatment of deep-seated *S.aureus* infection due its excellent tissue penetration and ability to inhibit toxin production. Unfortunately, while constitutively expressed resistance mechanisms are evident using routine microbiological methods, inducible mechanisms may evade detection by both the manual and automated sensitivity testing techniques currently available.
This study compared detection of resistance to Clindamycin in *S.aureus* using standard and molecular methodologies, with a view to assessing the clinical impact inappropriate sensitivity testing.
Methods: Forty-seven Erythromycin resistant *S.aureus* strains were collected randomly from patients attending a Scottish district general hospital. D tests and E tests were performed using standard methodologies. DNA was extracted from strains using the EZ1 BioRobot (Qiagen) and a tissue extraction kit (with a bacterial extraction protocol). DNA amplification and detection was achieved using an ABI Prism 7000 sequence detector and Sybr green universal detection dye (Applied Biosystems). Duplicate samples were analysed using a real-time PCR reaction designed to amplify a specific ermA, ermB, ermC and msrA resistance gene target.
Results:

- Of 42 MSSA isolates, ermA, ermC and msrA genes were harboured by 14, 24 and 4 isolates respectively
- 5/5 MRSA harboured the ermC gene
- VITEK 2 failed to detect Clindamycin resistance in all MSSA and 2/5 MRSA tested
- E tests failed to detect Clindamycin in all MSSA and 4/5 MRSA strains
- As predicted, D tests were negative in 4 strains which were PCR positive for the msrA gene which does not confer clindamycin resistance

- The D test successfully detected Clindamycin resistance in all strains PCR positive for erm genes associated with MLS (macrolide, lincosamide, streptogramin B) resistance in *S.aureus*

Conclusion:

- VITEK 2 and E test are inappropriate methods for detection of Clindamycin resistance.
- The D test should be performed on all strains phenotypically resistant to Erythromycin.
- The prevalence of erm genes in phenotypically Erythromycin sensitive strains requires urgent investigation.

In vitro antibacterial susceptibility & drug interaction studies

R2315 The antimicrobial susceptibility of 83 strains of *S. aureus* against 14 antimicrobials from Belarus

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Objectives: The aim of this study was to evaluate the antimicrobial susceptibility of 17 MSSA and 66 MRSA clinical isolates from Minsk, Belarus against 13 antimicrobials.

Materials and Methods: Bacterial isolates from the collection held in Belarus were tested against the following agents; clindamycin (CLD), gentamicin (GEN), amikacin (AMK), vancomycin (VAN), teicoplanin (TEI), ciprofloxacin (CIP), fusidic acid (FUS), daptomycin (DAP), rifampicin (RIF), linezolid (LIN), quinupristin/dalfopristin (Q/D), erythromycin (ERY) and tetracycline (TET). MICs were performed using CLSI agar dilution methodology using cation-adjusted Mueller Hinton agar, supplemented with 50 mg/L calcium for Daptomycin. The number of strains resistant according to CLSI breakpoints were calculated.

Results: The range, MIC50 and MIC90 (mg/L) and percent resistant for each drug are shown in the table below. Using CLSI breakpoints all isolates were susceptible to VAN, TEI, DAP, LIN and Q/D, greater than 50% of MRSA strains were resistant to GEN, ERY and TET. MRSA were more resistant than MSSA. Using MIC90 data rifampicin was the most active agent against both MRSA and MSSA; ERY was the least active agent.

	MRSA (n = 66)				MSSA (n = 17)			
	range	MIC_{50}	MIC_{90}	% R	range	MIC_{50}	MIC_{90}	% R
Clindamycin	0.12–32	0.25	32	22	0.25–0.5	0.25	0.5	36
Gentamicin	0.25–128	64	128	81	0.25–128	0.5	16	42
Amikacin	2–128	4	16	11	2–32	4	4	6
Vancomycin	1–4	1	1	0	1	1	1	0
Teicoplanin	1–4	1	1	0	1–2	1	1	0
Ciprofloxacin	0.5–32	1	2	11	0.5–16	1	1	6
Fucidic acid	0.03–4	0.06	2	nd	0.03–1	0.06	1	nd
Daptomycin	0.12–1	0.5	1	0	0.12–0.5	0.5	0.5	0
Rifampicin	⩽0.008–128	0.015	0.12	5	⩽0.008–0.015	0.015	0.015	0
Linezolid	1–2	2	2	0	1–2	2	2	0
Quin/dalfo	0.25–0.5	0.5	0.5	0	0.25–0.5	0.5	0.5	0
Erythromycin	0.25–256	256	256	85	0.25–256	0.25	256	47
Tetracycline	0.5–128	1	128	55	0.5–64	1	64	42

Conclusion: As expected MRSA strains were generally more resistant than MSSA however, aminoglycoside, macrolide/lincosamide and tetracycline resistance was common. Glycopeptides and the newer agents retained good activity against *S.aureus* from Belarus.

R2316 Urinary tract infection pathogens and their susceptibility to antimicrobials at a university hospital, Setif, Algeria: a 7-year study (2000–2006)

F. Sahli, N. Radji, A. Touabti (Sétif, DZ)

Objectives: To determine the most frequent pathogens causing urinary tract infection isolated at the microbiology laboratory of the Setif university hospital in Algeria during 7 years and we report their susceptibility (S) rates to antimicrobials.

Methods: 3227 isolates were collected from urine specimens during 7 years (2006–2007). Strains were identified by standards methods. S to antimicrobial agents was tested according to the CLSI guidelines as well as the detection of the extended spectrum betalactamase phenotype (ESBL).

Results: The most frequent pathogens were *E. coli* (59.4%), *Klebsiella* (12.9%), *Proteus* (8%), *Pseudomonas aeruginosa* (4.89%), *Enterococcus* (3%) and *Staphylococcus aureus* (2.23%). For the commonly isolated Enterobacteriaceae, antimicrobials S rates were: cefotaxim (89.4%), imipenem (100%), ciprofloxacin (86.6%) and triméthoprim/sulfamethoxazole (sxt) (52.2%). ESBL phenotype rates were 6.6% for *E. coli*, 8% for *Proteus* and 30% for *Klebsiella*. The S rates for *Pseudomonas aeruginosa* were as follow: piperacillin (57.9%), ceftazidime (64.9%), imipenem (94.8%), ciprofloxacin (69.7%) and colistin (100%). *Enterococcus* was susceptible to ampicillin in 62.2%, tetracycline in 20.6% and vancomycin in 100%. For *Staphylococcus aureus*, oxacillin was active at 71.8%, ofloxacin 82.2%, sxt 85.5% and vancomycin 100%.

Conclusion: Enterobacteriaceae and specially *E. coli* remained the most frequent urinary tract infection pathogens. The most actives antimicrobials agents were imipenem and third generation cephalosporins (3GC) although emerging BLSE phenotype had reduced the 3GC activity particularly on *Klebsiella* isolates. Fluoroquinolones were still actives but their S rates decrease progressively. Using adequate antimicrobial therapy with broad spectrum agents will not solve the multidrugresistant bacteria problem if strict measures aren't applied to avoid such isolates spread.

R2317 *Pseudomonas aeruginosa* infection and antimicrobial susceptibility in hospital patients, Setif, Algeria

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Objectives: to report the antimicrobial susceptibility (S) rates of *Pseudomonas aeruginosa* isolates from different specimens in Setif hospital

Methods: 399 *Pseudomonas aeruginosa* strains were isolated between January 2000 and September 2005 in the Setif hospital microbiology laboratory. Strains were identified by standards methods. S to antimicrobial agents was tested according to CLSI guidelines.

Results: most of our strains were isolated from respiratory sites (23%), wounds (19%), urine (15%), blood (9%) and chirurgical wounds (9%). Antimicrobial susceptibility rates were as follow: pireracillin (50%), ceftazidime (74%), imipenem (95.6%), gentamicin (70%), amikacin (91%), and ciprofloxacin (83.5%). Prior antibiotherapy was inadequate in 67% including cefotaxim or ceftriaxone; this contributes to select the most resistant strains and clinic deterioration. We note that susceptibility rate to ceftazidime had increased from 50% in 2000 to 74% in 2004 and 2005. For imipenem, there was no resistance until 2004.

Conclusion: Most of third generation cephalosporins used commonly haven't any activity against *Pseudomonas aeruginosa* and once again, almost third of our strains were resistant to ceftazidime. In critical ill patients, antibiotherapy prior S tests results must include one or more antimicrobials actives against *Pseudomonas aeruginosa* according to local S data.

R2318 Drug susceptibility testing of *Mycobacterium tuberculosis* strains by using two different methods

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Objective: The emergence of multi-drug-resistant tuberculosis points out how important timely identification of *Mycobacterium tuberculosis* complex strains and their susceptibility testing may be to achieving effective management of the disease and prevention of its spreading. Currently, a number of susceptibility testing methods have been used in many laboratories. In the present study we aimed to compare the agar proportion method with the E-test method for susceptibility testing

of *Mycobacterium tuberculosis* strains isolated from the patients in our hospital.

Methods: A total of 45 isolates were tested for isoniazid, rifampin, streptomycin and ethambutol susceptibility using an indirect-proportion method and E-test method. The isolated strains were identified by morphological, cultural and biochemical characteristics (microscopy, time of growth, type of colonies, catalase, niacin-test). H37RV strain was incuded in the test as reference strain. The E-test method was performed on 7H11 agar with OADC, and the standard proportion method was performed on Lowenstein-Jensen agar. E-test method gave correct results from the eighth day, with stability of the zone of inhibition until the 21st day.

Results: We found a good correlation between the two methods. The correlations between the Etest and agar proportion methods were 98, 100, 100, and 100% for isoniazid, rifampin, ethambutol, and streptomycin, respectively. The results are summarised in Table 1.

Table 1. Comparison of susceptible and resistant *M. tuberculosis* strains by the E-test and agar proportion methods

Agent	No. of isolates with indicated result by:				% Agreement
	Agar proportion method		E-test method		
	Susceptible	Resistant	Susceptible	Resistant	
Isoniazid	40	5	41	4	98
Rifampin	43	2	43	2	100
Ethambutol	44	1	44	1	100
Streptomycin	43	2	43	2	100

Conclusion: Our results showed that there were no statistically significant differences in susceptibility testing results between E-test and the proportion method. The results thus suggest that E-test method can be routinely used instead of the proportion method because the average times required for obtaining the final result with E-test method are less than the proportion method.

R2319 Detection of meticillin-resistant Staphylococci isolated from nasal specimens using PCR

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Background: The acquisition of mecA gene by Staphylococci is contributed with resistance to methicilin. Nasal carriage among hospital staff are believed to be a potential source of nosocomial infections. The aim of this study is to determine the frequency of mecA gene in meticillin resistant Staphylococci isolated from hospital staff nasal specimens in Shahre kord city of Iran using PCR.

Methods: A total of 204 nasal swabs from hospital wards staff were collected. Conventional microbiological methods were performed for Staphylococcal isolation and identification. Antibacterial susceptibility pattern of the *Staphylococcus* isolates to oxacillin (meticillin) were tested using agar screen method. The presence of mecA gene in meticillin resistant Staphylococci were tested using duplex PCR.

Results: *Staphylococcus aureus* and coagulase negative *Staphylococcus* isolates were cultured from 25.4% and 74% 0f nasal specimens respectively. In antibacterial susceptibility testing, twenty-three(44%) of *Staphylococcus aureus* and 70 (46%) of coagulase-negative staphylococcal isolates were phenotypically resistant to methicilin. Using PCR, mecA gene was detected in 52% of *Staphylococcus aureus* and 52.5% of coagulase-negative staphylococcal isolates.

Conclusion: This study showed that staphylococcal nasal carriage among hospital staff is a medical problem and the rate of mecA gene in hospital strains of staphylococcus isolates is significantly high. These resistant isolates may have an important role in distribution of antibiotic resistance in hospitals.

R2320 Comparison of coagulase-negative staphylococci isolated from patients and healthy individuals

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Introduction: More than forty species of the genus *Staphylococcus* have been identified so far. Coagulase-positive *S. aureus* has been frequently implicated in the aetiology of a series of infections. Over the last decade, coagulase-negative staphylococci (CoNS) have been recognised as aetiological agents of infectious processes. CoNS produce different virulence factors, although the differentiation between virulent and non-virulent strains is difficult since the virulence factors of these microorganisms are yet not well defined.

Objectives: The aim of the study was to identify the species spectrum, biochemical criteria and meticillin resistance of CoNS, isolated from healthy individuals, in-patients of surgical hospitals and out-patients.

Materials and Methods: The study was carried out in two Riga hospitals and a department for out-patients. The identification of isolated microorganisms from clinical samples was performed by the mini API system, Crystal system, and susceptibility testing – by the mini API system, the disk diffusion test, e-test.

Results: More than 400 strains of CoNS were investigated. The species spectrum of CoNS, isolated from in-patients, was as follows: *Staphylococcus epidermidis* 56.03%, *Staphylococcus haemolyticus* 14.18%, *Staphylococcus hominis* 10.2%, *Staphylococcus saprophyticus* 5.0% and others; from out-patients: *S. epidermidis* 48.73%, *S. haemolyticus* 36.97%, *Staphylococcus sciuri* 5.04%, *Staphylococcus simulans* 4.20% and others; from healthy individuals: *S. epidermidis* 55%, *Staphylococcus capitis* 16.6%, *S. haemolyticus* and *Staphylococcus cohnii* 6.6%, *Staphylococcus warneri* and *S. saprophyticus* 5.0% and others.

The results of the tests of utilisation and degradation of specific substrates revealed a significant difference between nosocomial and healthy strains in 2 tests – maltozide and methyl alpha, beta glucoside utilisation.

Meticillin resistance in CoNS from in-patients and out-patients was 51.23% and 19.3%, respectively. Bacteraemia was caused only by MRCoNS.

Conclusions: More virulent were *S. haemolyticus*, *S. hominis*, *S. sciuri*. Meticillin-resistant strains occurred to be more invasive and were the only causative agents of bacteraemia.

R2321 In vitro activities of tigecycline against *Aeromonas*, *Vibrio*, and *Salmonella* species, Taiwan

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Background: *Aeromonas*, *Vibrio*, and *Salmonella* species are endemic pathogens associated with a variety of clinical infections in Taiwan. There are few reports regarding in vitro activities of tigecycline against these pathogens.

Methods: A total of isolates of *Aeromonas*, *Vibrio*, and *Salmonella* species were tested. These organisms were isolated from various clinical specimens (blood, stool, and pus).

Bacteria (no. of isolates tested)	MIC (μg/ml)			No. (%)		
	Range	MIC_{50}	MIC_{90}	S	I	R
A. hydrophila (n = 81)	0.06–2	0.25	0.5	81 (100)	0 (0)	0 (0)
A. caviae (n = 63)	0.12–4	0.25	0.5	62 (98)	1 (2)	0 (0)
A. sobria (n = 57)	0.12–1	0.25	0.5	57 (100)	0 (0)	0 (0)
S. choleraesuis (n = 63)	0.12–2	0.5	1	63 (100)	0 (0)	0 (0)
S. typhimurium (n = 63)	0.25–2	0.5	0.5	63 (100)	0 (0)	0 (0)
Other *Salmonella* spp. (n = 72)*	0.12–1	0.5	0.5	72 (100)	0 (0)	0 (0)
V. parahaemolyticus (n = 41)	0.06–0.12	0.06	0.12	41 (100)	0 (0)	0 (0)
Vibrio cholerae non O1 (n = 26)	0.03–0.12	0.06	0.06	26 (100)	0 (0)	0 (0)
V. vulnificus (n = 15)	0.03–0.12	0.03	0.03	15 (100)	0 (0)	0 (0)
Other *Vibrio* species (n = 10)	0.03–0.12	0.06	0.12	10 (100)	0 (0)	0 (0)

*Including *Salmonella* serogroup O7 (n = 13), O8 (n = 14), and O9 (n = 45).

MICs of tigecycline were determined by using the broth dilution method (CLSI). The results were interpreted by the MIC criteria provided by U.S. FDA tigecycline susceptibility breakpoints listed for Enterobacteriaceae (S, $\leqslant$2 mg/L; I, 4 mg/L; R, $\geqslant$8 mg/L)
Results: All 81 *Aeromonas hydrophila*, 57 A. sobria, 198 non-typhoid salmonella, and 92 *Vibrio* isolates were susceptible to tigecycline. One out of 63 *A. caviae* isolates revealed intermediate to tigecycline.
Conclusions: Nearly all isolates of *Aeromonas*, *Vibrio*, and *Salmonella* species tested were susceptible to tigecycline. Further in vivo studies should be conducted to confirm the clinical efficacy of tigecycline for the treatment of infections due to these organisms.

R2322 Detection of ESBLs: comparisons of MicroScan, Etest and double disc tests

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Objectives: ESBLs are enzymes that mediate resistance to extended-spectrum cephalosporins and monobactams except cephamycins and carbapenems.ESBLs can be difficult to detect because they have different levels of activity against various cephalosporins. The ability of MicroScan (MS) panels NBC 33 (DadeBehring, California,USA) to detect ESBLs was compared with E-test ESBL strips and double disc (DD) tests.
Methods: 27 *E. coli*, 14 *P. mirabilis* and 9 *K. pneumoniae* non-urinary clinical isolates were tested. Microdilution susceptibility testing by MS panel NBC33 was performed according to manufacturer's instructions. For DD test a plate is inoculated as for a routine susceptibility test, discs containing cefotaxime and ceftazidime 30 ug are applied, at a distance of 25 mm, either side of one with co-amoxiclav 20+10 ug. ESBL production is inferred when the zone of either cephalosporin is expanded by the clavulanate. Etest ESBL strips (AB Biodisk, Sweden) was perfomed according the manufacturer's instructions, ESBL production is inferred if the MIC ratio for cephalosporin alone: cephalosporin + clavulanate MIC is >8. β-Lactamases AmpC producing was confirmed by DD test using cefotaxime 30 ug and cloxacillin 10 ug. Controls included *K. pneumoniae* ATCC 700603 as positive control and *E. coli* ATCC 25922 as negative control.
Results: In two strains of *E. coli* and two of *K. pneumoniae* producing ESBLs and β-lactamases AmpC MS doesn't detect the presence of ESBLs that were relieved only by DD tests. Two strains of *E. coli* were false positive with MS for the ESBLs producing, not confirmed by DD tests and Etest strips, in a strain of *E. coli* producing β-lactamases AmpC MS asks a confirmatory test to detect ESBL that wasn't confirmed by DD tests and Etest. All the ESBLs in *P. mirabilis* were confirmed.
Conclusion: 46 of the 50 strain(92%)were concordant with all methods.2 *E. coli* ESBL+ by DD were considered possible producers by MS and an alternative method was suggested by Alert system, while Etest gave out of range response. Two strains of *K. pneumoniae* ESBL+ with MS, ESBL− with DD and out of range with Etest. Anyway, despite this four discordances, there were no categorical differences in the reported β-lactam and mono-bactam antimicrobics. The fact that the simultaneously presence of ESBLs and other β-lactamases (AmpCs, inhibitor-resistant TEMs, hyper-production of TEM and/or SHV) could mask ESBL production, resulting in false-negative confirmatory tests, remains a unresolved problem with phenotypic methods.

R2323 Phenotypes of resistance to macrolide and related antibiotics in *Streptococcus agalactiae*

V. Vuksanovic (Podgorica, ME)

Introduction: For *Streptococcus agalactiae*, as one of the most important causes of neonatal infection and infections in pregnant women, penicillin or its congeners is the treatment of choice. However, if there is a penicillin allergy, erythromycin and clindamycin are the major substitutes. Unfortunately, macrolide resistance among Gram-positive cocci has been increasing worldwide. The resistance of group B streptococci (GBS) is expressed as either a macrolide-restricted (M) phenotype or inducible and constitutive macrolide-lincosamide-streptogramin B (MLSB) cross-resistance phenotypes (iMLSB and cMLSB).
Aim: in this study determinants of GBS isolates in Montenegro were defined for the first time and compared with the reports of the other country.
Materials and Methods: A total of 298 GBS isolates from vaginal swabs, collected from March to May 2007, were investigated in bacteriological department of Institute for Public Health, Montenegro. Phenotype was determined with double disc test by placing a 2 μg clindamycin disc and 12 μg erythromycin disc as a part of the normal disc diffusion procedure and according to CLSI.
Results and Discussion: 247/298 (82.9%) of GBS were erythromycin susceptible and 43/298 (14.4%) had resistance to erythromycin with different phenotypes. M phenotype had 7/298 (2.3%) of strains and 36/298 (12.1%) had MLSB resistance: cMLSB 15 (5.0%) and iMLSB 21 (7.1%) of the strains. GBS had intermediate susceptibility in 2.7% of cases. The macrolide resistance rate of 14.4% of our GBS isolates was similar with report in Spain (14.7%) and lower than previous reports of 18% in Canada, 16% in North Carolina, 29.7% in Taiwan, 22.4% in Turkey.
Conclusion: As the macrolide resistance increase is evident in our country too, this antibiotic should be in treatment under the rigorous control.

R2324 The effect of testing parameter variations on the in vitro activity of iclaprim

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Objectives: Iclaprim belongs to the diaminopyrimidine antibiotic class and is a potent and selective inhibitor of microbial dihydrofolate reductase (DHFR). It exhibits an expanded spectrum of activity and is notably very potent against important Gram-positive pathogens, including meticillin-resistant *Staphylococcus aureus* and Group A and B streptococci. Intravenous iclaprim recently completed two pivotal Phase III trials in complicated skin and skin structure infections and a Phase II trial in Hospital- and Ventilator-Associated Pneumonia is currently ongoing. The purpose of this study was to evaluate the effect of varying the standard Clinical and Laboratory Standards Institute (CLSI) microbroth dilution test conditions on the in vitro activity of iclaprim.
Methods: Minimal inhibitory concentrations (MICs) were determined following reference CLSI conditions using four strains of *Staphylococcus aureus* including ATCC 29213 and ATCC 25923, *Enterococcus faecalis* ATCC 29212 and *Escherichia coli* ATCC 25922. The effect of varying the inoculum concentration (5×10^4, 5×10^5, 5×10^6 CFU/mL), the medium pH (pH 6.0, 7.0–7.2, 8.0), the calcium concentration (2.5, 25, 50 mg/L), the incubation conditions (oxic and anoxic condition, 5% CO2), medium supplementation (addition of 5% lysed horse blood) and the presence of increasing concentrations of human serum (5%, 10%, 20%) was investigated.
Results: The calcium concentration, a higher medium pH, a lower inoculum concentration, incubation in presence of 5% CO2, change of medium supplementation or the addition of human serum had no significant impact on the activity of iclaprim. Minor changes in the MIC values of iclaprim with maximal two dilutions differences were observed by increasing the inoculum concentration, lowering the medium pH, or incubation under anoxic conditions.
Conclusion: The activity of iclaprim was not significantly affected by variations in standard CLSI susceptibility testing methodology.

R2325 In vitro activity of daptomycin against vancomycin-resistant Enterococci in patients with bacteraemia

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Objectives: The incidence of resistant Gram-positive bacteria in nosocomial infections is increasing. Daptomycin is a newer antimicrobial

agent active against Gram-positive pathogens including vancomycin-resistant *Enterococcus faecalis* and *Enterococcus faecium*. The aim of this study was to assess the in-vitro activity of Daptomycin by performing susceptibility testing of a set of vancomycin-resistant Enterococci (VRE).
Methods: The collection included 32 VRE isolates (28 *E. faecium* and 4 *E. faecalis*) isolated from patients with positive blood cultures between 2005–2007 in our hospital. Identification to species level was determined with the use of VITEK-II system (BIOMERIEUX-FRANCE). Phenotypically all isolates were of Van A type (vancomycin MIC $\geqslant$256 mg/L, teicoplanin MIC $\geqslant$16 mg/L. They were also susceptible to linezolid and expressed various susceptibility patterns to other antibiotics. Daptomycin MICs were determined by E-test method (AB Biodisk, Solna, Sweden). $\leqslant$4 mg/L was used as a susceptible breakpoint as approved by CLSI.
Results: Daptomycin showed very good activity, with all but one enterococcal isolates being in the susceptible range. Daptomycin MICs for vancomycin-resistant *E. faecium*: 39.3% (11/28) at 2 mg/L, 35.7% (10/28) at 3 mg/L, 21.4% (6/28) at 4 mg/L and 3.57% (1/28) at 8 mg/L. Daptomycin MICs for vancomycin-resistant *E. faecalis*: 25% (1/4) at 1 mg/L, 25% (1/4) at 1.5 mg/L, 25% (1/4) at 3 mg/L and 25% (1/4) at 4 mg/L.
Conclusion: Daptomycin was proved to be highly active against VRE isolates from patients with bacteraemia and appears to be a very good therapeutic option in treating serious infections.

R2326 In vitro activity of mecillinam in combination with aminopenicillin/β-lactamase-inhibitor – Combinations against ESBL-producing Enterobacteriaceae

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Objective: Due to raised MICs at high inocula it has been suggested that the amidinopenicillin Mecillinam (MEC) is not stable to ESBLs and should not be used in severe infections. The same is true for amoxicillin/clavulanic acid (AMC) and piperacillin/tazobactam (PTZ), since their activity largely depends on species, type of β-lactamase and inoculum. MEC preferentially binds to PBP2 and amoxicillin as well as piperacillin to PBP3. The aim of this study was to test (i) if MEC combined with AMC or PTZ would result in synergistic in vitro activity, and (ii) whether the effect would be stable in the presence of a high inoculum.
Material and Methods: Forty-two consecutive ESBL-producers (thereof *E. coli*, n = 36) isolated in 2007 from urine samples were studied. ESBL production was detected by CLSI methods and an extended double disk diffusion synergy test. Clonal relationship was investigated by means of RAPD and ERIC-PCR. MICs and synergy testing [MEC/AMC; MEC/PTZ] was done by Etest® and fractional inhibitory concentration (FIC) indices were calculated (FIC = MICAB/MICA + MICBA/MICB). In a preliminary study inocula were adjusted to comprise 104 CFU/ml and 108 CFU/ml.
Results: Preliminary experiments: MICs for MEC were 1–64 mg/L at an inoculum of 104 CFU/mL, rising to 2–256 mg/L at 108 CFU/mL. For AMC, PTZ, and the combinations MEC/AMC and MEC/PTZ MICs were not inoculum-dependent. Resistant colonies within the MEC ellipse could be observed in some cases. If these colonies were retested, they proved highly MEC resistant, what did not affect FIC indices. Synergy testing was thus done with the higher inoculum. For MEC, AMC and PTZ alone MICs50 were 1.5 mg/L, 8 mg/L, and 8 mg/L. According to CLSI, 76%, 57%, and 67% strains were susceptible. Synergy testing: the MIC90A/B for MEC/AMC and MEC/PTZ was 1 mg/L each, and the MIC90B/A was 0.7 mg/L and 0.3 mg/L, respectively. A synergistic, additive and indifferent effect for MEC/AMC [MEC/PTZ] was found for 66.6% [92.8%], 28.5% [2.3%], and 5% [5%] strains, respectively.
Polyclonality was shown for 34/42 *E. coli* (>80%) and 3/5 *K. pneumoniae* isolates.
Conclusion: MEC has a good, but inoculum dependent, in vitro activity against ESBL-producing enterobacteriaceae. The susceptibility of AMC and PTZ cannot reliably be predicted. When administered combined, both combinations resulted in a significant MIC reduction due to a highly synergistic, inoculum independent effect.

R2327 Fosfomycine tromethamine as second agent for the treatment of acute uncomplicated urinary tract infections in all age groups in the Netherlands?

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Background: Uncomplicated urinary tract infections (UTIs) are common among female patients. According to the national guidelines of the Dutch College (NHG) for General Practitioners (GPs) the drugs of first and second choice as therapy for UTIs are nitrofurantoin and trimethoprim with resistance percentages of 2% and 23% respectively. The third choice is fosfomycine tromethamine (FT) for which unfortunately no current resistance data from the Netherlands are available. The aim of this study was to determine these resistance percentages.
Material and Methods: During a 2-year period urine samples were collected from a representative sample of 21 general practices spread over the Netherlands, the Sentinel Stations of the Netherlands Institute for Health Services Research (NIVEL).
Escherichia coli isolated from female patients visiting their GP with symptoms of an acute uncomplicated UTI were used. FT susceptibility was determined with E-tests. An MIC for FT of 64 mg/L or lower was considered susceptible, MIC-values of 96 mg/L or higher resistant. *E. coli* ATCC25922 was used as a reference strain.
Results: In total, 1705 *E. coli* strains were tested of which 11 (0.64%) were FT resistant. The MIC50 and MIC90 values in this population were 1 and 4 mg/L respectively. Within the inhibition zone of 162 susceptible *E. coli* strains, resistant mutant colonies were observed of which after repetition of the susceptibility testing 68 were resistant. In total, 79 (5%) strains were FT resistant. There was no cross-resistance observed between FT and other antimicrobial agents tested previously.
Discussion: The high in vitro susceptibility to fosfomycine tromethamine in this population and the lack of cross-resistance between fosfomycine tromethamine and other agents together with the extensive global clinical experience support the choice of the NHG-guidelines to include fosfomycine tromethamine as a second – instead of third – therapeutic option in general practice for uncomplicated UTIs.

R2328 Comparative in vitro activity of ertapenem against carbapenem-resistant Gram-negative bacteria isolated from blood cultures

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Objective: Infections caused by multidrug resistant (MDR) organisms are increasing. Carbapenems (imipenem and meropenem) are the antibiotics commonly used to treat these agents but the emergence of carbapenem resistance in those microorganisms represents a major public health concern. The aim of this study is to evaluate the in vitro activity of ertapenem and other 15 antibiotics against isolates recovered from blood cultures. Presence of extended-espectrum β-lactamases (ESBL), was also determined.
Method: 31 non-duplicate isolates were included in the study. All isolates were collected between June 2006 and October 2007.
Minimum inhibitory concentrations (MICs) were determined using a commercial broth microdilution method, in accordance with the CLSI guideline M7-A6 (NCCLS, 2003).
Antimicrobials tested included, among others, cefotaxime, cefepime, ciprofloxacin, aztreonam, amikacin, piperacillin-tazobactam and colistin besides imipenem and meropenem.
E-test® (AB Biodisk, Solna, Sweden) methodology was used to study ertapenem susceptibility. E-test® was also used to determine ESBL production.
Results: All isolates were chosen because their imipenem resistance. Only three isolates were susceptible to meropenem. Ertapenem MIC90

was >32 mg/L. Colistin showed the highest activity of all agents tested followed by amikacin, piperacillin-tazobactam and cefepime with resistance rates of 29%, 64.5% and 77.5% respectively.
None of the isolates produced ESBL.
Pseudomonas aeruginosa was the most common MDR Gram-negative bacillus isolated from bacteraemia.
Conclusions:
1. Ertapenem and meropenem showed similar activity against the carbepenem-resistant Gram-negative bacilli, analysed in this study
2. Ertapenem appears not to be a good option to treat carbapenem-resistant Gram-negative isolates
3. Colistin demonstrated potent in vitro activity against MDR microorganisms.
4. Carbapenem-resistant isolates were found to be resistant to all β-lactam antibiotics tested.

R2329 Antimicrobial susceptibility of *Escherichia coli* isolated from patients with uncomplicated urinary tract infections in Asia and Middle East region

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Objective: To test antimicrobial resistance patterns of *Escherichia coli* collected prospectively in 9 different countries in Asia and Middle East (Turkey, Saudi Arabia, Jordan, Korea, Indonesia, Philippines, Thailand, Pakistan, and Taiwan) and the Netherlands from the urine of 15–50 year old female patients with uncomplicated UTI.
Methods: Microtiter plates containing freeze dried dilutions of the antibiotics have been prepared by Trek Diagnostics. Only one isolate per patient was included. All isolates were re-identified in the University Medical Center Utrecht and minimal inhibitory concentrations (MICs) were determined by microdilution method according to CLSIguidelines. *E. coli* ATCC 35218 and *E. coli* ATCC 25922 were used as control strains for quality control.
Results: So far, we have analysed antimicrobial susceptibilities in 5 countries (Table 1) As compared to the Netherlands, substantial resistance of *E. coli* was observed in the centres of countries in Asia and Middle East. Third generation cephalosporins (cefixime and cefpodoxime) had superior in vitro activities as compared to the other oral betalactams and the quinolones tested.

Table 1.

Antibiotic	Korea (n = 73)			Thailand (n = 48)			Turkey (n = 78)			Jordan (n = 75)			Netherlands (n = 75)		
	%S	MIC_{50}	MIC_{90}	%S	MIC_{50}	MIC_{90}	%S	MIC_{50}	MIC_{90}	%S	MIC_{50}	MIC_{90}	%S	MIC_{50}	MIC_{90}
Augmentin	37.0	8	16	14.6	8	32	39.7	8	16	34.7	8	16	65.3	4	16
Cefaclor	72.6	4	>32	45.8	16	>32	67.9	8	>32	60.0	8	>32	90.7	2	8
Cefurixime	46.6	8	>32	35.4	8	>32	51.3	4	>32	52.0	4	>32	77.3	4	8
Cefixime	76.7	0.5	>32	52.1	1	>32	75.6	0.5	>32	73.3	0.5	>32	97.3	0.5	1
Cefpodoxime	80.8	0.5	>32	60.4	1	>32	85.9	0.5	>32	77.3	0.5	>32	97.3	0.25	0.5
Ciprofloxacin	64.4	0.25	>32	33.3	32	>32	62.8	0.12	>32	69.3	0.25	>32	96.0	⩽0.03	0.03
Ofloxacin	64.4	0.5	32	35.4	32	>32	62.8	0.5	>32	69.3	0.5	32	96.0	0.06	0.12

Conclusions: Third generation cephalosporins were superior to quinolones in their in vitro activity against *E. coli* isolated from uncomplicated UTI in the countries of Asia and Middle East region. Susceptibility of ciprofloxacin and ofloxacin, which have been long primary agents for UTI, decreased against *E. coli*, possibly due to overuse of these antibiotics. Results of the complete study will be presented at the ECCMID.

New antimicrobials

R2330 Effect of farnesol on planktonic versus biofilm cells of *Staphylococcus epidermidis*

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Objectives: *Staphylococcus epidermidis* is the most frequent cause of nosocomial sepsis and catheter-related infections, in which biofilm formation is considered to be the main virulence mechanism. Quorum-sensing system have been recognised as important regulators of virulence in many bacteria. The goal of this study was investigate the effect of farnesol, a quorum-sensing molecule, on planktonic and biofilm cells of four *S. epidermidis* strains and compare farnesol susceptibility of planktonic with biofilm cells.
Methods: Four strains of *S. epidermidis* were grown planktonically and as biofilms to determine the effect of farnesol. To biofilm study, farnesol was added before biofilm formation and after 24 hours of biofilm formation. To study the effect of farnesol on planktonic cells, the utility of a rapid colorimetric method that is based on the reduction of Alamar Blue to measure cell viability was tested as well as standard bacterial enumeration techniques. The growth inhibition effect of farnesol on biofilm cells of *S. epidermidis* was assessed using XTT assay that measures cellular metabolic activity and Crystal Violet that measure total biomass of biofilm.
Results: It was observed that farnesol has inhibitory effect as on planktonic cells as on biofilm cells. Modest concentrations of farnesol were sufficient to exhibit an antibacterial effect on planktonic cells and on biofilm cells (before biofilm formation). When farnesol was added after 24 h of biofilm formation, it was observed a small reduction of total biomass and metabolic cellular activity with the increase of concentration of farnesol.
Conclusion: The inhibitory effect of farnesol was more pronounced on planktonic and biofilm cells (before biofilm formation) than when farnesol was added after 24 h biofilm formation. Biofilm structure impairs the action of farnesol. The results indicate a potential application for farnesol as a novel therapeutic agent for the prevention of biofilm-related infections.

R2331 Enterocin C, a two-peptide bacteriocin produced by a *Enterococcus faecalis* strain isolated from human colostrum

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Objectives: The aim of this study was the identification and characterisation of new antimicrobials compounds produced by bacterial species isolated from colostrum obtained from healthy women.
Methods: Antimicrobial peptides EntC1 and EntC2 were purified from *Enterococcus faecalis* C901 by cation-exchange, hydrophobic-interaction and C2/C18 reverse-phase chromatography (RPC-FPLC). The N-terminal amino acid sequences were determined by Edman degradation. Molecular masses of the peptides were determined by Matrix Assisted Laser Desorption/Ionisation-Time of Flight Mass Spectrometry (MALDI-TOF). DNA sequencing of the genes encoding EntC1 and EntC2 was carried using the plasmid pEntC as the template. Detection of virulence determinants in *E. faecalis* C901 was carried out by PCR.
Results: *Enterococcus faecalis* C901 showed inhibitory activity against several bacterial species as *Actinomyces neuii*, *Enterococcus faecalis*, *E. faecium*, *Lactococcus lactis*, *Lactobacillus paracasei*, *Leuconostoc mesenteroides*, *Propionibacterium acnes*, *Staphylococcus caprae*, *Streptococcus anginosus* and *S. intermedius*. This antimicrobial activity was due to a bacteriocin, named enterocin C, which has been biochemically and genetically characterised. Enterocin C consists of two different peptides, EntC1 (39 aas, 4,284 Da) and EntC2 (35 aas, 3,899 Da), whose complementary action is necessary for full enterocin C activity. The genes encoding enterocins EntC1 (entC1) and EntC2 (entC2) as to the putative immunity protein to these enterocins (EntCI) were localised in a 9-kpb plasmid named pEntC. In addition, *E. faecalis* C901 presented genes encoding several factors that are involved in the colonisation and persistence of enterococci within a host.
Conclusion: Here we describe the purification and genetic characterisation of enterocin C, a two-peptide bacteriocin produced by *E. faecalis* C901 isolated from human colostrum. The presence of bacteriocin-producing strains in such biological fluid may constitute one of the mechanisms explaining the protective effect of breastfeeding against infectious diseases.

Epidemiology of MRSA, VRE and other Gram-positives

R2332 Bacteriological assessment of intravascular catheter-related bloodstream infections versus colonisations

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Background: Catheter-related bloodstream infections (CRBSI) due to multidrug resistant bacteria represent a leading cause of nosocomial infections.
Objectives: Bacteriological assessment of colonisation vs. CRBSI.
Methods: Prospective study upon all consecutive cases admitted in a surgical intensive care unit, between May 2006 and June 2007. In all cases either jugular or subclavian polyurethane or silver impregnated short-term central venous catheters were placed. Bloodstream infection was defined as definite if a) the same pathogen grew in at least one blood culture and from the distal segment of catheter or b) same pathogen grew in at least one peripherally and centrally drawn blood culture, the differential time to positivity of central vs. peripheral blood culture was >120 minutes. Colonisation was defined in the absence of criteria for bloodstream or localised infection with bacterial growth from the catheter (external culture and intraluminal lavage) measured through quantitative and semi quantitative methods. Bacteriologic assessment was performed using the automatic system BactT/Alert and resistance patterns were determined with API 20E, Api 20NE and ATB automatic methods under CLSI 2006 standards.
Results: There were 188 documented infections and colonisations related to intravascular catheters. Average duration of in-place catheter was 9 days with similar gender, underlying diseases and type of catheter distribution. The cumulative incidence of colonisation was almost equal regardless of the catheter type (24/100 vs. 25/100 patients with in-place catheter) but smaller rates of CRBSI were found if silver-impregnated catheters were used (2.6/100 vs. 8/100 patients, NS). The most commonly isolated organisms were Gram-positive cocci-159 strains-85% (*S. aureus*-27.7%, coagulase negative stafilococci-28.7%, enterococci-24%). Almost all staphylococci were meticillin resistant, highly resistant to clindamicin and gentamycin but glycopeptide resistance was never found. One third of enterococci were resistant to ampicillin and 89% to gentamycin. All the 33 strains of Gram-negative rods (17.5%) were multidrug resistant. Sixty eight percent of all strains were multidrug resistant.
Conclusion: *Staphylococcus* spp. and enterococci are the most frequent colonizers or pathogens related to intravascular catheters. The multidrug resistant bacteria represent a therapeutic challenge. New technologies are important tools in prevention of CRBSI.

R2333 Hospital infections caused by community-associated meticillin-resistant *Staphylococcus aureus* strains

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Objectives: Community-associated meticillin-resistant *Staphylococcus aureus* (CA-MRSA) strains have been implicated in serious hospital infections. CA-MRSA are unrelated to hospital-acquired MRSA (HA-MRSA) because they carry the staphylococcal chromosomal cassette mec type IV (SCCmec IV) and harbour genes encoding for Panton-Valentine leukocidin (PVL) at higher frequency. The aim of our study was to determine whether nosocomial MRSA infections were caused by CA-MRSA strains.
Methods: During a 6-month period (April-October 2007) a total of twelve non-duplicate isolates were collected from hospitalised patients with nosocomial infections at Hippokration G. Hospital. Eight isolates were recovered from skin and soft tissue infection, two from bronchial aspirate and two from blood. Identification and susceptibility testing were performed using the VITEK 2 automated system (bioMérieux, France). Furthermore, coagulase production was tested with the tube test. Meticillin resistance was assessed by disk diffusion method using discs of oxacillin at 30ºC and cefoxitin at 35ºC. The presence of mecA was confirmed by PCR analysis using specific primers. In addition, the identification of SCCmec type IV and PVL genes by PCR permitted the characterisation of isolates as CA-MRSA. Strains that were resistant to erythromycin and susceptible to clindamycin were examined for inducible resistance to clindamycin by disk diffusion test.
Results: All MRSA strains harboured the mecA gene. Six (50%) of the 12 isolates were SCCmec type IV and were classified as CA-MRSA. Genes coding for PVL were detected in three strains, two CA-MRSA and one HA-MRSA. Susceptibility results to antimicrobial agents are reported in the table. CA-MRSA strains showed greater resistance to clindamycin, ciprofloxacin and erythromycin compared to HA-MRSA however the number of strains tested was limited. All isolates were susceptible to linezolid, vancomycin, teicoplanin and trimethoprim-sulfamethoxazole. Two isolates exhibiting the phenotype $Er^{R}Cli^{S}$ presented inducible resistance to clindamycin.

Table. Characteristics of meticillin-resistant *Staphylococcus aureus* isolates

Strain	Source	Ward	Antibiotic susceptibility[a]									PCR		
			OXA	CIP	CLI	E	LZD	TEC	VAN	TET	SXT	*mecA*	SCC*mec* IV	PVL
1	Wound	Transplantation	R	S	S	S	S	S	S	R	S	+	+	+
2	Bronchial	Int. medicinc	R	R	R	R	S	S	S	S	S	+	+	+
3	Wound	Orthopaedics	R	R	R	R	S	S	S	S	S	+	+	−
4	Wound	Orthopaedics	R	R	R	R	S	S	S	S	S	+	+	−
5	Wound	Surgical	R	R	S	R	S	S	S	S	S	+	+	−
6	Blood	ICU[b]	R	R	R	R	S	S	S	S	S	+	+	−
7	Wound	Transplantation	R	S	S	S	S	S	S	R	S	+	−	+
8	Wound	Surgical	R	R	R	R	S	S	S	S	S	+	−	−
9	Wound	Surgical	R	R	S	R	S	S	S	S	S	+	−	−
10	Wound	Neonatal	R	S	S	S	S	S	S	S	S	+	−	−
11	Bronchial	Neurological	R	R	S	S	S	S	S	R	S	+	−	−
12	Blood	Neonatal	R	S	S	S	S	S	S	S	S	+	−	−

[a]OXA, oxacillin; CIP, ciprofloxacin; CLI, clindamycin; E, erythromycin; LZD, linezolid; TEC, teicoplanin; VAN, vancomycin; TET, tetracycline; SXT, trimethoprim–sulfamethoxazole.
[b]ICU, intensive care unit.

Conclusions: Our results demonstrated that CA-MRSA has infiltrated in healthcare settings, being often involved in serious infections. Our study is preliminary and needs further investigation in order to improve our knowledge about the molecular and epidemiological impact of CA-MRSA in our hospital.

R2334 Impact of meticillin-resistance on the mortality in *S. aureus* VAP: a systematic review

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Objective: To estimate the impact of meticillin-resistance on mortality in ventilator-associated pneumonia (VAP) due to *Staphylococcus aureus* (SA).
Methods: PubMed, Scopus and bibliographies of the eligible studies were searched. We calculated pooled odds ratio (OR) and 95% confidence intervals (CI) by using the DerSimonian-Laird random effects model.
Results: Eight articles were included. Crude in-hospital mortality was higher in patients with VAP due to meticillin-resistant SA (MRSA) than meticillin-sensitive SA (MSSA) (OR: 1.79, 95% CI 1.21–2.65). This was also the case for the crude intensive care unit (ICU)-mortality (OR: 2.49, 95% CI 1.54–4.06). However, 3 of the selected studies, which adjusted for potential confounding factors, including adequacy of empirical treatment and severity of illness, demonstrated no difference in in-hospital mortality between patients with MRSA and MSSA VAP. This was not the case for another eligible study that also adjusted, but for confounders others than the above.
Conclusion: The limited available evidence seems to suggest that meticillin-resistance is associated with death among persons acquiring SA VAP. However, although supported by even more limited data, adjustment of risk factors suggests that this association may be not causal, but likely due to confounders, such as the adequacy of empirical treatment and severity of illness.

R2335 Investigation of vancomycin-resistant enterococci faecal carriage in intensive care units of a Turkish teaching hospital in a two-year period

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Objective: Vancomycin resistant enterococci constitute a major problem in nosocomial infections. The purpose of this study is to access the prevalance, resistance patterns, antibiotic susceptibilities of enterococci isolates and investigate the risk factors of carriage among the ICU patients in our hospital.
Methods: During two year period; between April 2005 and April 2007; rectal swab cultures were collected from patients in ICU. Samples were collected from patients in first 72 hours after hospitalisation and then repeated every 7 days until patient's discharge. Samples were cultured in VRE agar bases which contain 6 μg/ml vancomycin and 1 μg/ml meropenem. Identification was performed by conventional methods and Rapid ID 32 Strep (bioMerieux). Vancomycin resistance was detected by E test and Van gene characterisation was performed by a multiplex PCR. Antibiotic susceptibility patterns of strains for fosfomycin and linezolid was detected by disk-diffusion methods according to CLSI criteria.
Results: In two year period 21 VRE were isolated from 112 patients (18.75%). VRE faecal carriage period range was 1–72 weeks. Association between VRE colonisation and use of antimicrobials was investigated. Faecal carriers were using two or more antibiotics. Mostly used antibiotics were 3rd and 4th generation cefalosporins, vancomycin, levofloxacin and piperacillin-tazobactam. Fosfomycin and linezolid susceptibilities were investigated. All of VRE isolates were susceptible to linezolid and only one strain (4.5%) was resistant to fosfomycin. Van gene characterisation was performed in 10 strains and all of them were Van A type.
Conclusion: VRE colonisation must be monitored and risk factors should be determined in ICU's. Use of broad spectrum antibiotics is an important risk factor for VRE colonisation. Invitro linezolid and fosfomycin are effective on VRE strains.

R2336 The three-year presence of glycopeptide-resistant *Enterococcus faecalis* in the hospital

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Objectives: In the investigated hospital vancomycin resistant enterococci (VRE) were not frequently isolated up to the 2003. However three strains of vancomycin resistant (VR) *Enterococcus faecalis* were isolated in 1998 from patients of Intensive Care Unit (ICU) and they reappeared in 2003 in other ward. Between 2003 and 2006 years, 14 VR-*E. faecalis* were isolated from different patients of transplantant wards. All strains were recovered from urine. The aim of our work was to compare VR-*E. faecalis* strains derived from different years and wards.
Methods: The PFGE method, which is reference among molecular methods useful in epidemiological investigation was applied.
All 17 strains were defrosted, multiplied and their DNAs were examined for the presence of vanA, van B, vanD or vanG genes by PCR. The species status of each strain was confirmed by demonstration of the presence of the ddl gene specific for *E. faecalis*. PFGE was performed on bacterial DNA digested with SmaI in agarose discs. Products of digestion were separated using CHEF-DR II (Bio-Rad) and the obtained patterns of bands were compared.
Results: It was shown that all examined strains were *E. faecalis* and all harboured vanA gene. Comparison of obtained PFGE patterns revealed that two strains isolated in 1998 from different patients of ICU were identical, suggesting infection with the same strain. The third strain differed. Among 14 strains of VR-*E. faecalis* isolated in 2003–2006, seven displayed very similar patterns although they were isolated in different years and from different patients (3 in 2003, 2 in 2004 and 2 in 2005). The patterns of these strains differed from patterns of strains from 1998. All strains were obtained from patients of the two closely collaborating wards where people after transplantations were hospitalised. All eight strains were susceptible to ampicillin and highly resistant to aminoglycosides (HLAR). The patterns of bands after PFGE in the case of reminding seven strains were unrelated. Among strains from 2006, no pattern similar to that obtained for seven strains from 2003 to 2006 was found.
Conclusions: The results indicated that in 1998 there was a small outbreak in the ICU ward (2 patients infected or colonised with the same VRE). The results suggested also that the single strain of VR-*E. faecium* was present for three years in two closely related hospital wards.

R2337 *Corynebacterium striatum*: a multidrug-resistant emerging pathogen

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Objectives: *Corynebacterium striatum* is a Gram-positive organism that commonly colonizes the skin and mucous membranes. Over the last few years, this organism has been increasingly recognised as a cause of wound and low respiratory tract infections. The aim of the study was to evaluate epidemiology and antimicrobial susceptibility of *C. striatum* clinical isolates collected at our laboratory in 2006 and 2007.
Methods: Isolates of *C. striatum* obtained from routine specimens were characterised at the Microbiology Laboratory of the Ospedale di Circolo (Varese, Italy). Identification was achieved using the BBL Crystal Gram-Positive ID system (Becton Dickinson Diagnostic Systems, Sparks, MD). Antimicrobial susceptibility was quantitatively evaluated by the Etest method (AB Biodisk, Solna, Sweden). Results were interpreted according to the M45-A document (Clinical and Laboratory Standards Institute, 2006).
Results: Overall, 128 isolates were obtained during the two-year period, most of them from skin and soft tissue specimens (76.5%), but also from vascular catheter (8.6%), low respiratory tract (6.2%), and blood (3.1%). Strains were frequently isolated from aged and/or compromised patients admitted to different wards. The majority of isolates were characterised by multi-drug resistance, including penicillin, gentamicin, erythromycin, tetracyclines, expanded-spectrum cephalosporins, and ciprofloxacin. Teicoplanin and vancomycin as well as newer antimicrobial agents (linezolid, daptomycin, and quinupristin-dalfopristin) were consistently active in vitro against *C. striatum* isolates.
Conclusion: *C. striatum* should be considered an emerging multidrug-resistant nosocomial pathogen in aged/compromised patients and in long-term hospitalised patients. Drugs recently introduced to treat skin and soft tissue infections appear to represent an effective therapeutic option in order to limit the use of glycopeptides.

R2338 The prevalence of vancomycin and gentamicin-resistant enterococci isolated from human normal flora in Iran

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Objective: Enterococci are natural inhabitants of the gastrointestinal tract of humans and animals, but are also found in other anatomical sites including the vagina and oral cavity, and in plants and insects. Multidrug resistance is common among enterococci and presents a formidable treatment problem. High-level gentamicin resistance (MIC $\geqslant$ 500 mg/ml) in enterococci is predominantly mediated by aac(6′)-Ie-aph(2″)-Ia and vancomycin resistance with vanA genes.
Methods: 172 faecal samples collected from healthy volunteers were inoculated into BHI broth containing 6 μg vancomycin and 32 μg gentamicin. After enrichment the isolates were subcultured on mEnterococcus agar containing 6 μg and 32 μg of vancomycin and gentamicin respectively. The isolates were identified to the species level by biochemical tests and identification was confirmed also by PCR analysis. The MIC of isolates were performed by E test method. Identification of resistance genes were performed by PCR.
Results: Out of all isolates 15% gentamicin resistant enterococci (GRE) strains and 0.6% vancomycin resistant enterococci (VRE) strain have

been isolated. GRE isolates were highly resistant to gentamicin and the MIC of the VRE isolate was 256 μg. The only resistance genes were detected for gentamicin and vancomycin resistant isolates were aac(6′)-Ie-aph(2″)-Ia and vanA respectively.
Conclusion: The rate of vancomycin resistance were low in healthy population of our country. This indicative that the prevalence of vancomycin resistance enterococci as a normal flora is rare in the communuity. In the contrary the gentamycin resistance isolates were more prevalent in gut flora

R2339 *Staphylococcus aureus* nasal carriage among diabetic and non-diabetic volunteers

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Introduction: *S.aureus* is one of the most important human pathogens. It is a common cause of hospital and community-acquired infections worldwide, the incidence of which has been rising with increasing emergence of drug resistant strains.
Purpose: This study aims to estimate the extent of *S.aureus* carriage, particularly MRSA, among diabetic and non-diabetic residents of Fokida, a rural area in central Greece, by performing nasal swabs.
Methods: We conducted a cross-sectional study involving 82 adults. Samples were collected from the anterior nares using sterile cotton wool swabs. Nasal specimens were examined for the presence of *S.aureus* by standard quantitive culture methods including the tube coagulase test, the Microscan system and the Kirby-Bauer disk-diffusion test. Epidemiological information concerning risk factors for nasal carriage was also obtained. These included history of antibiotic usage in the past month, previous hospitalisation, history of chronic illnesses and residence in nursing home.
Results: A total of 82 individuals were examined. These comprised 40 diabetic (48.8%) and 42 non-diabetic (51.2%) persons with ages ranging from 14 to 85 years (mean age 56.68±17.04 years). None of the participants were ever hospitalised or had any other risk factor. Overall nasal carriage of *S.aureus* in this study population was 31.7% (26/82). From 82 nasal swabs collected, 4 MRSA isolates (4.88%) were identified, 2 of which were from diabetic persons. *S.aureus* positivity was noted in 12 of the 40 diabetic patients (30%) and 14 of the 42 healthy adults (33.33%). Heavy colonised carriers were 12 persons (46.1%) and half of them were diabetics.
Conclusions: Our study showed that there was no association between *S.aureus* carrier state and diabetes mellitus. Among the diabetic subjects, *S.aureus* colonisation was not associated with level of HBA1c and glucose control.
The findings also revealed that nasal carrier rates among residents of Fokida (31.7%) are higher as compared to those among the area's hospital personnel (20%), according to a previous study of ours. The prevalence of MRSA in our study population was found to be 4.88%.

R2340 Prevalence and characterisation of caMRSA in West Austria

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Objectives: Community-acquired meticillin-resistant *Staphylococcus aureus* (caMRSA) occur to play a significant role in clinical and outpatient care facilities.
The aim of the present study was to investigate the occurrence of caMRSA at the University Hospital of Innsbruck, in outpatient care facilities of small district hospitals and among patients of practitioners in Tyrol (Austria).
720 MRSA were collected from the University Hospital of Innsbruck in the period from January 2003 till November 2007. We used the CDC guidelines (http://www.cdc.gov/ncidod/dhqp/ar_mrsa_ca.html) to frame the "probable isolates of caMRSA". 174 MRSA from the University Hospital of Innsbruck were chosen for the study. Also 46 MRSA that were collected from outpatients in the period from August 2006 till November 2007were detected for caMRSA prevalence. All isolates were identified by standard microbiological procedures.
Methods: These strains were investigated for the presence of the lukS-lukF gene determinant of Panton-Valentine leucocidin (PVL). lukS-lukF-positive strains were characterised by resistance profile, SCCmec-cassette, accessory gene regulator (agr) and molecular genetic method (ribotyping). The patients' records were screened for possible risk factors.
Results: PVL-genes were detected in 23 MRSA: 13 at the University Hospital (1.7%), 5 from outpatient care facilities of small district hospitals (10.9%) and 5 among patients of practitioners in the Tyrol (10.9%). The mean age was 37.5 years and median age 27 years. PVL-genes were detected in 29% associated with cutaneous abscess and wound strains and 9%– with pneumonia.
The majority of strains (19) carried SCCmec-cassette IV, 1 strain SCCmec-cassette I, 1 strain SCCmec-cassette II, 2 strains SCCmec-cassette III. The major agr-type was type-1 (n = 16), 4 strains harboured agr-type 3, two strains – agr-types 3 and 4 each, and one could not be determined. Ribotyping revealed 9 different patterns, ribotype 2 was most prevalent.
Conclusions: The high prevalence of caMRSA in this study demonstrates an important health concern in Tyrol. The characteristics of the determined caMRSA-strains are comparable with the European average. The prevalence was higher than the European average (0.03% to 1.5%), which was evaluated 2002. The determination of caMRSA in the microbiological routine should be considered. In addition, practitioners should be educated to pay attention to the threat of caMRSA.

R2341 Prevalence of *Staphylococcus aureus* in healthcare workers in a general hospital in Komotini, Greece

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Objectives: The aim of present study was to determine the prevalence of *Staphylococcus aureus* carriage in the noses and on the hands of healthcare workers in our Internal Medicine Department and to detect the antibiotic susceptibility of *S. aureus*.
Material and Methods: Samples were obtained from nasal mucous (using swab) and hand skin (using imprints of each hand on blood agar) of total 210 employers in our department, during a three-month period. *S.aureus* isolates were identified by standard methods and typed by pulsed-field gel electrophoresis.
Results: 146(69.5%) females and 64(30.5%) males, with mean age 42±12.8 years, were included in the study. 156(74.3%) subjects were medical and nurse staff and 54(25.7%) were administrative personnel. Of total 630 samples, *S. aureus* isolated from 234(37.1%). 159 strains isolated from 53 women and 75 from 25 men. The rate of *S. aureus* in females and males were 36.3% and 39.1% respectively. There was no found statistical difference between males and females. The rate of *S. aureus* in medical stuff and administrative personnel were 45% and 19% respectively, $p < 0.001$. *S. aureus* isolated of 153 (65.4%) samples from nasal mucus and 81 (34.6%) from hands. Nasal carriage had 34 women (23.3% of women) and 17 men (26.6% of men). Hand carriage had 16 women (10.95% of all women) and 11 men (17.2% of all men). Meticillin resistant *S. aureus* was found in 42(6.7%) samples. Most of the *S. aureus* strains were susceptible to tested agents except erythromycin and azithromycin. Vancomycin and teicoplanin were active against all isolates even MRSA.
Conclusion: The prevalence of *Staphylococcus aureus* is quite high among the healthcare workers in our department (nearly half of our staff) but similar with colonisation rates reported in previously studies. The usually hygienic methods such as hand disinfection after each patients contact, masks use when is appropriate, must performed from all workers in hospitals to protect patients from nosocomial infections. Alcohol hand rub must be placed at every bedside in hospitals and promotional materials must be used to remind health workers and visitors to use the hand rub.

R2342 Clonal analysis and toxin gene carriage of meticillin-resistant *Staphylococcus aureus* isolated from three hospitals in western Greece

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Objectives: Meticillin-resistant *Staphylococcus aureus* (MRSA) is associated with an increasing number of both community and hospital-associated infections (CA/ HA-MRSA). The aim of this study was to identify clonal types correlated with the presence of genes encoding TSST-1 (tst) and Panton-Valentine leukocidin (lukS and lukF, PVL) among MRSA collected in three hospitals receiving patients from Western Greece.
Methods: In total 692 *S. aureus* isolates were characterised by conventional methods, followed by the determination of oxacillin MIC by the Etest. PBP2a production was investigated by a Latex agglutination test (bioMerieux). The presence of mecA, tst, lukSPV and lukFPV genes (encoding PVL) and agr groups were defined by PCRs. Clonal types were identified by PFGE of chromosomal DNA SmaI digests and named by their PFGE/agr types. CA-MRSA were isolated from patients without any predisposing risk factors.
Results: A total of 315 MRSA (mecA-positive) were isolated from different patients during one-year period, including 114 (36.2%) strains from children. The great majority (228, 72.4%) was CA-MRSA while 87 strains (27.6%) were HA-MRSA, derived mainly from the department of Orthopaedics. The PFGE analysis revealed 11 PFGE types (named A, B, C, E, G, F, J, K, L, M and N) common among the hospitals. Clonal and PCR analysis are shown at Table 1. Among the HA-MRSA four new clones were identified (J/3, L/2, M/3 and N/1) which were PVL and tst-negative. Among CA-MRSA clone C/3 carrying PVL genes predominated.
Conclusions: In Greece we are encountering an increasing rate of *S. aureus* infections, mainly due to the spread of CA-MRSA producing PVL. The identification of new clones in the participating hospitals reinforces the aspect of the continuous MRSA evolution.

Table 1. Clones and toxin genes among MRSA isolated during one-year period

Toxin	HA-MRSA (87/100%)										CA-MRSA (228/100%)										Total
	A/3	B/1	B/3	C/1	C/3	E/1	F/1	G/1	New types		A/3	B/1	B/3	C/1	C/2	C/3	F/1	G/1	G/2	K/4	
PVL(+)	–	–	–	–	39	–	–	–	–		3	1	–	1	1	209	–	–	1	–	255 (81%)
PVL(–)																					
tst(+)	–	1	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	1 (0.3%)
tst(–)	3	30	3	2	–	3	1	1	4		3	2	1	3	–	–	1	1	–	1	59 (18.7%)
Total	3	31	3	2	39	3	1	1	4		6	3	1	4	1	209	1	1	1	1	315
%	3.5	35.6	3.5	2.3	44.8	3.5	1.1	1.1	4.6		2.7	1.4	0.4	1.8	0.4	91.7	0.4	0.4	0.4	0.4	100%

R2343 Four-year survey of meticillin-resistant *Staphylococcus aureus* producing Panton-Valentine leukocidin in northern Greece

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Objectives: Meticillin-resistant *Staphylococcus aureus* is a major cause of hospital and community-acquired infections, such as surgical wound, skin and soft tissue infections and pneumonia.
The aim of this study was to define the prevalence and clonal distribution of PVL-positive MRSA among patients with staphylococcal infections in northern Greece.
Methods: Five hundred twenty eight *Staphylococcus aureus* isolates were collected during the 4-year period between January 2004 and September 2007 at the University Hospital of Alexandroupolis, Greece. Isolates were identified by conventional methods and the automated system VITEK 2 (BioMerieux, France). Susceptibility tests were performed by the disk diffusion method and determination of MIC by VITEK 2. The presence of PVL gene was detected by PCR, while the clonal relatedness was tested by PFGE.
Results: One hundred seventy three isolates(33%) were meticillin resistant. Among them, seventy three (42%), were resistant to kanamycin, tetracycline, intermediate susceptible to fusidic acid and carried the PVL gene. PFGE analysis revealed that all these isolates belonged to the clone C (ST 80), that predominates in Europe. The distribution of PVL positive strains were 43%, 51%, 35%, 37% for the years 2004, 2005, 2006, 2007 respectively.
Conclusion: In Greece the majority of PVL-positive strains express a common phenotype of antimicrobial agents(resistance to oxacillin, kanamycin, tetracycline and fusidic acid). Thus, isolates recovered from musculoskeletal infections with this phenotype must be examined for the presence of PVL gene, in order to establish prevention measures.

R2344 Intrafamilial spread of Panton-Valentine-positive *Staphylococcus aureus*: a Spanish case

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Background: Panton-Valentine leukocidin is a toxin associated with severe cutaneous infections and highly necrotising pneumonia. This toxin acts as virulence factor in some *Staphylococcus aureus* strains wich may be transmissed by person-to-person contact.
Objectives: To describe a family outbreak of skin infections, including furuncules, abscesses and cellulitis by *Staphylococcus aureus* Panton-Valentine positive, during 24 months.
Methods: The family affected is composed by mother, father, six children between the ages 6–17 years who lived at the same home. The outbreak also affected a 75 years old woman who spent a lot of time with the family, and the first son girlfriend. Detailed information regarding the history of infections of each patient and related diagnostic results and treatment were collected from Family Physician, Dermatologist and Paediatrician who treated the cases.
Samples were collected from infected skin areas affected and some of the patients and their contacts were screened for nasal and pharyngeal *S.aureus*.
All the *S.aureus* isolates were identified by conventional and molecular methods.
PCR was made to detect genetic sequences encoding mecA gene, PVL, enterotoxins, exfoliative toxins, alfa, beta, gamma and delta hemolysins, LukE-LukD leukotoxin and different adhesins. Isolates were typed by pulsed field gel electrophoresis (PFGE) and later Multilocus SequenceTyping (MLST) to ascribe the detected PVL-positive clone.
Antimicrobial susceptibility was studied with AST-P536 Vitek test.
Results: The cases were: furuncules, cellulitis and abscesses of diverse location, doing a whole of sixteen episodes. Three needed surgical incision and drainage, and seven episodes needed hospitalisation.
All clinic samples yielded *Staphylococcus aureus* PVL positive in pure culture. All strains were only penicillin resistant (PRSA). Four nasal swabs and five pharyngeal swabs were positive for PRSA (PVL+/PVL-:4/5). None carryied meticillin resistant *S.aureus*. All clinical isolates showed the same phenotypic and genotypic profile.
All family received treatment with cloxacillin and topic mupirocin.
Conclusion: This is the first case of intrafamilial spread of *Staphylococcus aureus* Panton-Valentine positive described in Spain. It is of concern the possible mupirocin resistance development after repeated treatment. Finally, it could be of public health importance to control the spread of infection, because the outbreak may involve non hospitalised persons.

R2345 Molecular characterisation of *Staphylococcus aureus* from Dutch general practice patients

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Objectives: Meticillin-resistant *Staphylococcus aureus* (MRSA) is an increasing problem, both in the community (community-associated

(CA)-MRSA) and in the hospital environment (hospital-associated (HA)-MRSA). In order to investigate the prevalence, the antibiotic susceptibility patterns and the population structure of *S. aureus* in the community, nasals swabs from patients without infectious symptoms visiting their general practitioner (GP) were taken and analysed.
Methods: GPs from 30 practices, situated all over The Netherlands, send nasal swabs from their patients to the laboratory for isolating and identification of *S. aureus*. The antibiotic susceptibility pattern to several classes of antimicrobial agents was determined according to CLSI guidelines, and the genetic background of the isolates was determined using spa typing and the algorithm based upon repeat pattern (BURP).
Results: From 617 out of 2,691 (23%) swabs, *S. aureus* was isolated. Of the 617 isolates, 595 were available for further analyses. The prevalence of resistance to penicillin, ciprofloxacin, macrolides, tetracyclin and fusidic acid was 68%, 1%, 5%, 3% and 6%, respectively. All isolates were sensitive to cefuroxime, clindamycin, imipenem, linozelid, meropenem. moxifloxacin, teicoplanin, trimethroprim/sulfamethoxazole and vancomycin. No MRSA isolates were found.
Typing revealed 244 spa types (of which 48 were new types), and 173 were clustered into 23 spa-clonal complexes (spa-CCs) (490 isolates). The remaining isolates could not be classified into spa-CCs. Up to 55% of the isolates were associated with a genetic background common to the endemic CA- or HA-MRSA clones, e.g. clonal complex (CC)1, CC5. CC8, CC22, CC30, and CC45. The remaining isolates were mainly associated with CC7, CC12, CC15, CC26, CC101 and CC121, commonly observed among MSSA worldwide.
Conclusions:
1. The *S. aureus* prevalence among general practice patients was 23%.
2. No MRSA isolated were observed in the population.
3. The low resistance percentages suggest that the increased resistance of *S. aureus* in hospitals is very likely not due to a reservoir outside the hospital.
4. Over half of the MSSA isolates had a genetic background common to endemic MRSA clones.
5. Several other MSSA lineages, not related to MRSA, were prevalent among the general practice patients.

R2346 The population structure of *Staphylococcus aureus* isolates of ICU patients in the Netherlands between 1996 and 2006

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Objectives: In The Netherlands, the meticillin-resistant *Staphylococcus aureus* (MRSA) prevalence is increasing. This increase could be due to a genetic macro-evolution of the meticillin-susceptible *S. aureus* (MSSA) isolates in The Netherlands. MRSA originated through the transfer of the mobile resistant element staphylococcal cassette chromosome mec (SCCmec) into MSSA. In order to investigate the changes in the population structure of *S. aureus* and the macro-evolutions of MSSA in The Netherlands, MSSA isolates from ICU patients were analysed.
Methods: *S. aureus* isolates (n = 856) from ICU patients were collected in The Netherlands between 1996 and 2006. They were yearly collected from January until July in two university hospitals and twelve general hospitals. The minimal inhibitory concentration (MIC) of oxacillin was determined by a micro-dilution method according to CLSI guidelines. The genetic background of the isolates was determined with spa typing and the algorithm based upon repeat pattern (BURP).
Results: Twenty-one isolates (2.5%) were resistant to oxacillin. Of the 287 spa types observed, 58 new spa types were identified and three new spa repeats were found among the new spa types. Among the *S. aureus* isolates, fourteen spa-clonal complexes were found. Ten isolates could not be clustered, and were singletons. Three of the singletons were classified as ST109. Seventy isolates were excluded from the analyses. A genetic background common to MRSA clones, e.g. CC1, CC5, CC8, CC22, CC30 and CC45 was observed among 46% of the isolates (n = 393). The remaining isolates were associated with CC7, CC15, CC25, CC26, CC51, CC97 and CC101.
Conclusions:
1. Half of the MSSA isolates (46%) had a genetic background common to MRSA clones.
2. The prevalence of oxacillin resistance was higher in this study than the prevalence found in The Netherlands. These isolates will be further analysed using SCCmec typing.
3. Several clonal complexes not related to MRSA clones were prevalent in the hospitals.
4. Further investigations to the changes over time and the virulence factor Panton Valentine leukocidin (PVL) are necessary.

R2347 Molecular heterogeneity of meticillin-resistant *Staphylococcus aureus* isolated in hospitals of Belarus

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Objectives: The emerging spread of meticillin-resistant *Staphylococcus* spp. poses a significant threat to public health. A thorough understanding of the molecular epidemiology and evolution of meticillin-resistance in *Staphylococcus* spp. is required to control and prevent diseases due to these organisms. The aim of the study was to examine the SCCmec types and clonal composition of MRSA isolated in Belarus.
Methods: 212 clinical isolates of *Staphylococcus* spp. including 71 MRSA, obtained from different hospitals during multicentre resistance monitoring in Belarus in 2006–2007 yy., were under study. It was detected by M-PCRs the presence of genes controlling resistance to meticillin and mupirocin (mec A, mup A), SCC mec types I–V, genes for toxic shock syndrome toxin (tsst), exfoliative toxins A and B (etaA and etaB), γ-hemolysins (hlg g), leukocidin E (luk E), leukocidin Panton-Valentine (pvl), genes for capsules of serotype 1, 5, 8 (cap1, cap5, cap8).
Results: Mec A positive isolates comprised 33% (71 isolates) of all tested *S. aureus*. Two strains (0.9%) harboured the gene of resistance to mupirocin. The majority of the tested MRSA possessed SCCmec type III (69%, n = 49) with the prevalence of IIIa subtype (63%). SCCmec types I, II, IV, V were detected less frequently in 10, 8, 8, 5% isolates correspondingly. Most of the MRSA strains (85%) carried hlg g and luk E – the genetic determinants of γ-hemolysins, leukocidin E production. 1.1% of MRSA were positive for etaA gene controlling synthesis of exfoliative toxins A. One of the virulence factors of *S. aureus* is the capsular polysaccharide. Most MRSA produced capsules of either serotype 5 (28%) or serotype 8 (65.5%). However, none of the studied strains possessed the cap1 genes. Studied MRSA strains did not hold the Panton-Valentine leukocidin and toxic shock syndrome toxin genes.
Conclusion: High isolation frequency of MRSA (33%) with the prevalence of the SCCmec type III (69%) and capsule serotype 8 clone was detected. The evolution and spread of this clone have to be monitored closely to prevent the clone's escape to non-hospital environment.

R2348 First detection of Chilean clone in clinical isolates of meticillin-resistant *Staphylococcus aureus* in the Colombian-Caribbean region

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Objective: MRSA has become a leading cause of nosocomial infections worldwide and it is beginning to prevail in the wider community as well. The aim of this study was to perform the molecular characterisation of MRSA clinical isolates from hospitals at the Colombian Caribbean Region.
Methods: Twenty three isolates recovered between October/05 and January/07 were identified by MicroScan® PC1A. Antimicrobial susceptibility tests were performed according to CLSI: disk diffusion with cefoxitin and oxacillin agar tests were used to confirm the presence of MRSA. PCR using specific primers was used to determine the mecA gene, SCCmec type and the presence of PVL. Genetic relatedness was assessed by PFGE, strain CHL93 (Chilean clone) was included for comparisons.

Results: The isolates were recovered primarily from ICU chiefly of skin or soft tissue. Nineteen (83%) were oxacillin resistant by disk diffusion, but only seven (30%) were positive by confirmatory tests (CLSI). These confirmed isolates presented resistance to clindamycin, ciprofloxacin, gentamycin and erythromycin; all were sensitive to vancomycin and linezolid, also mecA gene and SCCmec type I were detected. Isolates had no lukF-PV and lukS-PV genes. PFGE analysis of MRSA isolates demostrated a clone with 4 identical isolates and 1 narrowly related in the same institution, 3/5 were collected in ICU and 2 in Internal medicine ward from different patients. The clone showed a pattern profile equal to Chilean clone, reported before in 2003, which is found circulating in our region.
Conclusions: The presence of a multidrug resistance clone of HA-MRSA (SCCmec type I), with similar profile to the Chilean clone suggests the clonal spread and is emerging as an important cause of nosocomial infections in Colombian Caribbean hospitals.

Epidemiology of MDR-Gram-negatives

R2349 Molecular epidemiology of VIM-producing *Klebsiella pneumoniae* in a Greek hospital

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Objectives: Carbapenem-resistant *K. pneumoniae* strains, mainly due to metallo-β-lactamase (MBL) production, are widespread in Greek hospitals. MBL-producing *K. pneumoniae* were sporadically isolated in the Athens Navy Hospital since 2003. Following an outbreak that occurred from May to June 2006, due to the spread of one MBL-producing *K. pneumoniae* clone, all carbapenem-resistant *K. pneumoniae* isolated in this hospital from 2003 to November 2007, were sent for typing to the Microbiology Laboratory of the National School of Public Health.
Methods: Twenty-three carbapenem-resistant isolates, from an equal number of patients hospitalised in different wards, were studied. Identification and susceptibility testing were performed using the VITEK 2 system. All strains were tested for MBL production by the imipenem-EDTA disk synergy test. MBL production was confirmed by detection of blaVIM and blaIMP genes with PCR. All isolates were typed by PFGE and compared to the outbreak clone.
Results: All isolates were resistant to all major antibiotic classes and gave positive imipenem-EDTA disk synergy test. Genes of the blaVIM type were detected in all isolates. Typing revealed the spread of two major genetic types, present in all wards during the study period, one of them being identical to the outbreak clone.
Conclusions: Our study reveals the persistence of two VIM-poducing clones in the Athens Navy Hospital over the study period. One of these clones was responsible for the 2006 outbreak. Isolation rates of carbapenem-resistant *K. pneumoniae* decreased significantly after the outbreak, due to implementation of strict infection control measures. Surveillance, including typing, of carbapenem-resistant *K. pneumoniae* is of major importance, in order to prevent further spread of these strains.

R2350 Epidemiology and clinical impact of cefotaxime resistant *E. coli*, *Klebsiella* spp. and *Enterobacter* spp. causing bloodstream infection, skin and soft tissue including surgical site infection and closed space infection

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Objective: Cefotaxime resistant (CTX-R) Gram-negative bacilli (GNB) is endemic in Indian hospitals. The association with community and nosocomial (NO) occurrence and treatment outcome of these infections remains poorly characterised.
Methods: We analysed the prevalence and treatment outcome of 672 patients with GNB (*E. coli* (EC) n = 436, *Klebsiella* spp. (KS) n = 145, *Enterobacter* spp. (ES) n = 91) seen during Jan-June 2005 (six months) at our centre. Among them, 483 had soft tissue including surgical site infection (SSTI), 137, bloodstream infection (BSI) and 97, closed space infection (CSI) (peritoneal, pleural fluid and meninges). Records were missing for <1%. Acquisition of infection was clinically defined as community acquired (CA), when obtained as outpatient with no record of hospitalisation upto 1 year and do not have any features of healthcare-associated or NO (seen >72 hours after admissions). Antimicrobial susceptibility was performed by Kirby Bauer disc diffusion method against five antimicrobial classes. CTX-R was defined as ⩽27 mm zone diameter with a 30 μgm CTX disc and multi-drug resistant (MDR) if resistant >2 classes.
Results: Overall CTX-R infection rate was 56.8%; NO and CA rates were 32.2% and 24.6% respectively. CTX-R among NO/CA isolates were: EC 73/26%, KS 26/25% and ES 26/32%. CTX-R isolates caused 110 post surgical SSTIs. Among CTX-R isolates, resistance to ciprofloxacin was 85%, gentamicin 74%, pipercillin/tazobactum 70% and amikacin (53%). Overall MDR rate was 27% and 18.6% in CA-CTX-R isolates. All isolates were susceptible to imepenem and meropenem.. Confidence interval (CI) and Odds Ratio (OR) for clinical outcome parameters such as delayed improvement, early failure and early death were: 2.54 (1.44, 4.50); 0.49 (0.22, 1.07) and 2.86 (1.28, 6.37) respectively, occurring significantly more in SSTI and BSI caused by CTX-R isolates. In the CA-CTX-R infections CI/OR for these cited parameters were: 8.00(2.28, 28.05); 0.02 (0.06, 0.82) and 0.93 (0.9, 0.10) respectively Overall 36 (5.4%) patients died including 17 with BSI. Eleven patients with MDR CTX-R infections died. Inappropriate antimicrobial choice was observed in 36% of CTX-R infections with 25 deaths. All patients with CSI survived.
Conclusion: CTX-R GNB causing has emerged in the community, with high NO prevalence and a three-fold increase in mortality. Carbapenem resistance was not observed. As high rates of MDR exist clinicians need to be aware that appropriate treatment choice could impact outcome and post surgical. SSTIs are largely preventable via good infection control practice.

R2351 Clinical analysis and mortality risk factors with proved *Acinetobacter baumannii* bacteraemias

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Objectives: *Acinetobacter baumannii* is an important nosocomial pathogen because of high risk of mortality and increasing antibacterial resistance. We aimed to evaluate the predisposing factors, epidemiology, antibiograms and clinical features and mortality risk factors of *A. baumannii* bacteraemias.
Methods: A retrospective analysis of 51episodes with *A. baumannii* bacteraemia over two year period (2005–2006) at a tertiary hospital in Ankara, Turkey was performed. Hospital acquired infections were diagnosed according to the CDC criterias. Conventional methods were used for the identification of *Acinetobacter* isolates. Disc diffusion method was used antimicrobial susceptibility test of microorganisms. Multidrug resistance (MDR) was defined as resistance to all studied agents (including piperacillin/tazobactam, cefepime, ceftazidime, aztreonam, ciprofloxacin, gentamicin, tobramycin), but we allowed susceptibility to amikacin, netilmicin, imipenem and meropenem.
Results: The mean age of the patients with *A. baumannii* was 60±14 years (range:1–91 year). Predisposing factors detected in patients infected with *A. baumannii* were urinary catheter insertion (92%), central venous catheter (53%) and mechanical ventilation (43%), consecutively. The mean duration time between hospitalisation and occurrence of infection were 13.9±10.1 days. Clinical pneumonia was detected on 25 of the patients due to *A. baumannii*. An overall mortality rate was 61%. Mortality rate of the patients with clinical pneumonia was 63%. Among *A. baumannii* isolates 53% were detected as MDR. MDR infection resulted in higher mortality rate than non-MDR cases (1.7 times) but this difference was not statistically significant. Older age (⩾60 years), mechanical ventilation, central venous catheter, insertion of urinary catheter, being a ICU patient, infection with a MDR isolates

were analysed by univariate and multivariate test. Being an ICU patients was found a risk factor for mortality on univariate analysis ($p < 0.05$). However only infection with a MDR isolates was found as a risk factor independently associated with mortality ($p < 0.05$).
Conclusion: These data suggest that, the infections due to MDR isolates of A.baunannii may cause a high mortality rate especially in ICU patients. Performing strict infection control measures might be critical for the manegement of these infections.

Antibiotic usage

R2352 Tissue penetration and clinical efficacy of gatifloxacin, a respiratory quinolone, in adult patients with penicillin-intermediate *Streptococcus pneumoniae* and penicillin-resistant *Streptococcus pneumoniae* sinusitis

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Objectives: The clinical efficacy of Gatifloxacin (GFLX), a respiratory quinolone, in adult patients with acute bacterial sinusitis (ABS) was examined. Tissue penetration to nasal discharge was also examined in support of clinical efficacy.
Methods: Subjects were ABS patients aged ⩾18 years without underlying disease or pregnancy. At first visit, purulent or mucopurulent nasal discharge in middle nasal meatus was collected for bacterial cultivation. The clinical efficacy was determined based on the volume and viscosity of nasal discharge after oral administration of Gatifloxacin 400 mg/day b.i.d. Nasal discharge in middle nasal meatus two to seven days after administering GFLX was collected with aspirating tube and stored at −80ºC. The concentration of GFLX was analysed by gas chromatograohy.
Results: Fourteen male and 37 female patients ranging aged 19 to 74 years were included, those in their thirties accounting for 45%. The clinical efficacy of GFLX was remarkably good in 11 patients, good in 33 patients, somewhat good in two patients, and no response in five patients. GFLX was effective in total 86.3% of patients. Six of 51 patients had not responded to Telithromycin administered at first visit, however, GFLX administered at second visit was effective in all of them. In 32 of 34 patients (94.1%) with PISP and PRSP, GFLX was clinically effective with remarkably good or good response. Tissue penetration of GFLX to nasal discharge was examined in 9 patients. Nasal discharge sample was collected 1.5 to 12 hours after administering GFLX. The Concentration of GFLX in nasal discharge was 0.69–7.04 mu g/g. The antibacterial activity of GFLX against *S. pneumoniae* was MIC90 0.06–0.5 mu g/mL, against Hemophilus Influenzae was MIC90 ⩽0.06, and against *Moraxella catarrhalis* was MIC90 – 0.06–1 μg/mL.
Conclusion: Efficacy ratio of penicillin and cephem antibiotics against PISP and PRSP is around 50–60%. GFLX showed high efficacy ratio, 86.3%, with good antibacterial activity and high tissue penetration maintained for long time. Our results suggest that GFLX is first-line antibacterial agent of ABS therapy.

R2353 Daptomycin monotherapy in the treatment of vancomycin-resistant enterococcal bacteraemia

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Objectives: Daptomycin (DAP) is indicated for *Staphylococcus aureus* bacteraemia, but has not been sufficiently studied in bacteraemia caused by vancomycin-resistant enterococci (VRE), a difficult-to-study disease state common in the intensive care unit (ICU). The aim was to retrospectively evaluate DAP monotherapy in patients with VRE bacteraemia.
Methods: We performed a retrospective study of patients treated for VRE bacteraemia between January 2004 and July 2007 by reviewing medical records. Patients were included if they received DAP monotherapy and had a blood culture positive for VRE at the time DAP was started. Patients receiving concurrent antibiotics for VRE bacteraemia and those with negative blood cultures prior to receiving DAP were excluded. The primary endpoint was microbiological cure, defined as negative blood cultures for VRE at the end of therapy. Clinical outcomes at the end of therapy were also evaluated with standardised definitions and adverse events were assessed: cure or improvement were considered positive, and worsening or death within 7 days of the end of DAP therapy were considered negative. Descriptive statistics were used and data are reported as median (range), except where noted.
Results: 31 patients were included. DAP was initiated 23 days (1–281 days) into the hospital stay, for a duration of 13 days (1–42 days). 20/31 patients (64.5%) were in the ICU when presentation of bacteraemia occurred, with an ICU stay of 35 days (2–298 days). Prior to DAP therapy, bacteraemia was present for 3 days (0–7 days) without effective therapy for VRE (i.e. no antibiotics active against VRE, or linezolid failure occurred). *Enterococcus faecium* was cultured 28 times, *E. faecalis* was cultured 5 times, and *E. raffinosus* was cultured once; 3 patients had multiple species cultured. All isolates tested were susceptible to DAP. The dose given was 6 mg/kg (4.0–6.7 mg/kg). Blood cultures tested negative 2 days (0–24 days) after initiation of DAP therapy. Microbiologic eradication was observed in 25 patients (80.6%) and positive clinical outcomes were seen in 18 patients (58.1%). Mild creatine phosphokinase (CPK) elevations were seen in only two patients (CPK 243 U/L and 252 U/L; normal range 20–230 U/L) and both spontaneously resolved while on DAP.
Conclusion: In this descriptive study, DAP monotherapy was effective in clearing bacteraemia caused by VRE.

R2354 Prophylaxis of surgical site infections – Institutional Project Phase II

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Objectives: Surgical site infections (SSI) are among the most frequent and expensive Hospital acquired infections and are associated with higher morbidity, disability and lethality. We report the second phase (2003–2007) of an institutional project, aiming at reduction of SSI.
Methods: Prospective direct and indirect surveillance of SSI; development of Guidelines for Hospital hygiene and Antibiotic prophylaxis in Surgery; Audit of Antibiotic prescriptions; Calculation of Antibiotic consumption in DDD/100 patient-day (pd); Microbiological methods: according to the BSOPs, HPA, UK, 2005 (-wound, screen-, environment-specimens) and to the CLSI, USA, 2005 (Disk diffusion Antimicrobial susceptibility testing method).
Results: Guidelines for Hospital hygiene (standard and regarding the care for patient: -before, -during and -after the operation) and Guidelines for Antibiotic prophylaxis were actualised upon a consensus in 2003. Three 3-month audits of antibiotic prescriptions were performed. The first one (2003) showed a usage of cephalosporins of higher generations as a difference from the accepted rules, while in 2004/5- and 2006- audits the choice of antibiotic and antibiotic dosage were correct. A relatively high antibiotic consumption of 48.2–50.0 DDD/100 pd was calculated. The cephalosporins of first generation were the most commonly used antibiotics (29.5–33.4 DDD/100 pd), followed by the group of imidazoles (4.5–5.4 DDD/100 pd). Two unfavourable tendences took place: a decrease in the usage of penicillines (from 5.3 to 2.1 DDD/100 bd) and an increase of the cephalosporins of third generation (from 2.0 to 3.3 DDD/100 bd). The most frequent pathogens from the wounds of patients were *Staphylococcus aureus*, followed by enterobacteriae and anaerobes. Dynamic of Antimicrobial resistance showed a significant increase in 2004 vs. 2003, when MRSA raised 4-fold (up to 20%) and carbapenem-resistance in *Pseudomonas aeruginosa* – 2.5-fold (up to 35%), as a part of a national-wide increase in resistance; the rate of ESBL-producing Enterobacteriaceae was also high – 33%. Microbiological control tests did not show significant breaks in hospital hygiene.
Conclusion: The rate of SSI was decreased to <5% during the project, due to the consensus-accepted guidelines by the personnel of the Clinic for Surgery. However, further work would be necessary to decrease

antibiotic use and to tailor the correct time and duration of antibiotic prophylaxis.

R2355 Analysis on the transition of change in antimicrobical use following preventive intervention in the perioperative antimicrobial prophylaxis in a hospital

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Objectives: To confirm short-term effects of intervention in perioperative antimicrobial prophylaxis using computed prescription system
Methods: This was a prospective, before-after comparative study of the intervention conducted at an 860-bed university hospital in Korea, from January 2007 through September 2007. Computed intervention programme has been executed for the appropriate perioperative prophylactic antimicrobial use from May 2007. Upon antimicrobial prescription, computed intervention programme displayed pop-up providing recommended guidelines on the appropriate duration and type of antimicrobials and required to check the reason for prescribing. A small working group monitored the perioperative prophylactic antimicrobial use by department, division and class every two weeks and gave feedback to the departments and surgical doctors. The effect of intervention programme was analysed in comparing antimicrobial use density (AUD) in September 2007 with AUD in January 2007 (pre-intervention period) and the average AUD of last year. The transition of change in the usage of antimicrobials on overall and surgical purpose was analysed in detail, by monthly doses and antimicrobial class.
Result: Average monthly AUD for surgical patients (surgical AUD, 715) was higher than overall AUD, 516 in 2006. After intervention programme, surgical AUD dropped 23.0%, from 695 in January to 536 in September 2007. Likewise, overall AUD went down 8.8% from 503 to 459. Due to changes in doses by antimicrobial class, surgical AUD for 3rd generation cephalosporin decreased 53.3%, from 187 of annual average in 2006 to 87 in September 2007 and reduced 44.4%, from 157 in January to 87 in September 2007. For aminoglycoside class, surgical AUD fell 44.2%, from 211 of annual average in 2006 to 118 in September 2007, and diminished 48.5%, from 229 in January to 118 in September 2007. This shows that antimicrobials in these two classes have led the changes in overall antimicrobial use. Meanwhile, 2nd generation cephalosporin relatively increased 10.8% and 5.9% in the same comparison as above.
Conclusion: Regarding the transition of change in antimicrobial use before and after computed intervention programme, 3rd generation cephalosporin and aminoglycoside classes use in surgical patients were remarkably reduced, which attributed to the decrease in overall antimicrobial use.

R2356 Antibiotic prescriptions at the central pharmacy of a Hungarian town

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Background and Objectives: In Hungary, all antibiotics are prescription only medicines. As during the dispensation process at pharmacies several data are not electronically registered (e.g. patients' age, dosage), retrieve from prescription databases has limitations. Therefore, we aimed to perform a study where a more sophisticated aspect of antibiotic use could be determined.
Methods: The setting was the central pharmacy of a Hungarian town with a population of 30,000. The manual review of all prescriptions redeemed during six separated weeks in 2007 was performed. The following data was copied out from antibiotic (Anatomical-Therapeutic-Chemical class J01) prescriptions: the patient's age, gender, the doctor's individual code, the product name, form, the indication and the prescribed dosage.
Results: In total 26,638 prescriptions were reviewed, of which 1,032 (3.9%) were related to antibacterials. The antibacterial prescriptions were originated from 176 different doctors. The age-distribution of patients were the following: 0–14 years: 197 patients (19.1%), 15–65 years: 673 patients (65.2%), above 65 years: 162 patients (15.7%). Among children we observed a male (55.0%), above 15 years of age, a female dominance (65.0%). Parenteral therapy was indicated only in 6 cases (0.6%), oral liquid products were also prescribed in minority (135 cases-13.1%). The three most common diagnoses in children were tonsillitis, pharyngitis and rhinopharyngitis. In 15% of cases antibacterials were prescribed for otitis. In adults acute bronchitis was the most frequent diagnose, followed by pharyngitis and tonsillitis. Infection of the urogenital tract was the indication in 22% of cases. The top list of antibacterials were cefuroxime, amoxiclav and chlarithromycin in children and amoxiclav, cefuroxime and ciprofloxacin in adults. In adults, the prescribed antibacterial dosages showed good accordance with the World Health Organisation's (WHO) defined daily doses except amoxicillin, amoxiclav and cefuroxime, where doctors prescribed higher doses.
Conclusion: The manual review of individual prescriptions enabled us to perform an in-depth analysis. We have found that in several cases antibacterials were indicated for infections where the need of antibacterial treatment is questionable. We showed the lack of under dosing, and highlighted the discrepancy between WHO defined doses and prescribed dose in case of amoxicillin, amoxiclav and cefuroxime.

R2357 Daptomycin for the treatment of *Staphylococcus aureus* bacteraemia

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Objective: To examine the outcomes of patients (pts) with *Staphylococcus aureus* bacteraemia (SAB) treated with daptomycin; approved in US 2006 at 6 mg/kg/qd.
Methods: Pts with a positive blood culture for *S. aureus*, and evaluable for outcome (cure, improved, failure) were identified in a registry – The Cubicin® Outcomes Registry and Experience (CORE^{sm}) 2005 and 2006 programme years. Outcome was assessed at the end of daptomycin therapy using protocol-defined criteria. Pts with blood cultures positive for any other pathogen or for endocarditis were excluded. Success was defined as cure or improved.
Results: Of 238 pts with SAB meeting the selection criteria, 179 (75%) were evaluable; successful outcomes were reported in 157 pts (88% overall; 89% meticillin-resistant *S. aureus* [MRSA] n = 119, 85% meticillin-susceptible *S. aureus* [MSSA] or meticillin susceptibility unreported, n = 60). 54% of pts were males and 35% were >65 years of age. Comorbidities included diabetes (37%) and sepsis (20%). 26% received daptomycin in an intensive care unit. 35% of pts had an initial CrCl <30 ml/min, 65% of these were receiving dialysis. The most common concurrent infection types were skin and soft tissue (21%) and osteomyelitis (11%). Other antibiotics were given prior to daptomycin in 83% of pts, most commonly vancomycin (72%). The success rate of daptomycin was 88%, irrespective of prior vancomycin exposure. The most common reason for stopping prior antibiotic therapy was failure (31%). Concomitant antibiotics ($\geqslant$1 dose) were used in 57% of pts, most frequently rifampin (26%) and vancomycin (25%). The receipt of concomitant rifampin or vancomycin did not effect success (83% with and 89% without rifampin or vancomycin, P = 0.3). The median final daptomycin dose and duration of therapy was 6 mg/kg (60% received $\geqslant$6 mg/kg) and 15 days (range, 1–84), respectively. Eight adverse events in 8 (4%) pts were possibly related to daptomycin; three of these met the regulatory criteria for seriousness. The mortality rate was 6%, n = 11, no deaths were reported as being possibly related to daptomycin.
Conclusion: In this population, daptomycin was effective for SAB. No apparent difference in success rates was seen between patients with MRSA infections and those with MSSA infections. Previous treatment with vancomycin did not influence the outcome of patients treated with daptomycin, nor did concomitant use of the antibiotics rifampin or vancomycin.

R2358 Unlicensed and off-label antibiotic use in a paediatric city hospital in Russia

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Objectives: To determine the proportion of unlicensed and off-label antibiotic prescriptions in hospitalised paediatric patients.
Methods: All antibiotic prescriptions to children admitted to neonatology unit and three medical units for toddlers, school-age children and teenagers were prospectively recorded from 1st of January to 30th of June 2006. Categories of unlicensed use included: 1. unlicensed medicines imported from foreign countries; 2. adult preparations that is not suitable for children; 3. modifications to licensed drugs formulation; 4. use of chemicals as drugs; 5. medicines prepared or modified by hospital pharmacy. Prescriptions were considered off-label in the following cases: 1. use for indication not described in the licence; 2. use when product is contraindicated; 3. administration outside the age range; 4. use of wrong dose, frequency or alternative routs of administration. Package inserts and National Drug Register edited in 2004 were used as reference sources.
Results: Four hundred sixty nine patients aged from five days to 18 years (55.3% female, median age 8.9 years) were included in the study. A total of 469 antibiotic prescriptions were written for the patients. Antibiotics were prescribed to 51% of children in neonatology unit, to 34%, 41% and 56% of toddlers, school-age children and teenagers, respectively. In 93% of cases antibiotics were written by attending paediatrician, followed by doctor on call (3.2%) and clinical pharmacologist (3.2%). There were no cases of unlicensed antibiotic use recorded. The incidence of off-label prescriptions was the highest in neonatology wards (49%) and decreased with age (28%, 10% and 11% in toddlers, school-age children and teenagers, respectively). Off-label use due to unproved indication was registered in 38% of cases; it was related to dose and frequency of administration, age limitations in 36% and 26% of prescriptions, respectively.
Conclusion: The practice of off-label antibiotic use was quite common in hospitalised children in Russia especially in neonatal wards. These findings underline the importance of research on antibiotic use in paediatric population as well as additional efforts to improve the quality of their prescribing within the licence.

Molecular bacteriology

R2359 Analysis of factors of virulence and LPSs from *Vibrio cholerae* serotypes isolated from Slovak rivers

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Objectives: The analysis of genes of virulence and lipopolysaccharide analysis of *V. cholerae* serotypes O1 and non-O1 was the purpose of our work.
Methods and Results: Pathogenic potential of *V. cholerae* is represented by several virulence factors – toxins. We analysed virulence factors in O1 and non-O1 (O135, O34) serotypes of *V. cholerae* isolates using PCR method. There were analysed 9 genes of virulence in our isolates: ompW, ompU (genes determined porins), toxR (gene det. regulating factor) tcpA, zot (gene det. zonula ocludens toxin), ace (gene det. accessory cholera enterotoxin), hlyA/b (gene det. hemolysin), st (gene det. thermo-stable toxin).
As all Gram-negative bacteria, their cell walls contain characteristic lipopolysaccharide (LPS) structures – endotoxins. LPS is composed of three parts: lipid A, polysaccharide core and O-specific oligosaccharide. The O-oligosaccharide containing of mono- or oligosaccharide repeating units with enormous structural variation determines the serological specificity of the bacterium and also demonstrates vibriocidal activity. We have used two serotypes of non-O1 *V. cholerae*: O135 and O34. There were chosen 4 isolates of each serotype for cultivation, isolation and analysis of LPS endotoxins. Proportion of saccharides, proteins and nucleic acids in raw LPSs was determined by spectrophotometry. Molecular weights of the elements (lipid A, polysaccharide core, O-specific chain as an antigen) were determined by SDS-PAGE and HPLC. Then, the dialysis and acid hydrolysis of samples of LPSs were followed. The highly purified O-specific oligosaccharide chain was obtained by the size exclusion chromatography. The highly purified O-specific oligosaccharide chain (O-antigen) was analysed by NMR and IR spectroscopy. Saccharide composition of O-antigen was determined by gas chromatography.
Conclusion: Our results provide a confirmation of diversity of analysed serotypes on the basis of genotype variability and LPS variability, of a degree of coincidence with determined serotypes and of informational databasis about one important element of immunogen or antigen complex of *Vibrio cholerae*. The detailed examination of LPS structures is necessary for preparation of defined glycoconjugates (O-antigen bound to a protein vehicle) and could contribute to progress in creating a new stable, safe and sufficiently immunogenic vaccine.
This work was supported by the Slovak Research and Development Agency APVV No. 0032–06.

R2360 Genetic diversity of *Vibrio* pathogenicity island in *Vibrio cholerae* isolates of clinical origin in Iran

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Objective: Two important virulence factors of *Vibrio cholerae* strains includes cholera toxin and toxin coregulated pilus (TCP) encoded on *Vibrio* Pathogenicity Island (VPI). This region which is 41.2 kb in size encodes 29 proteins and including genes necessary for biogenesis of TCP which plays an important role in the colonisation of bacterium in the gut, biofilm formation and CTX prophage attachment. The aim of this study is to investigate the presence and diversity of the VPI in the *Vibrio cholerae* isolates of clinical origin from Iran.
Methods: 20 *Vibrio cholerae* isolates investigated in this study using biochemical and serological tests and subjected to analysis using Long-PCR analysis of the central region of the VPI including the complete TCP gene cluster. PCR products were subjected to Restriction Fragment Length Polymorphism (RFLP) analysis using EcoRI, EcoRV, ClaI, BglI and DdeI restriction enzymes. Left and right segments of VPI cluster were also subjected to analysis using 6 pairs of primers which spans the major gene clusters in these regions and also the integrase and joining sites of the cluster.
Results: Serogrouping revealed that 25% of isolates belonged to the O1 Ogawa, and 75% to the O1 Inaba serogroups. PCR analysis and subsequent RFLP of PCR products showed that 85,100,100, 76,100,100 and 80% of isolates possesed LJ, aldA, tagA, toxT, acfB-C, int and RJ regions, respectively. Long-PCR analysis and subsequent RFLP of product revealed that 100 and 100% of isolates possesed tcpI-F, tcpQ-R and tcpQ-F, tcpF-R, respectively, according to the standard strain ATCC 14035.
Conclusion: Results of this study emphasizes that, the integrity of TCP gene cluster is necessary for toxin coregulated pilus production and function and subsequent CTX prophage acquisition by *V. cholerae* and showes the low diversity of the left and right segments of this cluster. It seems that the ability of VPI for excision from the genome of the *V. cholerae* and some point mutations in the binding region of the primers stands for the absence of some of the fragments in a low percent of isolates and our data are in consistent with the hypothesis that the VPI can have a mosaic structure in some *Vibrio cholerae* strains and genotye diversity is due to the circulation of virulence genes.

R2361 Sequence-based bacterial identification from clinical samples: one year experience

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Introduction: Identification of clinically relevant bacteria relies on automated systems in clinical laboratories, assuming differences in

morphology, growth, metabolic and enzymatic activity. However, these methods are generally less accurate than genotypic methods, particularly when applied to poorly described, rarely isolated or phenotypically aberrant species. Genotypic approaches can even lead to the recognition of novel pathogens and non-cultured bacteria.
Objectives: The present study aimed at evaluating the use of MicroSeq 500 for the 16S rRNA sequence based identification of bacteria in isolates and primary clinical specimens with doubtful automatic identification results.
Methods: We analysed 6 isolates from an external quality control programme and 27 clinical samples (isolates and primary samples) by direct sequencing using MicroSeq 500 16S rDNA microbial identification system (Applied Biosystems).
Results: All QC samples showed the correct sequence based identification, and all clinical samples resulted in a clinically compatible bacterial identification. Of these, one isolate from a meningitis patient, showed as *Streptococcus suis* by conventional methods. As the primary CSF sample was still available it was analysed by an in house optimised 16S rRNA based broad range real-time-PCR and showed a positive result. We then applied MicroSeq 500 to the primary CSF sample, with the results confirming the automatic identification.
We correctly identified the genus of three *Brucella* spp. strains but could not reach species identification. This was due to low variability in the 500bp region sequenced that had been associated with failure to discriminate the species in this genus. We were unable to differentiate between *Mycobacterium chelonae*/*M. abcessus*, which is a well documented limitation of this method.
Conclusion: The present results indicate that sequence based identification represents a useful method for bacterial identification, whose performance could be improved by the use of pyrosequencing, with advantages both in turn-around-time (TAT) and the low TAT impact of the use of other target regions.

R2362 Improving diagnosis of Bordetella pertussis to improve patient management

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Objectives: Bordetella pertussis is a serious potentially life threatening infection particularly in unimmunised infants. There has also been an increase in the number of people who, although fully vaccinated, loose their immunity over time. Due to the fragile nature of the organism and the prolonged time required to culture the organism it is significantly under reported. It was proposed that introducing real-time PCR as a clinical service to detect Bordetella pertussis and Bordetella parapertussis (a related less severe infection) would improve the management of patients with this infection.
Methods: Pernasal swabs or naso-pharyngeal aspirates from patients with a clinical suspicion of whooping cough were collected and cultured immediately as per routine laboratory protocol. In our 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 reaction which amplifies a specific B.pertussis or B.parapertussis gene target allowing detection amplified DNA.
Results: In the first year of introducing real-time PCR as a clinical service the turn-around time of sample processing improved from 5–7 days using standard culture methods to 24 hrs with a maximum of 3 days if a specimen was collected on a weekend. The sensitivity of the assay was also improved with 4 cases from a total of 13 positive patients that would not have been detected when culture was used alone. Clinicians were also more likely to take specimens for pertussis testing due to the improved service this resulted in cases of dual infection with RSV being detected were a positive virology result alone may have resulted in no further investigations.
Conclusion: The use of real-time PCR has allowed both a faster turn around time in processing patient samples, increased sensitivity and more appropriate isolation procedures for in-patients.

R2363 Antibiotic resistance of commensal *Escherichia coli* and *Lactobacillus* isolates from the healthy gut microflora

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The aim of our study was to investigate the antibiotic resistance of commensal *E. coli* and *Lactobacillus* isolates in the gut of healthy voluntaries during the period 2006–2007 years from Yerevan.
The resistance of 235 *E. coli* and 57 *Lactobacillus* isolates from the faecal microflora of healthy voluntaries to the next antibiotics in the following concentrations were tested: tetracycline (Tc) 15 μg/ml, doxycycline (Dc) 15 μg/ml, amoxicillin (Ac) 25 μg/ml, chloramphenicol 30 μg/ml (Cm), streptomycin 50 μg/ml (Sm). Statistical analysis was performed using the CHITEST (null hypothesis).
Antibiotic resistance profiling has demonstrated that the *E. coli* strains have a substantial level of resistance to commonly used antibiotics. In 2006 the proportion of resistant *E. coli* isolates was 18% to Dc, 12% to Tc, 23% to Ac. The results for 2007 year showed that in healthy volunteers the number of antibiotic resistant *E. coli* isolates increased significantly in comparison with 2006 year (36% to Dc, 32% to Tc, 25% to Ac). The resistance to tetracycline group antibiotics was almost two fold greater in 2007. The sensitivity to Ac dropped insignificantly. The proportion of multiresistant isolates (resistant to at least to two antibiotic's groups) remained same for the investigated period. The increase was observed for single antibiotic resistant isolates (19.4% in 2006 and 23.4% in 2007 respectively).
Compared with the *E. coli* isolates the quantity of resistant *Lactobacillus* isolates is low in the gut microflora of healthy persons and the quantity of *Lactobacillus* isolates, having the same resistance to antibiotics as *E. coli* isolates in individual is less.
Thus it could be concluded that there is an increasing trend in antibiotic resistant commensal spreading in the gut of healthy subjects. This could represent the risk factor for antibiotic resistance gene dissemination by the commensal as it have been shown that the antibiotic resistance factors (plasmids) may circulate in a broader environment and include the bacteria of diverse origin and taxonomic position. In addition, *E. coli* strains efficiently exchange genetic material with pathogens such as genera *Salmonella*, *Shigella*, *Yersinia*, and *Vibrio* as well as pathogenic *E. coli*.

R2364 CagA and other key genes in the cag pathogenicity island of *Helicobacter pylori* isolated from dyspepsia patients

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Objective: *Helicobacter pylori* is a bacterium associated with upper gastrointestinal diseases in humans. Several studies have tried to establish an association between known virulence markers and clinical outcomes, in many cases the results has been conflicting. Cytotoxcin associated gene A (cagA), E (cagE) and certain vaculating cytotoxin (vacA) genotypes are associated with peptic ulcer disease (PUD). The present study compared the distribution of vacA allotypes and cag PAI status in Iranian patients isolates.
Methods: From November 2006 to July 2007, two hundred and ten patients referred to the Taleghani hospital for endoscopy.197 gastric biopsy specimens of 169 none ulcer disease (NUD) and 28 peptic ulcer disease (PUD) were distinguished qualify for next steps. The samples were cultured by standard methods. PCR was performed for ure C (glmM) gene to identify *Helicobacter pylori* strains. Isolation of cagE, cagM, cagG, oipA and vacA allotypes were done by PCR.
Results: Among NUD and PUD, *H. pylori* was isolated in 148 of 169 (75.1%) and 21of 28 (75%) respectively. PCR amplification detected cag A gene in 103 (69.6%) of NUD patients and in 66.7% of PUD patients. cagT, cagG, cagM, cagE and oipA genes were observed in *H. pylori* isolates from NUD patients in 29.7%, 10%, 0.2%, 39.4% and 50% respectively. Also these genes were found in 23%, 9%, 33% and 42% of PUD patients respectively. Among analysis of vacA allotypes,

s1 and m2 genotype were predominant types among *H. pylori* strains (36.2%). CagM was observed in 4% of the *H. pylori* isolates in PUD and was more than isolated strains in NUD(0.2%).
Conclusion: The data show that, infection by *H. pylori* is an important cause of gastritis in Iranian patients. Also the results note that, there was no association between cagA, cagG, cagM, cagE and oipA genes and clinical outcomes. The result of this study showed none of the markers are helpful in predicting the clinical presentation of a *H. pylori* infection, however, for full confirmation of our data and explanation of possible factors of *H. pylori* to the pathogenesis of the diseases further data to be collected and evaluated.

R2365 A molecular study on *Enterococcus faecalis* in primary and secondary endodontic infections

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Objectives: This study were aimed to investigate the presence of *Enterococcus faecalis* in primary and secondary endodontic infections using real-time PCR (RT-PCR) and to determine the statistical importance of the presence of *E. faecalis* in a Turkish population with endodontic infections.
Methods: *E. faecalis* was investigated from 40 samples from patients who were attended at the Endodontic Clinic of the Dental Faculty of Ataturk University in Erzurum, Turkey. Samples were taken from 40 root canals, 20 with acute periradicular abscesses (primary endodontic infections) and 20 with failed endodontic treatments (secondary endodontic infections). DNA was extracted from the samples using a QIAamp® DNA mini-kit and analysed with RT-PCR. 16S rRNA-directed species-specific primers were a forward 5′-3′ CCGAGTGCTTGCACTCAATTGG and a reverse 5′-3′ CTCTTATGC-CATGCGGCATAAAC. Ubiquitous primers directed to 16S rRNA were a forward 5′-3′ TTAAACTCAAAGGAATTGACGG and a reverse 5′-3′ CTCACGACACGAGCTGACGAC. As a positive control, we used an *E. faecalis* strain from the bacterial stocks of our laboratory in the Department of Microbiology and Clinical Microbiology. The bacterium had been previously identified based on fatty acid profiles using the MIDI Sherlock® Microbial Identification System (MIS) (MIDI, Inc., Newark, Delaware, USA). A proportion test and Fisher's exact Chi-square test were performed for statistical analyses using SPSS for Windows, 13.0.
Results: RT-PCR allowed for the detection of *E. faecalis* in 15 of 20 (75%) samples taken from patients in whom endodontic treatment had failed, and in 5 of 20 (25%) samples of pus aspirated from acute periradicular abscesses. *E. faecalis* was detected in 20 of total 40 patients, which revealed statistically that it may exist in 62% of all endodontic infections when the proportion test was applied ($z = -1.645$, $alfa = 0.05$). Additionally, *E. faecalis* was found significantly more often in patients with secondary endodontic infections than in patients with primary endodontic infections, based on Fisher's exact Chi-square test ($p = 0.004$).
Conclusion: Our data suggest that *E. faecalis* is a frequently-found bacterial agent in Turkish patients with endodontic infections, and is more often associated with secondary endodontic infections than primary endodontic infections.

R2366 Prevalance and molecular epidemiology of *Staphylococcus aureus* isolates carrying Panton-Valentine leukocidin genes during a five-year period (2003–2007) at a tertiary care centre in Izmir, Turkey

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Objective: Panton-Valentine Leukocidin (PVL) is accepted as a marker of disease severity in staphylococcal infections. In this study we have determined the prevalance of PVL genes among *S.aureus* isolated from clinical samples over a five year period.
Methods: 102 MRSA and 156 MSSA isolates which were obtained from, blood, wound, urine and respiratory tract samples of hospitalised patients were selected randomly from a collection of strains. PVL genes were screened by a multiplex PCR protocol which detects pvl (luk S and luk F) and nuc genes.
Results: PVL genes were detected in 0, 2 (4.3%), 4 (10.3%) and 2 (4.3%) of MSSA isolates in 2003–2004, 2005, 2006 and 2007, respectively. None of the MRSA isolates taken into the study contained pvl genes. An increment in the prevalance of pvl-positive isolates was noted in 2006. PFGE analysis revealed a small cluster of clonally related isolates in General Surgery patients with SSI.
Conclusion: PVL prevalance is low in MSSA and nil in MRSA strains isolated in our hospital. This is probably due to the fact that none of the MRSA isolates is community-acquired. PVL positive MSSA are distinct from the predominant MRSA clone in our hospital as revealed by PFGE analysis.

R2367 *Vibrio cholerae* among stool samples of patients with diarrhoea and PCR detection of two housekeeping genes hly and recA

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Objective: *Vibrio cholerae* is a noninvasive, Gram-negative bacterium responsible for severe epidemics of cholera and endemic diarrhoea in many parts of the world, especially developing countries. *V. cholerae* strains may produce a variety of atypical biochemical reactions which may pose some difficulties in rapid identification and recovery of these bacteria from patients. The objective of this study was to evaluate the use of PCR amplification of hly and recA housekeeping genes as a screening tool for identification of *V. cholerae* and comparison with PCR amplification of 16s-23s rRNA intergenic region and also with the time consuming biochemical conventional methods.
Methods: Among several stool samples referred to our laboratory, *V. cholerae* was recovered from 24 samples using conventional biochemical methods for identification of *V. cholerae*. The identity of *V. cholerae* was confirmed by PCR analysis of an intergenic region between 16s-23s rRNA, specific for *V. cholerae*. Serogrouping of isolates was performed by polyvalent and monospecific antisera according to the guidelines of centre for disease control (CDC). Detection of two housekeeping genes was investigated using primers which specifically designed for these regions.
Results: Among 24 *V. cholerae* isolated, 21% identified as Ogawa and the remaining 79% as Inaba serogroup. PCR analysis of the 2 housekeeping genes (hly and recA) revealed the presence of these genes in 100% and 92% of isolates, respectively. *V. cholerae* ATCC 14035 was used as a positive control in each assay.
Conclusions: The results of this study further confirms the use of amplification of 16s-23s rRNA region for PCR detection of *V. cholerae* strains from stool samples of patients with diarrhoea. Detection of the hly housekeeping gene can also be used as a molecular marker for identification, although the use of recA which is also a housekeeping gene is not recommended for identification of *V. cholerae* isolates from stool samples.

R2368 Detection of *Neisseria meningitidis*, *Streptococcus pneumoniae* and *Haemophilus influenzae* in cerebrospinal fluid in children using PCR

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Bacterial meningitis is a medical emergency requiring early diagnosis. Adequate treatment requires rapid detection of the bacteria in the cerebrospinal fluid (CSF). Traditional laboratory methods take up to 36 h or more and preadmission antibiotic treatment reduces the chance of positive results.
Objective: in order to improve bacterial meningitis diagnosis in our laboratory, we undertook a prospective study to evaluate the accuracy of PCR of CSF for the detection of *N. meningitidis*, *S. pneumoniae* and *H. influenzae* compared to conventional methods.
Methods: we include in this study 145 CSF specimens collected between December 2005 and November 2007. Specimens were classified

into five categories on the basis of laboratory findings. Category 1 (n = 31): culture was positive for *N. meningitidis*, S. Pneumoniae or *H. influenzae*. Stains were identified using conventional methods, serogroups of *N. meningitidis* were determined using anti-sera from Sanofi Diagnostics Pasteur, serotypes of *H. influenzae* were determined using antisera from Difco. Category 2 (n = 12): culture was negative, but direct smear and/or antigen detection for *N. meningitidis*, S. Pneumoniae or *H. influenzae* were positive. Antigen detection was performed by the latex agglutination kit Pastorex Meningitis (BIO-RAD). Category 3 (n = 44): CSF was abnormal with a high cell count but all other investigations were negative. Category 4 (n = 3): culture was postive for another bacterial species. Category 5 (n = 55): cases of viral meningitis. All CSF samples were tested by PCR using three primers correspondig to three genes: crgA for *N. meningitidis*, ply for *S. pneumoniae* and bexA for *H. influenzae*.

Results: PCR were positive at *N. meningitidis* in 11 cases, at *S. pneumoniae* in 18 cases and at *H. influenzae* in 6 cases. Among category 1, 2 and 3, samples with PCR positive results were respectively 23, 7 and 5. PCR was negative in all samples classified in category 4 and 5. Among category 1, PCR was negative in 2 cases of *N. meningitidis*, 3 cases of *S. pneumoniae* and 3 cases of *H. influenzae*. Sensitivity and specifity were 73.2% and 95.2% respectively. The low rate of sensitivity (failure of PCR in culture proven cases) could be due to the presence of an inhibitory for PCR and/or to the small quantity of bacteria in the CSF sample.

Conclusion: Our preliminary results suggest that use of PCR in meningitis diagnosis is useful. To improve sensitivity, we must use a method that separate bacteria from inhibitors.

R2369 16S rDNA PCR and sequencing: the only way for identification of *Actinobaculum massiliae* out of human clinical samples

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Objective: The identification of *Actinobaculum massiliae*, a pathogen which may affect the urogenital tract, is difficult due to the absence of suitable biochemical assays. We investigated *A. massiliae* isolates identified by 16S rDNA sequencing on morphology, growth conditions and variant commercial phenotypic methods.

Methods: A total of 17 *A. massiliae* isolates were identified by 16S rDNA sequencing since 2005 in our routine diagnostic laboratory. 14 of the 17 isolates were recovered from the urogenital tract, one from the abdominal wall, one from an abscess, and one from the auditory canal. Growth conditions as well as colony morphology were studied on blood agar with 5% sheep blood, schaedler agar with 5% sheep blood, chocolate agar and in thioglycolat broth. All of the plates were incubated aerobic, in a 5% CO2 atmosphere and under anaerobic conditions at 37ºC. In addition, Gram stains were performed and phenotypic analyses were done with API 20Strep, API Coryne, API Rapid ID 32Strep and API Rapid ID 32A (bioMérieux) systems.

Results: All of the 17 isolates (each fragment 1200bp) were unambiguously identified by 16S rDNA sequencing. 14 isolates were further investigated by re-culturing on the recommended agar plates in different atmospheres. Characteristic cultures were observed on schaedler agar as nonhaemolytic, greyish to white sometimes frayed colonies with a rise in the middle of the colony. Gram stains showed coccoid straight rods and were not distinct branched Gram-positive. With none of the used phenotypic test methods reliable identification could be achieved.

Conclusion: 16S rDNA sequencing is the only method for unambiguous identification of *A. massiliae*. This might be the cause of rare detection rates for this organism. The typical colony morphology and the appearance in Gram stain may be helpful for identification; however, establishing of a commercial system for reliable detection *A. massiliae* is needed.

Molecular typing

R2370 Pulsed-field gel electrophoresis and CTX-RFLP analysis of *Vibrio cholerae* strains isolated from patients in Iran

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Objectives: The multiple copies of CTX prophage are tandemly arranged in El Tor strains of *V. cholerae* but the number and arrangement of the CTX elements and the repetitive sequences are known to vary in different toxigenic strains and together with the Pulsed-field gel electrophoresis (PFGE) is a useful basis for study of diversity and characterisation of isolates.

Methods: PFGE performed according to a standard protocol of Centers for Disease Control (CDC) for *V. cholerae*. Composition and gene arrangement of the CTX genetic element were compared in 36 *Vibrio cholerae* strains isolated in outbreaks from Iran. Southern blot hybridisation using ctx and zot probes and Long-PCR amplification of the region between ig1-F and attB2-R which harbouring the entire CTX prophage in the genome of the *V. cholerae* performed to analyse the content and arrangement of the CTX genetic element, and the results interpreted in combination with the PFGE patterns, for assessing the clonality of isolates.

Results: Southern blot hybridisation with the specific probes revealed 3 profiles for each of the probes with the bands ranging from 4.2 to 8.3 kb in size and showed the presence of 1(30%, profile A), 2 (17%, profile B) and 3 (53%, profile C) copy numbers among the isolates. The results showed 3 Long-PCR amplification patterns including products with 6.9, 5.6 and 2.6 kb in sizes. Restriction Fragment Length Polymorphism (RFLP) analysis of patterns showed different CTX element content and arrangements. Pulsed-field gel electrophoresis (PFGE) was performed and two pulsotypes were found among the isolates. Majority of isolates (75%) with different CTX genomic arrangements and copy numbers showed a single pulsotype.

Conclusion: The isolates belonging to pulsotype 2 showed different CTX genomic arrangements, whereas the isolates with PFGE pulsotype 1 showed a single form of CTX arrangement. The results from this study showed that the variations in the content, arrangement and copy number of the CTX genetic element may occur in the same clonal isolates of *V. cholerae*.

R2371 Typing of MRSA with chromID™ MRSA medium and DiversiLab™ system

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Objectives: MRSA isolates can be responsible for severe infections both in hospitals and in the community. Molecular typing of these isolates provides critical information for epidemiology investigations. The goal of this study was to verify the compatibility of the chromID™ MRSA as a culture medium for the rapid screening of MRSA isolates with the DiversiLab™ system.

Methods: A total of 35 isolates recovered from 6 different body sites and collected in 13 countries were included in this comparison study. Cultures were performed on chromID™ MRSA and Columbia Blood Agar media. DNA was extracted from each culture using the UltraClean™ Microbial DNA Isolation Kit. The DiversiLab Staphylococcus kit was used for rep-PCR of non-coding intergenic repetitive elements in the genomic DNA. The amplicons were then analysed using the DiversiLab system including a microfluidics chip. Data analysis was performed with the web-based DiversiLab software using the Pearson Correlation (PC) coefficient. The fingerprints derived from bacterial cultures on Colombia blood agar were compared to those obtained with ChromID MRSA medium.

Results: The 35 strains were distributed in 12 clusters containing from 1 to 14 isolates. The 2 culture media generated identical fingerprints for each given strain. No correlation between body site or geographical origin was observed. As an example, the predominant cluster contained

14 isolates collected in different countries including Chile, Argentina, Spain and Switzerland.
Conclusion: The DiversiLab™ system has proven useful in strain-level discrimination of MRSA strains. This study shows that chromogenic medium chromID™ MRSA is compatible with the DiversiLab™ system and could help to improve the total turnaround time for MRSA fingerprinting.

R2372 Clustering of antibiotic resistant and susceptible Iranian *Helicobacter pylori* strains using RAPD-PCR profiling

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Objectives: *Helicobacter pylori* (Hp) infects the majority of the adult population in developing countries including Iran and is associated with gastrointestinal diseases such as gastritis, peptic ulcer disease (PUD) and gastric cancer. Antibiotic resistance is a key factor in the failure of Hp eradication therapy. Hence characterisation of susceptible and resistant strains and identification of their clonal population is critical. This study conceived to provide information on the diversity of Metronidazole and Clarithromycin resistant Hp strains in Iran.
Methods: The susceptibility testing of 50 Hp isolates were performed and interpreted according to the guidelines from the Clinical and Laboratory Standards. Two set of primers were designed to detect point mutations in the 23S rRNA gene responsible for clarithromycin resistance and RFLP was done to reveal point mutations via digestion of PCR products with BsaI, MboII. Another set of primers were used for detection of rdxA gene deletion responsible for metronidazole resistance. All of the strains were typed by RAPD-PCR.
Results: Approximately 17.6% and 59.4% of strains were resistant to clarithromycin and metronidazole respectively. One (3.3%) strain was resistant to both antibiotics. RAPD-PCR revealed amplified fragment sizes ranging from 2kb to 0.17kb. With 80% similarity 18 clonal populations were identified based on RAPD profiling. Most (80%) of the strains of cluster 5 were resistant to metronidazole whereas 78% of the strains in cluster 6 were susceptible to both antibiotics.
Conclusion: These results indicate that clinical *Helicobacter pylori* strains in terms of antibiotic resistance are highly diverse. It seems the characteristics of the resistant and susceptible strains can be predicted by clustering of strains using RAPD-PCR profiling.

R2373 Clonality of the blaVIM harbouring *Klebsiella pneumoniae* clinical isolates in a tertiary hospital in Greece

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Objectives: Hippokration General Hospital is among the first to isolate imipenem resistant *Klebsiella pneumoniae* due to the production of a VIM type metalloenzyme in Greece in 2002. Since then and despite the various infection control measures such strains are constantly isolated in this hospital. The purpose of the study is to elucidate the molecular epidemiological characteristics of the VIM producing *K. pneumoniae* isolates recovered from patients in the hospital during the period 2003–2006.
Methods: Eleven *K. pneumoniae* found imipenem resistant by the VITEK-II method (Biomerieux-FRANCE), routinely used in the hospitals laboratory, isolated during the study period, were selected. Susceptibility testing was reconfirmed by the Disk Diffusion method according to CLSI recommendations. Susceptibility to imipenem was further evaluated by E-test. Production of extended spectrum β-lactamases was determined by the double disk synergy test (DDST) and metallo-β-lactamase production by the EDTA-synergy test. PCR was used for detecting genes of the bla-VIM family. Isolates were genetically compared by PFGE.
Results: At the disk diffusion method all 11 isolates displayed susceptibility or intermediate resistance to imipenem with an MIC ranging between 0.064 and 4 mg/L in E-test. Ten isolates displayed a positive EDTA-synergy test, and the presence of the blaVIM gene was confirmed by PCR. Five of these isolates demonstrated a positive DDST test indicating a simultaneous presence of an ESBL in these strains. PFGE allowed the isolation of 3 types of clones among the 10 isolates. Clone A included 5 isolates, clone B: 4 and clone C: 1 isolates. Isolates of both clones A and B were isolated during the entire period whereas clone C was isolated in 2006. Further analysis of the MBL-determinant (bla-VIM1) revealed the presence of integron 1 in all 3 clones. The cassette region contained bla-VIM1, aacA7, dhfrI and aadA1 in all occasions.
Conclusions: Although a new blaVIM harbouring clone seem to have been introduced quite recently, imipenem resistance in *Klebsiella* in this hospital seem to be due to the persistence of two clones, a fact that indicates the need of intensifying the infection control measures in this hospital.

R2374 Rapid PCR-restriction fragment length polymorphism method to differentiate among *Campylobacter* clinical isolates

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Objectives: Thermophilic campylobacters, particularly *Campylobacter jejuni* and *Campylobacter coli*, are the most isolated bacteria causing diarrhoeal diseases in humans. Accurate routine *Campylobacter* speciation is needed to attribute human illness to different *Campylobacter* species and decide the most indicated antibiotic treatment. However, identification of these bacteria using phenotypic characters can be difficult; therefore the objective of this study was the development of a rapid and easy to perform identification system based on PCR-RFLP analysis to distinguish *C. jejuni* from *C. coli*.
Methods: PCR-RFLP of 23S rRNA gene was performed on 44 clinical isolates provided by La Ribera and Nou d'Octubre Hospital (Valencia, Spain) and 2 reference strains, *C. jejuni* ssp. *jejuni* DSM 4688 and *C. coli* DSM 4689 (German Collection of Microorganisms and Cell Cultures). The isolates were cultured on blood agar and incubated at 37ºC under microaerophilic conditions for 48 h. The strains were characterised by morphology and Gram stain, and subsequently identified by PCR (Fermér and Engvall, 1999).
For PCR-RFLP analysis, an internal region of the 23S rRNA gene of about 2.6-Kbp was amplified according to Hurtado and Owen (1997). Restriction endonucleases were selected by computer analysis using REbsites (http://rebase.neb.com). PCR products (10 μl), showing a single band of the expected size, were digested with 10 U of the restriction enzyme HpaII (MBI Fermentas®) in a final volume of 20 μl at 37ºC for 3 h. Restriction fragments were separated on 2.6% (wt/vol) agarose gel electrophoresis in TAE buffer at 90V for 3 h and visualised after staining with ethidium bromide. In order to assess reproducibility, all the strains were analysed at least two times in different experiments.
Results: A 2.6-Kbp PCR product of the 23S rDNA was amplified in all the strains. Digestion with restriction enzyme HpaII yielded specific patterns for *C. jejuni* (625, 560, 455, 360 and 225 bp) and *C. coli* (670, 625, 560, 455 and 225 bp). These results were almost identical to the predicted fragments based on the nucleotide sequence data. A total of 39 out of the 44 clinical isolates studied were identified as *C. jejuni* and the remaining 5 as *C. coli*.
Conclusion: The RFLP method proposed is able to differentiate between *C. jejuni* and *C. coli* in one work day using only a pair of primers and a single enzyme; therefore it can be a simple, rapid and useful method for routine identification.

R2375 *Pseudomonas aeruginosa* strains DNA typing by semi-automated repetitive-sequence-based PCR

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Background: Pulsed-Field Gel Electrophoresis (PFGE), based on the separation of long strands of DNA by length, is a highly discriminatory

method. It is traditionally considered to be "the gold standard" for gene mapping and epidemiological studies. However, it presents some disadvantages since it takes several days to perform with specialised equipment and often laborious techniques. A typing method, not time consuming, is therefore needed to assess outbreaks in real time.
Objectives: the aim of this study was to evaluate the usefulness and the performance of the semi-automated repetitive-sequence-based polymerase chain reaction (rep-PCR) which examines non-coding highly conserved repetitive sequences, interspersed throughout the bacterial genome for typing *Pseudomonas aeruginosa* strains.
Method: the typing of 82 *Pseudomonas aeruginosa* strains by the DiversiLab system® was realised on the basis of an 95% similarity threshold, and results were compared with PFGE. Twenty-nine of these strains were unlinked strains, 16 were issued from 5 epidemic cases, 7 from an epidemiological study, 5 from members of two families and 25 from water, aerosols and patient isolates of the same hospital.
Results: a good correlation was observed between the semi-automated rep-PCR method and PFGE: the 29 strains were typed as different and most of the other strains were linked in the same way as with the PFGE method. However, two cases are discordant: one for which the source of contamination was not the same with the two methods and one for which results were not reproducible.
Conclusion: even if further studies must be performed to confirm the discriminatory power and the reproducibility of the rep-PCR method, this simple and fast technique seems to be appropriate to type *Pseudomonas aeruginosa* strains.

Molecular biology, including diagnostics – others

R2376 Comparison of Seeplex VRE detection kit with chromID VRE agar for the detection of vancomycin-resistant enterococci in faecal specimen

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Objectives: As vancomycin-resistant enterococci (VRE) is increasing as a nosocomial pathogen, rapid and accurate detection of VRE is important. However, conventional culture methods take long time for detection of VRE. Recently, Seeplex VRE Detection kit with internal control (Seegene, Seoul, Korea) and chromID VRE agar (bioMérieux, Marcy l'Étoile, France) were developed and we compared performance of these two methods with conventional blood agar plate (BAP) based culture after enrichment in Enterococcosel broth (EB).
Methods: For 10 days in September 2007, 180 rectal swab specimens were received for detection of VRE and they were inoculated in 1 mL of EB containing 6 μg/mL vancomycin. After 35.18 h incubation, DNAs from 50 μL of bacterial pellets in black pigment-producing EB were extracted and amplified for detection of van A and van B genes by multiplex PCR using Seeplex VRE Detection kit. Amplified DNA products were identified on 2% agarose gel electrophoresis. Blackened EBs were also inoculated on chromID VRE agar containing 6 μg/mL vancomycin and BAP containing 30 μg vancomycin and teicoplanin discs. After 35.18 h incubation, VRE suspected colonies from each plate, were submitted to identification and antimicrobial susceptibility test.
Results: 131 out of 180 rectal swabs produced black pigments in EBs and of them, 41 samples were positive for VRE by at least one method. When these 41 samples were considered true positive for VRE, the results were listed in Table 1.
Sensitivity of Seeplex VRE Detection kit, chromID VRE agar, and BAP were 92.7% (38/41), 90.2% (37/41), and 75.6% (31/41), respectively. The average time for reporting VRE positive specimen of them was 2 days, 3.4 days (range, 2–5), and 3.7 days (range, 2–6). Other bacterial species seen as VRE-like colonies on chromID VRE agar were Gram-negative bacilli, Gram-positive bacilli, *Enterococcus gallinarum*, *Enterococcus casseliflavus*, Pediococcus pentosaceus, yeast, and unidentified colonies.
Conclusions: Seeplex VRE Detection kit revealed the highest sensitivity and the shortest time for reporting VRE. chromID VRE agar revealed good performance but had longer reporting time up to 5 days. Therefore, Seeplex VRE Detection kit can be the best choice of methods for VRE surveillance.

Table 1. Comparison results of Seeplex® VRE Detection kit with those of chromID VRE® agar and BAP based culture

Method	Number of results (131 swabs in total)			
	True positive	False positive	True negative	False negative
Seeplex® VRE Detection	38	0	90	3
chromID® VRE	37	18	72	4
BAP	31	38	52	10
Total	41		90	

R2377 Usefulness of immunocytochemical p16ink4a expression in routine screening of a tested female population

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Objectives: This study aimed to compare the detection of P16ink4a protein staining and HPV DNA testing for human papillomavirus (HPV) detection in cervical swab samples in population of Bosnian women.
Methods: P16ink4 biomarker (Ventana) was applied on 31 HPV DNA cervical swab samples of 97 examined women which were detected by high risk HPV Hybride Capture-2 (HC2), Digene and research polymerase chain reaction (Roche, primer set PGMY 09/11). Age groups were defined as: 20–24 yr., 25–29 yr., 30–34 yr., 35–39 yr., 40–44 yr., 45–49 yr., and >50 yr. old. Cervical swab samples and slides were collected for December 2004 to January 2005 at Sarajevo clinics and shipped to Johns Hopkins University.
Results: Positive cytoplasmic immunostaining by P16ink4a biomarker showed prevalence 9.27 (9/31) of total HPV DNA positive (HC2 and PCR). The overall prevalence for high risk HPV by HC2 was 22.68% (22/97). Total of both high risk and low risk HPV prevalence by PCR was 29.89% (29/97).
Conclusion: Despite a good correlation of the results of HC2 and P16ink4a and PCR and P16ink4a, we cannot draw any firm conclusion regarding the usefulness of this technique in routine screening.

R2378 A novel Gap-PCR for detection and differentiation of BK and JC virus infections

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Introduction: BK virus is a causative agent of nephropathy in renal transplant recipients and can cause premature graft failure, while JC virus is aetiologic agent of multifocal progressive leukoencephalopathy (PML). Despite similarities at the genetic level (more than 75% homology) among JC virus and BK virus, differentiation of them regarding to aetiologic differencies is intransitive.
Methods: In this study we utilised discrepancy in genetic level of BK virus and JC virus for designing of a Gap-PCR that finally would create two different fragments in length from concerned virus. Positive Control sera and urines containing BK and JC viruses were used in this study. Viral DNA extraction was performed by a commercial kit and then was amplified by designed Gap-PCR. Product has been running on agarose gel to detection of infection and coinfection of JC and BK viruses.
Results: The performance of this new assay for detection and differentiation of BK and JC viruses was evaluated with 1 duplicated urine and serum samples from 40 renal transplant reciepents who underwent on renal transplantation at least 6 month previous assay. To establish the sensitivity, a serial dilution of plasmid containing whole BK virus genome was used. Finally this new assay found sensitive and

simple for detection and differentiation of BK and JC viruses infection and coinfection.
Conclusion: The acceptable sensitivity and very high simplicity of the Gap-PCR, which allows the easily screening of transplant recipients and HIV positive patients for recurrence of BK virus and JC virus, make this new assay especially suitable for this purpose. While previous studies usually have been used multiplex PCR and hybridisation methods for differentiation of JC and BK we designed two primers that amplified and differentiate simultaneous both viruses without need to other complicated steps.

R2379 Use of GenoType® Enterococcus test to improve enterococci detection correctness

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Objectives: Enterococci as cause for bacteraemia, wound infections, endocarditis, urinary tract infections and infections of CNS are associated with intra-hospital infections. In some cases infections are caused by enterococci with multi drug resistance (MDR). Vancomycin is important to treat MDR enterococci patients. Some enterococci species exhibit natural resistance to vancomycin. Therefore it is highly important to identify enterococci species correctly and to interpret results from drug susceptibility testing. Our aim was to compare molecular biology method for identification of enterococci species with phenotypical detection methods.
Methods: This study was performed on 40 enterococci cultures obtained in 2 Latvian hospitals: clinical hospital "Gailezers" and State Agency of TB and Lung Diseases. We used a DNA strip assay (GenoType® Enterococcus; Hain Lifescience GmbH) designed for the simultaneous detection of the most frequent enterococcal species, *Enterococcus faecium*, *E. faecalis*, *E. gallinarum*, *E. casseliflavus*, and *E. flavescens*, and of the vancomycin resistance genotypes vanA, -B, -C1, and -C2/3. The assay is based on the specific amplification step followed by reverse hybridisation on nitrocellulose strip. Mini Api® rapid ID 32 Strep (bioMerieux) and BBL Crystal™ GP ID Kit (Becton Dickinson) were used as phenotypical methods for culture identification. Susceptibility to vancomycin was tested by disc diffusion and E-test.
Results: Of the 40 tested enterococci species, 26 (16 *E. faecalis*, 10 *E. faecium*) were identified as same species with the phenotypical and genotypical methods, 2 *E. faecium* identified by molecular method and by BBL Crystal™ but as *E. casseliflavus* by Mini Api®, 1 *E. faecium* was identified by molecular method and by Mini Api® but was identified as *E. gallinarum* by BBL Crystal™. 2 *E. faecium* identified by molecular method but as *E. gallinarum* by Mini Api® and as *E. casseliflavus* or *E. casseliflavus*/ *E. gallinarum* by BBL Crystal™. Other species represented *E. avium* or *E. raffinosus*. Phenotipicaly identified *E. casseliflavus*/ *E. gallinarum* showed susceptibility to vancomycin. 1 vancomycin resistant *E. faecalis* isolate was having vanA by the GenoType® and was conformed by disc diffusion and E-test.
Conclusion: Our results shows that correctness of enterococci identification only by phenotypical testing methods can lead to wrong diagnosis and genotypical methods should be used when drug susceptibility testing mismatch with species identified.

R2380 Seroepidemilogical study of HTLV and its clinical impacts in thalassaemia, haemophilia and haemodialysis patients in Hormozgan

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Background: Transmission of blood borne infections is common among multitransfusion patients such as thalassaemia, Hemophilia and haemodialysis patients. HTLV (Human T-Lymphotropic Virus) is one of the Retroviruses which is transmitted by blood and its products. In Iran HTLV is endemic only in Khorasan. This virus causes (HAM/TSP) and (ATL). In this study we consider the seroepidemiological patterns of HTLV infections in thalassaemia, Hemophilia and haemodialysis patients in Hormozgan in South of Iran in order to assess the possibility of the virus trasmision and its clinical impacts.
Methods: In this study, 210 patients, including 163 thalassaemias, 40 haemodialysis patients and 7 hemophilia (98 female, 112 male) with the age range of 9 to 79 were analysed. Their plasma was tested for detection of Ab against HTLV and patients were examined for its clinical manifestations.
Results: Anti-bodies against HTLV were detected in 2.38% (5 patients) which is higher than its prevalence in healthy individuals. All of positive samples were belonging to Thalassaemia patients. Anti-body positive patients were symptomatic clinically as well.
Conclusion: This study shows that multitransfusion patients were infected probably via blood transfusion or haemodilyse and existence of this virus is not limited only to the Khorasan.

R2381 Highly prevalent protistan intestinal parasites not detected by use of the formol ethyl acetate concentration technique is revealed by the routine use of PCR- and culture-based detection

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Objectives: The detection of protistan intestinal parasites (PIP) traditionally relies on examining faecal concentrates obtained by the formol ethyl acetate concentration technique (FECT) including Ziehl-Neelsen staining for (oo)cysts. However, this procedure does not consistently allow for the detection of trophozoites. For some common parasites, e.g. *Dientamoeba fragilis* and *Blastocystis*, regular cyst stages either do not exist or are hardly recognisable in faecal concentrates. Moreover, in case of increased gastrointestinal passage due to PIP, only trophozoites may be shed in the stool, which is why the examination of fresh, warm stools or stools fixed in e.g. sodium acetate-acetic acid-formalin (SAF) has been undertaken. However, these methodologies are not routine practice or even possible in all diagnostic laboratories, which is why other methods are wanted for. PCR technology has enabled sensitive, high-throughput assays for detecting species-specific DNA from parasites such as e.g. *Entamoeba*, *Giardia*, *Cryptosporidium* and *Dientamoeba* extracted directly from unpreserved faeces. In-vitro culture in Jones' medium for 48 h has proven inexpensive and sensitive regarding the detection of *Blastocystis*.
This study sought to examine the diagnostic relevance of integrating supplementary analyses based on PCR and culture in routine diagnostic parasitology.
Methods: In a period of two months 1000 fresh faecal samples were randomly selected and examined by three methods: The FECT including Ziehl-Neelsen staining, *Blastocystis* culture, and PCR for *Entamoeba histolytica*, *Entamoeba dispar*, *Giardia intestinalis*, *Cryptosporidium* sp., and *D. fragilis*.
Results and Conclusions: The study is ongoing, and results will include a comparison of FECT vs. PCR for the above mentioned protozoa and *Blastocystis* culture. Results will include semiquantitative (FECT, PCR) and qualitative scores (culture). Preliminary data show that the negative predictive value of the FECT is <80%, which is mainly attributable to the fact that this method is inapt for the detection of *D. fragilis* and *Blastocystis*, which seems to be remarkably prevalent parasites in Denmark. The study will reveal data essential for the discussion and contemplation of the relevance of supplementing the FECT with PCR for protozoa and *Blastocystis* culture.

Diagnostic/laboratory methods (other than molecular)

R2382 Application of the chromID ESBL medium in a routine microbiological laboratory

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Objectives: The chromID ESBL medium (bioMerieux) applicability assessment in routine ESBL-positive strain detection in a hospital laboratory.
Methods: 223 strains of G– bacilli, isolated from different clinical materials of hospital wards patients, were used: Enterobacteriaceae (n = 201), *Acinetobacter* (13), *Pseudomonas* (7) and *Stenotrophomonas* (2). From among the Enterobacteriaceae rods, 105 were ESBL+. The strains were inoculated onto the assessed medium and onto a Columbia Agar with blood. VITEK GNI+ cards were used for identification, ESBL production was determined using GNS 650 cards and verified using the double disk test. All strains grown on the assessed medium underwent double identification and ESBL production determination.
Results: Identification The growth of 138 out of the 223 strains inoculated onto the chromID ESBL medium was observed. All of the *Enterobacter* (n = 15), *Klebsiella* (n = 95) and *Escherichia* (n = 5) colonies were pigmented as expected, all the non-fermenting rod colonies were not pigmented. The growth of 2 *Citrobacter freundii* and of 1 *Proteus mirabilis* strains was colourless.
ESBL detection On the tested medium, out of the 105 ESBL+ Enterobacteriaceae, 103 strains developed colonies (98%). No growth of 1 *Klebsiella pneumoniae* and 1 *E. coli* strains was attained, in spite of the ESBL+ phenotype detection for both of them.
On the assessed medium, 119 (59.2%) Enterobacteriaceae strains developed colonies, whereas there were 105 (52.2%) ESBL+ strains in this group. It was found that 16 ESBL-negative Enterobacteriaceae developed on the assessed medium. This was mainly concerning *Enterobacter cloacae*, where 13 out of 17 strains developed, and only 1 of them was ESBL+.
Out of 22 non-fermenting rods (all of them being ESBL–), 19 developed colourless colonies.
Conclusion: Obtained results show that the chromID ESBL medium sensitivity makes it applicative in ESBL+ strain detection. However, a growth of a certain number of ESBL-negative strains, as well as identification of cases of colourless Enterobacteriaceae growth, entails a necessity to verify ESBL production using some other method, and to identify the bacteria giving colourless growth.
Using the medium in question in most cases leads to shortening of the examination time, as well as to cost-cutting, thanks to the limitation of the amount of tests necessary only to the strains growing on the evaluated medium.

R2383 Comparative evaluation of performance and convenience of the RapID NH (OXOID™) with the API-NH (BioMérieux™)

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Objectives: Evaluate the RapID NH (OXOID™) for the identification of microorganisms of the family of Neisseriaceae and *Haemophilus* and other related species in comparison with the API-NH (BioMérieux™) for both performance and convenience in our laboratory daily routine.
Methods: This study compares 88 clinical stains that have been sampled from January 10 to October 24 2007 (53 *H. influenzae*; 13 *Moraxella catarrhalis*; 5 *H. parainfluenzae*, 13 *N. gonorrhoeae*, 2 *N. meningitidis* and 2 *Haemophilus* spp.). All samples have been processed according to manufacturer's instructions provided with the kits. We analysed the identification results and used a scored grid (1 to 5) for evaluation of 10 parameters to assess the adaptability/versatility/practicality of each kit in daily use. We also used 2 quality control strains *Neisseria lactamica* NTCC 10617 and *Haemophilus parainfluenzae* ATCC 7901.
Results: Eighty six of the 88 strains studied and both quality control strains coincided in the identification to the species level for both techniques. The mean score for the evaluation grid was 3.72 for the RapID® NH and 2.95 for the API™ NH. The mean inoculation time for one panel was 1 minute for RapID® NH and 2.25 minutes with the de API™ NH. The data base of the RapID® NH also includes 8 additional genera in addition to the *Neisseria* spp., *Haemophilus* spp. and *Moraxella* spp., widening its range and potential use.
Conclusions: The identification results for *Neisseria* spp., *Haemophilus* spp. and *Moraxella* spp. have been comparable for both techniques. As to the overall convenience of use, the RapID® NH characteristics were more adaptable to our laboratory conditions.

R2384 Prevention of false-positive reactions in whooping cough ELISA

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Objectives: Since 1991 is has been known that heat inactivation of serum results in very high false-positive values when analysing for the presence of antibodies to pertussis toxin (PT) by ELISA [Tada, Dev. Biol. Stand. 1991, 73: 175–184]. Since PT is widely agreed to be the antigen of choice for whooping cough ELISA, prevention of false-positive reactions is crucial when analysing sera with unknown treatment. No reports so far have addressed this issue. Furthermore, heat could present a major problem e.g. in tropic areas.
Methods: An indirect ELISA based on pertussis toxin for detection of antibodies in human serum was optimised for the removal of false-positive results occurring at analysis of heat treated sera. Heat treated sera (56ºC, 30 minutes) with known low contents of PT-antibodies were used, as well as non-treated sera from patients with confirmed whooping cough.
Results: When analysing heat treated sera with known low contents of PT-antibodies in the non-optimised PT-ELISA, the resulting estimates for PT-antibody contents were similar to, or even higher than, the values obtained when analysing sera from patients with confirmed whooping cough. Dilution curves of the heat treated sera moreover produced markedly different slopes than those obtained with non-treated sera, as was also seen by Lopez in 1998 [Lopez, Diagn. Microbiol. Infect. Dis. 1998, 30: 21–24].
An ELISA-method based on Maxisorp microtiter plates (Nunc®, Denmark), using 1% milk in blocking solution and 0.1% milk in sample dilution, was seen to be a stable assay with elimination of the false-positive results. PT-antibody values obtained with heat treated sera were identical to values obtained with the corresponding non-treated sera.
Conclusion: False-positive results for PT-antibodies arising from heat-inactivation of sera were eliminated by a blocking step with 1% milk. Studies involving measurements of PT-antibodies in historic sera should involve such a blocking step, since historic sera might have been subjected to routinely heat inactivation. Moreover, in settings where sera have been heat-inactivated for safety reasons, an accurate analysis of PT-antibodies is now feasible.

R2385 Comparison of antifungal MICs for yeasts obtained using EUCAST in a reference laboratory and Etest in ten different hospital laboratories

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Objectives: The determination of MICs for yeasts in routine laboratories often relies on Etest® because it is easy to perform and it has shown a good correlation with reference techniques in liquid media. However, this correlation was established in specific studies performed by reference laboratories. We took the opportunity of the surveillance programme of candidaemia set up in 2003 to compare the E-tests results performed

on a routine basis in ten different hospitals and the EUCAST results, obtained in a reference laboratory unaware of the hospital results.
Methods: A total of 722 isolates were included in the analysis (374 *C. albicans*, 118 *C. glabrata*, 72 *C. parapsilosis*, 58 *C. tropicalis*, and 100 other yeast species) and tested for amphotericin B (AMB), fluconazole (FCZ), itraconazole (ITZ), voriconazole (VRZ), flucytosine (5FC), and caspofungin (CAS). Etest® was not performed for each antifungal for each isolate depending on the hospitals. Both on-scale and off-scale values were included in the analysis. Low off-scale values were left unchanged and high off-scale values were converted to the next higher concentration. For comparison purpose, the Etest® MICs were raised to the next corresponding EUCAST concentration. Agreement was defined as differences of no more than 2 dilutions between results obtained by the two techniques. Categorical agreement were also calculated based on breakpoints previously published (Cuenca-Estrella M. et al, Clin Microbol Infect 2005, 11, 486–92).
Results: See the table.

	n	% agreement	
		+/1 dil	+/2 dil
AMB	681	47.4	75.0
FCZ	694	54.2	71.8
ITZ	198	39.4	70.7
VRZ	612	72.9	86.4
5FC	332	82.5	90.4
CAS	249	36.5	73.9

The best correlation was observed with 5FC. The good correlation for AMB could be explained by the narrow range of MICs. The correlation for azoles ranged from 71% for ITZ to 86% for VRZ. The results by yeast species did not show significant differences excepted for susceptibility of *Cryptococcus neoformans* to 5FC for which the correlation was <40%. Categorical agreement ranged from 59.6% for ITZ to 91.0 for 5FC. Major and very major discrepancies occurred in less than 10 and 2%, respectively. Differences between hospitals were not statistically significant.
Conclusion: This study confirms that results obtained with Etest®, even if performed in non reference laboratories, show a good correlation with results obtained by EUCAST reference method. Regular quality controls should be implemented to maintain the performance of the Etest®.

R2386 Correlation of antibodies to antigens of Epstain-Barr virus with systemic lupus

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Objectives: We have examined a correlation of Epstein-Barr virus (EBV) infection with Systemic lupus (SLE). We determined antibodies on EBV specific antigens and heterophilic antibodies in SLE patients and control group.
Methods: The SLE group consisted of 50 patients treated at the Clinic for Rheumatism or at the Rheumatologic Counseling Office of the same Clinic. Mean age of the group was 48 years. The female-male ratio was 47/3. The control group consisted of 21 patients with degenerative arthritis and 29 clinicaly healthy individuals, all of the similar age and gender structure as the SLE group. Each serum samle was tested by Elisa for: Enzygnost anti-EBV/IgG, Enzygnost anti-EBV/IgM, anti-VCA/IgG, anti-VCA/IgM, anti-EA-D/IgG, anti-EBNA-1/igG and heterophilic antibodies.
Results: Anti-EBV/IgG antibodies were positive in 50 (100%) SLE patients and 39 (78%) controls. SLE and control groups were negative for EBV/IgM antibodies. Anti-VCA/IgG and anti-EBNA-1/IgG antibodies were positive in 50 (100%) SLE patients and 39 (78%) controls. SLE and control groups were negative for anti VCA/IgM antibodies. Anti-EA-D/IgG antibodies were positive in 35 (70%) SLE patients and 8 (16%) controls. Heterophilic antibodies were positive only in two SLE patients.
Conclusion: There was no significant difference in frequency of anti-EBV antibodies in two groups ($p = 0.585$). However, the percentage of high antibodies concentrations in serum was significantly higher in SLE patients than in control group ($p = 0.0001$). The frequency of anti-VCA and anti-EBNA-1 antibodies was similar in both groups, while the frequency af anti-EA-D antibodies was significantly higher in SLE.
EBV infection is closely related to SLE, whether as a triggers or as precursors of EBV infection.

R2387 Validation of the Abbott Architect syphilis TP test against the Dade Behring Enzygnost syphilis ELISA and the Fujirebio *Treponema pallidum* particle agglutination test

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Objective: The performance of the recently introduced automated Abbott Architect syphilis TP test (ARS) was compared with the Dade Behring Enzygnost syphilis ELISA (DBS) and the Fujirebio *Treponema pallidum* particle agglutination test (TPPA) as a screening test for syphilis.
Methods: Specificities of the ARS, DBS and TPPA were assessed in 1227 prenatal and 775 diagnostic sera obtained in a low prevalence setting for syphilis. To determine the sensitivities of the ARS, DBS and TPPA, 227 sera were selected from patients previously diagnosed positive and 5 sera from patients previously diagnosed negative for syphilis with increasing TPPA titres ($\geqslant$80). Sera with concordant test results were considered as true negatives or true positives. Equivocal DBS results were considered as negative. For discrepant sera a previous diagnosis of syphilis and a syphilis line immunoassay (LIA) were used as the reference.
Reproducibility of the ARS was assessed by calculation of intrarun and interrun coefficients of variation (CV) from 3 repeated determinations of 5 negative (index <0.2), 5 low positive (1.0 < index < 5.0) and 5 positive sera (index >10.0).
Results: In 12/1938 (0.6%) truly negative sera discrepant results were observed. Five sera were positive in DBS only, 6 samples were positive in TPPA only and 1 serum was positive in TPPA and equivocal in DBS. Three prenatal sera were positive in ARS only and indeterminate in LIA and therefore classified as indeterminate. In 9/293 (3.0%) truly positive sera discrepant results were observed. One serum was negative in ARS only, 5 sera were equivocal in DBS only, 2 samples were negative in DBS only and 1 serum was negative in TPPA and equivocal in DBS. Specificities of ARS, DBS and TPPA were 100% (99.8% with indeterminate sera included), 99.4% and 99.4%. Sensitivities of ARS, DBS and TPPA were 99.7%, 97.3% and 99.7%. ARS intrarun and interrun CV's were 2.3% and 38.3% for negative sera, 3% and 5% for low positive sera and 1.4% and 7.3% for positive sera.
Conclusions: The ARS showed excellent specificity and sensitivity as compared to DBS and TPPA and was highly reproducible. Therefore ARS can reliably used as a screening test for syphilis. Its fully automated design makes ARS very suitable for large scale testing.

R2388 Efficient automated purification and accurate quantification of cytomegalovirus DNA

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Objectives: An efficient combination of preanalytical processing steps and molecular diagnostic assays is required for reliable diagnosis and accurate quantification to monitor CMV infections and determine antiviral therapy. The aim of this work was to evaluate the combination of automated viral DNA extraction using a BioRobot® EZ1® workstation and real-time PCR-based CE-marked artus® CMV PCR Kits for the detection and quantification of CMV from human plasma samples.

Methods: Serial dilutions of human EDTA plasma spiked with CMV were purified using the BioRobot EZ1 workstation in combination with the EZ1 Virus Mini Kit v2.0. The extracted viral nucleic acids were analysed using artus CMV PCR Kits on different platforms, using LightCycler® 1.1/1.2/1.5, Rotor-Gene™ 3000, and ABI PRISM® 7000 SDS instruments. The limit of detection (LOD) was determined by probit analysis. In addition, the linear range, robustness, and effect of interfering substances (haemolysis, icterus, and lipaemia) were defined.
Results: The LOD ($p = 0.05$) for the combination of the BioRobot EZ1 workstation with artus CMV PCR Kits was determined to be 67.2 copies/ml on the LightCycler1.1/1.2/1.5, 21.8 copies/ml on the Rotor-Gene 3000, and 38.3 copies/ml on the ABI PRISM 7000. The linear range for this combination has been determined to cover concentrations from at least 3.16×10^2 – 1×10^7 copies/ml. The robustness of the BioRobot EZ1 workstation in combination with artus CMV PCR Kits was about 99%. No inhibition or lack of sensitivity was observed due to the presence of interfering substances in plasma samples.
Conclusion: The combination of automated sample preparation using the BioRobot EZ1 workstation and the EZ1 Virus Mini Kit v2.0 with sensitive detection using artus CMV PCR Kits allows reliable diagnosis and monitoring of CMV infections in routine diagnostic laboratories.
Trademarks: QIAGEN®, artus®, BioRobot®, EZ1® (QIAGEN Group); LightCycler® (Roche Group); Rotor-Gene™ (Corbett Research); ABI PRISM® (Applera Corporation or its subsidiaries).
Disclaimer: EASYartus CMV: The combination is currently for Research Use Only. Not for use in diagnostic procedures. The CE-marked combination for diagnostic use will be available in 2008. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

R2389 An analysis of two sets of paired BACTEC aerobic/F and anaerobic/F blood culture vials with or without an agar-broth biphasic selective fungal culture vial for the detection of fungaemia

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Objectives: It is important to know our own laboratory's diagnostic ability for detecting fungaemia which is a nosocomial infection with high mortality. We evaluated the utility of a second set of paired BACTEC Plus Aerobic/F and Anaerobic/F blood culture vials for the detection of fungaemia and also analysed the benefits of adding an in-house (modified after the BACTEC Myco/F Lytic medium) agar-broth biphasic selective fungal culture vial.
Methods: This is a retrospective study of patients admitted to the Singapore General Hospital over a 27-month period, from April 2004 to June 2006, who had at least one positive blood culture for fungi using the BACTEC vials or the in-house selective fungal culture vial. Comparative analyses were made exclusively on blood culture sets in these configurations: two sets of paired BACTEC vials (2–2), and two sets of paired BACTEC vials with one in-house fungal culture vial (2–2-1).
Results: There were 86 two sets of paired BACTEC (2–2) vials with at least 1 set of either paired vials positive for fungi. 53.5% (46/86) had both sets positive. This implied the addition of a second set of paired BACTEC vials had increased the fungi recovery rate by 87.0%. There were 23 two sets of paired BACTEC vials with one in-house fungal culture vial (2–2-1) that had at least 1 vial positive for fungi. 26.1% (6/23) had positive cultures in both the BACTEC and selective fungal culture vials. 43.5% (10/23) had a positive culture only in BACTEC and 30.4% (7/23) had a positive culture only in the selective fungal medium. There is poor agreement (kappa, measure of agreement = –0.558) between the two culture media for the diagnosis of fungaemia.
Conclusion: The use of a second set of paired BACTEC blood culture vials increased the detection of fungaemia by 87%. In spite of this, the addition of one vial of our in-house selective fungal culture medium would detect 30% more fungaemia. Hence, this finding lends strong support for the use of a selective fungal culture medium to complement even two sets of paired BACTEC Aerobic and Anaerobic vials to enhance the diagnosis of fungaemia.

R2390 Diagnostic significance of procalcitonin in assesing septic complications after major gynaecological surgery

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We wished to establish the value of procalcitonin (PCT) in assessing early postoperative sepsis in patients after major gynaecologic surgery. After major surgery traditional inflammatory parameters (C-reactive protein – CRP, white blood cell count – WBC, percentage of granulocytes, erythrocite sedimentation rate) can be elevated on account of tissue trauma caused by surgery.
Methods: The prospective study included 88 patients from the University Clinical Hospital Maribor, Clinical department of Gynecologic Oncology, 47 of these after a minor gynaecologic operative procedure acc. to Piver I, 35 after a major procedure acc. to Piver II and III with no postoperative infectious complications, 6 patients with postoperative sepsis. In all patients inflammatory parameters (PCT, CRP, ESR, WBC and percentage of granulocyte) were determined on postoperative days 1–10.
Results: PCT levels under 0.5 ng/ml were noted throughout the 10 postoperative days in patients after minor Piver I operation and in 77% of patients after major surgery (Piver II and III) while 17% of these had transitional levels between 0.5 and 1ng/ml on the second day and 6% had 1–2ng/ml on the second day after the procedure. After the procedure all 6 patients with septic complications had levels well above 2ng/ml, already on the second postoperative day(median 23ng/ml). The CRP level was elevated in all study patients. The concentration corresponded to the extent of tissue trauma during the procedure, low levels were observed post Piver I, and higher post Piver II and III (median 45 mg/dl vs. 135 mg/dl, $P = 0.03$). The maximum values of CRP in non-septic group were reached on the second postoperative day, then CRP slowly returnd to normal values in the folowing days. Septic patients had significantly higher CRP levels than those without septic complications, although significant difference between septic group and the group after major surgery(Piver II, III) group was not observed before the forth postoperative day. On the other hand, the significant difference in PCT level between septic patients and patients after major surgery without septic complications was observed already on the second postoperative day.
Conclusion: Procalcitonin proved a good diagnostic parameter for early detection of bacterial sepsis in gynaecology, particularly when its postoperative level exceeded 2 ng/ml, since procalcitonin is not reflecting surgical trauma.

R2391 Bacterial pathogens isolated from patients with bloodstream infection

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Objectives: To determine the frequency of pathogens isolated from 1483 patients with Blood Stream Infections (BSI) and to investigate their resistance patterns, between January 2005 and July 2007, in "Dr. V. Babes" Hospital of Infectious and Tropical Diseases, Bucharest.
Methods: Blood cultures were performed using the BacT/ ALERT 120 automated system. ATB/ Expression and VITEK 2 Compact automated system (bioMerieux, France) was used for identification and resistance testing of pathogens, according to NCCLS 2005–CLSI 2007. Internal quality control was provided by using *S. aureus* ATCC 25923, *S. pneumoniae* ATCC 49619, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853.
Results: A total of 3561 blood cultures were screened for aerobic and anaerobic bacteria. Bacteraemia occurred in 176 cases (4.9%). Out of 176 isolated strains, 107 were Gram-positive (60.8%) and 69 were Gram-negative (39.2%). Among Gram-positive, the most prevalent was Coagulase-Negative Staphylococci 34.6%, followed

by *Staphylococcus aureus* 26.2%, *Streptococcus pneumoniae* 14.0%, *Enterococcus* spp. 4.7%. The most frequent of streptococci were *Streptococcus pyogenes* and *Streptococcus bovis* (3.7% each). Among the Gram-negative *Escherichia coli* was 47.8%, *Klebsiella pneumoniae* 15.9%, *Salmonella* spp. 13.0%. In lower rates we found: other Enterobacteriaceae, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Neisseria meningitidis*, *Haemophilus influenzae* and anaerobic bacteria. All Gram-positive isolates were susceptible to glycopeptides. 20/ 37 of CoNS were MRSCN, 13/ 37 resistant to fluoroquinolone, 12/ 37 to gentamicin. 9/28 of *Staphylococcus aureus* were MRSA, 4/28 were resistant to aminoglycosides, 5/28 to fluoroquinolones. In *S. pneumoniae* isolates, 1/15 was reported with high level resistance to penicillin. *E. coli* and *K. pneumoniae* strains produced ESBL in 12.1% and 45.4% respectively. Most of *A. baumannii* and *P. aeruginosa* strains were susceptible to carbapenems.

Conclusions: 1. CoNS (21.0%) were the leading cause of BSI, followed by *E. coli* (18.7%), *S. aureus* (15.9%), *S. pneumoniae* (8.5%). 2. All Gram-positive isolates were susceptible to glycopeptides. 3. 45.4% *K. pneumoniae* was ESBL positive. 4. Carbapenems had a good activity against Gram-negative bacilli.

R2392 Optimisation and evaluation of an immunomagnetic separation method using a monoclonal anti-LPS antibody for the detection of *Legionella pneumophila* serogroup 1 in environmental water samples

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Objectives: Rapid and sensitive techniques are urgently needed to detect the presence of *Legionella* in environmental water samples. The main objective of this study was to develop an immunomagnetic separation (IMS) method aimed to improve the recovery by plate culture of *L. pneumophila* serogroup 1 (Lp1) from environmental water samples of different origins.

Methods: IMS was performed with Dynabeads (Dynal, France) coated with different antibodies (Abs) including monoclonal Abs directed towards the Lp1 LPS and the mip protein (Microbiodetection, France) and a commercial polyclonal anti-LPS Ab (Dynal). The test was first evaluated on a panel of 38 epidemiologically unrelated strains of Lp1 from different origins. The performance of the test was evaluated for each Ab by comparing the rate of bacteria recovered by combining IMS and culture to that obtained after culture alone. The best antibody was then applied to the detection by IMS of Lp1 cells in environmental samples taken from hospital water circuits and air cooling towers.

Results: The anti-mip Ab was not found adequate to capture Lp1 cells with regards to its low and late expression in the course of the bacterial growth cycle. By contrast, the monoclonal and the polyclonal anti-LPS Abs were both found able to capture most of the Lp1 isolates since 32 out of 38 isolates were recognised by each Ab. However, the monoclonal Ab was found more specific than the polyclonal one to detect *Legionella* in samples contaminated with other bacterial species such as *Pseudomonas aeruginosa*. This latter point was confirmed when the method was applied to environmental samples taken from hospital water circuits and air cooling towers. In these samples, the method combining IMS and culture led to the detection of higher amounts of Lp1 as compared to culture alone.

Conclusion: The method that combines IMS using a monoclonal anti-LPS Ab and culture was found promising to selectively capture Lp1 cells from water samples highly contaminated by various micro-organisms, leading to an improved sensitivity of the culture assay.

R2393 Evaluation of VITEK 2 ID NH for identification of fastidious organisms in a paediatric hospital

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Objectives: to investigate the accuracy and performance of a rapid and automated commercial identification system (VITEK 2 ID NH card), using a clinical set of fastidious organisms, especially *Haemophilus* and *Campylobacter* which frequently encountered in paediatric community acquired infections.

Methods: A total number of 129 strains and 2 quality control strains (102 *Haemophilus* and 17 *Campylobacter* strains as well as 10 *Neisseria* strains provided by National Meningitis Reference Laboratory) were used for assessing the performance of VITEK 2 ID NH Colorimetric Card (bioMérieux, Marcy l'Etoile) in comparison with conventional methods and biochemical API system.

All *Haemophilus* and Neisseriae isolates had been identified using API NH and manual disc factors (xv, x, v), *Campylobacter* species using the Api Campy and hippurate hydrolysis as gold standard compared with VITEK 2 ID NH card.

Results: A total of 118 (91.5%) isolates were correctly identified at species level without additional tests while 8 (6.2%) were correctly identified at the genus level with low discrimination between two species, thus requiring supplemented testing. Three (3) isolates were identified correctly at genus but were misidentified at species level. Only 3 isolates of *H. influenzae* were misidentified as *H. parainfluenzae* and one *C. jejuni* strain as *C. coli*. No misidentifications were observed at genus level for those fastidious species.

Final identification results

Species	Correct ID	Correct with suppl. testing	Mis ID species	Mis ID genus	no ID
H. influenzae (80)	(71) 88.8%	(6) 7.5%	(3) 3.7%	0.0%	0.0%
H. parainfluenzae (22)	(19) 86.4%	(3) 13.6%	0.0%	0.0%	
N. lactamica (5)	(5) 100%	0.0%	0.0%	0.0%	0.0%
N. meningitidis (4)	(4) 100%	0.0%	0.0%	0.0%	0.0%
M. catarrhalis (1)	(1) 100%	0.0%	0.0%	0.0%	0.0%
C. jejuni (13)	(12) 92.3%	0.0%	(1) 7.7%	0.0%	0.0%
C. coli (4)	(4) 100%	0.0%	0.0%	0.0%	0.0%
Total	**91.5%**	**6.2%**	**2.3%**	**0.0%**	**0.0%**
	97.7%		**2.3%**	**0.0%**	**0.0%**

Conclusions: The VITEK 2 ID NH set is an easily used, promising new tool for the identification of fastidious species, providing rapid results within 6 hours, demonstrating a very good accuracy for various genus, including *Haemophilus*, *Neisseria* and *Campylobacter* spp.

R2394 Evaluation of a Latex Agglutination test for the detection of *Helicobacter pylori* in stool specimens

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Objectives:

1. To evaluate the usefulness of a new latex stool antigen assays: PYLOGEN, (CerTest Biotec, Zaragoza, Spain) in the diagnostic of *Helicobacter pylori* infection and to confirm *H. pylori* eradication after treatment.
2. To compare their accuracy with a Immunocromatographic antigen assay: *H. pylori* Letitest (leti diagnósticos) and with two ELISA assays Premier Platinum HpSA (Meridian Diagnostics, Cincinnati, OH), Amplified IDEIA Hp StAR (DakoCytomation, Cambridge, UK).

Methods: We evaluated stool samples from 38 patients diagnosed with *H. pylori* infection and from 9 patients without infection. To confirm *H. pylori* eradication we evaluated 57 patients who received *H. pylori* treatment. Eradication was confirmed with 13C-urea breath test 6 weeks later. The assays were performed according to the recommendations of the manufacturer. Sensitivity, specificity, correlation and kappa values with UBT were calculated.

Results: PYLOGEN test detected *H. pylori* antigen in 30/38 samples from study group (78.9% sensitivity), *H. pylori* Letitest in 35/38 (92.1%), Premier Platinum HpSA in 33/38 (86.8%) and Amplified IDEIA Hp StAR 37/38 (97.3%). All tests were negative for samples from control group patients (100% of specificity).

In the 57 patients evaluated 6 weeks after eradication therapy, the overall agreement between urea breath test and the antigen tests were: PYLOGEN 89.4% (k 0.683), Letitest 80.7% (k 0.468), Amplified IDEIA Hp StAR 94.7% (k0.348) and Premier Platinum HpSA EIA 73.7% (k 0.837).
Conclusions: PYLOGEN is latex agglutination assay, quickly and easy to perform, with a good sensitivity to determine *H. pylori* infection and a good specificity. Compared to the 13C UBT, PYLOGEN and Premier Platinum HpSA EIA show a comparably good correlation to assess the success of eradication therapy.

R2395 Rapid culture based detection of *Streptococcus agalactiae* on vaginal/rectal swabs

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Objectives: To develop and test a rapid method based on direct agglutination of a selective Todd-Hewitt broth to detect group B streptococci (GBS) on routine vaginal and/or rectal swabs obtained for perinatal screening.
Methods: A total of 447 swabs for the detection of GBS were cultivated in Todd-Hewitt + antibiotics enrichment broth overnight at 35ºC. The next day 2 drops (100ul) of the broth were extracted and a direct latex agglutination (Streptex®, Remel Inc.) was performed for the detection of Lancefield group A, B, C, D, F and G streptococci. On the same broth, subcultures at 35ºC overnight were made on sheep blood agar (n = 447) and chromogenic agar plates (STREPTO B ID® bioMérieux, n = 168) to search for GBS. Agglutination was then used again to identify all types of colonies.
Results: Overall, direct agglutination gave 81 broths (18.1%) positive for a group B streptococcus. Subcultures confirmed GBS in 80 (98.7%) of the samples, only 1 colony type could not be identified on sheep blood agar after 24 hours. Out of the 366 broths presenting none or other streptococcal groups on direct agglutination, only 1 presented a GBS after subculture on sheep blood and chromogenic agar.
Conclusion: Direct agglutination of the Todd-Hewitt + antibiotics enrichment broth containing vaginal/rectal swabs offers a rapid (within 24 hours), sensitive and specific method for the detection of GBS colonisation in perinatal screening. This cost-effective procedure can easily be performed in any clinical microbiology laboratory.

R2396 Evaluation of the new VITEK-2 ANC identification card in a clinical laboratory

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Objectives: The purpose of this study was to evaluate the ability of the new VITEK2 ANC card (bioMérieux, Marcy l'Etoile, France) to identify anaerobic and coryneform bacteria in comparison to reference methods.
Methods: a total of 319 strains belonging to the 63 species of anaerobic and coryneform bacteria listed in the ANC card database were investigated. Strains included 263 anaerobic clinical strains isolated in the laboratory of Bacteriology, University Hospital Centre, Nancy, France and 56 coryneform clinical strains isolated in the laboratory of Bacteriology, University Hospital Centre, Strasbourg, France. These isolates were previously identified by conventional methods. The new VITEK 2 ANC cards were used according to the manufacturer's recommendations. The cards were filled with organism suspensions prepared in 0.45% aqueous NaCl to turbidity equivalent to a McFarland #3 standard. Inoculated cards were incubated in the VITEK2 for approximately 6 h. Identification results were generated by a computer-assisted algorithm. In the case of conflicting results, 16S rRNA and rpoB gene sequencing methods were used for genetic identification.
Results: The new VITEK 2 ANC card was able to identify 314 (98.4%) strains to the genus level and 281 (88%) to the species level. Nine (2.8%) strains were misidentified and two (0.6%) could not be identified.
Conclusion: The new VITEK2 ANC appears to be a useful and reliable method for the rapid identification of anaerobic and coryneform bacteria.

R2397 *Klebsiella oxytoca* as a causative agent of antibiotic-associated haemorrhagic colitis

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Objectives: *Klebsiella oxytoca* has been isolated from stool samples of patients with *Clostridium difficile*-negative antibiotic-associated haemorrhagic colitis. However, the pathogenic role of this organism has not been fully elucidated yet.
The aim of our study was to investigate the presence of toxin producing *Klebsiella oxytoca* in patients with antibiotic-associated haemorrhagic colitis.
Methods: 2500 stools specimens from patients with the clinical diagnosis antibiotic-associated haemorrhagic colitis and 85 stool specimens of a healthy control group were examined in the period from June to November 2007 in Tyrol (Austria).
The specimens were cultured on MacConkey agar and investigated for the presence of *K. oxytoca* by standard microbial procedures. The cytotoxic effect of *K. oxytoca* strains was tested on Hep-2 cells and Vero cells, the amount of cytotoxicity was determined by Lactat-Dehydrogenase release. The *Klebsiella* strains were characterised phenotypically by antibiotic susceptibility testing and were typed by Pulsed-Field Gel-Electrophoresis. In addition, all stool samples were investigated for *Clostridium difficile* toxin by ELISA.
Results: 119 of 2500 stool specimens (4.8%) of patients with antibiotic-associated haemorrhagic colitis yielded *K. oxytoca*. In the control group only 2 of 85 specimens (2.4%) were positive for *K. oxytoca*. The rate of cytotoxic strains among *K. oxytoca* was 46% in patients with antibiotic-associated haemorrhagic colitis compared with none in the healthy control group.
In addition we could demonstrate that Vero cells are superior to Hep-2 cells for investigating *K. oxytoca* strains for toxin production.
Eleven percent of the stool specimens were positive for *Clostridium difficile* toxin.
Conclusions: Our study demonstrates that beside *Clostridium difficile* cytotoxigenic *K. oxytoca* are a causative agent of antibiotic-associated haemorrhagic colitis. Thus, cytotoxigenic *K. oxytoca* should be included in routine microbiologic diagnostic of stool specimens. Furthermore we recommend Vero cells for determination of the cytotoxic effect of *K. oxytoca* strains.

R2398 Septum sonication of removed ports for diagnosis of related bloodstream infections

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Objectives: Venous access ports are used in patients receiving long parenteral treatments. Diagnostics specific for port-related infections have not been enough developed. In these infections microorganisms are typically present forming biofilms. The aim of this study was to analyse the role of septum vortexing-sonication technique to diagnose port-related bloodstream infections (PRBI).
Methods: All the devices removed in our hospital during a 27-month period (august 2005 to October 2007) were prospectively studied. We compared the septum sonicate-fluid culture with the port internal lumen swabbing and the culture after sonication of the catheter tip. We considered a confirmed port infection when the same microorganism was isolated from blood cultures and in any port culture [for coagulase-negative staphylococci (CNS), cultures were considered positive if there were at least 1000 cfu/ml in any of the sonication techniques, or 15 cfu for the chamber swabbing. For other microorganisms any count was considered significative].
Results: 240 ports were removed from 240 patients. Ports were removed due to end of use in 187 (77.9%) cases, because of suspected PRBI in 29 (12.1%), because of mechanical complications in 19 (7.9%)

and because of local infectious complications in 5 (2.1%). Finally, 27 patients met criteria for PRBI. Most of these patients (92%) were receiving antimicrobial therapy when port was removed. The most common isolated microorganisms were: Gram-negative bacilli (30.4%), CNS (21.7%), yeasts (21.7%) and *Staphylococcus aureus* (17.5%). Sensitivity of septum sonication (78.6%) was better than that of chamber swabbing (67.9%) ($p > 0.05$), and than that of catheter tip culture (51.8%) ($p < 0.05$). The specificity of the septum sonicate-fluid culture, chamber swabbing culture, and catheter tip culture were 97.1%, 95.7% and 93.7%, respectively. Positive and negative predictive values for the septum sonicate-fluid culture were 66.7% and 97.1%, respectively. The ROC curve analysis showed that the best threshold for the septum sonicate-fluid culture was 110 cfu/ml (S:78%, E:93%) for any isolated microorganism.

Conclusions: According to data from the present study, the septum sonicate-fluid culture was the most sensitive method for the microbiologic diagnostic of PRBI. The high negative predictive value of this technique permits to exclude port as origin of the bloodstream infection if culture is negative.

R2399 Value of antibodies to the *C. albicans* enolase for the diagnosis of invasive candidiasis in non-neutropenic critically ill patients

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Objective: Non-neutropenic critically ill patients with *Candida* colonisation are at high risk for invasive candidiasis. We evaluated the usefulness of serum antibodies against *C. albicans* recombinant enolase in the diagnosis of invasive candidiasis in non-neutropenic critically ill patients as well as in those with *Candida* score <3 and ⩾3. In a previous study, a *Candida* score ⩾3 accurately selected patients who would benefit from early antifungal treatment [1].

Methods: 381 patients (547 sera) with risk factors for fungal infection were recruited from an ongoing prospective observational multicentre study ("CAVA Project"). Besides surveillance screening cultures and other work-up studies, antibodies directed to *C. albicans* recombinant enolase were determined on weekly serum samples by a commercially Candida Enolase ELISA IgG kit (Lab. Vircell®, Granada, Spain) [2], according to the manufacturer's instructions. Study groups were proven invasive candidiasis (n = 20), *Candida* colonisation with Candida score ⩾3 (n = 132) or Candida score <3 (n = 147), absence of *Candida* colonisation (n = 82).

Results: *Candida* antibodies were documented in 182 patients (47.8%) (250 sera), which corresponded to 11/20 patients with invasive candidiasis (24/55 sera), 60/132 with Candida score ⩾3 (82/174 sera), 82/147 with Candida score <3 (112/222 sera), and 29/82 (32/96 sera) without colonisation.

Positive antibody test	Positive serial serum samples, no. (%)			
	One	Two	Three	Four
Invasive candidiasis, n = 11	4 (36.3)	4 (36.3)	0	3 (27.3)
Candida colonisation				
Candida score ⩾3, n = 60	48 (80)	5 (8)	6 (10)	1 (2)
Candida score <3, n = 82	62 (75.6)	16 (19.5)	2 (2.5)	2 (2.5)
No *Candida* colonisation, n = 29	28 (96.5)	0	1 (3.5)	0

Conclusion: In serial serum samples, *Candida* enolase antibodies showed a good correlation with suspicion of proven *Candida* infection. This assay, however, should be used together with other techniques for definite diagnosis of invasive candidiasis in non-neutropenic critically patients.

Reference(s)

[1] Leon C et al. Crit Care Med 2006;34:730–7.
[2] Mendoza J. (Lab. Vircell®, Granada, Spain).

R2400 Evaluation and comparison of organism identification and antimicrobial susceptibility results using direct and conventional testing methods for clinically significant positive blood culture isolates

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Objective: To compare the direct inoculation of positive blood culture organisms into an automated system (VITEK 2), compared to conventional overnight incubation, organism identification and antimicrobial testing methods.

Methods: For a 3-month period, 61 clinical isolates at Monklands hospital were analysed using direct inoculation into VITEK 2 identification and antibiotic susceptibility cards.

Results: Of these isolates, 3 were unsuitable for VITEK 2; of the remaining isolates 98.3% provided the same identification profiles as the conventional testing method. The most frequent organisms associated with septicaemia were *E. coli* (33%), meticillin sensitive *Staphylococcus aureus* (15%), meticillin resistant *Staphylococcus aureus* (12%), and *Streptococcus pneumoniae* (13%). Overall the Gram-positive isolates (GP) demonstrated 100% correlation between both direct and conventional testing, however *Streptococcus pneumoniae* had a comparison rate of 50%. Gram-negative (GN) isolates other than *E. coli* demonstrated 100% correlation, and *E. coli* isolates had 95% correlation between the testing methods. Analysis of the time taken for completion of both organism identification and antibiotic susceptibility testing (AST) for the direct testing method showed the time (hours) of identification of isolates and sensitivity (respectively) as 5.25±0.28/9.44±0.27 (mean±sem; n = 9) for *Staphylococcus aureus*, 5.54±0.63/8.07±0.53 (n = 7) meticillin resistant *Staphylococcus aureus*, 5.50±0.48/9.5±0.51 (n = 4) coagulase negative Staphylococci, 4.31±0.39/9.66±7.30 (n = 4) *Streptococcus pneumoniae*, 4.54±0.14/6.53±0.22 (n = 19) *E. coli*, 7.00±8.88/9.38±0.02 (n = 3) Non-fermenting organisms and other Gram-negative organisms 4.34±0.35/7.78±0.76 (n = 8). The time taken for organism identification and sensitivity using the conventional methods of overnight culture ranged between 22.16–24.88/24.59–27.81±0.75. The overall MIC agreement between the testing methods was 96.9% ($p < 0.05$), with minor error rate (mE) of 1.2, major error rate (ME) of 1.4 and very major error rate (VME) of 0.2 for all organisms tested.

Conclusion: The direct testing of positive blood cultures using the automated Vitek system was 18 hrs quicker than conventional testing, with high overall MIC agreement for sensitivity testing.

R2401 The diagnosis of central venous catheter-related bloodstream infection by differential time-to-positive culture is affected by the time between bed to Bactec

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Objectives: Differential time to positive (DTP) blood culture is a simple method to differentiate non-catheter-related from catheter-related bloodstream infection (CR-BSI). A DTP of >2 hours is indicative of a CR-BSI. In most studies where DTP is measured, the blood cultures have been transported immediately to the laboratory. In clinical practice the transport time may exceed 12 h. The aim of this study was to examine if storing of blood cultures in room temperature before incubation influences the DTP.

Methods: Fresh human blood was added to BACTEC + Aerobic blood culture bottles, which were weighed to assess the amount of added blood. Overnight cultures of *S. epidermidis* ATCC, *S. aureus* ATCC 29213, P. auruginosa ATCC 27853 and *E. coli* ATCC 25922 were prepared in BHI broth. Tenfold serial dilution was performed in PBS. For each species three bottles per dilution (n = 9) were inoculated with 0.2 ml aliquots of

each dilution ($1:10^4$–$1:10^6$). The initial inoculums of each bottle (cfu/ml blood) were enumerated by viable count. Three bottles with different inoculums were immediately placed in a detector (BACTEC 9420). The remaining bottles were placed in room temperature for 12 h (n = 3) and 24 h (n = 3). Each analysis was repeated at least once.
Time to positive blood cultures (TTP) and cfu/ml blood of the initial inoculums were plotted and a regression analysis was performed. DTP between 100 and 1000 cfu/ml blood of the initial inoculums and the ratio of initial inoculums to obtain a DTP = 2 h were calculated.
Results: See the table.

Species	Time in room-temperature (h)	TTP at 100 cfu/ml blood (initial inoculum) (h)	TTP at 1000 cfu/ml blood (initial inoculum) (h)	DTP between 100 and 1000 cfu/ml blood (initial inoculum) (h)	Difference in initial inoculum size to obtain DTP = 2 h
S. aureus	0	11.54	9.50	2.04	9.5 times
	12	7.15	5.59	1.56	19 times
	24	3.90	2.80	1.10	66 times
S. epidermidis	0	15.74	13.62	2.12	8.7 times
	12	13.21	11.16	2.05	9.4 times
	24	11.33	9.52	1.81	13 times
P. aeruginosa	0	13.03	11.26	1.77	13 times
	12	8.48	6.89	1.59	18 times
	24	4.60	2.97	1.63	17 times
E. coli	0	8.91	7.91	1.00	99 times
	12	5.08	3.88	1.20	46 times
	24	1.89	1.34	0.55	799 times

Conclusions: The time in room temperature before incubation affects the DTP. Blood cultures should be placed in the positive-culture detector no later than after 12 h from sampling. DTP should be used with caution when *E. coli* is the causing organism of septicaemia.

R2402 Respiratory swab sampling for bacteria: comparisons of swab material, sampling site, and sample processing

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Objectives: Nasopharyngeal (NP) or nasal sampling is needed in investigating the aetiology of respiratory infections. We conducted a comparative study taking six nasal/NP swab samples to contrast two different sampling swabs, nasal with NP sampling, and the saline elution of the swab with the direct culturing of the swab.
Methods: The samples were collected from 36 children aged 6 months to 11 years. First, right nostril was sampled with a dacron swab which was placed into 0.5 ml of saline. After vortexing, 10 µl was streaked on culture plates. This specimen was termed the nasal dacron sample (N-dacron-NaCl). The second sample was taken from the right NP with a dacron swab which was processed as described above (NP-dacron-NaCl). The third sample was taken from the right NP with a dacron swab, but the swap was directly streaked on culture plates (NP-dacron). The three following samples were taken and processed in the same order and principle from the left nostril with a cellulose swab. Those specimens were termed N-cellulose-NaCl, NP-cellulose-NaCl, and NP-cellulose. The primary outcome was the number of bacterial colonies on blood agar plates.
Results: Comparisons of swab material, sampling site, and sample processing by cumulative logistic regression resulted in the following age-adjusted odds ratios. Saline elution vs. direct plating: NP-dacron-NaCl vs. NP-dacron 4.9 (P = 0.001); NP-cellulose-NaCl vs. NP-cellulose 11.0 (P < 0.001). Cellulose vs. dacron swab: N-cellulose-NaCl vs. N-dacron-NaCl 1.9 (P = 0.221); NP-cellulose-NaCl vs. NP-dacron-NaCl 3.4 (P = 0.024); NP-cellulose vs. NP-dacron 1.5 (P = 0.365). Nasopharynx vs. nostril: NP-dacron-NaCl vs. N-dacron-NaCl 0.6 (P = 0.308); NP-cellulose-NaCl vs. N-cellulose-NaCl 1.1 (P = 0.897).
Conclusion: Saline elution of the sample did not affect the viability of bacteria. Actually, significantly more colonies were obtained with this procedure than with direct plating of the swap. Most importantly, the saline elution provides sample material for several different microbiological analyses. In conclusion, we suggest NP swab sampling and swab elution into saline for the bacterial analyses of NP.

R2403 Immunological diagnosis of early *Pseudomonas aeruginosa* lung infection in cystic fibrosis patients

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Objectives: Serological methods to monitor Pa colonisation in patients with cystic fibrosis (CF) are advocated but the diagnostic value of a commercially available *P. aeruginosa* antibody test to detect early Pa colonisation has not been evaluated.
Methods: Colonisation of 235 CF patients (120 male) aged 10.5 years (range 1–30 years) was assessed by regular culture (every three months) of sputum or oropharyngeal secretions during four consecutive years (2003–2007). At the beginning of the study period all patients were negative for Pa.
Commercially available ELISA tests were performed to detect IgG and IgA against Pa lipopolysaccaride in sera once a year.
A subgroup of 46 patients (22 male) aged 10.5 years (range 2–24 years) showed culture conversion during the follow-up period and serology data of one year before (T-1) and one year after (T+1) the first infection, were compared.
Results: Serum antibody level (mean + SD) of immunoglobulin G (IgG) and immunoglobulin A (IgA) are showed in the table. IgG level at (T+1) was significantly higher when compared to (T-1).
In 13 patients (28%) at (T-1) the IgG levels were above the cut-off (6 U.I./ml), whereas the IgA levels were above the cut-off (8 U.I./ml) in 12 patients (26%).
At (T+1), 25 patients (54%) were positive for IgG antipseudomonas and 10 patients (22%) were positive for IgA.

	IgG (cut off: 6 UI/ml)		IgA (cut off: 8 UI/ml)	
	(T − 1)	(T + 1)	(T − 1)	(T + 1)
Mean±SD	4.18±3.49	9.69±7.03	3.89±8.55	11.69±22.7
p (T + 1 vs T − 1)	0.000025		0.1056	

Conclusions: Serology resulted in a higher number of positive patients than culture suggesting:
– higher diagnostic value of serology than culture,
– culture identification failures (because of lower bacteria densities in the airways in case of first infection),
– antibodies are markers of previous infection occurred several months or years before.

CF patients with positive serological tests and negative cultural tests might be monitored closely.

R2404 Usefulness of enzyme immunoassay as a screening method in syphilis serology

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Objective: The aim of this study is to evaluate the usefulness of the enzyme immunoassay (EIA) method bioelisa SYPHILIS 3.0 versus RPR and TPHA tests, in laboratory diagnosis of syphilis.
Methods: EIA was performed in 87 serums obtained in October and November 2007, selected by their positive result in RPR, TPHA or FTA-Abs in order to know if EIA test was able to screen them as positive samples. In our laboratory, FTA-Abs was performed only when RPR or TPHA test was positive. We considered true positives results when RPR and TPHA were positive, or RPR negative and TPHA and FTA-Abs positive and false positive when RPR or TPHA were positive and FTA negative.
EIA results were compared to a combination of RPR/TPHA/FTA-Abs that allowed considering true or false positive results.
Results: EIA versus current syphilis serology tests shows a sensitivity of 93.3% and a specificity of 97.6%.

	Syphilis serology RPR/TPHA/FTA-Abs (2 or more tests positive)		
EIA	+	–	Total
+	42	1	43
–	3	41	44
Total	45	42	87

Sensitivity: 93.3%; Specificity: 97.6%.

Conclusion: Although EIA may be a useful screening method in laboratories that perform a large number of tests in which automation becomes absolutely necessary, positive tests need to be confirmed by classical methods of nontreponemal and treponemal tests. Nontreponemal test are still useful as a measure of syphilis activity and in monitoring drug response.

R2405 Portuguese EQA results for serogical diagnosis of syphilis and brucellosis

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Objectives: Syphilis is a chronic infectious disease caused by spirochaete of the genus *Treponema*. It is usually transmitted by sexual contact or from mother to infant. Brucellosis is the most widespread zoonosis transmitted from direct contact from animals. As both diseases have a worldwide impact on human health it is necessary to monitor laboratory results. In 1989, the Portuguese EQA provider, Programa Nacional de Avaliação Externa da Qualidade (PNAEQ), created the Bacterial serology programme witch included toxoplasmosis, hydatidosis, rubella, syphilis, brucellosis and typhoid fever. Since 2002 the Bacterial Serology programme only includes syphilis and brucellosis. The main objectives of this programme are evaluating participant's results and consequently improve clinical laboratory performance nationally. In this work we evaluate PNAEQ's participant results in syphilis and brucellosis.
Methods: Results of three annual assays, consisting of syphilis (human sera), a brucellosis sample (animal sera) and clinical information.
For syphilis diagnosis we evaluated the data for treponemal tests (FTA-abs, TPHA, TPPA, EIA IgG, EIA IgM, EIA IgG and IgM, Imunoblot) and non-treponemal tests (VDRL, RPR, USR). For brucellosis diagnosis we evaluated the data for Wright and rose Bengal tests. The results were statistically treated accordingly with the answers given by the laboratories.
Results: Throughout the year 2006 there was reduction of 20% in the number of answers sent to PNAEQ and in 2007 a more active participation.
For negative syphilis samples there is accuracy above 84% and for reactive samples the mean accuracy is 79%. In Brucellosis the percentage of correct answers for non-reactive samples is higher than 90% but for reactive samples the mean accuracy is 70%. The behaviour of different reagents used by laboratories was also studied, that being heterogeneous in the different assays and different bacterial load. The majority of the results were compatible with the clinical information provided.
Conclusion: There was a more active laboratory participation in 2007. Treponemal tests for diagnosing syphilis had a better performance, as in rose Bengal test for brucellosis diagnose. A deeper study of reagent behaviour should be performed in order to improve the diagnosis of both syphilis and brucellosis.

R2406 Sympathetic skin response in patients with purulent meningoencephalitis

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Objectives: Sympathetic skin response (SSR) is defined as a minute change of skin potential after electrical stimulation. This test measures the change in voltage that originates from the surface of the skin and is attributed to sudomotor activity. The involvement of the autonomic nervous system has been demonstrated with this method in diseases affecting the central nervous system; we presumed either to be affected in meningoencephalitis. It was the aim of our study to investigate SSR in patients with purulent meningoencephalitis.
Methods: SSR was performed bedside by Medelec/TECA Saphire II machine in 40 healthy volunteers and before lumbar puncture in18 patients with purulent meningoencephalitis. At the time of testing 8 patients were unconscious. The surface disk electrodes were attached to the skin: for palmar/plantar SSR the active surface electrodes were placed on the middle of the palm/sole and the reference electrodes were placed on the dorsum of the hand/foot. The ground electrode was situated in the forearm.
A single square electrical impulse triggered on the volar side of the wrist was used to evoke SSR. The intensity of electrical impulse was 100–250 V, the duration was 100–200 μs. Responses were amplified with bandpass 0.01 and 3 kHz for an analysis time 10 s.
Results: SSR were recorded in all 40 healthy subjects, regardless of age and on all measured sites. The typical SSR consisted of triphasic potential, ussually with negative initial peak. On the contrary, there were absolute absence of all SSR in 8 patients, who were unconscious at the time of test. In 4 patients, there was absence of plantar SSR with prolongation of palmar SSR latency. Also, there were significant prolongation of palmar and plantar SSR latency and decrease of SSR amplitude in 6 conscious patients with purulent meningoencephalitis.
One month after discharge from hospital, all survived patients had normal palmar and planter SSR again.
Conclusion: SSR is a simple and bedside technique, with which we can predict severity of CNS infection. There is typical reversible absence of SSR in comatose patients and prolonged SSR latency and lower SSR amplitude in conscious patients with severe purulent meningitis.

R2407 Use of mass spectrometry to identify clinical *Fusarium* isolates

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Fusarium spp. have recently emerged as significant human pathogens. Species identification of these fungi is important both for epidemiologic purposes and for patient management, but conventional identification based on morphologic traits is hindered by major phenotypic polymorphism.
In this study, we subjected 62 isolates belonging to nine *Fusarium* species to both molecular identification (TEF1 gene sequencing) and MALDI-TOF analysis. Following stringent standardisation, the latter method appeared both reproducible and robust even when various influencing factors such as growth medium, temperature and time of incubation were evaluated. Spectrum comparison with a database of the most frequently isolated species correctly identified 57 isolates. As expected, the four members of species not represented in the database were not identified. Mass spectrometry and molecular identification agreed in 5 of the 6 cases in which morphological and molecular identification disagreed.
In conclusion, the constructed database and the standardised protocol were validated for identification of the most common clinical *Fusarium* isolates. Uncommon species must now be added to the database. MALDI-TOF yielded results within one hour, at a cost similar to that of the molecular method. MALDI-TOF may also prove useful for identifying other clinically important moulds.

Methods for antibacterial susceptibility testing

R2408 Detection of extended-spectrum β-lactamases among Enterobacteriaceae by use of VITEK 2 and manual detection procedure

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Objectives: Rapid detection of extended-spectrum β-lactamase (ESBL) producing Enterobacteriaceae is crucial in order to control spread and institution of optimal antibiotic therapy. Purpose of this study was to compare the performance of different routine methods.
Methods: Isolates for this study were selected on basis of a positive ESBL-screen test and/or resistance to cefuroxime with the VITEK 2 system (cards N 045 and N 046).
Over a period of 6 weeks (March-April 2007) 254 isolates were consecutively recovered from 215 clinical specimens (urine 54.9%, sputum 19%, other 25.1% and blood 1%). The following species were included: *E. coli* (n = 96), *E. aerogenes* (n = 39), *E. cloacae* (n = 34), *K. oxytoca* (n = 15), *K. pneumoniae* (n = 22) and *M. morganii* (n = 48).
On all 254 isolates the disk approximation method with cefotaxime (30 μg), ceftazidime (30 μg), aztreonam (30 μg) and amoxicillin-clavulanic acid (20/10) disks was used as reference method. Only 91 isolates were identified as ESBL producer. Routine performance of three different methods for ESBL detection was compared: the VITEK 2 system (Biomérieux, Marcy l'Etoile, France), E-test ESBL (AB BIODISK, Solna, Sweden; strips with ceftazidime and cefepime/clavulanic acid) and chrom IDTM ESBL (Biomérieux, Marcy l'Etoile, France). All analyses were performed in accordance with the guidelines of the manufacturer.
Results: The performance characteristics of the three methods are shown in the table.

Isolate	No.	VITEK 2		Chrom ID		E-test	
		Sens.	Spec.	Sens.	Spec.	Sens.	Spec.
E. cloacae	34	100	58.1	100	41.9	100	100
E. aerogenes	39	93.3	29.2	86.6	16.7	100	100
E. coli	96	100	86.3	73.3	90.2	100	92.2
K. oxytoca	15	100	50	91.7	33.3	84.6	100
K. pneumoniae	22	92.9	75	100	100	100	87.5
M. morganii	48	100	14.9	100	91.5	100	100
All isolates	254	97	50.1	83.5	69.9	98.9	96.3

The method with the highest sensitivity for the detection of ESBLs was the E-test (98.9%) followed by the VITEK 2 (97%), however specificity was more variable, ranging from 50.1% (VITEK 2) to 96.3% (E-test). The performance differed widely between the species investigated.
Conclusion: Considering the rather low specificity of the ESBL detection by VITEK 2 we recommend the use of confirmation for all organisms reported positive for ESBL production by VITEK 2.

R2409 Comparison of direct and standardised antimicrobial disc susceptibility testing on positive blood cultures

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Objectives: To investigate the accuracy of immediate antimicrobial disk diffusion susceptibility testing on blood cultures (=direct test, DT) compared to standardised testing on subcultured isolates (=reference test, RT).
Methods: A total of 573 positive blood cultures were investigated. RT was performed on subcultures according to the CLSI protocol, DT by swabbing the positive blood culture directly on the Mueller-Hinton II agar. Cultures consisting of mixed flora or isolates detected only on subculture and species with a prevalence of less then 10 isolates in 2 years were excluded from the study. Discrepancies in susceptibility results were classified as, minor errors (m): resistant or susceptible in DT and intermediate in RT or vice versa; major errors (M) resistant in DT but susceptible in RT; and very major errors (VM) susceptible in DT but resistant in RT.
Results: Species isolated were *S. epidermidis* (43%), *S. hominis* (11%), *S. aureus* (7%), *E. coli* (14%), *S. haemolyticus* (6%), *P. aeruginosa* (4%), *S. capitis* (3%), *K. pneumoniae* (3%), *E. cloacae* (3%), *E. aerogenes* (3%) and *S. warneri* (2%).
Direct and reference susceptibility testing showed an overall concordance of 91.9% (5.5% m, 2.2% M and 0.4% VM): 91.8% for Gram-positive isolates (5.0% m, 2.7% M and 0.5% VM) and 92.0% for Gram-negative isolates (6.8% m, 1.0% M and 0.2% VM).
When analysing all antibiotic/isolate combinations, very major errors occured most frequent for oxacillin (1.2%), clindamycin (1.0%) and mupirocin (1.2%) in DT for Gram-positives and for sulfamethoxazole (1.5%) in DT for Gram-negatives. Based on the bacterial species there was a significantly ($p < 0.05$) higher percentage of very major errors for *S. aureus* and *E. aerogenes* compared to the other common Gram-positive and Gram-negative pathogens, respectively.
Clustering of very major errors with prevalences >4% for specific antibiotic–isolate combinations was evident for aztreonam/amoxicillin-clavulanate – *E. aerogenes* (6.7%/6.7%), piperacillin–tazobactam – *K. pneumoniae* (5.3%) and oxacillin – *S. aureus* (4.9%).
Conclusion: There is a very good concordance between the results of direct susceptibility testing and reference testing even without standardisation of the turbidity of the inoculum in the DT. Therefore results of direct testing can be reported. However, they still need to be confirmed by the reference test in order to possibly correct categorical differences especially within the species *S. aureus* and *E. aerogenes*.

R2410 MICs of non-tuberculous mycobacteria using the TREK Vizion™ system compared to manual readings

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Objectives: o compare MIC readings of nontuberculous mycobacteria using the TREK Vizion System with visual readings.
Methods: Seventy-six isolates of nontuberculous mycobacteria (16 *Mycobacterium abscessus*, 6 *M. chelonae*, 10 *M. fortuitum* group, 2 *M. immunogenum*, 1 undescribed rapidly growing *Mycobacterium* sp., 1 *M. mucogenicum*, 24 *M. avium* complex, 3 *M. marinum*, 4 *M. simiae*, 5 *M. kansasii*, 2 *M. parascrofulaceum*, 1 *M. lentiflavum*, 1 *M. arupense*) were tested using Sensititre® (TREK Diagnostic Systems) 96-well MIC plates following the Clinical and Laboratory Institute (M24-A) including guidelines for antimicrobials tested. After incubation according to species, all plates were read visually using a Sensititre mirrored light box. Subsequently, the same 96-well plates were read in a blinded fashion using the Vizion System. The Vizion System projects a digital image of the growth in each well onto a touch screen monitor with the plate's antimicrobial template overlaid on the image. Touching or clicking the MIC well provided instant feedback of interpretations and the images were stored for later review. The results were automatically recorded and printed with interpretations. Comparisons were made between the readings using current recommended CLSI antimicrobial susceptibility criteria for interpretation of susceptible (S), intermediate (I) and resistant (R) isolates. Those with susceptibility interpretive category change between the two methods from S to R or R to S had major errors. Minor errors were those with susceptibility interpretative category change from S to I, I to S, I to R, or R to I.
Results: 96% of the MICs were the same interpretative category for visual and Vizion readings. Only 4% of the MIC comparisons had minor errors and there were no major errors.
Conclusion: The Vizion System demonstrated excellent correlation to visual MIC reads of nontuberculous mycobacteria. Additionally, the Vizion System allows more rapid MIC reads, thus streamlining laboratory workflow, providing a teaching and training tool for new

laboratorians and enhancing collaborations between laboratories using the stored images. The Vizion System also provides a built-in comprehensive epidemiology programme which allows inter and intra laboratory comparison of MICs and generation of antibiograms.

R2411 Comparison of 4 commercial methods for antimicrobial susceptibility testing of temocillin

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Objectives: Susceptibility testing for temocillin is currently performed by disk diffusion method (DD). E-test (AB Biodisk) and new VITEK 2 (VT) cards (bioMérieux) have been recently marketed for temocillin testing. Data concerning the accuracy of these commercial methods are limited. The aim of this study was to compare the accuracy of the VT, E-test and DD methods (paper disk from Beckton Dickinson and Neo-Sensitabs from Rosco) for determining susceptibility of *Escherichia coli* to temocillin.
Methods: We tested 153 *E. coli* clinical isolates, including ESBL-producing strains (54%): 70 CTX-M (CTX-M-2, CTX-M-9, CTX-M-15), 12 TEM (TEM-24, TEM-30, TEM-52), 6 SHV-12, 15 AmpC hyper-producers (AmpC HP) and 53 strains with wild phenotype or other resistance mechanisms. ESBL production was confirmed by combined disk method (Bio Rad), PCR and DNA sequencing. Strains were tested in parallel by VT (AST-N045 and AST-N046 cards), DD, E-test and agar dilution (AD) according to the CLSI guidelines as gold standard method.
Results: MICs obtained by E-test and VT were within 1 dilution of those obtained by AD in >99% of isolates. No differences were observed between AD, E-test and VT ($p > 0.5$). VT and E-test showed the lowest number of discrepancies (TABLE).

Table. Summary of results. Results interpretation based on breakpoints from Fuchs (MIC ⩽ 16 mg/L)

Method (critical zone diameters)	Susceptible (%)	MIC_{50} (mg/L)	MIC_{90} (mg/L)	Errors (%) Minor	Major	Very major	Total
Neo-Sensitabs (15–17 mm)	95			3	0	1	4
BD (16–18 mm)	74			15	5	0	20
VITEK 2	93	8	16	0	2	0	2
E-test	95	8	16	0	3	0	3
Agar Dilution	95	8	16	–	–	–	–

VT provided results in a mean time of 7 hours (6–10 hours). Errors were observed with isolates showing borderline temocillin MIC of 16 or 32 mg/L. No discrepancies were observed for strains with MIC < 4 mg/L.
Conclusion: E-test and the VITEK 2 system showed good accuracy for determination of MICs of temocillin in *E. coli* including ESBL and AmpC HP strains. For disk diffusion Neo-Sensitabs showed better discrimination between susceptible and resistant strains and lower number of discrepancies than BD disks.

R2412 Evaluation of the Etest and broth dilution methods for susceptibility testing of *Brucella melitensis*

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Objectives: In vitro susceptibility tests are not standardised for *Brucella* species and they are not rountinely performed. The main objective of the present study was to evaluate the degree of agreement of two in vitro tests (E-test and the broth dilution method) with several antibiotics including tigecycline againist *Brucella melitensis* (*B. melitensis*).
Material and Methods: 16 *B. melitensis* isolates were collected between May 2003-October 2004 from blood cultures of adults patients with acute brucellosis at Baskent University Adana Practice and Research Center. Samples of blood were cultured using an automated culture system (BACTEC 9050, Becton Dickinson, Maryland, USA). The antimicrobial agents used in this study were: tigecycline (Wyeth Research, Pearl River, NY, USA), rifampin, trimethoprim-sulfametoxazole, and levofloxacin (Sigma-Aldrich, USA).
E-test method: Bacterial inoculum was prepared 0.5 McFarland turbidity standart by Mueller-Hinton broth (Oxoid). The bacterial suspension was spread on Mueller-Hinton agar (Oxoid) plates supplemented with %5 sheep's blood agar and E-test strips (AB Biodisk, Solna, Sweden) were applied.
Broth dilution method:The MIC values of antibiotics were determined by the broth dilution technique, according to Clinical Laboratory Standarts Institue with brucella broth (Merck, Germany), using an inoculum of 104 colony-forming units (CFU). *Brucella* broth medium was used within 12 hours after preperation. Tigecycline solutions prepared fresh on the day of testing. The plates incubated at 350C in ambient air, and the results were read after 48 h.
Results: In our study, the rate of agreement obtained with levofloxacin, rifampin, co-trimoxazole, and tigecycline between the E-test and broth dilution methods were 25%, 18.75% 0%, and 18.75%, respectively. Statistically significant difference was found between the methods for MIC values of levofloxacin, rifampin, co-trimoxazole, and tigecycline ($p < 0.01$, $p < 0.001$, $p < 0.001$, $p < 0.05$) (Table)

Table: Median and standard deviation values for MICs of antibiotics determined by E-test and broth dilution method

	Median±standard deviation Median (min–max) n = 16	
	E-test method	Broth dilution method
Levofloxacin	0.093±0.045	0.157±0.067**
	0.079 (0.050–0.190)	0.120 (0.060–0.250)
Rifampin	1.219±0.256***	0.641±0.258
	1.000 (1.000–1.500)	0.500 (0.250–1.000)
Co-trimoxazole	0.025±0.045	0.184±0.137***
	0.014 (0.000–0.190)	0.120 (0.060–0.500)
Tigecycline	0.035±0.015	0.065±0.036*
	0.032 (0.020–0.060)	0.060 (0.020–0.120)

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Conclusion: The MIC values determined with E-test method were lower than the values determined with the broth dilution method. This situation should be considered when evaluating the results of E-test method for *B. melitensis*.

R2413 Validation of inoculation of the VITEK 2 AST cards from SA-select agar for *S. aureus*

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Objectives: To validate direct susceptibility testing of *Staphylococcus aureus* from the chromogenic SA-select agar (Bio-Rad) on the VITEK 2 system (bioMérieux).
Methods: Two Belgian regional hospitals (Imelda Ziekenhuis, Bonheiden (IZ) and H. Hartziekenhuis, Lier (HH)) prospectively analysed a total of 90 *S. aureus* strains, isolated from routine clinical samples (IZ n = 46 and HH n = 44). These strains were identified following local routine procedure in each lab.
Inoculation of Vitek AST-P536 cards was done in duplo from SA-select medium and from blood agar, as neutral medium and reference method, also cleared by bioMérieux for direct inoculation of VITEK AST cards. In each centre, the ATCC 29213 strain (MRSA) was also included. IZ used VITEK 2 and HH VITEK 2 compact instruments.
The degree of concordance of MIC values as well as that of categorical values (RIS) was assessed.
Results: A total of 90 samples and 2 ATCC strains (one on each site) were analysed. 2 Very major and 6 minor errors were encountered. No major errors were found. Most discordances were found in oxacilline and norfloxacin MIC values. The very major errors were observed in nitrofuratoin and fosfomycin testing, both presumably random errors.

Results of the ATCC strain 29213 showed no categorical errors on both sites.
Conclusion: The degree of discordance between direct susceptibility testing from SA-select agar and blood agar is acceptable. Therefore, SA-select agar can be used as a primary medium for inoculation of VITEK 2 AST cards.

Public health & community-acquired infections

R2414 A case of pyogenic liver abscess caused by *Pseudomonas aeruginosa*

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Introduction: Pyogenic liver abscess (PLA) is a condition with significant morbidity and mortality. Biliary tract pathology is the most common cause of PLA. The clinical manifestations of PLA are not spesific; they include fever, chills, abdominal pain, and vomiting. The diagnosis of PLA was based on the clinical findings and evidence from imaging studies, either abdominal ultrsonography or computed tomography (CT). Treatment with intravenous antibiotics and the application of catheter drainage or aspiration are the primary therapeutic strategies. *Pseudomonas aeruginosa* has long been regarded as a relatively rare pathogen of PLA, because of this reason we decided to submitt a case of PLA caused by *Pseudomonas aeruginosa*.
Case: A 28-year-old man presented to our hospital with a two week history of pain in the right upper abdomen, followed by fever, chills and vomiting. In his history, he was exposed to traffic accident and posttraumatic empyema was developed two months ago. On his physical examination the temperature was 38.6ºC, pulse rate 96/min, respiratory rate 28/min and blood pressure was 110/60 mmHg. Laboratory investigations revealed total leucocyte count of 11800/mm3 (polymorphs 88%), C reactive protein was elevated up to 63 mg/dl, ESR was 87 mm/h. He got a liver abscess at right hepatic lobe which was confirmed by abdominal ultrasound and CT diagnoses. Ultrasound-guided percutaneus aspiration of liver abscess was done soon after the cofirmation. The culture result of aspirate grew *Pseudomonas aeruginosa*. The patient received a 4 week corse of adequate antibiotics treatment (Imipenem 2 gr/day + Amicasin 1.5 gr/day) after the aforementioned aspiration procedure. A series of ultrasound were performed to follow the resolution of abscess during the treatment period. Finally, the lesion resolved completely without leaving any complication.
Conclusion: It is well known that PLA can be cause by several organisims. The most common pathogenic agents, which enter the liver by vasculer routes, are *Escherichia coli*, *Salmonella typhi*, *Proteus vulgaris*, streptococci and staphylococci, but anaerobs may also be present. In conclusion, PLA often presented with non-spesific symptoms and was commonly associated with diabetes mellitus and biliary pathology. It could be effectively treated by intravenous antibiotics and drainage under ultrasound or CT guidance.

R2415 Antibiotic resistance and virulence markers of human *Salmonella enterica* serovar Kentucky isolates from Slovakia

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Objectives: *Salmonella* Kentucky is well recognised as a serovar isolated from cattle, fish, poultry, and other animals as an uncommon human pathogen associated with occasional salmonellosis. The occurrence of S. Kentucky is rare in Slovakia. The aim of present study was to investigate the antibiotic resistance, presence of class 1 integrons, as well as the selected virulence markers (bacterial adherence, motility and production of biofilm) of 95 sporadic human isolates of this serovar during 2005–2006.
Methods: Antimicrobial susceptibility tests were performed by disk-diffusion method using 10 different antibiotic disks according to the method of CLSI. The intergron content was investigated by PCR method using a 5′-CS/3′-CS primer pair. Bacterial adherence to xylene was determined according to the method of Rosenberg et al. (1980), and the motility was performed in semisolid agar (Braga et al., 1994). Biofilm-forming abilities of isolates were assessed by absorbance at 570nm in the tissue culture plates (Stepanovic et al., 2004).
Results: Among 95 investigated strains multidrug resistance (MDR) was found in 88 (92.6%) strains. The most frequent (47.4%) MDR phenotype was: ampicillin, ciprofloxacin, gentamicin, streptomycin, sulfisoxazole, tetracyclin. Three strains were also resistant to extended-spectrum cephalosporin – ceftriaxon. The presence of class 1 integron with 1500 amplicon was detected in 46 MDR strains. One MDR isolate contained integron with amplicon of 2000 bp. Bacterial adherence to xylene (hydrophobicity) of the isolates under study was weak. Only 16.8% isolates were evaluated as positive. The motility was positive in 47.4% strains. Based on biofilm-forming the strains were classified as non-adherent (12.6%), weakly adherent (63.2%), moderately adherent (22.1%) and strongly adherent (2.1%).
Conclusion: Previously, S. Kentucky was considered as an unsuccessful pathogen because it was rarely associated with human illness. The increased occurrence in human isolates in Slovakia highlights the pathogenicity and the dissemination of this serovar and it will be important to monitor further its occurrence as well as its features.
This work was supported by Science and Technology Assistance Agency under the contract No. APVV-21–052602.

R2416 Isolation of sulfate reducing bacteria from dental unit waterlines

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Objectives: Dental Unit Waterlines (DUWLs) include a high number of long pipes with small diameter. Direct contact with municipal water, stagnant water, average temperature in DUWLs support the formation of microbial biofilms. Thus dental units might be contaminated by high levels of microorganisms. Aerobic, opportunist microorganisms such as *Legionella pneumophila* and also anaerobic sulphate reducing bacteria (SRB) may be present in biofilm. SRB reduce sulfur compounds to hydrogen sulfide (H2S) that is toxic and acidic product. H2S can cause corrosion, which leads to industrial loss, on metallic surfaces such as galvanised steel, stainless steel. In addition, H2S causes ulcerative colitis in human and, SRB trigger liver abscess and septicaemia. There is not any worldwide standard that have been devote to number of anaerobic SRB in DUWLs.
Methods: The aim of the present study was to determine the SRB distributions within different DUWLs. Water samples were taken from air-water syringe, high-speed drill and water source in 41 DUWLs. Most probable number technique and Posgate B medium were used for isolation and count of SRB.
Results: SRB were not determined in 21 (51%) inlet water source samples. However, SRB were recovered from all outlets of the DUWLs. While the highest determinable number of SRB in air-water syringe was 103 cells/ml, the lowest detectable count was 5 cells/ml. Also maximum concentration value of SRB in high-speed drill was established as106 cells/ml, minimum viable counts were 5 cells/ml.
Conclusion: We think that the presence of SRB in DUWLs may be undesirable since they can cause corrosion on metalic apparatus used in dentistry and also health problems in human gastrointestinal system.

R2417 A survey on 80 cases of botulism and its clinical presentations as a public health emergency, Tehran, Iran

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Botulism is a toxin-induced paralytic illness characterised by cranial nerve palsies and descending flaccid paralysis. Botulinum toxin is regarded as the most lethal substance known. The diagnosis in sporadic cases and even in small outbreaks is frequently missed. The aim of this study was to assess clinical presentations of recorded botulism as a public health emergency from Loghman Hakim hospital.

A descriptive study (Routine data base study) was conducted in 80 admitted botulism cases between 1996–2006 in Loghman Hakim hospital. The diagnosis of botulism was based on epidemiological data and on a clinical score of severity. The patients were classified into three forms: mild, intermediate and severe. SPSS 12 soft ware was used for stastistical analysis.
The patients included 40 (50%) male and 40 (50%) female aged 1–66 years (mean 30.66±15.2year). The suspected causative food was cheese in 25 patients (31%), sea-food in 20 patients (25%). The mean incubation period was 1.06±1.8 days (range 4 h–10 days) and the mean from the first symptom to arriving into hospital was 3.77±4 days (2 h–17 days) and the mean from the first symptom to starting antitoxin therapy 3.95±4.27 days (2 h–17 days). The first symptom was nausea and vomiting in 17 cases. 11 (14%) patients were classified as suffering from severe disease, 5 (6%) from intermediate disease and 64(80%) from a mild form. Confirmation of the diagnosis was obtained in 47 patients (58.75%). The most common toxin was A [22 cases (27.5%)], then E [15 cases (20%)]. All the patients were treated with antitoxin. All patients recovered without sequelae. Six patients had to be admitted to intensive care unit (ICU) and required ventilatory assistance. Only one patient (1.3%) died.
The mainstays of therapy are meticulous intensive care (including mechanical ventilation, when necessary) and promptly treatment with antitoxin.

R2418 Laboratory diagnosis of *Toxoplasma gondii* infection in population in northern Greece

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Objectives: Toxoplasmosis is usually an asymptomatic infection, but acute illness acquired during pregnancy may lead to severe sequelae in the foetus. The diagnosis of toxoplasmosis is usually established through serological methods. The aim of the study was to present the results from the serodiagnostic approach of toxoplasmosis in patients of different age, along with the results of prenatal and during pregnancy control against toxoplasmosis in women.
Methods: A total of 697 subjects (247 males, 450 females) from 2 months to 81 years old (486 patients, 130 women before pregnancy, 81 pregnant women in the first trimester) were studied and 805 serum samples were analysed. All serum samples were tested for *Toxoplasma* specific IgM and IgG antibodies and in subjects positive for IgM antibodies IgG-avidity was determined using Vidas System. Also, IgA antibodies were determined in selected samples using an ELISA method.
Results: IgM and IgG antibodies were not found in 500 subjects out of the 697 (range 71.7%). The overall seroprevalence of toxoplasmosis was 19.5% (96 patients, 23 women before pregnancy, 17 pregnant women). Increased IgM and IgG antibodies and high IgG-avidity were found in 28 subjects, out of the above 136, suggestive of latent infection with persistent of IgM antibodies. Results suggestive of a recent infection (positive IgM and IgG antibodies, low IgG-avidity) were found in 52 subjects (45 patients, 2 women before pregnancy, 5 pregnant women), range 7.5%. Also, increased IgM and IgG antibodies and equivocal IgG-avidity were found in 9 subjects, so that they were not able to distinguish recent from past infection. From the total 697 subjects examined 273 were women of reproductive age (15–40 years old) and in 58 of these immunity against *Toxoplasma* was observed (range 21.2%). Also, significant increase of the IgA antibodies in patients with recent infection was found, but these were negative in subjects with latent infection
Conclusion: Diagnosis of toxoplasmosis based on clinical appearance and serological tests is not always easy and definitive. The interpretation of the serological results is some times very difficult and all the available serological tests in more than one serum samples must be performed. Specifically, a detailed serological examination and correct interpretation of the serological results must be combined for the estimation of the clinical situation in pregnant women.

R2419 Fluoroquinolone resistance in Enterobacteriaceae strains isolated from community-acquired urinary tract infections

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Objectives: Community-acquired urinary tract infections (UTI) are among the most common bacterial infections in Greece. After quinolones became the first choice for empirical therapy of UTIs, their subsequent overuse resulted to increasing quinolone resistance among uropathogens. Aim of this study is to investigate the frequency and antibiotic susceptibility of quinolone resistant bacterial stains isolated from patients with community-aquired bacteriuria and compare it with urinary pathogens from hospitalised patients.
Methods: During a 3-years period (2005–2007) a total of 1703 bactrial strains were isolated from urine samples submitted for culture in our hospital laboratory from the community (632) and from hospitalised patients (1071) with UTI. Cultures and bacterial identification were obtained by conventional methods. The sensitivity to antibiotics was tested by Kirby-Bauer disk diffusion method according CLSI criteria.
Results: Of the 1703 bacterial strains studied (*Escherichia coli* 1372, *Klebsiella pneumoniae* 197, *Proteus mirabilis* 134), 15.6% were found to be quinolone resistant. Quinolone resistance for all Enterobacteriaceae was 21.1% for hospitalised patients (HP) and 6.2% for community patients (CP). Quinolone resistance for *E. coli* was 17.4% for HP and 5.05% for CP, for *K. pneumoniae* 38.1% for HP and 18.9% for CP and for *P. mirabilis* 24.7% for HP, 9.7% for CP. Susceptibility percentage of the 1703 quinolone resistant isolates to other antimicrobial agents was for HP and CP respectively as following: For *E. coli* ampicillin (AM) 7.1–10.7%, amoxicillin-clavulanate (AMC) 49.3%-53.6%, piperacillin-tazobactam (TZP) 75.4%-85.7%, cefuroxime (CXM) 61.3%-82.1%, sulfamethoxazole-trimethoprim (SXT) 19%-17.9%, ceftazidime (CAZ) 71.1%-82.1%, cefepime (FEP) 73.2%-85.7%, gentamicin (GM) 76.6%-71.4%. For *K. pneumoniae* AM 0%-0%, AMC 16.4%-14.3%, TZP 19.7%-28.6%, CXM 14.8%-42.9%, SXT 1.6%-0%, CAZ 18%-42.9%, FEP 26.2%-42.9%, GM 65.6%-85.7%. For *P. mirabilis* AM 0%-0%, AMC 13%-0%, TZP 91.3%-100%, CXM 8.7%-0%, SXT 0%-0%, CAZ 13%-0%, FEP 95.7%-100%, GM 30.4%-25%.
Conclusions: High resistance rates to fluoroquinolones were observed in uropathogen bacteria isolated not only from hospitalised patients but also in bacteria associated with community-acquired urinary tract infections in Greece. Strict antibiotic policy concerning UTI in community patients, is needed.

R2420 Mechanisms of resistance to oxyimino-cephalosporins in clinical *Escherichia coli* isolates recovered from food-producing animals in France

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Objectives: To characterize the β-lactamase genes encoding resistance or diminished susceptibility to ceftiofur (a third-generation cephalosporin) and/or cefquinome (a large-spectrum cephalosporin) in 24 *Escherichia coli* isolates recovered in France in 2005–06 from sick animals (21 calves, two piglets, one pig).
Methods: Antimicrobial susceptibility to 15 b-lactams and 13 other antibiotics was determined with the disk diffusion method. A double-disk synergy test was carried for detection of ESBL. The presence of genes encoding TEM, SHV, OXA1/4, phylogenetic group 1, 2, 9 CTX-M and six families of plasmid-mediated AmpC β-lactamases as well as the existence of mutations in the chromosomal ampC gene were investigated by PCR and sequencing in the 24 *E. coli* isolates studied.
Results: The following ESBLs and plasmidic AmpC-type cephalosporinases were detected (number of isolates): CTX-M (13 including 12 CTX-M-1), TEM-52 (1), and CMY (2 including 1 CMY-2). Mutations in the ampC gene were identified in 17 isolates at positions –88, –82, –73, –42, –28, –18, –1, +20, +34, +58, +70, or +81. Mutations at positions –18 (GA) and –42 (CT), resulting in the formation of a strong promoter and

overexpression of AmpC, were observed in five isolates. Furthermore, OXA-1 was observed in four strains moderately susceptible or resistant to cefepime. PFGE performed with XbaI endonuclease showed that the 24 isolates tested belonged to 22 distinct genotypes; two pairs of isolates, containing CTX-M-1, showed indistinguishable PFGE patterns, indicating a potential clonal dissemination among animals from different farms.

Conclusion: Our data underscore the diversity of genes encoding resistance to oxyimino-cephalosporins in *E. coli* strains isolated from calves and pigs. The emergence of CTX-M-1-producing isolates in food-producing animals in France is of major concern to both public and animal health.

R2421 Measles and mumps epidemic outbreaks in two geographic areas of Catalonia, 2007

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Objectives: In Catalonia, a two-dose MMR vaccination programme for children, free of charge and on a voluntary basis, was launched in 1988. Catalonia is free of indigenous measles since 1999. In 2007 was set the goal of mumps elimination but just in 2007 occurred the most important measles and mumps outbreaks on the last ten years. This report summarizes the differences between measles and mumps cases reported in two geographical areas of Catalonia (Vallès Occidental and Vallès Oriental) with 1.207.464 inhabitants.

Methods: Mumps and measles suspected cases should be reported promptly to the Epidemiological Surveillance Unit. Confirmation of cases requires PCR testing of urine for measles or oral fluid for mumps, or specific IgM serum antibody, or to be epidemiologically linked to a laboratory confirmed case. Epidemiological data and vaccination status had been collected individually from all cases. Correct vaccination status was defined as two doses of MMR vaccine, the first dose after 12 months of age and the 2n dose at least one month later.

Results: Measles outbreak finished in June 2007 with 18 confirmed cases; all of them were not or incorrectly vaccinated; 10 were less than 4 years old; 6 were nosocomial transmitted, 3 on personal staff.

Until September 2007 were reported 83 mumps confirmed cases, predominantly teens; 73% were correctly vaccinated. Most mumps cases coincided with summer vacation. Delay in diagnosis and failure to report in a timely manner occurred in some cases. Despite control effort and highly vaccinated population secondary cases on families and schools were detected but not nosocomial transmission.

Conclusion: The most important differences on epidemiology of the measles and mumps cases were mean age and vaccination status. The majority of mumps cases were correctly vaccinated. Healthcare providers should suspect measles or mumps, dignose by using laboratory testing and report cases immeditly. Furthermore mumps should be suspected independent of vaccination history. When indigenous measles or mumps cases are eliminated the goal must be to interrupt the virus transmission from imported cases.

R2422 Comparision of the result of sinus track culture and bone culture in chronic osteomyelitis

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Purpose: The infection of bone that contains bone marrow called osteomyelitis, which was caused by different microorganisms. In this study, we aimed to determine the diagnostic value and accuracy of cultures of material from a sinus track compared with those of cultures of bone specimens have been controversial.

Material and Method: Prospective study conducted at Hospital University of Dicle, a 1090-bed university based hospital located in Diyarbakir, Turkey. Between May 2005 and September 2006 sinus track cultures were compared with those of bone cultures from 43 patients with chronic osteomyelitis.

Results: The patients' mean age was 30.6±3.6 year, and 29 (67%) were male and 14 (33%) were female. Their mean ESR, C reactive protein (CRP) and WBC were found 82.7 mm/hour ±4.4, 122.4 mg/dl±11, 11099.3/mm3 ±640 respectively. The high level of CRP and ESR were determined meaningful ($p \leqslant 0.005$), but total leucocyte number was not meaningful by statistically. Organisms isolated from bone cultures were staphylococcus 72.5% (29/40), *Escherichia coli* 10% (4/40), *Pseudomonas aeruginosa* 10% (4/40), *Proteus mirabilis* 7.5% (3/40) respectively. Cultures of sinus track and bone specimens gave identical results in 62.7% of patients, with slightly better concordance in chronic osteomyelitis caused by staphylococcus 78% than by all other bacterial species.

Conclusion: The value of bone culture in the therapy of osteomyelitis must be emphasised; it is the only reliable means of determining the responsible agent, and then basing antibiotic therapy on this. The correlation between sinus track and bone cultures was 37.3%. That means, the failure in the treatment at four patients in 10.

R2423 Risk factors and molecular typing of community-acquired urinary tract infections with ESBL (+) *Escherichia coli*

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Objectives: ESBL-producing *E. coli* is a well known aetiologic agent of nosocomial infections. The incidence of community-acquired ESBL-producing *E. coli* infections is increasing. The aim of this study is, to determine the risk factors for urinary tract infections (UTI) caused by ESBL-producing *E. coli* and define the molecular types of ESBLs.

Methods: Thirty-three patients who have community acquired UTI with ESBL-producing *E. coli*, between 1st May 2006 and 1st Jan 2007, included in the study. Control group was consisted of 70 patients who have UTI with non-ESBL producer *E. coli*. Data were collected by the help of a standardised survey form. Strains which were detected as ESBL producer by phenotypic tests, controlled by PCR.

Results: In univariate analysis, female gender ($p = 0.01$), previous hospitalisation ($p = 0.01$), urinary system abnormalities ($p < 0.01$), antibiotic consumption in the previous three months ($p < 0.01$), ciprofloxacin use ($p = 0.04$), aminopenicillin use ($p = 0.01$), having diabetes mellitus ($p = 0.03$) were found to be significant as risk factors. Any kind of antibiotic consumption in the previous three months (OR, 11.42; CI 95%, 1.97–66.20; $p = 0.007$) and female gender (OR, 0.81; CI 95%, 0.007–0.82; $p = 0.034$) were found to be significant in logistic regression analysis. ESBL enzymes detected were 85% CTX-M, and 61% TEM. No SHV enzyme was detected.

Conclusion: Community acquired infections with ESBL-producing *E. coli* worth of concern. Judicious use of antibiotics is essential in the control of infections caused by resistant microorganisms. It may be possible to stop the spread of such resistant organisms by appropriate antibiotic consumption policies and infection control measures, both in the community and hospital.

Emerging infectious diseases

R2424 A case of Steven-Johnson syndrome caused by combined use of lamotrigine and fluoksetin

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Introduction: Steven Johnson Syndrome (SJS) is a rare, life threatening acute allergic presentation with target lesions and blebs of epidermis. Aetiology frequently comprises the use of sulfonamides, nonsteroid antiinflamatory drugs, antimalarial agents and anticonvulsant medication. However, combine duse of these drugs have been caused an increase in the blood levels of each other by competing with glucuronidation metabolism and leads to severe skin reactions. This report presents a SJS case caused by combined use of lamotrigine and fluoksetin.

Case: A 41-year-old man applied to emergency clinic with skin eruption, especially on the face and trunk, and lesions around the mouth. The history of him revealed lamotrigine and fluoksetin use for the last three weeks for major depression. On his physical examination the

temperature was 38.5ºC, pulse rate 100/min, respiratory rate 18/min and blood pressure was 130/80 mmHg. Dermatological examination revealed severe bilateral conjunctivitis, erosion of the lips with hemarrogic crusts, pale erythematous papules, bullae and erosion on the ears, gingivitis, and severe erosions of the pharynx. Laboratory investigations revealed total leucocyte count of 8000/mm3 (polymorphs 80%), C reactive protein was elevated up to 143 mg/dl, ESR was 78 mm/h. Liver function tests such as AST: 230 U/L and ALT: 224 U/L were elevated. There was no bacterial growth in throat, urine, faecal and blood cultures. First of all the drugs which were used, was stopped. Additional treatment involved fluid replacement, prednisolone (1 mg/kg/day), antibiotic eye drop and oinment, mupirosin oinment for skin, bicarbonate and clorhexidine mouth wash for oral mucosal lesions, wet dressing for epidermal surfaces. When his biochemical values and general condition improved, and there were no new lesions, the steroid dosage was gradually reduced and completely stopped. He was discharged following complete recovery on the 15th day of treatment.

Conclusion: SJS, a rare mucocutaneous reaction, has an incidence of one in two or three million per year. Despite many aetiological factors, usually certain drugs rank the first. The risk arises most frequently within the first 2–8 weeks of antiepileptic treatment. In conclusion, clinicians should bear in mind the possibility that the drugs increase the blood levels of each other, especially those which compete with glucuronidation metabolism, may lead to severe skin reactions.

R2425 Laboratory diagnosis of Crimean-Congo haemorrhagic fever in Iran in the year 2007

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Objectives: Crimean-Congo Haemorrhagic Fever (CCHF) is a viral zoonotic tick-born disease with a mortality rate up to 50% in humans. The CCHF Virus (CCHFV) is from genus Nairovirus and family Bunyaviridae. CCHFV is transmitted to humans by the bite of infected tick and by direct contact with blood or tissues from infected humans and livestock.

Methods: In the year 2007, sera were collected from Iranian CCHF probable cases and sent to the National Reference Lab, Pasteur Institute of Iran. These sera were analysed with Immunological (specific ELISA) and molecular (RT-PCR) assays.

Results: Among 128 probable human cases, 53 were confirmed cases. Between confirmed ones, 42 were IgM positive (37 among them were also RT-PCR positive) and 11 cases were only RT-PCR positive. Just one case led to death during the year 2007(up to 14 November), comparing with the year 2001 in which 11 death between 66 confirmed cases were observed.

Conclusion: CCHF is in the most important viral haemorrhagic fevers in Iran. In this year, 16 out of 30 provinces of Iran have been affected by CCHF. Sistan-Balouchestan in the southeast of Iran, because of being near the border of endemic countries (such as Pakistan, Afghanistan), has the highest rate of CCHF. In this study, it was demonstrated that CCHF was seen much more in the age range of 21–40 and is more common in professions related to livestock such as butchers, slaughterers, farmers, etc. Therefore, it seems, informing the groups of high risk professions has been efficient. Fortunately, with a precise surveillance and laboratory detection, the mortality rate in the year 2007(till 14 November) is decreased up to 2% in contrast to 17% in the year 2001.

R2426 Bacteraemia after transrectal ultrasound guided prostate biopsy in the era of multidrug resistance: impact of a new preventive protocol

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Objectives: Although the incidence of bacteraemia after transrectal ultrasound guided prostate biopsy (TRUSGPB) is low under antibiotic prophylaxis (0.5%), emergence of resistant microorganisms is becoming worrisome. We had to implement a new preventive protocol because a high incidence of bacteraemia was observed under the old preventive protocol (OLDPP). We assessed the incidence of bacteraemia and characteristics of isolated microorganisms, before and after the setting up of a new preventive protocol (NEWPP) in patients undergoing TRUSGPB.

Methods: this study was performed at an University hospital in Barcelona, Spain, in which a mean of 200 TRUSGPB are performed annually. Description of preventive protocols, analysis of the incidence of bacteraemia before and after setting up a NEWPP, and description of isolated microorganisms and antibiotic resistance patterns were done. During the 2nd period patients were prospectively followed after the procedure.

Results: OLDPP (Jan 2006-Feb 2007): amoxicillin/clavulanate 500 mg tid the day before, the day of the procedure and one day after; NEWPP (Mar 2007-Nov 2007): cefoxitin 2 g one hour before the procedure and ciprofloxacin 750 mg po bid the day before, the day of the procedure and 3 days after the procedure; a dipstick urinalysis (i.e., leukocyte esterase test and nitrite test) was performed before the procedure, and patients with positive results were not biopsed. Incidence of bacteraemia after TRUSGPB with OLDPP vs NEWPP: 9/204 (4.4%) vs 0/105 (0%), ($p = 0.022$); Isolated microorganisms in blood cultures with OLDPP: *Escherichia coli* 6 (66.6%), *Klebsiella pneumoniae* 2 (22.2%) and *Morganella morganii* 1 (11.1%). *Enterobacter cloacae* was also isolated in one case; five (55%) of the isolates were quinolone resistant and 4 (44.4%) produced extended spectrum betalactamases and were also resistant (CMI > 16/8) or intermediate to amoxicillin-clavulanate (CMI = 16/8). With the NEWPP 2 (1.9%) cases of low-grade fever without bacteraemia were recorded. 29 (27%) cases were not biopsed because of positive dipstick urinalysis.

Conclusions: Before TRUSGBP, excluding patients with positive dipstick urinalysis is an advisable practice. Antibiotic prophylaxis should take into account local resistance patterns. Cefoxitin could be used as prophylaxis in centres with high prevalence of extended-spectrum betalactamase enterobacteriaceae

R2427 Emergence of Enterobacteriaceae producing VIM-1 metallo-β-lactamases in a tertiary hospital in Spain: clinical and epidemiological characteristics

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Objectives: The emergence of carbapenemases in Enterobacteriaceae is becoming a matter of concern. We report the clinical and epidemiological data of patients infected or colonised with Enterobacteriaceae producing VIM-1 metallo-β-lactamases (MBL).

Methods: Retrospective descriptive study of patients infected or/and colonised with MBL producing Enterobacteriaceae in a 450-bed Hospital from December 2006 to May 2007. Clinical and epidemiological data were collected by means of charts and hospital data base review. Identification and susceptibility of isolates were done by MicroScan system (Urine ComboS1 and NC36 panels). To confirm carbapenem resistance, imipenem and ertapenem disc diffusion, imipenem and imipenem+EDTA E-test and the cloverleaf test were performed.

Results: During the study period 12 MBL-producing Enterobacteriaceae were isolated. One of them was isolated from a flexible endoscope in a rutine control. Data from one patient could not be obtained. Finally, 10 patients were studied. Six had *E. cloacae*, 3 *K. oxytoca* and 1 *K. pneumoniae*. Six (60%) were women. Mean (SD) age was 73 (14) years. Seven (70%) patients had chronic underlying conditions. Three (30%) patients had bacteraemia, 3 (30%) skin and soft tissue infection, 2 (20%) IV catheter colonisation, 1 (10%) respiratory infection and 1 (10%) peritonitis. All episodes were nosocomial acquired with a mean (SD) previous hospital stay of 27 (17) days (range 2–55). During the previous month, 8 (80%) patients had indwelling urinary catheter, 6 (60%) IV catheter, 5 (50%) had previous surgery, and 4 (40%) mechanical ventilation. Seven (70%) patients received antibiotics during the previous 2 months. Four of them received >3 Gram-negative active

drugs and 3 patients were treated with carbapenems. Empirical treatment was inappropriate in 5 of 5 patients. However, none of them dead under empirical treatment. Antibiogram guided treatment was amikacin in 3 cases (one monotherapy, one combined with cotrimoxazole and one with colistin) and tygeciclin monotherapy in 2 cases, with good clinical outcome in all of them. Crude mortality rate was 60% and related mortality rate 1 (10%). Mean (SD) stay was 47 (28) days (range 17–102).

Conclusions: Emergence of VIM-1 MBL-producing Enterobacteriaceae in Spain is reported. Isolates are nosocomial acquired. Most of the patients have chronic conditions, receive multiple antimicrobials previously, and undergo invasive procedures during prolonged hospital stay.

R2428 Epidemiological, clinical and laboratory findings in human Leptospirosis

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Objectives: Leptospirosis is a worldwide zoonosis of protean manifestations. Frequently it is underdiagnosed because of the nonspecific symptoms of the disease. The aim of our study is to describe epidemiological, clinical and laboratory features of patients with leptospirosis.

Methods: We retrospectively reviewed medical records of 63 patients with leptospirosis admitted to our hospital during 2003–2006. The diagnosis was based on the clinical manifestations of the disease and was subsequently confirmed serologically by ELISA method.

Results: We observed an increase of the number of cases from six cases in 2003 to thirty-four cases in 2006. During this period most of the cases were diagnosed in the second part of each year and the peak incidence occurred in the months August and November. The majority of the cases were from rural areas (68.25%) and patients were infected during occupational activities. A male gender distribution was found and the median age was 47 within the range 22–72 years.

The most frequent signs and symptoms were fever in 80.95% of the cases, nausea and vomiting in 71.42% of the cases, headache and myalgia in 63.45%, respectively 52.38% of the cases. The icteric form (total BIL >3 mg/dl) and the conjunctival suffusion were observed in 36.50%, respectively 26.98% of the cases.

Laboratory findings showed transient thrombocytopenia ($<100\times10^3$) in 32 cases (50.79%), creatinine >2 mg/dl in 19 cases (30.15%) and elevated liver enzymes in 28 cases (44.44%). There had been nine severe forms with the decrease of the prothrombin index. (IP) under 60%, two cases have deceased. The pulmonary involvement was confirmed by chest X-ray examinations in 29 cases (46.03%)

Conclusions: The frequency of the leptospirosis was 5.33 times greater in 2006 than in 2003. The possible explanation can be the greater rainfall and/or the underdiagnosis of the diseases. The diseases affected mainly men from rural areas. Clinical data showed the prevalence of the anicteric form of the disease. The most frequent involvements were hepatic, pulmonary and renal. Having knowledge of the epidemiological, clinical and laboratory characteristics of the leptospirosis is important for the diagnosis and right treatment.

R2429 Optimal diagnostic approach in a Q fever outbreak situation

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Background: Over the last years an increase of outbreak activity of Q fever in Germany has been recognised. The diagnosis of acute Q fever largely depends on serology as culture of the organism is an extremely hazardous procedure. However, the delayed development of specific antibodies often leads to late identification of Q fever cases or misinterpretation of disease.

Objectives: To tackle this challenge we compared different serological tests in patients with acute Q fever and evaluated the polymerase chain reaction (PCR) as an additional tool in symptomatic seronegative patients.

Methods: During the large Q fever outbreak in Jena in 2005 with 321 reported cases we tested several consecutive serum samples from 41 hospitalised patients with signs of acute Coxiella burnetii infection. 22 patients met the criteria of an acute Q fever (febrile illness, elevated C-reactive protein (CRP), chest X-ray changes, residency in the outbreak area, initial positive serology or at least seroconversion during follow up) and were included in the study. The initial serum of all 22 patients was than tested with a commercially available complement-fixation-test (CFT), an enzyme-linked immunosorbent assay (ELISA) and in initially seronegative cases with PCR (modified by Fenollar et al. 2003).

Results: The sera were collected between the first and seventh day of illness. In two cases (9.1%) the CFT of the initial serum sample revealed a positive result. The ELISA, in contrast, had a sensitivity of 59.1% (13 cases). The introduction of PCR as an additional test in seronegative cases increased sensitivity by using ELISA in combination with PCR up to 77.3% (17 cases).

Conclusion: In outbreak situations the combination of an ELISA with additional PCR in seronegative patients provides a useful diagnostic tool for early recognition of Q fever cases. The still popular CFT should be replaced by an ELISA system.

R2430 Management of bacteraemia due to carbapenem resistant non-carbapenemase-producing *Klebsiella pneumoniae*

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Background: Carbapenem resistance in *K. pneumoniae* is still uncommon, but may emerge due to several mechanisms. Here we report on two cases of bacteraemia caused by multidrug-resistant (MDR) *K. pneumoniae* producing an Extended-Spectrum Beta-Lactamase (ESBL) and showing resistance or reduced susceptibility to carbapenems.

Methods: MICs and MBCs of carbapenems were determined as recommended by the CLSI. Susceptibility to other agents was determined by disk diffusion. Serum bacteriostatic (SBA) and bactericidal (SBC) activity against the *K. pneumoniae* isolated from the same patient were determined according to CLSI. Carbapenemase production was assessed by spectrophotometric assay. Genes encoding ESBLs and outer membrane K35, K36 and K35 proteins were investigated by PCR and sequencing.

Results: Two patients admitted to the same surgical ward developed a post-surgical bacteremic infection due to an MDR *K. pneumoniae* which was susceptible to imipenem (MIC, 2 mg/L – MBC, 8 mg/L), but intermediate to meropenem (MIC, 8 mg/L – MBC, 16 mg/L) and resistant to ertapenem (MIC, 32 mg/L). Concerning other agents, the isolates were only susceptible to amikacin, tigecycline and polymyxin B. Carbapenemase production was not detectable, while both isolates produced an ESBL (CTX-M-15) plus two broad-spectrum β-lactamases (TEM-1 and SHV-1) and were deficient in OmpK36 due to insertional inactivation of the gene. Before isolation of the MDR klebsiellae, one patient received empiric therapy with ceftriaxone (5 days) and, susbsequently, piperacillin/tazobactam (4 days), while the other patient received meropenem (5 days). When the results of blood cultures were available, one patient was treated with imipenem 1g tid plus amikacin 1g od, and the other with imipenem 1g tid plus tigecycline 50 mg bid. Both regimens were able to resolve fever and bacteraemia, in 36 and 24 hours respectively. At the steady state, peak and through SBA were 1:2 or higher in either case.

Conclusion: Infections due to noncarbapenemase-producing MDR *K. pneumoniae* producing multiple β-lactamases associated with porin loss retaining susceptibility to imipenem, were successfully treated with imipenem in combination with either amikacin or tigecycline.

R2431 A retrospective study of the isolates of *Nocardia* spp. from 1997 to 2006

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Nocardia infections, although low incidence, have experienced an increase in recent years. To find the current state of *Nocardia* spp. infection in our environment, we have analysed retrospectively the isolates of these microorganisms in the last ten years (1997–2006) at the Hospital del Mar and the Hospital de la Esperanza of Barcelona.
Methods: Clinical samples cultivation was carried out by conventional methods using a-BCYE addition when filamentous Gram-positive bacilli were seen in the microscopic examination.
Nocardia spp. isolates were identified by Gram characteristic, colony morphology, growth at 45ºC, urease and nitrate tests and antibiotic profile susceptibility. The molecular identification was carried out at the National Microbiology Reference Center (Instituto Carlos III) by restriction enzyme analysis after 16S RNA PCR. The susceptibility study was performed by disk diffusion in Mueller agar supplemented with 5% of blood, adjusting the inoculum to 0.5–1 of the McFarland scale. It also conducted E-test in cases where the disk results were not clear.
Results: In the period under review 41 isolates of *Nocardia* were isolated, 8 came from soft tissue and 33 from respiratory samples. The patients, 34 men and 8 women, were aged between 33 and 90 years (76.2% older than 60 years). The molecular identification was carried out in 16: 2 *N. brasiliensis*, 1 *N. paucivorans*, 1 *N. farcinica*, 1 *N. carnea*, 5 *N. abscessus* and 6 *N. cyriacigeorgica*. Of the 25 isolates that are available only phenotypic identification, 9 would correspond to the type VI, 1 and 2 type II to type V and the remaining 13 were not reached a definitive identification. Trimetoprim-sulfametoxazol (T/S) was active on 38 of 39 isolates tested.
Conclusions: The number of *Nocardia* spp. isolates has experienced a slight increase over the past years. The patient characteristics (immunosuppression, bronchial pneumonopathy chronic treatment with corticosteroids) correspond to the data of most of the series published. Trimetoprim-sulfametoxazol retain its antibacterial activity, 97.4% susceptibility in our series.

R2432 *Enterobacter cloacae* in bone and joint infections: analysis of 22 cases from a university referral centre in Greece

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Objectives: *Enterobacter cloacae* is a rare pathogen in bone and joint (BJ) infections. Current study assessed epidemiological and clinical features of 22 patients followed at a referral BJ infection centre in Athens, Greece
Patients and Methods: All cases of bone and joint infections that were clinically, radiologically, laboratory and microbiologically assessed, were retrospectively analysed via an electronic data-base registry. From a total of 574 records, 22 (4%) were microbiologically documented (cultures from pus, intra-operative tissues, bone and foreign material samples) as BJ infections caused by *E. cloacae*. Treatment success was the clinical, radiological and microbiological resolution of the BJ infection while failure referred to all cases that did not fit this definition. Demographics, surgical operations, antimicrobial treatment, outcome and infection relapses were evaluated.
Results: *E. cloacae* was isolated from intra-operative bone tissues (n = 5, 23%), pus (n = 15, 68%), synovial fluid (n = 1, 5%) and blood (n = 1, 5%). Mean age of patients was 51.5 years (range 17–76). Men predominated (14/22, 63.6%), as well as comorbidities (i.e diabetes mellitus, cardiovascular disease and rheumatoid arthritis) (13/22, 59%). Almost half of the cases (45.5%) had a history of car accident related to the infection. The time from the infection onset to treatment ranged from 0–24 (mean 5) months. BJ infection was located to joints (n = 4), long bones (n = 17) and lumbar spine (n = 1). Only 3/22 (13.6%) had a medical device (osteosynthesis) while 13/22 (59%) underwent surgical debridement. Ciprofloxacin was administered in 77% of cases at a high dose (1500 to 2000 mg/day) for a mean of 7.3 months (2–16). Treatment success was assessed in 12/22 (54.5%) of cases. There were 2 relapses of the infection during a mean follow-up of 11.2 months (SD + 10.9).
Conclusions: Although rare, *E. cloacae* is a difficult to treat pathogen in BJ infections, possibly because these infections are often post-traumatic and affect patients with comorbidities. Moreover, despite long and adequate treatment as well as surgical debridement, patients with *E. cloacae* BJ infections presented with an important rate of failures and relapses of the infection. Good collaboration of orthopaedists and ID specialists on the field is mandatory in order to improve therapeutic approach in these patients.

R2433 Neurolisteriosis. Review of 22 cases

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Objectives: To analyse the epidemiologic and clinical features of central nervous system (CNS) infection by *Listeria monocytogenes* (LM) in a general hospital in the last 11 years and review of the literature.
Methods: We reviewed the medical and laboratory records of all patients diagnosed of CNS infection by LM between January 1996 and September 2007. Inclusion criteria were: adults, with blood or cerebro-spinal fluid (CSF) positive cultures for LM and symptoms of SNC infection.
Results: 22 patients were recruited, men 52%, mean age 64 years (range 28–90). The number of cases has been increasing during these years. 10 patients (45.5%) were not immunocompromised. The most frequent clinical form was meningoencephalitis with 14 patients (64%). The clinical picture was different depending on clinical presentation. Blood cultures were done in all patients and were positive in 86% of them. CSF culture was done in 19 patients (86%), 53% of them were positive for LM. In CSF, lymphocytes dominated in 58% of patients, 74% had a protein level over 0.45 g/L and only 21% had glucose levels below 40 mg/dL, but with a CSF/plasma ratio below 0.5 in 15 patients (79%). Gram stain was done in 17 cases and was negative in all of them. A computed tomography brain scan was performed in 91% of patients, being altered in 30% of them. Magnetic resonance imaging scan of the brain was obtained in 59%, showing abnormal images in 63% of cases. Mean time between admission and diagnosis was 2.3 days (range 0–6). Mean time between admission and treatment was 1.1 days (range 0–4). A correct double antilisterial treatment was prescribed in 11 cases (50%). Mean duration of treatment was 27.6 days (range 1–70). Any of the patients with brain abscess needed surgical treatment. General mortality was 36% (6 patients), 4 of them (18.2%) directly related to LM infection. Surviving patients presented neurological sequelae in 4 cases (18.2%). Mean survival was 10.8 days (range 1–38).
Conclusion: LM must be considered in CNS infection especially in the elderly and immunosupressed patients. Its incidence in not immunocompromised patients is increasing. Early diagnosis and empirical treatment including an antilisterial drug are very important for prognosis. Domination of lymphocytes in CSF differential cell count and a CSF/plasma glucose ratio below 0.5 can suggest LM and it can be advisable to include an antilisterial drug empirically. CSF Gram stain is usefulness in many cases.

R2434 *Listeria monocytogenes* bacteraemia. Review of 19 cases

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Objectives: To analyse the epidemiologic and clinical features of bacteraemia caused by *Listeria monocytogenes* (LM) in a general hospital and review of the literature
Methods: We reviewed the medical and laboratory records of all patients diagnosed of LM bacteraemia between January 1996 and September 2007. Inclusion criteria were: adults (older than 18 years old), with blood positive cultures for LM without symptoms or signs of infection in other regions.

Results: 19 patients were recruited, 58% were men. Mean age 61 years (range 27–94). 12 (63%) were older than 60 years of age. The incidence has increased from 1 case in 1996 to a maximum of 5 cases in 2003. Only 4 patients (21%) were not immunocompromised, but all of them were older than 60 years. The most frequent immunocompromising conditions were: pregnancy 3 cases, non-haematological malignancies (4), haematological malignancies (3), immunosuppressive treatment (2), and steroids treatment (4). The clinical picture was fever in 10 cases (53%) and chills in 5 patients (27%). 75% of patients presented symptoms for less than 2 days before hospital admission (mean 2 days, range 0–7). Mean time between admission and diagnosis was 1.7 days (range 1–3). The 3 cases associated to pregnancy manifested in the third trimester with fever and abdominal pain. All of them lead to premature birth, but only one newborn was infected.
Mean time between admission and treatment was 1.8 days (range 1–3). The majority of patients, 15 cases (79%) received an antilisterial treatment (Ampicillin 13 patients, in 4 cases associated with gentamicin and one case associated with cotrimoxazole, amoxicillin-clavulanic acid 2 patients). Ceftriaxone was the first treatment in one patient. 3 patients died before receiving any treatment. Mean duration of treatment was 18 days (range 1–31). Mortality rate was 42.1% (8 cases), 3 patients directly attributed to LM infection. Mortality rate between patients receiving an antilisterial treatment was 27%. Mean survival was 14 days (range 3–30).
Conclusion: LM bacteraemia incidence is increasing, probably caused by the increase in the number of patients with immunosuppressant conditions and the increase in life expectancy. In the elderly or immunocompromised patients with symptoms suggesting bacteraemia without symptoms or signs of infection in other regions LM infection must be suspected and an empirical treatment with an antilisterial drug must be administered as soon as possible.

R2435 Listeriosis infection in Kazakhstan

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In Republic Kazakhstan it is widely distributed Listeriosis registered among people, agricultural animals, rodents and also birds. *Listeria* strains were isolated in various products, mainly an animal origin, including in the food stuffs ready to the use.
Materials and Methods: The material from patients for the period 1993–2003 is investigated. Properties 188 *Listeria* strains are investigated. Sensitivity to 21 antibacterial preparation *L. monocytogenes* strains of various origins is determined.
Results: For the period of 1993–2003 207 patients with laboratory confirmed Listeriosis diagnosis are revealed. Patients had various clinical picture and accordingly various diagnoses. In 7% of cases at patients contact to pets has come to light, connection of disease with foodstuff in 27% is marked: 2 families Listeriosis are fixed, in everyone were ill simultaneously on 3 members of families. Two patients had professional character of disease (women from poultry farm and men-farmer). Four cases of bacteriological confirmed Listeriosis of newborn are marked also.
The age structure of Listeriosis patients was from 1 month till 65 years, middle age has made 19 years. 30% from number of all patients have fallen to a share of children in the age of from 1 month till 14 years; 61% – on age from 19 till 40 years, 10% – on age from 55 till 65 years. At research of material from people the percent of isolation *Listeria* from tests of pathological material was the following: from pharynx – 45%, from blood – 31%, from contents of initial skin affect – 14%, from urine – 10%.
Irrespective of source of isolation all cultures are submitted as *L. monocytogenes* I serovar. Cultures had typical cultural-morphological and biochemical properties, only some distinctions in ability or speeds of fermentation of separate sugars and spirits were marked.
Resistance to cephalosporins, sulfonamide, polymyxin, and lincomycin and nitrofurans preparation was (common for all investigated *Listeria* strains of various origins. The most active in relation to *Listeria* strains were amoxicillin, ampicillin, gentamycin, streptomycin, tetracycline and erythromycin.
Conclusions: Listeriosis infection has a wide distribution in Republic of Kazakhstan among people of various ages. Listeriosis strains basically have typical properties are submitted *L. monocytogenes* I serovars.

Infection control

R2436 Does the use of probiotics/synbiotics in patients undergoing abdominal surgery prevent postoperative infections? A systematic review of randomised controlled trials

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Objective: Advanced surgical techniques and improved perioperative care have considerably lowered postoperative morbidity. However, infection following abdominal operation remains a considerable morbidity factor. Probiotics are food supplements containing, beneficial to the host, live bacteria. These bacteria inhibit the growth of pathogens and support microbial balance of the intestine towards a healthier flora. Prebiotics are indigestible sugars that stimulate the growth or activity of certain bacteria of the gastrointestinal flora, to the benefit of the host. We sought to evaluate the current literature and identify the potential benefit, if any, of perioperative administration of probiotics/synbiotics to patients undergoing abdominal surgery.
Methods: We searched PubMed, Scopus, Web of Science and Cochrane library to identify randomised controlled trials (RCTs) that studied the perioperative administration of probiotics/synbiotics in patients undergoing abdominal surgery.
Results: Eight RCTs studying 753 patients were included in our study. The incidence of postoperative infectious complications (intra abdominal abscess, wound infection, bacteraemia, cholangitis, liver abscess) were less frequent in patients receiving probiotics compared to the control group. In addition, in 1 study delayed gastric emptying was less frequent in the group receiving probiotics compared to the control group. Two studies report that septic morbidity between the group of patients receiving synbiotics and the control group did not differ significantly. Two studies report shorter length of hospital stay in the group of patients receiving symbiotics; however, 5 other studies do not report any inter-group difference regarding the length of hospitalisation.
Conclusion: The use of probiotics and/or synbiotics may have beneficial effect in the reduction of postoperative infections after abdominal surgery. However, available data do not permit the derivation of safe conclusions. RCTs focusing in specific abdominal surgery, using the same synbiotic supplement and the same therapeutic route are warranted to further evaluate this promising preventive option that may decrease morbidity, length of antibiotic therapy, hospital stay, and pressure for emergence of antimicrobial resistance.

R2437 Investigation of an outbreak of *Stenotrophomonas maltophilia* bacteraemia in an haemato-oncology unit: unexpected genetic diversity of isolates

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Introduction: *Stenotrophomonas maltophilia* (Sm) is ubiquitous in aqueous environment. Several nosocomial outbreaks have been reported, of which only 2 in haemato-oncology (HO) units. Between 1998 and 2006, 36 Sm bacteraemia occurred in our hospital (median 3/year, range 0–7) of which 14 in the HO unit (median 1.5/year, range 0–3). During a 5-week period (12.06–01.07), 3 new cases were diagnosed in the HO unit. An investigation was conducted to search a possible cross-transmission and a potential source.
Methods: Clinical and epidemiological data of the 3 patients with Sm bacteraemia were recorded. Environmental sampling of their rooms and of a fourth room of the same unit was done twice one month apart (02.07–03.07). One liter of water and swabs were taken from showers and faucets in each room (total 16 samples). For the 2nd sampling, swabs

of sink siphons were also analysed. Isolates were identified to the species level by VITEK® 2 (bioMérieux, France) GN card. All Sm isolates were typed by pulsed-field gel electrophoresis (PFGE) using XbaI enzyme.
Results: The 3 patients were profoundly neutropenic at the time of Sm bacteraemia. All had received myeloablative chemotherapy. The 3 Sm isolates had 3 distinct PFGE patterns. One patient presented a second Sm bacteraemia 3 months later with a strain genetically identical to the first episode, suggesting a relapse. The first environmental investigation revealed that 3/16 samples yielded Sm. These 3 samples originated from 2 rooms, of which only one had been occupied by a patient with Sm bacteraemia. The second investigation revealed that the 4 siphons were contaminated with Sm. PFGE genotyping showed that each environmental isolate was unique. Moreover, no environmental isolate shared the same genotype with one of the clinical isolates. Of interest, Sm isolates recovered in 02.07 and 03.07 were different. No intervention was done, and so far (11.07) no new case occurred in the HO unit.
Conclusion: Molecular typing proved that there was no cross-transmission of Sm among patients. Environmental isolates were all different from clinical isolates, suggesting that the source of infection was not the water supply. However, as the isolates recovered during the first and the second investigations were genetically different, one can postulate that the environmental Sm flora is changing rapidly.

R2438 Infections after cardiac surgery

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Objective: the aim of this study is to know the rates of NI (Nosocomial Infection) in patients operated on open heart surgery: valve replacement and by-pass grafting.
Patients and Methods: The 1871 patients operated on open-heart surgery in the hospital of Basurto between January 2000 & September 2007 were included. Preoperative protocol regarding infection control includes: shower with 4% chlorhexidine soap the night before surgery and repeated the day of surgery. Shave just before surgery. Antibiotic prophylaxis: Cefuroxime 1.5 gr. IV beginning 5 minutes before anaesthesia induction. A second intraoperative dose is administered if the operation lasts for longer than 6 hours, large estimated blood loss or fluid administration, since January 2006. The infection control team makes infection surveillance. All the patients undergoing open-heart surgery are prospectively studied since the day they are operated until the end of the episode. A computer based surveillance system INOZ designed for incidence studies of NI is used during admission and continued 1 year after discharge. CDC definitions of nosocomial infection are used. All the nosocomial infections not only surgical site infections (SSI) are recorded.
Results: Age and sex: 1270 (67.87%) men, mean age 66.4 years. Mean preoperative stay in the hospital 5.2 days (SD: 8.5). NNIS (National Nosocomial Infection Surveillance) score 0: 126 patients, score 1: 1200 patients, score 2: 527 patients and score 3: 18 patients. Nosocomial infections: 374 patients acquired 535 NI, 91 of them were Surgical Site Infections (SSI): 26 incisional superficial, 28 deep incisional and 37 organ space. NNISS score 0: 1.6%, score 1: 3.7%, score 2: 6.6% and score 3: 38.9%. Microorganisms: *S. aureus* 11, Coagulase-negative Staphylococci (CNS) 32. *P. aeruginosa* 10, *Enterococcus* spp. 5. Urinary tract infections (UTI): 136. *E. coli* (45), *E. faecalis* (26), *P. aeruginosa* 18 and *C. albicans* (16) were the most frequent aetiological agents. Respiratory infections: 178 patients. Pneumonia: 37 cases. Bacteraemia: 46 cases: 8 catheter related (*S. aureus* 3), 21 secondary (*P. aeruginosa* 5, *E. coli* 4) and 17 primary bacteraemias (*Enterococcus* spp. 5).
Conclusions: Cumulated incidence of SSI dropped from 5.86% to 2.70% after the introduction of the second intraoperative dose of antibiotic prophylaxis. Coagulase-negative Staphylococci are the most common bacteria isolated from Surgical Site Infections following cardiac surgery during the study period.

R2439 Impact of a new perioperative antibiotic prophylaxis policy on the antimicrobial resistance: experience in a comprehensive cancer centre

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Objectives: The use of inappropriate or broad-spectrum antibiotics in perioperative prophylaxis is a significant cause of antimicrobial resistance in hospital.
To reduce this problem, the adherence to shared guidelines represents a key step. Aim of our study was to evaluate the effectiveness of the compliance with newly introduced guidelines in decreasing the antimicrobial resistance of hospital bacteria.
Methods: At the European Institute of Oncology, until 2002 each division followed an internal perioperative antibiotic prophylaxis schedule. In November 2002 new guidelines were published, establishing, for each surgical procedure, the appropriate choice of antibiotic, the way, timing and duration of administration, and the alternative drugs in case of allergic patients. Meetings with the clinicians, to discuss and share the new indications, training courses for all the medical and nursing staff were organised, and periodical checks to verify the compliance with these indications were performed. To evaluate the effect of this new policy, we compared, in surgical patients, the antimicrobial resistances of the isolated bacteria, both pathogen and colonising, before and after the introduction of the guidelines.
Results: With regard to compliance with the new guidelines, the ratio of totally correct prophylaxis increased from 39.7% to 54.8% (ratio of correct active principle from 52.9% to 96.7%).
The antimicrobial sensitivity of isolated bacteria increased overall from 72% to 76% (Wilcoxon, $p = 0.0019$); in particular, a significant increase in the susceptibility to tested antibiotics occurred for *Staphylococcus aureus*, Coagulase Negative staphylococci (CoNS) and *Pseudomonas* species; on the contrary, for Enterobacteria there was an increase of the resistance, and for the other Gram-negative and the Enterococci no significant variations were found (Tab. 1).

Table 1. Percentage of antibiotic susceptibility in 2002 and 2005 and significance of the variation

	% Susceptibility						
	Entero-bacteria	Other Gram(–)	*S. aureus*	CoNS	Enterococci	*Pseudomonas* species	Total
2002	80	63	81	34.5	68.5	68	72
2005	78	66	85	37	65	74	76
p	0.0001	0.4900	<0.0001	0.0005	0.8501	0.0001	0.0019

Conclusions: The introduction of guidelines for perioperative prophylaxis and the compliance with their indications were fundamental to regulate the appropriate use of antibiotics, but it is also necessary that these guidelines are shared with all the staff. It is also essential to train all healthcare staff about the right use of the drugs, with training activities, organised by the Infections Control Committee in co-operation with clinicians, microbiologists and pharmacologists, regarding the diagnosis and treatment of infections and the need to contain and face the increase of antibiotic resistance.

R2440 Imipenem resistance among *Pseudomonas aeruginosa* isolates: risk factors and impact of resistance on clinical outcomes in a Tunisian burn unit

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Pseudomonas aeruginosa is an important cause of nosocomial infections particularly in burned patients. Imipenem is one of the most effective drugs against *P. aeruginosa*, but imipenem resistance had occurred and has increasingly been reported in *P aeruginosa*.
This study was conducted to determine the risk factors for acquisition of imipenem resistant *P. aeruginosa*(IRPA)and the impact of this resistance on clinical outcomes at a Tunisian burn unit.

Patients hospitalised in the burn unit from January 2005 to December 2006 were included in this study. The features of patients with IRPA isolates were compared with those of patients with imipenem susceptible *P. aeruginosa* isolates (ISPA). Demographic features, total burn surface area (TBSA), burn depth, antimicrobial used 15 days before, presence of IRPA in the unit at the same period and previous ISPA isolated from the patient were included in the risk factors analysis. We compared length of hospitalisation and mortality in the two groups of ISPA and IRPA.
P. aeruginosa was recovered from 64 patients in this period, 25 were IRPA and 39 were ISPA.
There was no significant difference between patients with ISPA and patients with IRPA in terms of age, TBSA burn depth and length of hospitalisation. The percentages of septic choc were 64% in IRPA group compared with 47% in ISPA group. The mortality rate among patients infected with IRPA was significantly higher: 40% versus to 17.9% with ISPA (relative risk= 2. 23, $p = 0.05$).
Previous ISPA isolate from the patient (OD=14. 8, $p = 0.02$) and hospital admission in the previous year (OD=8.25, $p = 0.002$) were independent risk factors for acquisition of IRPA.
However, the presence of IRPA in the unit at the same period and antibiotic use before isolation were not significantly associated with IRPA in our study ($P > 0.05$).
The imipenem resistance among *P. aeruginosa* strains had a significant impact on mortality in our study. The presence of ISPA in a patient before isolation of IRPA is a significant risk for acquisition of IRPA; *P. aeruginosa* has the ability to develop drug resistance during therapy. Carapenem exposure led the microorganism to this resistance. Therefore, the judicious use of these antibiotics might be an important strategy to prevent the imipenem resistance.

R2441 Antimicrobial effects of Persica© mouth wash on *Helicobacter pylori* growth

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Introduction: The resistance of different bacteria to the current antibacterial agents, toxicity of the antibacterial agents and the cost of the treatment has led to the development of new active molecules against the bacteria. Since ancient times medicinal plants have been used for the treatment of bacterial infections. In present study we investigated antibacterial activity of a commercial compound of Persica© mouth wash on growth of *Helicobacter pylori*.
Material and Methods: Persica© mouth wash integrated of Meswak, Mint and Yarrow plants extracts and some tannin compound. Agar dilution was done by brucella agar and incubated in anaerobic jar for 72 hour. *Helicobacter pylori* 124823 used as a standard bacteria. Results compared for clinical and standard bacteria.
Results: Antibacterial activity of Persica© mouth wash against *Helicobacter pylori* emerged on 1/20 dilution of stock. This dilution is conventional dilution presented as common prescribing dilution. There was not difference on effect of drug between standard and clinical samples.
Discussion: One of the sources of *Helicobacter pylori* infection is mouth and dental plaque. Deletion of this bacterium from mouth and dental plaque can prohibit probable reinfection of bacteria in stomach. In present study we found Persica© as an effective plant drug for inhibition of *Helicobacter pylori*. Maybe further investigation reveal fact of this inhibitory effect.

R2442 Colonisation of hospital water distribution system by *Legionella pneumophila*

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Introduction: Legionnaires' disease is an important cause of community and hospital-acquired pneumonia. The latter occurs after colonisation of hospital water distribution system. Several countries, including Greece, now mandate routine environmental surveillance for *Legionella* in hospitals, regardless of occurrence of cases. This study describes the problems of *Legionella* colonisation encountered in a Greek hospital after implementing water surveillance cultures and their resolution.
Material and Methods: During a 14-month period 100 water samples from cooling towers, boilers, taps and showers were cultured for *Legionella* by the methodology of ISO 11731 (1998). The corrective measures that were undertaken and the cost of these activities were recorded. For the detection of infection a rapid urine *Legionella* antigen test (Binax, Inc.) was used.
Results: Out of 100 water samples, 32 were positive for *Legionella pneumophila* serotype 1. Colonisation of hospital water ranged from 500 to 350,000 CFU/L and the highest values were obtained from the water boilers. Preventive and control measures were successfully undertaken: shutting down of the warm water sullpy, hyperchlorination, installation of filters in high risk wards and finally replacement of the water heating system followed by heat shock and a constant elevation of the warm water temperature (>55ºC). There were 4 cases of pneumonia caused by *Legionella* but all were community-acquired. Main problems encountered were high total cost, high energy consumption, damages of water pipelines, increased temperature of cold water and adverse publicity.
Conclusions: This is the first description of hospital water colonisation with *Legionella* in Greece. The problem was solved promptly and because of the high levels of surveillance, there were no cases of nosocomial infection.

R2443 The role of media fill in the evaluation of radiopharmaceutical manufacturing process at the European Institute of Oncology

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Objective: Aim of this study is to evaluate the microbial contamination in the ordinary labelling of process to produce a sterile therapeutic radiopharmaceutical. The manufacturing and handling of radiopharmaceuticals are potentially hazardous, for this reason the radiopharmaceuticals are usually released before sterility testing, while it is carried out after complete decay. In this case the continuous assessment of the effectiveness of the aseptic procedures has main importance.
Methods: Radioactive drugs sterility is defined by the absence of viable and actively multiplying microorganisms when tested in the specified culture media. In order to obtain a validation of aseptic technique we carried out a labelling simulation by the Media Fill Test. Applying this method, operators simulate every step of the process using culture medium instead of actual radiopharmaceutical product. Triptic Soy Broth (TSB) has been used as nutrient medium because supports the growth of a wide spectrum of microorganisms.
We created a written procedure to follow in simulation of 90Y-DOTATOC compounding. It specifies all working conditions and requirements that need to be insured to obtain a good evaluation about process sterility. Media fill test has been subdivided into 4 phases from the simulation of reagents preparation to the dispensing operations until ward delivery. All the operations involving radioactive material need to be performed inside the hot cell, while all other steps (e.g. reagents preparation) can be done under a laminar flow hood. At the end of the procedure the processed medium is incubated at 35ºC for 14 days. If the compounding procedure is adequately performed there will be no microbial growth.
Results: Microbiological analysis carried out on all vials obtained at the end of each step showed no microbial growth. This is the reason why it is possible to consider the test satisfied.
Conclusion: Application of media-fill test allowed both to validate operative modality used for 90Y-DOTATOC handling and to attest the ability of operators who worked it. Additionally a correct quality control of the radiopharmaceutical iv preparations allows control and prevention of clinic infections.
Results are shown in media fill final report attached to the batch-record.

Clinical epidemiology of nosocomial infections (POWI, VAP, UTI, BSI, . . .)

R2444 Colonisation with meticillin-resistant staphylococci and multidrug-resistant Gram-negative bacilli in hospitalised patients: prevalence and risk factors

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Background: To determine the frequency of and risk factors for colonisation of hospitalised patients by meticillin-resistant *Staphylococcus aureus* and coagulase-negative staphylococci (MRSA and MRCNS), or multidrug resistant Gram-negative bacilli (MDR-GNB) and multidrug resistant nonfermentative Gram-negative bacilli (MDR-NFGNB) classified as if they were resistant to at least 3 different antibiotic groups, a one-day prevalence survey was conducted.
Methods: A total of 440 patients (172 were hospitalised within 72 hours after admission, 268 were for more than 72 hours) were screened, using nasal and cutaneous swabs. For each patient, the following variables were recorded: age, sex, length of hospital stay, hospitalisation before admission, the presence of anti infective therapy and invasive procedures or devices.
Results: The prevalence of MRSA, MRCNS, MDR-NFGNB and MDR-GNB colonisation was 9.9%, 34.3%, 1.7% and 0.6% among patients hospitalised within 72 h; 36.6%, 81.3%, 14.6% and 11.9% among patients hospitalised for more than 72 h, respectively.
Conclusions: There was a significant difference ($p < 0.001$) in colonisation of these bacteria between patients hospitalised for more than 72 h and within 72 h. Prolonged length of hospital stay, hospitalisation before admission, surgery and anti infective therapy with betalactams and glycopeptides for MRSA, MRCNS strains; the presence of urinary and central venous or arterial catheter and anti infective therapy with betalactams and carbapenems for MDR-NFGNB and MDR-GNB were found to be independent risk factors for colonisation. Identification of the risk factors for colonisation is the first step in formulating an effective strategy to prevent hospital infections.

R2445 *Acinetobacter* spp. in the maternity hospital: is there a relation between neonatal and maternal isolates?

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Introduction: Multi-resistant *Acinetobacter* spp. has become an important cause of nosocomial infection worldwide. The purpose of this study was to define and compare the species profile and antibiotic resistance pattern of both neonatal and maternal clinical isolates.
Materials and Methods: All clinical isolates of *Acinetobacter* spp. isolated between January, 2001 and December, 2006 were analysed.
Results: A total of 343 *Acinetobacter* spp. were isolated, 138 (40.2%) were neonatal isolates and majority 102 (74%) were blood isolates. While 184 (90%) of maternal isolates were genitourinary isolates, 85 (41%), 65 (31.7), and 33 (16.1%) were HVS, urine and episiotomy wound isolates respectively. *Acinetobacter baumannii* was the commonest identified special both in neonatal and maternal isolates form 64.7% (185) of species while *Acinetobacter calcoaceticus* form 33.2% (95) of identified species. 77.3%, 86.5%, 45% *Acinetobacter baumannii* were resistant to ampicillin, cefuroxime and cefotaxime respectively and only one isolate was resistant to imipenem and tazocin and no resistance detected to amikacin and meropenem. The *Acinetobacter calcoaceticus* had the same antibiotic profile as *Acinetobacter baumannii*.
Conclusion: The species profile and the antibiotic resistance pattern for both neonatal and maternal isolates were comparable and may indicate that the neonatal isolates mainly acquired in the hospital may be related to maternal community strains. At the same time we did not elicit the trend of multi-resistant *Acinetobacter* spp. that is reported in the literature as main problematic nosocomial isolates during the last six years.

R2446 *Pseudomonas aeruginosa* bacteraemia: 303 cases

C. Ezpeleta, J. Alava, J. Unzaga, V. Cabezas, C. Busto, E. Gomez, I. Atutxa, R. Cisterna (Bilbao, ES)

Objective: *P. aeruginosa* bacteraemia (PAB) is a serious infection associated with high mortality, being one of the most common nosocomial pathogen. Underlying diseases, source of infection and inappropriate antibiotic therapy are associated with mortality. The aim of this study is to asses clinical epidemiology of PAB in our hospital.
Methods: Prospective study of all patients with PAB. Study period Nov 1993-July 2007. Blood cultures are performed by means of BACTEC 9240. The Infection Control team studies every patient with positive blood cultures. Variables under surveillance are age, sex, underlying illnesses, predisposing conditions, source of bacteraemia, nosocomial/community acquired, antibiotic susceptibility, treatment and outcome. A computer based surveillance system (SEPSIS-DATA) is used.
Results: 303 cases, 203 males (67%). Age: 67.6% >60 years. Underlying illnesses: Neoplasia 42.24%, Diabetes 16.17%, COPD 13.2%, HIV 7.59%. Hospital acquired 48.84%. Source of bacteraemia: Primary 27.0%, Urinary tract 20.13%, Respiratory 21.12%, abdominal 11.22%, skin: 6.60%, Catheter related 5.94%, surgical site 2.64%. Predisposing conditions: mechanical ventilation 9.9%, ICU 11.5%, surgery 13.5%, urinary catheter 25.4%, antibiotic 48 h before bacteraemia: 40.59%, intravascular catheter 49.17%, immunosuppressive therapy 26.73%, neutropenia: 16.17%. Time to positivity of blood cultures 80.2% less than 48 h. Polymicrobial bacteraemia: 20.8%. Treatment and outcome: Inappropriate antibiotic therapy 20.1%. Crude mortality: 32.34%. Sensitivity to antibiotics: Amikacin 94.72%, Imipenem 90.76%, Ceftazidime 88.45%, Gentamicin 87.46%, and Ciprofloxacin 78.88%. Nosocomial bacteraemia: 148 cases, 63.5% males, 55.4% older than 60y. Underlying illnesses: neoplasia 45%, VIH 7.4%. Source of bacteraemia: primary 31.7%, urinary 18.9%, respiratory 18.24%, catheter related 10.14%, surgical site 5.4%, abdominal 8.11%. Crude mortality nosocomial acquired: 37%.
Conclusions: The incidence of *Pseudomonas aeruginosa* bacteraemia in our hospital remained stable over last years. Neoplasia was the most common underlying disease. The most active antibiotics are Amikacin, Imipenem and Ceftazidime. The rate of multidrug-resistant *P. aeruginosa* has increased in the last years. For these reasons, patients with *Pseudomonas* bacteraemia might receive empirical antibiotics that are inactive against *Pseudomonas*, especially before identification and antibiotic susceptibility results become available.

R2447 Risk factors for nosomial infections in an intensive care unit

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Objectives: Knowledge on risk factors (RF) for the development of nosocomial infection (NI) is crucial to establish preventive measures; our aim is to assess RF for NI in an Intensive Care Unit (ICU) at a second level hospital.
Patients and Methods: A prospective and observational study of NI cases on the ICU during a 12 month period; epidemiological and clinical variables as well as information about risk factors related to NI were assessed (underlying conditions, severity at admission, rate of urgent surgeries, invasive techniques, ICU length of stay, mechanical ventilation, etc.). A logistic regression statistical analysis was done to identify RF for different groups of NI.
Results: 806 patients were assess during the study period (33% women; mean age 66.4±14.86 years). Mean ICU length of stay was 4.6±6.72 days. Mean APACHE II score was 11.41±8.25. Reasons for ICU admission were: coronary disease 65%, other medical reasons 27%, surgical patients 5% and traumatisms 2%; 40% of patients required central intravenous line (CIL), 20% mechanical ventilation (MV), 48%

urinary catheter, 18% surgery and 5% urgent surgery. In the statistical logistic analysis RF associated to NI were: a) for nosocomial pneumonia, APACHE II >15 (OR = 6.17; CI 95%, 1.39–27.33), MV duration >5 days (OR = 9.98; CI 95%, 1.81–55.04) and tracheotomy during more than 2 days (OR = 14.73; CI 95%, 3.13–69.27); b) for urinary infection, urinary catheter duration >3 days (OR = 4.03; CI 95%, 1.60–10.18) and ICU admission >10 days (OR = 3.55; CI 95%, 1.41–8.91); c) for catheter related bacteraemia, neutropenia (OR = 5.46; CI 95%, 1.33–22.39) and CIL duration >6 days (OR = 35.89; CI 95%, 16.31–79) and d) for surgical site infection, surgical re-intervention (OR = 42.61; CI 95%, 3.25–558.64), urgent surgical re-intervention (OR = 30.89; CI 95%, 4.31–221.39) and ICU admission >10 days (OR = 4.94; CI 95%, 1.19–20.47).

Conclusions: Mean RF for NI were identified in our cohort and were related to severity at admission, invasive techniques (urinary catheter, CIL and MV) and ICU length of stay. Knowledge on these RF for NI is crucial to establish preventive measures mainly in terms of the care associated to invasive techniques.

Travel medicine, tropical & parasitic diseases

R2448 Epidemiological investigation of hydatidosis infections in Iran

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Objectives: Hydatidosis is an important parasite infection, which has a global spreading. This zoonose disease as an endemic one, in Iran, has caused mortality and economic damages. Aim of this study is to determine prevalence of hydatidosis infection in human and livestock populations during recent five years (2002–2007) and to propose guidelines for prevention and control of it.

Methods: In this analytical-descriptive study, rate of infection to hydatid cyst in slaughtered livestock in 28 provinces of Iran (in term of detected infected organs) and also prevalence of human hydatidosis in mentioned provinces were evaluated and analysed epidemiologically in the five year period.

Results: Average rate of infection to hydatid cyst in slaughtered livestock was determined as 6.73%. 4,298,882 livers and lungs in 63,851,561 slaughtered animals were taken out from consumption cycle due to hydatid cyst infection. Lung infection was 1.8 times more than liver one. Among 28 studied provinces, Khorasan and Yazd with 18.71% and 2.4% ranked highest and lowest infection rates respectively. In addition, economic damage caused by omitting infected organs in this five-year period was estimated to be about 76 million dollars. Average prevalence of human hydatidosis during years 2002 to 2007 was determined 0.61 (in each 100,000 persons). Khorasan province with 615 cases out of 2083 ones (29.52%) and Hormozgan province without any cases took the first and the last positions respectively.

Conclusion: Health and economic significance evaluations and epidemiologic studies for each infection are the first steps for prevention and controlling and also a confident starting point for facing with infection. Livestock hydatidosis has been endemic in Iran and have had an ever-increasing rate during studied five-year period. As this infection is zoonose disease, proposed suggestions could be useful.

R2449 Epidemic typhus with concomitant *Staphylococcus hominis* bacteraemia in an immunocompetant patient after tourist trip to India and Nepal: case report.

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Background: Epidemic typhus is an acute febrile illness, caused by *Rickettsia prowazekii* and transmitted by louse *Pediculus vestimenti*, that may present with a broad spectrum of clinical manifestations. Epidemic typhus occurs in Central and South America, Africa, northern China, and certain regions of the Himalayas. *Staphylococcus hominis* is a harmless commensal on human skin. We present a case of *Rickettsia prowazekii* infection with concomitant *Staphylococcus hominis* bacteraemia in an immuncompetant patient who returned from tourist trip in India and Nepal.

Case presentation: A 30-year-old female was admitted with 2-week history of high-grade fever of 40C, general weakness, chilly sensation and sweating. A week before hospitalisation she returned from 19-day tourist trip in India and Nepal. At the beginning of fever she took 8-day oral amoxicillin therapy, during which she observed one-day maculopapular rash on the arms and legs. Before the trip patient obtained vaccine prophylaxis against hepatitis A and B, typhoid fever, poliomyelitis, tetanus and diphtheria. During trip, she took regularly antimalarial chemoprophylaxis with atovaquon-proguanil which was interrupted by herself in Nepal, so initially malaria was considered. Clinical examination revealed mild splenomegaly. Laboratory analysis yielded erythrocyte sedimentation rate of 48 mm/h, microcytic anaemia and moderately elevated C-reactive protein and aminotransferases level. A chest X-ray showed diaphragm-pleural adhesions. An ultrasonography discovered restrained left pleural effusion. Serologic blood tests for HIV, EBV, *Mycoplasma pneumoniae* and Bordetella pertussis were negative. Malaria was excluded and all bacterial as well as fungal cultures of urine and stool were negative. Blood cultures yielded *Staphylococcus hominis* and empiric antibiotic therapy with ciprofloxacine was introduced. This treatment was confirmed as appropriate by further sensitivity data and continued for 10 days. The Weil-Felix agglutination test was positive in titer 1:800, so diagnosis of epidemic typhus was established and oral doxycycline therapy initiated. The patient was discharged from the hospital in good general condition.

Conclusion: *Rickettsia prowazekii* infection should be considered in Europeans with acute febrile illness, especially following travels to endemic regions. Moreover, the other concomitant infections are also possible.

R2450 *Trichomonas vaginalis*: a point prevalence study in a high-risk population in Havana, Cuba

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Objectives: To estimate *Trichomonas* prevalence, risk factors and its association with past and current sexual transmitted diseases (STD's) in an urban, high risk population, in Havana City, Cuba.

Methods: A random, point prevalence study in a population with a high risk for STD's in urban areas of Havana City, Cuba. 375 healthy young adults, 294 females and 81 males, with an age range from 14 to 35 years old gave consent to participate and been tested for STD's. Participants older than 35 years, younger than 14 and without previous sexual intercourse and those who were under current broad spectrum antibiotics or in the last 4 weeks were excluded. A cervical smear in women and an urethral smear in men was used for molecular detection of *Trichomonas* using an established real-time PCR procedure.

Results: Prevalence of *Trichomonas vaginalis* was 5.7%. Among the subjects positive for *Trichomonas*, females were mostly affected (95.2%;20/21). There was no relation with age and *Trichomonas* was equally found among all age groups. The current infection with *Trichomonas* was related with past STD infection(OR:3.3;p = 0.037). Previous vaginitis complaints (OR:2.2;p = 0.119) and pelvic inflammatory disease episodes (OR:1.8;p = 0.238) were not significantly related. The age of first sexual intercourse was highly associated(OR:2.9;p = 0.012) with the diagnosis of *Trichomonas* in the study group; similar as with having two or more sexual partners in the last five years (OR:2.9;p = 0.06). *Trichomonas* infection was a predictor for concurrent *Chlamydia* and gonorrhoea infection (OR:1.8 and 17.5, respectively).

Conclusion: Infection with *T. vaginalis* was found to be common in sexual active women, but age was not a significant factor of infection. *Trichomonas* was not associated with other gynaecological co-morbidities but was a marker of STD co-infections. Because positive samples were equally distributed among all age groups, strategies to approach the problem may greatly differ from other STD's.

R2451 Macroscopic and light microscopic manifestations of the cyst of *Coenurus cerebralis* in sheep brain

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Objectives: Coenurosis is caused by the larval stage of *Taenia multiceps*. The larvae, *Coenurus cerebralis*, form cysts in cerebrum, cerebellum and spinal cord of sheep, goats, cattle, horses, and have been also reported in humans. When the parasite settles in the central nervous system of intermediate host, it develops as a cyst growing slowly and becoming 5–6 cm in 6–8 months. The presence of freely roaming dogs (fox, jackal) on grazing land greatly contributes to the existence of the diseases. Dogs fed with offal including sheep head maintain the *C. cerebralis–T. multiceps* cycle. Severity of the disease may be attributed to the magnitude of the cyst in the brain. Because of the pressure of cyst/cysts, the circling, the commonest sign becomes. When the animal is forced to rotate, it circles to the affected side. Head deviation, to lag behind the flock and visual impairment are the other common signs. This study aims to call especially medical doctors attention to Coenurosis, a zoonotic disease and to show morphological features of the *C. cerebralis* cyst.
Methods: One of the sheep dead due to complaints similar to those mentioned above was used in the present study. When the animal sent for a necropsy to the Veterinary Control and Research Centre in Erzurum, Turkey was dissected, a cyst 3.5 cm in diameter in the brain was inspected. The cyst and brain tissue were evaluated at the macroscopic level. After whole cyst was removed from the brain, it was fixed in 10% formalin, and dehydrated in graded alcohol series, and embedded in paraffin for light microscopic examination. Paraffin sections were stained with Haematoxylin-Eosin (H-E).
Results: Hyperaemia and petechial haemorrhages were present on the brain. Whitish specks (0.3–0.4 mm in diameter) on the transparent cyst wall were observed with naked eye, representing invaginated scolices. In H-E stained slides, scolices from the germination membrane and their rostellums with a double crown hooks were examined microscopically.
Conclusion: However the cases of man are rare, coenurosis has to be kept mind. Preventive measures are very important for this mortal zoonosis.

R2452 Prevalance rate of *Cryptosporidium* infection in haemodialysis patient admitted to hospital, Mashhad, Iran, 2006–2007

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Introduction: *Cryptosporidium parvum*, an intracellular protozoan parasite, is a significant cause of gastrointestinal disease worldwide. Transmission can occur from an infected person, animal or faecally contaminated environment. The clinical manifestations of cryptosporidiosis are dependent on the immunologic state of the host. Infection among immunocompetent hosts results in diarrhoea that is typically self-limited. In immunocompromised hosts, however, the infection may be protracted and life-threatening with no reliable antimicrobial therapy.
Results: In this study we compared the prevalence rate of *Cryptosporidium* infection in hemodialysis patients and control group (patients family member and general population). Stool specimens of 50 adult outpatient chronic hemodialysis patients and 100 healthy individuals were examined for the presence of *Cryptosporidium* oocysts by using a modified acid-fast staining method and formalin-ether sedimentation technic. 6 of 50 (12%) dialysis patients were infected with *Cryptosporidium*. This was significantly higher than 1 of 100 (1%) cases in the control group respectively ($p = 0.006$).
The prevalence rate of *Cryptosporidium* infection did not correlate with patients' age, duration of dialysis, cause of renal failure, history of kidney transplantation, history of diabetes mellitus, history of diarrhoea or history of taking antibiotics during last mount. As hemodialysis patients are candidates for renal transplantation, general preventive measures against acquiring *Cryptosporidium* infection must be considered and these patients must be screened for their carrier state before transplantation. We also should prevent transmission to other dialysis patients by isolation of the index case until successful treatment is accomplished.

R2453 Serum copper, zinc, magnesium and selenium levels in patients with snake bite

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Objective: The aim of this study is to compare the serum Copper (Cu), Zinc (Zn), Magnesium (Mg) and Selenium (Se) levels of patients with snake bite and healthy individuals.
Material and Methods: In this study, 40 patients with snake bite, and 90 healthy individuals in the control group were included. From totally 130 individuals, 5 ml of venous blood was taken after fasting 10 hours at night. Serum samples were decomposed by centrifugeting at 5200 turn for 10 minutes. Serum samples were diluted with deionised water. Cu, Zn, Mg and Se levels were measured in all serum samples by using Unicam 929 Atomic Absorption Spectrophotometer. All data entry and analysis were performed using SPSS for Windows Version 10.0 programme.
Results: The average age of 40 patients with snake bite was 40.1 ± 19 years, of whom 18 were males (45%), 22 were females (55%). The average age of 90 healthy individuals was 29.3 ± 8.8 years, of whom 45 were males (50%), 45 were females (50%). Serum Cu, Zn, Mg and Se levels of patients with snake bite was found to be 75.8 ± 21 μg/dl, 86.9 ± 25 μg/dl, 1200.6 ± 562 μg/dl and 96.2 ± 44 μg/dl respectively. Serum Cu, Zn, Mg and Se levels of healthy individuals were found to be 57.1 ± 17 μg/dl, 55.8 ± 13 μg/dl, 1430.9 ± 292 μg/dl and 118.4 ± 56 μg/dl respectively. A statistically significant ($p = 0.0001$) increase at serum Cu and Zn levels and decrease in Mg and Se levels of patient with snake bite were detected, when compared with the control group.
Conclusion: Snake bite is associated with significant increase at serum Cu and Zn levels and decrease in Mg and Se levels of trace elements. Further studies on the measurement of trace element may be useful to understand the course of snake bite.

R2454 Biofilm production and antimicrobial susceptibility of enteroaggregative *Escherichia coli* causing traveller's diarrhoea

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Objective: The main objective of this study was to investigate the biofilm production and antimicrobial susceptibility of 75 enteroaggregative *Escherichia coli* clinical isolates causing traveller's diarrhoea.
Methods: The study population included adults who referred a diarrhoea episode related to a visit to a developing country and had EAEC isolated after attending at Hospital Clinic, University of Barcelona, from January 2005 to December 2006. Bacteria were isolated in different selective media and identified by conventional biochemical tests. All recovered *E. coli* were analysed by specific PCR to establish the *E. coli* enteropathotype and the presence of the aggR gene, a transcriptional factor which regulates the expression of some virulence factor. The antimicrobial susceptibility was established by mean of commercial antibiotic panels (Sensititre® TREK Diagnostic Systems Limited), whereas the production of biofilm was determined using a microtitre plate assay.
Results: The Laboratory of Microbiology received 900 for investigation of TD between January 2005 and December 2006. EAEC was isolated from 79 of those samples which represents 8.8% (nº of patients submitting samples). Consistent data were available for 75 patients who were included in the analysis. Antimicrobial susceptibility tests were available for 50 isolates. Of those, 40 (80%) were resistant to ampicillin; 20 (40%) to nalidixic acid; 11 (22%) to ciprofloxacin, 44 (88%) to tetracycline; 41 (82%) to cotrimoxazol and 13 (26%) to chloramphenicol. It is important to highlight that 10% of the EAEC isolates were resistant to third generation cephalosporins, all of them carried the blaCTXM-15

and were isolated from patients to India. Forty-nine (49%) out of 75 EAEC isolates produced biofilm. When the presence of the AggR gene (35, 46.7%) was determined in the biofilm-forming EAEC group versus non-biofilm-forming EAEC group, was the most prevalent virulence factor in the biofilm-forming EAEC group (70% vs 9%, p = 0.01).
Conclusions: These results show that biofilm may be considered as an important virulence factor, strongly associated with the presence of AggR among EAEC isolates causing diarrhoea in travellers and that the EAEC causing traveller's diarrhoea show a high level of resistance to ampicillin, tetracycline, trimethoprim and nalidixic acid. It is the first time that the presence of the blaCTXM-15 is described in EAEC clinical isolates.

R2455 Synthetic *Plasmodium falciparum* GPI: TLR recognition and structural requirements

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Plasmodium falciparum malaria is confined to blood stage parasites. Host immune response to parasite antigens is essential for parasite clearance but also contributes to disease manifestation. Plasmodial glycosylphosphatidylinositol (GPI) contributes to malaria pathology by induction of excessive cytokine release via Toll-like receptor (TLR) 2. Since GPI is a promising anti-disease vaccine candidate, identification of structural requirements for TLR-activation is relevant.
Applying synthetic *P. falciparum* GPI-glycan analogues we identified GPI moieties required for immune activation. On RAW264.7 macrophages, GPI substructures lacking the diacylglycerol moiety were still stimulatory. However, at least four mannose residues were required for TNF-alpha induction. Integration of lipidated GPI into erythrocyte membranes induced increased TNF-alpha and IL-12 compared to pure compound.
We conclude that the glycan moiety is the immune-stimulatory moiety while the acyl chains confer optimal presentation of the molecule on cell surfaces.

R2456 Leishmaniasis in Libya: epidemiological survey in Sirt

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Objectives: The cutaneous leishmaniasis in Libya to be, sometimes at least, a rural zoonotic infection. We conduct a pilot study covering a 4 months period (October 2006 to February 2007) in the Alhish Hospital, Sirt in Libya.
Patients and Methods: The diagnosis of cutaneous leishmaniasis based on a clinical presentation, or positive parasitic smear. Information gathered for each patient, including age, sex, geographic location, previous history of leishmaniasis, a stay in an endemic area, the month of consultation, lesion location, and the number and size of lesions. We also note treatment and outcome.
Results: During this 4 months period, information on 84 (78 adult and 6 children) patients with lesions of cutaneous leishmaniasis was collected. Distribution according to age showed that leishmaniasis affected adult in 92.8 percent of the cases. Among the 84 patients were 26 female and 58 male. All of our patients lived in an endemic area. The patients gave a history of cutaneous leishmaniasis in other family members in 3.5 percent of the cases. The face was the most commonly affected site (89%). Clinical diagnosis confirmed by the parasitologic smear. Giemsa stain revealed amastigotes, which appear pale blue.
Discussion: Leishmaniasis is quite prevalent in Libya. The distribution is both in endemic areas and sporadic throughout the country. Coetaneous leishmaniasis (CL) cases were recorded during this period (4 months) in Elhisha, Sirt, an endemic area of CL in Libya. The age distribution of cases showed that the age group 17–30 years was most affected. Most case was in Elhisha (54.42%) and Tawargha (32.14%) may because of presence of reservoir and the lakes. New case in new area (Algadahia) indicate the spread of the disease to East. Various therapeutic regimens were discussed. Only patients with severe lesions (approximately 21%) were given 3 to 4 weeks' treatment with intravenous Pentostam, to which all except 4 responded satisfactorily with minimal side effects.

Resistance & mechanisms of action of antifungals

R2457 Antifungal susceptibility testing of yeast isolates from nine hospitals in Bulgaria and their species distribution – a two-year study

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Objectives: The aim of this study for a second year is to evaluate the species distribution and antifungal susceptibility pattern of yeast isolates from nine medical centres in Bulgaria.
In the current study was 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 were additionally confirmed with the referent broth micro-dilution method CLSI (formerly NCCLS) M27-A2.
Methods: A total of 424 clinical yeast isolates were collected in the clinical routine of nine participating medical centres from January 2006 to November 2007. All yeast strains were isolated from diverse body site, of immunocompetent and immunocompromised patients, including HIV infected ones. Etest MICs were determined with Mueller-Hinton agar containing 2% glucose and 0.5 mg per l methylene blue dye and were noted after 24 and 48 h of incubation at 35ºC.
Results: The most frequently isolated species was *Candida albicans* (70%). Among the non-*albicans* species, *C. glabrata* (8.5%) was the most prevalent, followed by *C. parapsilosis* (5.9%), *C. tropicalis* (3.5%), *C. krusei* (2.8%), and other *Candida* non-*albicans* strains (9%). Low susceptibility to fluconazole was detected among *C. albicans* strains (2.3%, MIC >256 mg l^{-1}), *C. glabrata* (28%, MIC 16–32 mg l^{-1}; 36%, MIC >64 mg l^{-1}), *C. krusei* (27%, MIC 32–64 mg l^{-1}; 73%, MIC >256 mg l^{-1}). No resistance to fluconazole and voriconazole was detected in *C. parapsilosis*, *C. tropicalis* and *C. lusitaniae* strains. Resistance to voriconazole was detected only in *C. glabrata* (12.8%, MIC >32 mg l^{-1}) and *C. albicans* (2.3%, MIC >32 mg l^{-1}) strains. Voriconazole showed good activity to most of the tested isolates with MICs in the range of 0.008–0.5 mg l^{-1}.
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 fluconazole susceptibility detected in *C. glabrata* and *C. krusei* and their high prevalence.

R2458 Antifungal activity of *Thymus serpyllum* essential oil against *Candida albicans* and *Candida* non-*albicans* clinical isolates

M. Pavel, F. Alecu (Bucharest, RO)

The increase of fungal resistance to classical drugs, the treatment cost, and the fact that most available antifungal drugs have only fungistatic activity, justify the search for new strategies. Aromatic plants and their essential oils constitute the object of numerous studies, because alcohols and phenols, components of essential oils, have strong antifungal properties.
Objectives: The aim of this study was to establish the antifungal effect (fungistatic and fungicidal effect) of *Thymus serpyllum* essential oil.
Material and Method: We used aerial parts of *Thymus serpyllum* provided from a commercial lot. The essential oil was isolated by water distillation from dried plant material, using a Clevenger-type apparatus, according to the procedure described in the European Pharmacopoeia. The antifungal activity of the essential oil was evaluated against 7 *Candida albicans* isolates (5 isolates recovered from patient with clinical signs of oral candidosis (I to V) and two oral isolates from healthy carriers (VI-VII) and one *Candida glabrata* recovered from a patient

with oral candidosis associated to denture and diabetes (VIII). Antifungal activity. MICs, determined by the macrodilution broth method, and minimal lethal concentrations (MLCs) were performed according to the references of National Committee for Clinical Laboratory Standards for yeasts and filamentous fungi. Serial dilutions in ethanol ranging from 0.25 to 64 microl ml-1 were tested for essential oil. Equivalent dilutions for ethanol were tested separately.
Results and Discussion: Evaluation of MIC and MLC showed that the oil was active against all tested isolates. MIC values ranged from 1 to 2 $\mu l\,ml^{-1}$ for essential oil and 109 $\mu l\,ml^{-1}$ in case of ethanol. MLC for essential oil was 2 $\mu l\,ml^{-1}$ for isolates I-III and 4 $\mu l\,ml^{-1}$ for isolates IV-VIII while MLC for ethanol was 109 $\mu l\,ml^{-1}$. *T. serpyllum* essential oil exhibited significant antifungal activity and it is reasonable to consider that the activity of this oil can be related to the presence of thymol and carvacrol.
Conclusion: *T. serpyllum* essential oil has proved its potential to be used as a topical antifungal agent against fungi that are pathogenic to humans. This essential oil showed an important activity against *Candida albicans* and *Candida glabrata*.

Fungal infections

R2459 Fungal infections at a Coimbra burns unit: 2003–2007

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Objectives: Analyse the fungal infections, as well as the results of systemic antifungal therapy in Coimbra Burns Unit (Portugal), during the study period.
Methods: The authors retrospectively reviewed data of the patients who developed fungal infections, admitted to the Burns Unit, from January 2003 to November 2007. Epidemiological, microbiological and pharmacological parameters were collected. The data was analysed using the statistical programme SPSS.
Results: During the study period, 856 patients were admitted to the burn unit and 69 patients were enrolled in the study, however, only 41 had complete data for the statistical analysis. The characteristics of the patients admitted were: 62.9% male, mean age of 57.28 (± 87.6) years [11; 96] and mean total burned body surface area (TBSA) of 15.8 (± 18.06) % [1; 100], versus 56.1% male, mean age of 63.54 (± 21.3) years [16; 96] and mean burned TBSA of 28.39 (±16.1) % [5; 60], for the patients enrolled. The mean hospital stay in the Burns Unit was 16.72 days, whereas, 46.34 days for the enrolled patients and the mortality average was 15.2% and 36.6% (40.2%/year), respectively. Eight per cent of the patients developed fungal infections. *Candida* infection was the most common (82%), being *Candida albicans* the main isolated specie (56%). The antifungal agent most prescribed was amphotericin B liposomal preparation (44%). In the study population, no statistical significant difference was found when comparing the days of hospital stay required by the patients that used different antifungal treatment.
The mean cost of hospitalisation in Coimbra Burns Unit was 3272 € for the average patient versus 11,915 € for a patient with antifungal therapy.
Conclusions: In the study population, a higher mean age and also a greater mean percentage of TBSA was present in those patients who developed fungal infections. These patients also required more days in the hospital and had a higher mortality rate. Microbiological data should be used frequently to monitor the evolution of the most frequent fungal infections and in order to choose an adequate antifungal drug since these agents contribute to higher costs of treatment.

R2460 Epidemiology and outcome of candidaemia at an acute-phase community hospital

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Objectives: To clarify the frequency of candidaemia, isolated *Candida* species, and prognosis at an acute-phase community hospital, we retrospectively analysed the clinical and microbiological data concerning the cases of *Candida* species detected from blood between June, 2003 and February 2006.
Patients and Methods: In cases that candida species were isolated, we recorded age, sex, underlying disease, identification and susceptibility for isolated candida species, doses of antifungal agents, duration of the therapy, presence or absence for ophthalmic examination, and outcome. Microbiological examination was carried out by an examination company. Species identification was confirmed with the VITEK YBC system or API 20 C AUX or API 32ID. The susceptibility testing was performed by the use of a commercially prepared colorimetric microdilution panel (ASTY®).
Results: In 329 patients with bacteraemia and/or candidaemia, 15 patients (4.6%) were candidaemia. The mean age was 79.5 years. There were 8 men and 7 women. The underlying diseases were malignancy (n = 7), cerebral infarction (n = 3), chronic renal failure (n = 1) and others (n = 4). The isolated organisms were *C. albicans* (n = 5) *C. parapsilosis* (n = 5), *C. tropicalis* (n = 2), *C. glabrata* (n = 2) and non-identified candida species (n = 1). In the 15 patients, a central venous catheter (CVC) had been inserted which was removed before determination of the species identification. In 12 patients that culture examination of CVC tip was carried out, the same *Candida* species in the blood was detected in 4 patients (33.3%). Thirteen of the 15 patients with candidaemia (86.7%) were not performed the ophthalmic examination for candida endophthalmitis. The patients with candidaemia were treated with a daily dose of 100 mg to 400 mg of fluconazole (n = 10), and of 250 mg of micafungin (n = 1). Two patients received no treatment. Two patients died before the species identification. Nine of the 15 patients (60.0%) died within the 30 days following a positive blood culture. The susceptibility of isolates to fluconazole was sensitive (n = 11) and susceptible dose/delibery-dependent (n = 4).
Conclusions: In community hospitals, it is important that physicians are cognizant of the possible occurrence of candidaemia in the patients with CVC. Furthermore, if candidaemia occurred, appropriate antifungal therapy and the ophthalmic examination for candida endophthalmitis are also important.

R2461 In vitro susceptibility of clinical isolates of *Candida kefyr*

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Background: Several surveillance programmes in Europe and USA have detected an increase in the prevalence of fungal infections caused by *Candida* species other than *Candida albicans*. Among these species, *C. kefyr* has been reported recently as an a possible emerging pathogen. We have reviewed the antifungal susceptibility profile of the collection of clinical isolates of *C. kefyr* in order to give any insight on the management of this pathogen.
Methods: A total of 34 isolates of *C. kefyr* received in our institution between 1995 and 2007 was evaluated. The isolates were identified by routine physiological tests. The MICs of amphotericin B (AMB), flucytosine (5-FC), fluconazole (FLC), itraconazole (ITC), voriconazole (VRC), ravuconazole (RVC), posaconazole (POS), caspofungin (CAS), micafungin (MCF) and anidulanfungin (AND) were determined according to the recommendations proposed by the European Committee on Antifungal Susceptibility Testing for fermentative yeast (EUCAST-definitive document).
Results: Of the 34 strains, a total of 26.5% was isolated from blood cultures, 17.6 from vaginal exudates, 14.7% from oropharyngeal exudates, and 29.4% from other sites. A high number of isolates were received in the last four years (19/34, 55.8% since 2004). All of the strains were considered sensitive to AMB (Geometric Mean, GM, 0.22 mg/L and MIC range: 0.06–1 mg/L). Of note, three isolates exhibited an AMB MIC=1 mg/L. Only one isolate was resistant in vitro to flucytosine (MIC $\geqslant$32 mg/L). All the azole compounds showed great activity against *C. kefyr* isolates, with GM values of 0.25, 0.029, 0.015, 0.024 and 0.015 for FLC, ITC, VRC, RVC and POS respectively. In addition, echinocandins demonstrated excellent activity against all of the strains tested. (MIC values lower than 0.12 mg/L)

Conclusions: *C. kefyr* seems to be an emerging pathogen. Most of isolates are susceptible in vitro to all antifungal agents currently licensed.

R2462 Complement evasion by *Candida* is dependent on glucose concentration

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Objectives: Pathogenic fungi represent a major threat to immunocompromised hosts, leading to severe, and often lethal, systemic opportunistic infections. Factor H (FH), a soluble plasma protein consisting of 20 short consensus repeats (SCRs), is the main fluid phase inhibitor of the alternative pathway of complement. C4b-binding protein (C4bp), having a polymeric structure composed of 6–8 identical alpha-chains and a single beta-chain, is the main fluid-phase inhibitor of the classical and lectin pathways of complement. Both proteins can be acquired onto the surface of various human pathogens conveying resistance to complement destruction and thus contributing to their pathogenic potential. We have recently shown that *Candida albicans* evades complement by binding both FH and C4bp. Preliminary data suggest that a glucose transporter molecule is involved in this binding. The aim of this study was to assess whether complement evasion via this mechanism is dependent on the glucose concentration present.
Methods: Immunofluorescence, fluorescence activated cell sorting and specific constructs of the proteins investigated, as well as antibodies directed against particular parts thereof have been used to identify and characterise these virulence factors.
Results and Discussion: We now show that binding of factor H and C4bp is strongly dependent on the glucose concentration present and discuss important implications, e.g. for diabetics.
Conclusion: By acquiring complement regulators from the host, yeasts are able to evade the destructive action of complement. This acquisition, however is dependent on the glucose concentration present.

R2463 Evaluation of disc diffusion in susceptibility testing of anidulafungin against *Aspergillus* isolates

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Objective: We studied the applicability of a disk diffusion (DD) method for testing the susceptibility of anidulafungin against *Aspergillus* spp. in comparing with the broth microdilution (MD) reference method (CLSI M38-A).
Material and Methods: We tested the antifungal susceptibility of 65 *Aspergillus* strains (*A. flavus*, *A. terreus*, *A. fumigatus*, *A. niger*, *A. glaucus*) isolated from clinical samples. DD method was done using anidulafungin disks prepared in house (according the M44-A document of the CLSI). Two final concentrations were assessed: 1 μg and 2 μg/disk. Blank paper disks were impregnated with 20 μl of each anidulafungin suspension (concentrations 50 μg/ml and 100 μg/ml), containing 1% DMSO and 0.1% P-80 (Jones et al. Diagn Microbiol Infect Dis, 2007). The media tested for DD method was Mueller-Hinton agar (MH) (Difco). The inoculated plates were incubated at 35ºC and read the inhibition zones diameters at the point of marked decrease in fungal density (IZ-2) at 24 and 48 h. The MD test was done according M38-A document of CLSI for filamentous fungi. Stock inoculum suspensions were prepared from 7-days-old cultures grown on potato dextrose agar and ajusted spectrophotometrically with saline solution to ~80% transmittance. The diluted inoculum sizes achieved a final concentration of ~10^4 CFU/ml. For the MD method we read the MEC (minimum effective concentration) at 48 hours. *A. fumigatus* ATCC 204305, *A. flavus* ATCC 204304, *C. parapsilosis* 22019 and *C. krusei* 6258 were included in each susceptibility test for quality control and assessment of reproducibility.
Results: The results are expressed in Table 1, as *Aspergillus* spp. (number of isolates) anidulafungin GM (geometric mean) of MEC (minimum effective concentration in μg/ml) for MD at 48 h; AM (arithmetic media) of IZ-2 in mm on MH 24/48 hours.

Table 1.

	MD		DD 1 μg				DD 2 μg			
	48 h		24 h		48 h		24 h		48 h	
	Range	GM	Range	AM	Range	AM	Range	AM	Range	AM
A. fumigatus (28)	⩽0.03	⩽0.03	25–37	31.3	25–35	29.1	25–45	36.5	25–41	31.8
A. flavus (18)[a]	⩽0.03	⩽0.03	22–36	30.8	22–30	27.8	22–37	32.3	22–35	29.1
A. terreus (17)[b]	⩽0.03	⩽0.03	23–32	29.9	23–32	27.9	24–36	31.5	24–35	29.9
A. niger (2)	⩽0.03		25–28		25–28		26–32		26–32	
A. glaucus (1)	⩽0.03		30		28		35			
Total (65)	⩽0.03	⩽0.03	22–37	30.3	22–35	28.4	22–45	33	22–41	30.4

[a] No growth observed in 2 strains at 24 hours.
[b] No growth observed in 3 strains at 24 hours.

Conclusions:
1. All the strains of *Aspergillus* spp. tested with anidulafungin got MECs ⩽0.03 μg/ml and IZs-2 ⩾22 mm at 24 h with disk of 1 μg on MH agar.
2. Disk of 2 μg produced very large zones, so the optimal concentration for anidulafungin is 1 μg/disk.
3. DD could be an useful method for testing the susceptibility of anidulafungin against *Aspergillus* spp. already at 24 h of incubation, although additional studies are needed to know the real applicability.

R2464 DHPS genotypes in *Pneumocystis jirovecii* infected patients from Spain

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Objectives: The absence of a reliable method to identify *Pneumocystis jirovecii* resistance to sulfa or sulfone drugs, has led to develop molecular techniques based on identification of punctual mutations at codon 55 and 57 on the Dihidropteroate Synthase gene (DHPS). Several studies suggest that these mutations are associated with the failure of prophylaxis and with the worsening of the *Pneumocystis* pneumonia (PCP) prognosis. The aim was to establish the frequency of DHPS mutations in Spanish patients infected by *P. jirovecii*.
Patients and Methods: The study includes 95 subjects were DHPS gene was typed. Among patients included, 35 of them suffered of *Pneumocystis* pneumonia (PCP) and 60 were colonised subjects without evidences of immunosuppression.
P. jirovecii was identified by nested PCR at mtLSUrRNA gene. DHPS polymorphisms were identified by Restriction-Fragment-Length-Polymorphism; digestion with Acc I and Hae III identify mutations at positions 55 and 57 respectively.
Results: The analysis provides a 22.1% rate of DHPS-gene mutations in our population, with the presence of all possible polymorphisms described. All polymorphism were found in a similary rate in both groups. The presence of mutations was(20% in colonised vs 25.7% in PCP patients.
The statistical analysis does not show differences due to age, sex; exposures to steroid treatment, or smoking habit when patients infected by *P. jirovecii* with or without mutations were compared.
Conclusions: This study shows the presence of DHPS mutations in our area, not only confined to patients who have followed chemoprophylaxis.
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R2465 Smoking influence in *Pneumocystis jirovecii* colonisation from patients with chronic obstructive pulmonary disease

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Objectives: Chronic obstructive pulmonary disease (COPD) is characterised by a continuous airflow limitation due to chronic bronchial inflammation. Currently, it has been described the colonisation by *Pneumocystis jirovecii* in patients with COPD, as well as reservoir and source of transmission. Several studies carried out in HIV infection patients have shown that smoking is a risk factor for developing

Pneumocystis pneumonia (PCP). On the other hand, in a mouse model of infection pneumonia has been found that nicotine reduces the rate of infection/colonisation by this microorganism. This fact can be explained because nicotine reduces s-adenosil-Methionine (AdoMet) levels, which is a key nutrient in the metabolism of *Pneumocystis*. However, the role that smoking can play in *Pneumocystis* colonisation remains unknown. To assess the relationship between smoking and *Pneumocystis* colonisation in patients with COPD.

Patients and Methods: From a previously study to determinate the prevalence of *Pneumocystis* colonisation in patients with chronic lung diseases, we selected those diagnosed with COPD based on GOLD guide and from the smoking habit information was available. Sputum samples from 238 patients were study. The presence of P Jirovecii colonisation was detected by specific nested-PCR based on mitochondrial region (mtLSUrRNA).

Results: A 15.5% of patients (37/238) were colonised by *P. jirovecii* being 75.6% (180/238) active smokers. From the smokers patients, 18.8% (34/180) were colonised by *Pneumocystis* compared with 3 out of 58 non-smokers (5.1%); $p = 0.02$. To evaluate the role of other factors favouring colonisation multivariate analysis was performed including sex, age, smoking habit, steroids consumption, levels of lymphocytes, presence of emphysema and FEV1%. From all these variables only active smoking habit was an independent risk factor for pneumocystis colonisation in patients with COPD (OR 3.6, 95% CI: 1.07–12.6).

Conclusion: Smoking habit produces a ciliary dysfunction being a risk factor for *Pneumocystis jirovecii* colonisation in patients with COPD.

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R2466 Prevalence and causative agents of tinea capitis among primary school students in Erzincan, Turkey

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Objective: Tinea capitis is a worldwide-spread infection of the scalp and hair caused by dermatophytes. Children between 3 and 9 years old are at highest risk and outbreaks have been noted in schools and day care centres. A variety of clinical presentations is recognised as being either inflammatory or non-inflammatory and is usually associated with patchy alopecia. Since the clinical appearances can be subtle, mycological examinations should be done to ensure accurate diagnosis. In the present study we aimed to determine the prevalence and the agents of tinea capitis among the primary school students in Erzincan, Turkey.

Method: A screening study was performed in 94 schools, covering a total of 19173 children with 9827 boys and 9346 girls, aged 6–14 years. Of the students 13855 were going to the schools in the city centre, and the others (5318 student) in the rural area.

All children were clinically examined and selected on the basis of symptoms indicative of tinea capitis infection. From both clinically evident and suspected cases skin scraping and hair samples were taken for microscopy and culture in order to confirm the diagnosis.

Samples were cultured onto Sabouraud Dextrose agar, incubated at 30ºC for up to 30 days and checked for fungal growth every week. At the same time, direct examination with KOH-DMSO was made to detect fungal structures. Grown isolates were identified using conventional methods.

Results: According to the clinical examination, 34 students were pre-diagnosed as tinea capitis, but only the 14 of them (9 boys and 5 girls) were confirmed by microscopy. In 11 of the cases, the clinical form was tinea capitis superficialis, and in the remaining 3 it was Kerion celsi. Most of the tinea capitis cases were from rural regions (11 out of 14). Causative agents were isolated in only 7 of the 14 samples of which microscopy were positive. The isolated 7 strains were identified as follows: Two were *M. canis* (28.5%), 2 were *M. audouineii* (28.5%), 2 were *T. tonsurans* (28.5%) and 1 was *T. violaceum* (14.5%).

Conclusion: In the study tinea capitis prevalence among the primary school students in Erzincan province was found as 0.073%. This result was generally in accordance with the previous studies made in the same region. But *T. tonsurans* was the first isolate in Eastern Anatolian while it was not reported as a causative agent in the previous studies. In contrary to the previous studies *T. schönleinii* could not be isolated in the present study.

R2467 Protothecal endocarditis in a dialysis dependent patient

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Background: Protothecosis is an uncommon infection in humans caused by achlorophyllic algae of the genus Prototheca. The majority of infections caused by these ubiquitous organisms involve the skin and subcutaneous tissue, and rarely cause systemic disease. Here we report a case of protothecal endocarditis in a patient dependent on dialysis via a native arteriovenous fistula.

Case report: A 56 year old female haemodialysis patient presented with fever, rigors and a rising C-reactive protein. Her extensive past medical history included chronic renal failure secondary to polycystic kidney disease, Crohn's disease with short bowel syndrome and subsequent total parenteral nutrition via Hickman line, recurrent Hickman line sepsis, polycystic liver disease and portal hypertension for which a TIPSS procedure was carried out and long-standing leukopenia of unknown cause. She had echocardiographic evidence of aortic valve vegetations. Prototheca wickerhamii was isolated from multiple blood cultures via the BacT/Alert continuous-monitoring system and identified to species level by the Rapid ID™ Yeast Plus System (Remel). Susceptibility and synergy testing was carried out by the Mycology Reference Laboratory, Bristol. She responded well to the administration of liposomal amphotericin 3 mg/kg/day and oral doxycycline 200 mg/day reflected by a drop in C-reactive protein from 100 to 15. Surgical valve replacement was considered unnecessary. No obvious source of infection was found.

Conclusion: Systemic protothecal infection is uncommon with only one other case of protothecal endocarditis reported in the literature. This case highlights several risk factors for infection and provokes some interesting questions regarding appropriate management. We report a good clinical response to treatment with intravenous liposomal amphotericin and oral doxycycline.

R2468 Isolation from blood cultures and susceptibility testing of *Candida* sp. strains during three years in a tertiary hospital in Greece

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Objectives: To study the isolation from blood cultures and the susceptibility of *Candida* sp strains to antifungal drugs in a tertiary Hospital in Greece, during a three year period (1/1/2004–31/12/2006).

Methods: During the study period blood cultures from 15723 patients were sent to the laboratory. The vials of the blood cultures were incubated in BACTEC 9240 (BD) and BacT/ALERT 3D (bioMerieux) automated systems. Positive vials were subcultured on conventional media. The strains were identified by VITEK 2 (bioMerieux) automated system. Susceptibility testing was performed on RPMI agar (E&O) by E-test (AB Biodisk).

Results: During three years' period, blood cultures from 2605 patients were found to be positive (2605/15723, 16.57%). *Candida* sp strains were isolated in 71/2605 (2.7%) of these patients. The origin of the strains was from Internal Medicine Department 32/71 (45.1%), Surgical Department 24/71 (33.8) and Intensive Care Unit (ICU) 15/71 (21.1%). *Candida albicans* was the predominant strain (29/71, 40.8%), followed by *C. parapsilosis* (15/71, 21.1%), *C. glabrata* (13/71, 18.3%), *C. tropicalis* (7/71, 9.8%), two strains of *C. famata*, two strains of *C. lusitaniae* and *C. krusei*, *C. dubliniensis* and *Candida* sp one strain each. Susceptibility was tested in 50 strains. The resistance rate of the isolated strains was: itraconazole 29%, fluconazole 22%, voriconazole 7%, fluocytosine 6%, while there was no resistance to caspofungin and amphotericin B.

Conclusions:

1. The rate of candidaemia in our hospital is approximately 3% of the patients with positive blood cultures.

2. *C. albicans* represented 40% while non-*albicans Candida* composed 60% of the total.
3. There was a high resistance rate to itraconazole and fluconazole, but also resistance of lower rate was observed to voriconazole and fluocytosine, while there was no resistance to caspofungin and amphotericin B.
4. In cases of candidaemia antifungal therapy according to the susceptibility test of the isolated strains is warranted.

AIDS and HIV infection

R2469 HIV-related morbidity in the HAART era

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AIDS related hospital admissions and morbidity have decreased after the introduction of HAART.

Objective: To assess the changes in hospital admissions and morbidity with the improvement of HARRT.

Methods: We studied the hospital admissions and morbidity over three different periods pre-HAART (January 1992 to December 1994), early-HAART (January 1995 to December 1998) and post-HAART (January 1999 to October 2006), in HIV infected patients followed at our centre. Results are expressed for periods pre-HAART, early HAART, and post-HAART respectively. Mean (SD) CD4/mL were: 16±5, 37±11 and 118±33 ($p < 0.0001$). Undetectable plasma viral load (%) were: 0, 16 and 43 ($p < 0.0001$). Patients with a prior diagnosis of AIDS were: 91%, 68% and 36% ($p < 0.001$). The rate (%) of women/heterosexuals was: 6/2, 11/5 and 21/11 ($p < 0.01$) and, that of IVDA (%) was: 96, 88 and 75 ($p = 0.03$). Hospital admissions due to AIDS-defining illnesses decreased ($p < 0.001$) with a significant increase in the rate of respiratory tract infections ($p < 0.005$), digestive tract ($p < 0.01$) and liver diseases ($p < 0.001$). The proportion of AIDS-defining illnesses decreased after HAART ($p < 0.01$), whereas the rate of liver diseases increased ($p < 0.001$).

Conclusions: The HAART era has been associated with a progressive decrease in hospital admissions due to AIDS-defining conditions, and a steady enhancement of the spectrum of admissions by non-AIDS-defining conditions has increased.

R2470 CCR and HERV polymorphism in HIV-1 infection: Polish population analysis

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Objectives: Polymorphism in many loci affects susceptibility to HIV-1 infection. The allelic forms of chemokine receptor CCR5 and CCR2 genes are known to be of importance. However, the exact significance of a particular allele in different populations and groups of patients is not always established. Some of human endogenous retrovirus (HERV) sequences have structural and/or functional similarity with HIV sequences. Because of that there is possibility of positive or negative effect on course of HIV-1 infection. Some of HERVs (HERV-K113 and HERV-K115) encode protein within the env gene, termed Rec. This protein is a functional counterpart to the Rev protein of HIV.

In our investigation we determined frequency of CCR5-delta32, CCR2–64I alleles and HERV-K113 and HERV-K115 sequences in the HIV-1-positive patients in Lower Silesia Region of Poland in comparison to exposed uninfected people (EU) and healthy, unexposed people from control group from that region.

Methods: The genotype was determined by PCR method. In the case of CCR2 two pairs of primers and PCR-RFLP method was used. The genotype was determined by PCR method with three reaction for HERV-K113 and four for HERV-K115.

Results: Frequency of CCR5-delta32 and CCR2–64I alleles in Polish population was found to be 11.9% and 12.8%, respectively (healthy, non-infected, non-exposed to HIV individuals). In HIV-1-infected patients frequency of CCR5-delta32 allele was reduced (7.9%). On the contrary, higher CCR5-delta32 allele frequency was found in the group of exposed, uninfected individuals (16.7%). Comparable frequency of CCR5-delta32 allele was found in homosexual and drug using HIV patients (9.6% and 9.2%, respectively). In the case of heterosexually-transmitted infection the frequency was lower (4.4%). Frequency of CCR2–64I allele was found to be 12.5%. Frequencies of CCR2–64I, HERV-K113 and HERV-K115 in Polish population were found to be 12.5%, 13.6% and 8.4% respectively. In the case of these alleles no significant differences between control, exposed uninfected and HIV-1-infected groups were detected.

Conclusion: According to the obtained results individuals who had CCR5-delta32 allele were significantly susceptible to HIV-1 infection, especially in the case of heterosexual transmission. Presence of CCR2–64I allele didn't protect against HIV-1 infection. Neither CCR5-delta32 and CCR2–64I nor HERV-K113 and HERV-K115 had influence on progression of HIV-1 infection.

R2471 Detection of active polyomaviruses JC and BK in HIV-1-infected patients

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Objectives: Up to 90% of human population is infected with two human polyomaviruses JC (JCV) and BK (BKV). After primary infection, which occurs during childhood, both viruses persist in renal tissues and B lymphocytes. Reactivation of these viruses may be connected with immunodeficiency or immunosuppressive therapy. JCV is the causative agent of the progressive multifocal leukoencephalopathy in AIDS patients as well as in other immunocompromised hosts. BKV is associated with haemorrhagic cystitis in immunocompromised patients. The aim of the study was to evaluate frequency of active JCV and BKV infections and association between these infections and lymphocyte CD4+ count among HIV-1 positive patients.

Methods: Polyomavirus were detected in plasma, urine and cerebrospinal fluid with PCR-RFLP method. Viral DNA from samples was extracted and purified on Qiagen column according to the manufacturer's protocols. The primers pair amplified a 176-bp sequence from BKV genome and a 173-bp sequence from JCV genome. Digestion of the PCR products with the BamH1 prior to electrophoresis was used to discriminate between BKV and JCV sequences.

Results: Three groups of patients were studied. The first group was comprised of 53 HIV-1 positive patients with CD4+ cell count less than 200/mcl. In the second group 21 patients with CD4+ cell count 200–500/mcl were included. The third group was comprised of 22 patients with CD4+ cell count >500/mcl. JCV was detected in urine of 8 patients in the first (15%), 3 patients in the second (14.3%) and 5 patients in the third group (22.7%). BKV was detected in urine of 9 patients in the first (17%), 1 in the second (4.7%) and 1 in the third group (4.5%). Beside it, two patients in the first group had detectable polyomaviruses in plasma: in one case JCV (1.9%), and in the second BKV (1.9%). The viruses were not detected in cerebrospinal fluids of 10 patients with neurological symptoms. There was no statistically significant differences between activated JCV and BKV presence in tested groups. On the other hand, no polyomaviruses were found in the control group (20 healthy, not HIV-infected individuals).

Conclusion: According to the obtained results no correlation between the active infections of JCV and BKV and the level of CD4+ cells was found.

R2472 HIV infection presenting in older children: experience from Cambodia

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Objectives: Because HIV infection is poorly described in older children, we investigated social and clinical features in HIV-infected children aged 8–18 years from the cohort of 87 HIV-infected Cambodian children.

Methods: All children and their guardians have consented to participate in the study, have the basic examination including height, weight, social status, clinical examination and laboratory tests (blood tests, liver enzymes, basic biochemistry, CD4 count, TB check). HIV test was always confirmed at the National Institute of Public Health (referral laboratory in Phnom Penh) before the beginning of the treatment.
Results: Of 87 children treated for more than 3 years, 42 were in the age between 8–17 years (average 10.6 years). 11 of these children (26%) are outdoor patients and 31 (74%) are indoor patients living in House of Family and House of Smile – orphanages for HIV positive children in Phnom Penh. Males are 23 (55%) of the children, 31 (74%) of children are complete orphans and 11 (26%) are semi-orphans. None of the children has both parents alive. All the living parents are HIV positive and all of the death parents died of HIV infection.
CD4 count before the treatment was in the range from 2 cells/μl to 875 cells/μl with average 158 cells/μl. At the beginning of the treatment 40 children (95%) had respiratory infections and 27 (64%) of them had recurrent respiratory infections after beginning of the treatment. Chronic skin infection was present in 36 (86%) of children. TB of lungs was diagnosed and treated in 17 (40%) cases, 1 child had TB of bowels. Oropharyngeal candidiasis was present in 11 (26%) cases. 1 child is now treated for lymphoma.
Treatment was started with first line ART available in Cambodia. 28 (67%) of children are treating with combination of Stavudine + Lamivudine + Nevirapine. Those contraindicated to Nevirapine therapy are receiving Efavirenz. Length of the treatment varies from 1 to 40 months (average 25.6 months). CD4 count in treated children varies from 54 to 1699 cells/μl with average of 781 cells/μl which is 5 times more than on the beginning of the treatment.
Conclusion: All the children in our project who received HAART did extremely well and medical doctors as well as social workers are successful in their effort to help HIV-infected children in Cambodia.

R2473 Analysis of clinical variables for determining the caries risk in patients with HIV/AIDS

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Aim: The aim of the present study was to evaluate the caries risk in patients HIV+ by analysis of the following clinical variables: MDF index, stimulated salivary flow, buffer capacity, and presence of dental plaque.
Methods: Forty HIV-soropositive patients (20 men, 20 women) and 40 HIV-soronegative patients (20 men, 20 women) with a mean age of 58 years (range, 50–70 years) from the Special Patient Care Center, Paulista University, Brazil, were evaluated. After clinical examination, the MDF index and the presence of dental plaque were recorded. In addition, the stimulated salivary flow and the buffer capacity were measure by using a Dental Buff (Inodon, Brazil) system.
Results: In the HIV+ patients, 70% had low salivary flow (<1 ml/min), 22% intermediate (1–1.5 ml/min) and 8% regular (1.6–2.3 ml/min). In the control group, 25% had low salivary flow, 27.5% intermediate, and 47.5% regular. On the other hand, 35% of HIV+ patients had a low buffer capacity (pH < 4.5), 37.5% intermediate buffer capacity (pH 4.5–5.5), and 27.5% regular buffer capacity (pH > 5.5). In the control group, 22.5% had low buffer capacity, 5% intermediate, and 72.5% regular. In relation to MDF index, it was 22.12 in the HIV+ patients and 23.4 in the control group. The dental plaque index was 69.96% in the HIV+ patients and 67.05% in the control group. There was no significant difference when both groups were compared in relation to MDF and dental plaque indexes.
Conclusions: Salivary flow was low in the HIV+ patients. The pH of these patients was critical when compared to that of HIV− patients. In summary, the low salivary flow associated to low buffer capacity and to high plaque index renders to the HIV+ patients a high caries risk.

R2474 Kinetics of HIV-specific CD8+ T-cells in HIV-infected adults: one-year follow-up

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Objectives: HIV-specific CD8+ T cells play a significant role in immunopathogenesis of HIV infection. Further knowledge of their role could be applied in vaccination strategies and immunotherapy of HIV infection. The aim of this study was to analyse the frequency of HIV-specific CD8+ T cells in HIV+ patients during one year follow-up and assess the effect of antiretroviral therapy (ART) on the frequency of these cells.
Methods: In this prospective study, a total of 58 HIV+ patients were enrolled based on their HLA-A2 haplotype. This group included 33 patients on ART (group A), 13 previously non-treated patients in which ART was introduced after study enrollment (group B) and 12 patients without therapy (group C). The following parameters were analysed at baseline and after 12 months: CD4+ and CD8+ T cell count, HIV-1 viral load and the percentage of HIV-specific CD8+ T cells. HIV-specific CD8+ T cells were detected using staining with HLA*0201/gag tetramers and flow cytometry analysis.
Results: The table presents percentage of patients with detectable levels of HIV-specific CD8+ T cells and median viral load. The comparison of the median frequency of gag-specific CD8+ T cells showed no difference between all groups both at baseline (0.05%, 0.06% and 0.135% in A, B and C, respectively) and after 12 months (0.05%, 0.12% and 0.185%, respectively). Moreover, no significant changes were observed in the frequencies of gag-specific CD8+ T cells over the study period in any group. Finally, lack of correlation was found between the frequency of HIV-specific CD8+ T cells and CD4+ count as well as viral load.

Group	No. (%) of patients with detectable gag-specific CD8+ T cells		HIV-1 viral load (copies/ml)*	
	Baseline	12 months	Baseline	12 months
A (n = 33)	11 (33.3%)	13 (39.4%)	50	50
B (n = 13)	7 (53.8%)	8 (61.5%)	60400	50
C (n = 12)	8 (66.7%)	7 (58.3%)	9800	5135

*Median.

Conclusion: The results demonstrated that the higher percentage of non-treated HIV+ patients has detectable gag-specific CD8+ T cells in comparison to ART-treated patients. Also, the frequency of gag-specific CD8+ T cells is not significantly influenced by ART despite of its effect on the decrease of viral load.
Acknowledgement: The study was supported by the grants GAUK Nr. 18/06 and GACR Nr. 310/05/H533.

R2475 Prevalence of and risk factors for anaemia on the medical wards of a central hospital, Blantyre, Malawi

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Introduction: To study the prevalence and determinants of anaemia and to test if anaemia was a risk factor for morbidity and mortality in patients admitted to the medical wards of the Queen Elizabeth Central Hospital, Blantyre, Malawi.
Methods: All patients admitted to the medical wards between April and July 2007 were included. Patients' presenting clinical features, dietary intake, mid upper arm circumference (MUAC), full blood count (FBC), HIV status and HAART use, duration of hospitalisation and final diagnosis on discharge or death were registered. Proportions were compared using chi-squared tests, continuous data were analysed using Mann-Whitney's test. Multivariate logistic regression analyses were employed to look for independent risk factors for anaemia, duration of hospitalisation and mortality.

Results: 907 patients were included; in 676 complete FBC were available and included in the study. Median Hb in males was 11.0 g/dL (2.0–19.5) and 9.7 g/dL (2.5–18.4)) in females. According to the WHO criteria 490 patients were anaemic (72.5%). 348 patients were HIV positive (78.7%). Median Hb in HIV negatives positives was 12.5 g/dL (2.0–19.5) versus 9.2 (2.5–18.4) in HIV positives ($p < 0.001$). Within the group of HIV positive patients on HAART, median Hb and MCV was significantly higher in those on HAART for 6 months or more (Hb 11.3 [6.1–15.3]; MCV105.0 [61.0–131.0]) as compared to those on HAART for less than 6 six months (Hb 9.0 [2.9–18.4; MCV 94.0 [66.0–121.0]; $p = 0.002$ and $p = 0.001$ respectively). Median MUAC and dietary score were significantly lower in the anaemic group(213.0 [143.0–340.0] and 6.0 [4.0–11.0]) compared to the group with no anaemia (241.0 [135.0–529.0] and 6.0 [4.0–11.0]; $p < 0.001$). The discharge diagnoses TB (OR 3.1 95% CI [1.9–4.9) and sepsis (OR 2.3 95% CI [1.5–3.8]) were also associated with anaemia. Duration of hospitalisation was significantly longer in anaemic patients (7.0 [1–36] vs 5 [1–37] days in non anaemics; $p = 0.003$). Anaemic patients had a significant increased risk for in-hospital mortality (OR 2.3 [1.6–3.5]).

Conclusion: Anaemia as well as HIV are very prevalent in patients admitted to the medical wards of the QECH. In this cohort, HIV, malnutrition, TB and sepsis are all associated with anaemia. Anemic patients have an increased duration of hospitalisation and mortality when compared to non-anemic patients. Both HIV and TB are in essence treatable diseases and treatment with HAART was shown to 'protect' from anaemia.

R2476 Detection of human papillomavirus in HIV-positive Venezuelan patients

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Objective: The aim of this work was to evaluate the association between the HIV infection and the genotypes of HPV in genital and anal lesions.

Methods: Patients: Thirty eight patients with a diagnosis of HIV infection (thirty seven men and one woman) were rolled after informed consent and complete physical examination.

Nineteen biopsies of the genital area and eighteen anal swaps were obtained for HPV test. The amplification of 450 Pb fragment of HPV DNA included in the open reading frame of L1 region was performed using the degenerate primers MY09 and MY11. To determine specimen adequacy, the GH20/ PC04 human beta-Globins target was co amplified with HPV sequences. A known positive specimen and a negative specimen were included in each assay as controls. The typing of HPV was determinated by MPCR Amplification (Maxim Biotech, Inc.) that simultaneously detects the HPV types 6, 11, 16, 18 and 33. The MPCR DNA products were separate eletrophoretically on a 2% agarose gel containing ethidium bromide. The CD4 count and viral load were evaluated in 18 patients of the study group.

Results: HPV infection was detected in 35 HIV infected patients (95%). The 19 biopsies of genital area were histological diagnosed as condylomata acuminate. The location of these lesions was observed with more frequency in the perianal area (57.89%), followed of penis (36.84%) and vulva (5.27%). The HPV was detected in all specimens of condylomata acuminate, the type 11 was observed with more frequency (63.16%), followed by the type 6 (21.05%), three cases (15.17%) was none typified and neither case was observed co-infection with types of high risk oncogenic. In contrast, HPV 16 genotype was found in 16/18 of the anal swaps samples.

We did not find any correlation between CD4 cell count, HIV RNA viral load and the presence of specific HPV genotype.

Conclusion: In this study the results show a clear association of the low oncogenic risk types 6 and 11 and the development of the genital warts. Our data showed that in our population of HIV infected men, there is a high prevalence of HPV 16 genotype in anal samples. A screening programme to detect anal squamous intraepithelial lesions in high-risk individuals may be of value to prevent anal cancer.

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R2477 Increased expression of Werner and Bloom helicases in peripheral blood mononuclear cells of HIV-1 infected patients

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Objectives: WRN and BLM helicases are two crucial proteins involved in DNA metabolic pathways, such as cell repair, replication, and ricombination. We previously described increased levels of WRN and BLM gene expression in PBMC from HIV-infected persons (Bordi L, et al., Human Immunology 68, 2007). The role of WRN as cofactor for HIV-1 transcriprtion and replication has been also demonstrated by Sharma A, et al. (J Biol Chem 16, 2007). Aim of the study is to analyse the steady-state level of Werner (WRN) and Bloom (BLM) mRNA and protein helicases in PBMC and CD4 and CD8 positive T subsets obtained from a larger number of HIV-1 infected patients and HIV-seronegative healthy donors.

Methods: The mRNA expression level of WRN and BLM genes and wrn and blm proteins were investigated in ex-vivo PBMC obtained from 70 HIV-1-infected patients and 27 HIV-seronegative healthy donors (HD). WRN and BLM mRNAs were measured by real-time PCR; wrn and blm proteins were analysed by immunocitochemistry, both in total PBMC and in different subsets of PBMC (CD4-positive and CD8-positive T lymphocytes, CD14-positive and residual cells) isolated by magnetic beads.

Results: In total PBMC from HIV-1 infected individuals, 3.2-fold higher mean level of WRN mRNA (mean +SEM: 1.004 +0.07) has been detected in comparison to HD (0.315 +0.020) and this difference was significant ($p < 0.0001$). BLM mRNA mean levels were slightly higher than in controls (0.165 +0.0162 versus 0.419 +0.052, respectively), but the difference was equally significant ($p = 0.018$). WRN increase (but not BLM) resulted to correlate positively with CD4 and CD8 T cell absolute numbers. CD4 and CD8 T cells were the main subsets containing higher levels of WRN mRNA in HIV-infected patients (in comparison to HD), while no differences were observed within subsets from HD and HIV-infected persons for BLM. Analysis of wrn and blm proteins in different subsets are still in progress.

Conclusion: The increase in helicases expression observed in T cells from HIV-infected persons could be a mechanism aimed to prevent hyper-recombination, transformation and premature senescence in replication-competent cells, and therefore, it may be explained as a modification induced by the virus finalised to assure the host genomic integrity and an efficient viral replication.

R2478 Bone mineral density in HIV infection

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Background: Anti-HIV therapy is associated with an increased prevalence of osteopenia and osteoporosis. Nevertheless, the relationship between the duration of anti-HIV therapy and bone mineral density (BMD) is not established.

Objective: To determine the relationship, if any, between the duration of anti-HIV therapy and bone mineral density.

Methods: Prospective study of BMD in 108 HIV-infected patients, compared with 239 HIV-uninfected controls, from January 2002 to December 2006 at a teaching hospital (median follow-up of 3.86 years).

Results: A total of 108 HIV-infected patients were studied, 76 men (age: 38±11 years, CD4: 351±119/μL) and 32 women (age: 44±16 years, CD4: 368±127/μL), of them 57 initiated HIV therapy in the pre-HAART era (52.8%), 36 (33.3%) and 15 were naive (13.9%). None of the patients received treatment for osteoporosis. Osteoporotic fractures were not observed during follow-up. 66 patients (61%) had osteopenia, and 14 had osteoporosis (13%), with an OR of 5.9 and 3.2, respectively compared to controls. HIV treated patients had a 2.9-fold increased odds of a reduced BMD compared to naive HIV patients. HIV patients treated with PIs had a 4.3-fold increased odds of a reduced BMD compared to

patients who did not receive PI treatment, and a 6.8-fold increased odds of a reduced BMD compared to naive HIV patients. Factors associated with a reduced BMD in multivariant analysis were treatment with PIs [RR (95% CI), 2.27 (1.88–2.39), p = 0.03), and time on HIV therapy [RR for <2 years on HIV therapy: 2.33 (1.93–2.68), RR for 2–4 years on HIV therapy: 5.17 (4.52–6.38), and RR for 4 years on HIV therapy: 8.74 (7.06–10.12), p < 0.001 in all cases). Sex, body mass index, CD4 levels or, viral load were not related with the BMD.
Conclusions: HIV infected patients have an increased prevalence of osteopenia (6-fold), that, is associated with the time on anti-viral treatment, and with PIs' therapy.

R2479 An epidemiological and clinical survey of 298 HIV-positive patients in 3 centres, Tehran, Iran

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Objectives: The total number of registered HIV-infected cases was 13,702 by mid-September 2006 in Iran, and the most frequent route of transmission was injection drug use. There are more new HIV infections every year, so we have to know more about the epidemiology of HIV in Iran.
Methods: In this retrospective descriptive cross-sectional study, the subjects were the HIV positive patients who had been visited in two teaching hospitals (Rasoul-e-Akram and Bu-Ali)and Tehran West Clinic from March 2000 to March 2005. Demographic data, risk factors and clinical symptoms were determined in 298 HIV positive patients.
Results: The mean age of the patients was 33.2 years(±8.45). 269 cases(90.3%) were male and 29 cases(9.7%) were female.231 cases(77.5%) all of whom were IDUs with a history of shared drug injection, had a history of staying in prison. Injection drug use and unsafe sexual contacts were the possible major routes of transmission of HIV infection. The most common clinical diseases were Thrush and pulmonary tuberculosis. HBsAg and HCV RNA were detected in 7% and 29.5% of the patients respectively. There were 38(12.8%) PPD-positive patients. VDRL and FTA-abs in 2 cases were positive. The mean CD4+ count (±SD) was 500.31/mm3 (± 382.87).
Conclusion: Our findings suggest that drug injection inside prison carries a particular risk factor for HIV infection and we need HIV-related programmes to strengthen HIV surveillance and introduce harm reduction programmes to IDUs. Such programmes should be integrated with a condom promotion programme to support the well-being of young Iranian population.

Hepatitis

R2480 Hepatitis C virus infection in patients from the haemodialysis clinic of a medical university, Plovdiv, Bulgaria

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Objectives: It has been reported, that patients with end-stage renal disease on dialysis therapy form a high risk group for hepatitis C virus (HCV) infection. Risk factors such as number of blood transfusions and frequent exposure to parenteral interventions have been pointed. The aim of our study was to determine the prevalence of HCV infection in a caseload of Bulgarian patients on permanent hemodialysis and to assess risk factors for HCV in this group.
Methods: All subjects were tested for HCV antibodies by ELISA method, as well as for HCV-RNA by real-time PCR. Liver function tests (ALT) were performed by routine methods and epidemiological data (age, sex, ethnic group, duration on hemodialysis, number of blood transfusions) were collected.
Results: A total of 85 patients (mean age 52.6 years; male/female: 48/37), visiting three times weekly Hemodialysis clinic of Medical University in Plovdiv were enrolled. HCV antibodies were found in 41(48.2%) and viraemia – in 29 (34.1%) subjects, thus the overall prevalence of HCV (HCV antibody and/or HCV-RNA positivity) was 51.8% (44 patients). HCV viraemia was detectable in 63.4% of the anti-HCV-positive patients and in 6.8% of the anti-HCV-negative patients. Hence 3.5% of the whole study group were HCV infected, but could not be diagnosed by routine HCV antibody testing. Duration of dialysis and number of received blood transfusions were established as main risk factors for acquiring HCV infection.
Conclusion: HCV prevalence is high in hemodialysed patients and nosocomial spread of hepatitis C virus is suggested. These data indicate: (i) the need for strict adherence to infection control measures in dialysis units and (ii) the importance of screening by both PCR and serological methods at stated intervals to reveal all HCV infected patients.

R2481 Implication of basal core promoter/precore mutants upon genotype and clinical outcome in the hepatitis B virus

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Background and Aim: Hepatitis caused by HBV is a major health problem worldwide, with more than 350 million people affected. Understanding the implication of HBV's virologic characteristics is important in the management of the disease. Our aim was to study genotype prevalence and to determine basal core promoter/precore (BCP/PC) mutations in a cohort of 61 Andalusian patients with different clinical features.
Materials and Methods: 61 samples with viral load >1,500 IU/ml from 61 patients (73.8% men, 26.2% women) were studied. 88.5% were HBeAg– and 11.5% were HBeAg+. 59% patients were diagnosed of chronic hepatitis B (CHB), 31.1% of liver chirrosis (LC) and 9.8% were inactive carriers (IC). Viral load was measured by real-time PCR using the COBAS Ampliprep/Taqman technology (Roche Diagnostics). Genotypes were determined sequencing the surface gene using the Trugene HBV genotyping kit (Siemens). BCP/PC mutations were determined by semi-nested PCR amplification (using primers RMD26–Ci1 in the first round and RMD26–PC1 in the second round) and sequencing of the core gene using the 7-Deaza-dGTP-Cy5/Cy5.5 Dye Primer Sequencing Kit (Siemens). Statistical analysis were performed with SPSS version 14.0.
Results: 60.7% were genotype D, 34.4% genotype A, 3.3% genotype F, 1.6% genotype B and 1.6% genotype F. Only patients with CHB, LC and genotypes D and A were included in this study. BCP and/or PC mutations were detected in 83.4% of CHB patients, 95% of LC patients, 92% of genotype D and 81% of genotype A patients. The most abundant BCP/PC mutations are shown in Table 1.

Table 1

	CHB	LC	Gen.D	Gen.A
BCP				
T1753C	41.7%	52.6%	**59.5%**	**19%**
A1762T–G1764A	55.6%	73.7%	59.5%	42.8%
G1764A	8.3%	10.5%	2.7%	19%
C1766T	5.6%	21.1%	5.4%	19%
T1768A	**2.8%**	**31.6%**	10.8%	14.3%
PC				
C1857T–G1897A	2.8%	–	–	4.8%
G1896A	61.1%	57.9%	**78.4%**	**28.6%**

In the statistical analysis, we compared these mutations with genotypes and clinical status. Statistical significance ($p \leqslant 0.005$) was observed between T1753C, G1896A and genotype D and between T1768A and patients with LC.
Conclusions: 1 – HBV genotype D is the most prevalent in Andalusia, followed by genotype A. 2 – Double mutation A1762T–G1764A were the most frequent changes in the BCP region and G1896A in the PC region. 3 – Due to the predominance of genotype D in our population

and its association with mutations T1753C and G1896A, high prevalence of these are expected in those areas where genotype distribution is similar to ours. 4 – Although we could associate mutation T1768A and chirrotic status, more studies are necessary to confirm our data and to establish a clear relationship between this and other mutations with clinical outcome.

R2482 Changes in serum lipid profile during and after pegylated IFN and ribavirin therapy in chronic hepatitis C

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Objectives: chronic Hepatitis C is one of the most significant health problems worldwide. At present, the most effective therapy available for the treatment of HCV infection is the combination of pegylated IFN (pegIFN) and ribavirin and using such therapy is associated with a wide range of systemic side effects and complications such as metabolic changes. In fact, its use has been associated with a change in serum triglyceride and cholesterol levels.
Therefore, we designed this study to investigate changes in serum lipid concentrations during and after therapy with pegylated interferon in combination with ribavirin in these patients.
Methods: 71 patients with active chronic hepatitis C were enrolled in this study and received Pegylated interferon at a dose of 180 μg in combination with ribavirin at a dose of 1000–1200 mg, daily for 48 weeks. Blood samples were collected before, during (3 months after the start of), at the completion of and after (6 months after the completion of) IFN therapy.
Results: 65 male (91.5%) and 6 female (8.5%) with the mean age of 37.9 years were evaluated. mean serum triglyceride levels rose from 105 mg/dL to 129 mg/dL at the third month and 134 mg/dL at the completion of therapy ($p < 0.0001$). Afterward, mean serum triglyceride levels decreased to 130 mg/dL six months after stopping therapy.
Serum cholesterol levels had no significant changes during the same time period; however, decreased levels during the therapy were noted and returning levels to baseline were seen 6 months after stopping therapy. Thus, levels decreased from 154 mg/dL to 147 mg/dL at the third month and 145 mg/dL at the completion of therapy; subsequently, levels returned to baseline by 6 months after stopping therapy (mean 156 mg/dL).
Conclusion: We clarified that Serum triglyceride levels increases consistently in patients with chronic hepatitis C during therapy and returns to normal after it, while serum cholesterol level follows the opposite trend.

R2483 Hepatitis E in Cuba: the case report of an infected pregnant woman

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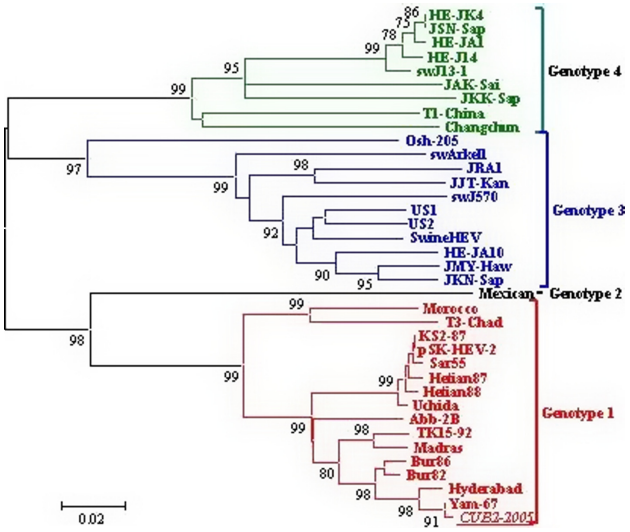
The HEV is transmitted primarily through the faecal–oral route in contaminated drinking water. It has been classified in the genus Hepevirus of the family Hepeviridae. Although there is only one serotype four major genotypes of HEV have so far been reported. Infection leads to high mortality rates among pregnant woman in their third trimester of pregnancy (20–25%). We report here the case of a 22 year-old Cuban pregnant woman with a gestational period of 35 weeks, admitted in 2005. She reported four days history of febrile episodes, asthenia, epigastric pain, nauseas, and vomiting. Her urine was dark-yellow and her faeces white. The patient had no previous jaundice, no international travel and no eats exotic food. Physical examination reveled icteric sclera, abdominal examination revealed an enlarged uterus according to gestational age and a fetal heart rate of 140 beats/min; the liver and spleen were not palpable. The results of biochemical indicators in blood showed 47.9 mmol/L creatinine, 358.1 mmol/L uric acid, 143.2 IU/L ASAT, 58.4 IU/L ALAT and 50 μmol/L of direct bilirubin. Coagulogram revealed a blood clotting time of 8 min, platelet counts 218×10^9 cells/L and retraction of the blood clot. The clinical signs improved and enzymatic tests returned to normal levels 4 weeks after onset of symptoms. The hepatitis A virus IgM, HBsAg, HBc IgM and anti-HCV were all negative. Anti-HEV IgM was found to be positive, total anti-HEV showed increasing levels in several determinations, which was a strong evidence of acute HEV infection. Viral genomes were detected in stool samples using primers specific for the open reading frame1, described by Zhai. PCR amplification of an RNA dependent RNA polymerase was used as a consensus genomic region for HEV genotyping. A phylogenetic tree (Fig. 1) was constructed for this sequence and the four major genotypes were defined. The Cuban strain CUB2–2005 clustered within the Asian genotype I and was closely related to Indian HEV strains. It shared 98% nucleotide homology with Yam-67 isolated in Yamuna Nagar.. This molecular characterisation of an indigenous HEV strain from the Americas showed for the first time the presence of HEV genotype I in this region. It is noteworthy that HEV shows a global presence and the genotype distribution, while dominant in a given geographic area, is not limited to it. The case reported in this letter had a satisfactory clinical course of her pregnancy and successfully delivered at 39 weeks.



Phylogenetic tree constructed on the basis of 307 nucleotides (RdRp) from ORF1 region using the Neighbour Joining method. Bootstrap values for 1000 replicates that were above 70% are shown in the internal nodes. The major branches represent HEV genotypes.

R2484 Recombination analyses in intrapatient populations of hepatitis C virus

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Objectives: Hepatitis C virus (HCV) is a major cause of liver disease worldwide and a potential cause of substantial morbidity and mortality in the future. HCV is characterised by a high level of genetic heterogeneity. Although homologous recombination has been demonstrated in many members of the family Flaviviridae, to which HCV belongs, there are only a few studies reporting recombination on natural populations of HCV, suggesting that these events are rare in vivo. Furthermore, these few studies have focused on recombination between different HCV genotypes/subtypes but there are no reports on the extent of intra-genotype or intra-subtype recombination between viral strains infecting the same patient. Given the important implications of recombination for RNA virus evolution, our aim in this study has been to assess the existence and eventually the frequency of intragenic recombination on HCV.

Methods: We retrospectively have analysed two regions of the HCV genome (NS5a and E1-E2) in samples from two different groups: (i) patients infected only with HCV (either treated with interferon plus ribavirin or treatment naive), and (ii) HCV-HIV co-infected patients (with and without treatment against HIV). The complete data set comprised 17712 sequences from 136 serum samples derived from 111 patients. Recombination analyses were performed using 6 different methods implemented in the programme RDP3.
Results: Recombination events were considered when detected by at least 3 of the 6 methods used and were identified in 10.7% of the amplified samples, distributed throughout all the groups described and the two genomic regions studied. The resulting recombination events were further verified by detailed phylogenetic analyses.
Conclusions: Recombination must be considered as a potentially relevant mechanism generating genetic variation in HCV and with important implications for the treatment of this infection.

R2485 Case report: chronic hepatitis B infection susceptible to adefovir despite the rtI233V mutation

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The mutation I233V in HBV polimerase has been associated with primary resistance to adefovir dipivoxil treatment (ADV). We report a chronic hepatitis B which responded to ADV although bearing I233V.
Italian man born in 1930, affected by HBeAg-negative chronic hepatitis B; HDV-HCV-HIV negative. He had a bronchial adenocarcinoma resection in 2001 and is on chronic treatment with interleukin-2 for the first week of each odd month since then. Hepatitis B was unsuccessfully treated with interferon in 1998. Treatment with lamivudine 100 mg qd, started in November 1999, initially suppressed viraemia but a viral breakthrough occurred at 3 years of therapy and lamivudine was stopped. In May 2003: HBVDNA was 20,680,000 IU/ml (TaqMan Roche); ALT 122 IU/L; the pol gene sequencing and clonal analysis showed that all clones analysed had both I233V and S159T mutations, Y141H and V142G were present in 6/11 clones, M204V in 6/11 clones (3 in association with L180M). ADV 10 mg qd was started in June 2003. At one year of treatment: HBVDNA was 971,000 IU/ml; ALT 50 IU/L; sequencing and clonal analysis were unchanged compared to baseline, except that M204V was detected only in 1/11 clones. ADV was stopped in September 2004 (15 months of therapy). In November 2004: ALT increased to 111 IU/L; HBV DNA decreased to 127,000 IU/ml; genotypic analysis showed that I233V, S159T, Y141H, V142G and M204V were present in almost all clones. ADV was restarted in February 2005 and it is ongoing. At the time of ADV restart ALT had spontaneously declined to 40 UI/L, and HBV DNA to 700 IU/ml; I233V, S159T, Y141H and V142G were present in almost all clones, M204V was not detected. During this second course of ADV ALT are always normal; HBVDNA is always <12 IU/ml; HBsAg is still positive.
HBVDNA had a 1.3 Log decline of at one year of ADV despite I233V mutation. HBVDNA further decreased when ADV was stopped and it is persistently undetectable during the 2nd course of ADV. It seems that the patient shifted from a highly replicative to an inactive carrier status. Other mutations detected (S159T, Y141H, V142G) might play a role in reducing viral fitness and possibly restore ADV antiviral activity. Complementarily, interleukin-2 regularly taken by the patient might have enhanced ADV activity and played a role in this unexpected "change of status".
The real impact of I233V mutation on ADV anti-HBV activity needs to be better defined, especially when in combination with other mutations.

R2486 First complete genome of a hepatitis C virus genotype 1g isolate

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Objectives: In our study, we present the first complete genome sequence of an HCV subtype 1g isolate. Hepatitis C virus isolates have been classified into six main genotypes and a variable number of subtypes within each genotype, mainly based on phylogenetic analysis. Although the most reliable analyses of genetic relationships among genotypes and subtypes are performed using complete genome sequences (or at least the full coding region), up to now only 31 out of 80 confirmed or proposed subtypes have at least one complete genome available.
Methods: Virus genome was isolated from a serum belonging to a Spanish woman that received a blood transfusion in 1996. Serum was obtained before pegylated interferon plus ribavirin treatment to which the patient didn't respond. Complete HCV-1g genome was obtained by direct sequencing of ten overlapping RT-PCR fragments. ClustalW implemented in MEGA was used to obtain a multiple alignment. All phylogenetic trees were constructed by maximum likelihood in PHYML with the nucleotide substitution model that best fit the data for which we used the procedure implemented in Modeltest. The robustness of the tree topology was assessed by bootstrap analysis with 1000 replicates implemented in PHYML.
Results: The nucleotide sequence of the HCV-1g genome (9490 nt) includes the complete coding region and partial sequences from both 5′UTR and 3′UTR. Phylogenetic and genetic distance analyses reveal that HCV-1g is the most divergent subtype among the HCV-1 confirmed subtypes. HCV-1g genome does not arise as a recombinant form of previous known genotypes or subtypes.
Conclusion: In light of this, we propose to change from provisional to confirmed the current status of its subtype-specific designation.

R2487 Different response to anti-HCV treatment may be related to immunological gene expression profile in chronically HCV-infected patients

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Objectives: Hepatitis C virus (HCV) is estimated to infect approximately 170 million people worldwide, being the leading cause of chronic liver diseases in many countries. Without an effective vaccine for HCV, our weapons in the fight against HCV infection are limited to antiviral treatments. Combination therapy with pegylated interferon alpha (peg INF-alpha) and ribavirin is currently the standard treatment for patients with chronic hepatitis C however, this treatment is still limited by a high non-response rate. The mechanism by which peg INF-alpha and ribavirin therapy induces resolution of chronic HCV infection is not fully understood. As immunological mechanisms behind response differences still not completely known, we approached to immunological gene expression profile of chronically HCV-infected patients. Gene expression was accessed in eight chronically HCV-infected patients (four responder and four non-responder patients) during peg IFN-alpha and ribavirin treatment.
Methods: Peripheral blood mononuclear cells were collected before treatment, and one month after starting therapy. RNA isolated from PBMC, was reverse transcribed and gene expression relative quantification was performed by real-time PCR, responders vs non-responders patients.
Results: Our results show differences in various genes expression levels between responders and non-responders patients in pre-treatment and one month after starting therapy. In pre-treatment we observed Increments (1–2 logs) on IL-12p40, IL-2, IL-5, IL-8, FAS, FASL, HLADRB1, GZMB, MADH3 and a lower expression of IL6, IL-17, IL-18, CD4 in responders vs non-responders patients. One month after starting therapy we observed a higher expression of IL-3, IL-6, IL-18, CD4, C3, CCR4, cox-2, HLA-DRB1, IKB2, MADH3 and a lower expression of IL-10, IL-12p35, IP-10, ITAC, MCP-1, MIP-1a in responders vs non-responders patients.
Conclusions: These results point out that response to therapy may be related to differences in expression of genes involved in effectors mechanisms of viral elimination, suggesting potential markers for future approaches. Further prospective large studies are needed to confirm these observations.

R2488 Genetic variability of hepatitis B virus "a" determinant in Iranian isolates

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Objectives: Hepatitis B virus (HBV) persistently infects more than 350 million people worldwide and can induce a spectrum of acute and chronic liver diseases. Because HBV is a DNA virus with a reverse transcription step, it is subject to a high mutation rate. HBsAg is the primary diagnostic marker for HBV infection. The "a" determinant of HBsAg is a highly conformational, immunodominant antigenic determinant and is common to all subtypes. There is very limited knowledge about the genetic variability of HBV strains circulating in the population of Iranian HBV infected patients. The aim of this study was to determine HBV subtypes and to analyse mutations in HBV "a" determinant among chronically infected patients from different regions of Iran.
Methods: 249 sera from HBV infected patients living in different regions of Iran were collected between January 2004 and July 2007. Serological markers of HBV infection were determined by available commercially ELISA kits. A 402 bp fragment of the HBV S gene including the "a" determinant was amplified, sequenced and then subjected for genetic variability analysis. Subtypes were also predicted from the sequence encoding the HBsAg by identifying the amino acids encoded at specific positions.
Results: The majority of Iranian isolates were ayw2 (239/249, 94.4%). Five isolates were ayw3 (2%), Seven isolates were ayw1 (2.8%) and One was detected as ayw4 (0.4%). Interestingly, one isolate was unknown subtype because it had atypical substitution at subtype specifying residues (127S). Various point mutations in the sequence of HBsAg have been found among Iranian HBV isolates. The most common escape mutant amongst Iranian patients was S143L (5.2%) and P120S (1.6%), T118A (0.8%), S136Y (0.8%) and T118K (0.8%) ranked next. Other mutations were also found with minor prevalence (0.4%) as P120T, T123N, T126S, A128V, G130N, P142L, D144E, G145R and P153Q.
Conclusion: Amino acid substitutions within the "a" determinant can lead to conformational changes. Some of these changes may cause important medical and public health issues such as vaccine escape, failure of hepatitis B immune globulin treatment in liver transplant patients, and failure of commercially available immunoassays to detect infected individuals. Our results showed high prevalence of HBsAg mutants in HBV infected Iranian patients and these mutants may play a role in HBV evolution against mass vaccination.

R2489 Do HBV-DNA levels affect liver histology in patients with chronic hepatitis B?

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Objectives: In hepatitis B patient hepatitis B virus DNA (HBV-DNA) in serum is correlated with the progression of the liver disease. In this study we aimed to determine the relationship between hepatitis B virus (HBV-DNA) levels and clinical and histological findings in patients with chronic hepatitis B.
Metods: A total number of 216 patients with chronic hepatitis B (CHB) (112 inactive carriers, 44 HBeAg positive CHB and 72 HBeAg negative CHB patients) who were admitted to our clinic between 1999 to 2005 were included into the study. CHB was diagnosed on patients who were HBsAg positive for more than six months and had HBV DNA levels greater than 105 copy/mL and whose alanin transferase (ALT) levels were elevated persistently or intermittently. Inactive HbsAg carrier state was accepted as presence of HBsAg for more than six months, having HBV DNA level less than 104 copy/mL and persistently normal ALT levels. Serum HBV-DNA levels and the other parameters relevant to liver function were determined in all patient. Liver biopsy was performed on 116 patients with CHB patients.
Result: The HBeAg positive CHB patients were significantly younger than the HBeAg negative group and inactive carriers ($p = 0.000$). The mean HBV-DNA levels were 2×10^3copy/ml, 3×10^8 copy/ml and 9×10^8 copy/ml in inactive carriers, HBeAg negative CHB patients and HBeAg positive CHB patients respectively. HBeAg positive CHB group had significantly higher HBV DNA levels than HbeAg negative CHB patients and inactive carriers ($p = 0.003$). The HBeAg negative group had more severe inflammation and fibrosis scores compared to the HBeAg positive groups ($p = 0.037$ and $p = 0.001$). There was not any correlation between HBV-DNA levels and necro-inflammation and fibrosis scores of neither HBeAg negative CHB patients or HBeAg positive CHB patiens. Alanine aminotransferase and aspartate aminotransferase levels correlated positively with HBV-DNA levels in HBeAg negative CHB patients. Similar correlation was not observed in HBeAg positive CHB patients.
Conclusion: Although HBV DNA levels were higher in CHB patients when compared with inactive carrieres, no correlation between serum HBV DNA level and fibrosis of liver was detected in CHB patients.

R2490 Efficacy of vaccination against hepatitis B in HIV-infected patients in northern Greece

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Objective: Several studies have shown that HIV-positive patients with HBV co-infection have higher HBV replication, lower rates of seroconversion to anti-Hbe and anti-Hbs, and show accelerated progression towards cirrhosis. Since HIV and hepatitis B share epidemiological risks, it is essential to prevent hepatitis B by vaccination. The aim of this study was to evaluate the antibody response rates to HBV by immunisation in HIV-infected patients as compared to HIV-uninfected healthcare professionals and to identify differences in responsiveness correlating to CD4 counts in the HIV-infected group.
Methods: 158 HIV-infected patients and 480 HIV-uninfected healthcare professionals, tested negative for hepatitis B surface antigen (HBsAg) and antibody to HBsAg (anti-HBs) received vaccination with hepatitis B vaccine (Engerix). All subjects received three standard doses of the vaccine at 0–1-6 months. Upon completion of vaccination anti-HBs levels were examined by ELISA (AXSYM, Abbott) and a positive response was defined as an anti-HBs titer of $\geqslant$10 IU/L. CD4 cells measurements were done by flow cytometry. Patients and healthy individuals were divided into two groups according to the magnitude of response to vaccination.
Results: Protective antibody titres were evident in 37% ($n = 58$) of patients with a mean CD4 cell count of 749.36 ($\pm$-259.1)/mm^3. Interestingly, in 13.8% (8/58) of those subjects, CD4 cells did not exceed 500/mm^3.
The remaining 63% ($n = 100$) showed inadequate seroconversion titres. Mean CD4 cell count among HIV-infected non-responders was 621.1 ($\pm$306.43)/mm^3, while 50% of those patients had a CD4 count of >500/mm^3. Statistical analysis using Student's t test, revealed that overall differences in CD4 counts between responders and non-responders are significant ($p = 0.0126$). Protective antibody titres were found in 88.2% of healthy controls.
Conclusion: Efficacy of current HBV vaccination schedule is low among HIV-infected patients. Despite significant differences observed in CD4 counts in relation to antibody response, the former cannot be used as the sole marker of immune system activation. Failure to generate an antibody reaction maybe due to other factors as inferred by the high percentage (11.8%) of non-responders in the control group. Alternative strategies such as higher hepatitis B vaccine doses, prolongation of the vaccination schedule, or both, as prescribed for many patients with non-HIV-related immune deficiencies, may be considered.

R2491 An outbreak of hepatitis A in northeastern Greece

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Objectives: Hepatitis A virus (HAV) is one of the most important causes of focally transmitted acute infectious hepatitis worldwide. In Greece, beside the sporadic HAV infections, outbreaks also occur, particularly in our area, the northeast part of the country. The aim of this study was to present the epidemiological, clinical and laboratory features of acute hepatitis A outbreak among adults in our area.
Material and Methods: An outbreak of acute hepatitis A occurred in our area during October and November 2007. 176 cases of acute hepatitis A reported to local Public Health Authorities in our city and 67(38.1%) cases concerned adult patients above 15 years of age. 64 patients hospitalised in our internal medicine department with the diagnosis of acute hepatitis A and we registered the demographical, clinical and laboratory findings and outcome. Acute hepatitis A cases were confirmed if patients had a positive IgM anti-HAV serology.
Results: Of a total 64 patient (37 were females and 27 males) 56(87.5%) patients were between 17–25 years of age. All the patients were inner inhabitants and living at the same area of the city. The most of them belonged to families with low socioeconomic level. Epidemiological investigation revealed drinking water as a common-source of the outbreak. Regarding clinical features main prodromal symptoms included weakness, fatigue, nausea, vomiting and anorexia. Fever was present in 51(79.7%) cases, artralgia and myalgia in 31(48.4%) and epigastric pain in 28(43.7%) cases. Hepatomegaly was present in all cases, splenomegaly in 26(40.6%) and lymphadenitis in 11(17.2%) cases. Jaundice was present in 61(95.3%) cases. Mean serum bilirubin level was 5 mg/dl, ranging from 1.6 to 14 mg/dl. The mean serum AST and ALT levels were 1100 and 1650 UI, respectively. The mean length of hospitalisation was 7±2.4 days. No death was registered.
Conclusion: The pattern of this common-source water-associated outbreak indicates that some regions of Greece, such as our area, are still considered of moderate endemicity of HAV infection. Methodical sanitary controls of water supply and vaccination of patients belonging to high risk groups are the keys to prevent outbreaks of HAV infections.

Virology (non-HIV/non-hepatitis)

R2492 Determination of inhibitory effects of buthionine sulfoximine on apoptosis induced by measles virus

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Background: Measles virus is a highly contagious agent which causes a major health problem in developing countries. We studied the effect of buthionine sulfoximine (BSO) on the replication of an AIK-HDC strain of measles virus (MV) and its induced apoptosis in Vero cell lines.
Methods: In this study, toxicity of BSO on Vero cells was investigated first, resulted in determination of sub-lethal or non-toxic concentration zone of BSO for cells. Next, antiviral effect of BSO at various time limits was evaluated and virus titer was determined at each stage either as 50% tissue culture infective dose (TCID)50 or by plaque assay method. Using specific antimeasles IgG, antiviral effect of BSO on measles virus replication cycle was evaluated through indirect immunofluorescence assay, meanwhile presence of viral RNA was investigated by RT-PCR and gel electrophoresis.
Results: According to the experiments, BSO, at concentration of 50 μM, markedly inhibited the cytopathic effect (CPE) induced by measles virus. BSO also significantly inhibited apoptosis induced by measles virus. BSO either influences replication of measles virus genome, or may inhibit virion formation.
Conclusion: These results suggest that the inhibition by BSO of CPE and apoptosis induced by measles virus may be associated with the effect of BSO on viral RNA genome. Therefore, it is suggested that measles virus infections can induce apoptosis through the activation of a common pathway that can be inhibited by BSO.

R2493 Human herpesvirus 6 infections in patients with allogeneic haemopoietic stem cell transplantation in a medical university in Warsaw 2004–2006

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Objectives: Human herpesvirus 6 (HHV-6) has been recognised as a potential significant pathogen in haemopoietic stem cell transplant recipients. Different clinical manifestations have been described including fever, skin rash, bone marrow suppression and encephalitis.
We want to measure prevalence of the HHV-6 DNA in group of allogeneic haemopoietic stem cells recipients hospitalised in Haematology, Oncology and Internal Medicine Clinics of Medical University in Warsaw.
Methods: A retrospective review of group of 26 adult recipients of allogeneic HSCT was made. Serum samples taken before transplant were examined for presence of specific anti-HHV-6 antibodies in IgM and IgG classes. After transplantation quantitative real-time PCR was used to determine viral load in 294 plasma samples in range 0–180 days.
Results: HHV-6 DNA was detected in plasma samples in eight (30%) of the 26 recipients between day 18 and day 41 of transplantation. All of them developed fever of unknown origin, and 50% had GvHD features. Three individuals from this group died during detectable HHV-6 viraemia. Additional two recipients showed a single positive PCR result in later period. Thus, infection with HHV-6 was confirmed in 10 (38%) of the 26 graft recipients.
Conclusions: There is a high frequency of detectable HHV-6 viral load in SCT recipients in Poland. Further investigation to monitor HHV-6 reactivation in graft recipients will be important to improve the outcome for these patients.

R2494 Serodiagnosis of human cytomegalovirus infection by completely automated chemiluminescent immunoassays: Architect CMV IgG and IgM

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Objectives: To evaluate the performances and suitability of new automated assays for the detection of IgM and IgG antibodies directed against human Cytomegalovirus (CMV).
Methods: The Architect CMV IgG and IgM assays are two fully automated chemiluminescent immunoassays that employ a viral lysate (IgG assay) or a combination of viral lysate and recombinant antigens (p52 and pp150, IgM assay) on the solid phase (paramagnetic particles) and, respectively, an anti-human IgG or IgM conjugate. The two assays were evaluated both on unselected and on repository sera in 12 Microbiology labs from 7 Italian regions, in comparison with other commercial tests. Samples were obtained from pregnant women (PW), from volunteer blood donors (BD), and from hospital in- and outpatients (HP).
Results: A total of 6,009 samples (3,590 from BD, 942 from PW, 1,477 from HP) have been analysed so far. Among BD the general seroprevalence (Architect) for IgG was 79.6% and for IgM 1.6%, with significantly lower rates (38.5%) for IgG and higher (2.7%) for IgM in the single Centre that tested only new donors and formerly seronegative regular donors. Seropositivity rates for IgG and IgM among PW and HP were 63.5% and 6.0% vs. 73.4% and 9.6%, respectively. Cumulative positivity rates for IgG increased with age, from <50% in subjects aged less than 20 years to >90% in subjects ⩾60 years. The Architect CMV IgG assay showed different concordance rates with the other tests, lower in comparison with Diasorin Liaison and higher with Abbott AxSYM. For the Architect CMV IgM assay the concordance with other methods was generally <90%, due to the high number of discordant reactives. Most IgM positive samples by any assay showed a high IgG avidity index. The distribution of negative results showed a good separation from the assays cutoff; for IgG (U/mL), the mean was 1.12+0.78 U/mL,

the median 0.79, the 99th percentile 4.65; for IgM (Index) the mean was 0.34+0.19 S/CO, the median 0.30, the 99th percentile 0.92.
Conclusion: CMV IgG seroprevalence increases significantly by age, positivity rates for CMV IgM range between <2% to 10% in different populations. The Architect CMV IgG assay seems more sensitive than other automated methods; the Architect CMV IgM is more specific than AxSYM and quite similar to other methods, though most IgM-positive specimens are characterised by a high IgG avidity, as expected according to literature. The Architect CMV assays are suitable for routine.

R2495 Prevalence of parvovirus B19 infections among patients with sickle cell anaemia and thalassaemia in Saudi Arabia

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Objectives: The present study was aimed at serological assessment of parvovirus B19 infection among sickle cell anaemia and thalassaemia patients in Al-Khobar and Dammam, Saudi Arabia.
Methods: the methods used are clinical assessment, serological measurement of parvovirus B19 antibodies using ELISA, viral DNA using PCR and an interview-based questionnaire.
Results: the age of the patients included in the study (N=138) ranged from 6 months to 61 years (mean 28 years). 40 samples (28.4%) have detectable levels of Parvovirus B19 IgG. 6 samples (4.3%) have detectable levels of Parvovirus B19 IgM antibodies. All IgM-positive samples had detectable B19 parvovirus DNA by PCR. Samples of sickle cell disease patients (N=138) were analysed for antibodies against Parvovirus B19 using the novel western blot assay (recomblot assay). 46 samples (33.3%) have detectable levels of Parvovirus B19 antibodies by recomblot assay.
Conclusion: establishing the extend of parvovirus B19 infection in sickle cell anaemia and thalassaemia patients will help in proper management of aplastic crisis in such patients and will also be useful for epidemiological purposes.

Mycobacterial infections (including diagnosis)

R2496 Features of tuberculous lymphadenitis – Experience of a single tertiary centre in Greece

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Objectives: Tuberculous lymphadenitis (TBL) is considered the most prevalent form of extrapulmonary tuberculosis. Incidence and clinical features of various forms of tuberculosis have changed during the last 40 years, mainly due to introduction of new drugs, admixture of populations and increased number of immunosupressed individuals.
We conducted a retrospective study to determine the clinical and laboratory features of TBL in an adult population evaluated for peripheral lymphadenopathy in a tertiary centre in Greece, where published data on tuberculosis are sparse.
Patients and Methods: Among 516 adult patients, who presented with peripheral lymphadenopathy between 1990 and 1995, 27 (5.2%) were diagnosed with TBL, following an excisional biopsy. TBL was the 3rd leading benign cause of lymphadenopathy in this population, following infectious mononucleosis and toxoplasmosis. Patients were evaluated by physical examination, complete blood counts, erythrocyte sedimentation rate (ESR), chest X-rays and PPD skin test.
Results: All patients were native Greeks with median age of 47 years (18–70); 70% were females. All presented with cervical/supraclavicular lymphadenopathy. A single patient was diagnosed by axillary lymph node biopsy, but small axillary and inguinal lymph nodes were found in 42% and 4% of patients respectively. Notably preauricular, postauricular and suboccipital nodes were not palpable in any case. Only 19% had concomitant fever, 15% weight loss and 7% sweats. The median size of the nodes was 2.5cm2 (0.5–25.0) and none had fistulas, while hard texture was present in 46% and tenderness in only 8% of the patients. Splenomegaly was noted in 4% and hepatomegaly in 15% of the patients. All patients had positive PPD test. Hemoglobin and platelet count were within normal limits, while, lymphocytopenia was observed in 29% of the patients. Median ESR was 34 mm (range 4–85). Chest X-rays did not reveal abnormal findings in any case. All patients were successfully treated with isoniazide and rifampicin for 9 months and ethambutol for 3 months.
Conclusion: TBL is a major cause of lymphadenopathy in adult patients in Greece. It should be considered especially in patients >40 years old with clinically significant, non-tender, cervical/supraclavicular lymphadenopathy and positive PPD. The expansion of immigrant population in Greece during the last decade should prompt for a further increase in the level of suspicion for this curable disease.

R2497 Genotype MTBDRplus for detection of rifampin and isoniacid resistance in clinical specimens of *M. tuberculosis*

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Objective: The present study sought to evaluate the use of the new Genotype MTBDRplus assay (Hain Lifescience GmbH, Nehren, Germany) directly from positive clinical specimens, comparing the results with those obtained by conventional phenotypic resistance studies.
Methods: 59 bacilloscopy-positive sputum samples from patients with suspected tuberculosis were subjected to the new Genotype MTBDRplus assay. This technique is based on DNA-strip technology, consisting of a multiplex PCR in combination with reverse hybridisation using nitrocellulose strips for detecting mutations related with Rifampin (RIF) and Isoniacid (INH) resistance comparing the result with those obtained phenotypically in Bactec MGIT 960.
Result: Of the 59 *M. tuberculosis* strains tested, 9 were phenotypically sensitive to INH and RMP, and displayed no resistance-linked mutations. Among the 50 sputum specimens showing some form of phenotypic resistance, 13 displayed RMP resistance, 14 INH resistance and 23 Multi-drug resistance (MDR). All of the phenotypically RIF resistances were detected by the test and only two of the INH resistant strains could not be detected. Sensitivity for RIF resistance is 100% in our study and 94.5% for INH.
Conclusions: The new Genotype MTBDRplus assay is a valid technique for detecting resistance to RMP and INH, providing within 6–8 hours a result that enables a more effective orientation of patient treatment.
Since the detection of resistance-related mutations did not cover 100% of possible cases of resistance, use of this new assay does not obviate the need for conventional phenotypic resistance testing.

R2498 Risk factors for indeterminate results of a tuberculosis-specific gamma interferon release assay in immunocompromised patients

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Introduction: Tuberculosis (TB)-specific interferon-g release assays (TIGRA) have been introduced as an alternative to the tuberculin skin test (TST) for the diagnosis of latent TB in the last few years. These tests use stimulation of T-cells with phythemagglutinin as positive control, which – in contrast to TST – allows identification of false negatives. Since little data exist on reliability of these tests in immunocompromised (IC) patients, we prospectively used the QuantiFERON-TB Gold in tube (QFT-IT, Cellestis) in IC patients to determine the rate of and identify risk factors for indeterminate results (IR).
Methods: Of 420 patients 240 (57%) were male and 180 (43%) female. 234 (56%) had either kidney or liver transplants, 50 (12%) were HIV positive, 77 (18%) had autoimmune diseases, 44 (10%) had primary immunodeficiencies and 15 (3.6%) were stem-cell transplant patients. 88 healthy students and healthcare workers served as control group. Data collection included demographic data, medical history, medication, and laboratory values. For statistical analysis the Chi square based measure

Cramer V was used to determine relationships of categorical variables and tested for significance in SPSS.
Results: 1% of healthy control subjects (1/88) had IR, significantly less than IC patients (11.4%, $p < 0.05$). IR rates were higher in female (30/180, 16.7%) than in male patients (18/240, 7.5%; $p < 0.001$). IR rates varied significantly dependant on disease groups: patients with autoimmune diseases had a significantly higher percentage of IR than organ-transplant patients (20.7% vs 7.6%; $p < 0.05$). The highest rate of IR was found in patients with stem cell transplants (8/15; 53%). IR rates in patients with immunosuppressive therapy (11%) were similar to those observed in patients with primary immunodeficiencies or HIV-infection (13%).
Conclusion: Although IR rates are higher in IC patients than in healthy subjects, QFT-IT still produces reliable results in more than 88% of this patient group. The study shows a significant gender difference of IR results using QFT-IT in IC patients that has not been reported previously and needs confirmation in future studies. Patients with organ transplantation had significantly lower rates of IR than patients with autoimmune diseases. Use of QFT-IT prior to immunosuppressive therapy seems to be especially important in stem-cell transplant patients. If confirmed the need for gender- and disease-specific cut-off values has to be evaluated.

R2499 Low body mass index is associated with tuberculosis infection in patients starting continous ambulatory peritoneal dialysis

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Background; Due to lack of the cell-mediated immune response that is responsible for killing intracellular organisms in uremic individuals, there is an 8 to 25-fold increased risk of tuberculosis in patients on dialysis due to renal failure, compared to the general population. Because tuberculosis is well managed if detected early, it is important not only to make an early diagnosis based on clinical suspicion, but to suspect tuberculosis of patients who are highly susceptible to the disease. This study was undertaken to investigate the epidemiology and risk factors associated with tuberculosis infection in patients starting continous ambulatory peritoneal dialysis.
Method; A retrospective review of 203 patients who initiated peritoneal dialysis from January 2000 to December 2005 at Severance Hospital due to chronic renal failure was performed. Diagnosis of tuberculosis was made based on the following; 1) if AFB was positive on sputum, body fluid smear, tissue stain, cultures or 2) caseous necrosis was present on a tissue sample or 3) if x-ray findings strongly suggested tuberculosis lesions and lesions improved after anti-tuberculous medication. To determine risk factors for tuberculosis, we analysed gender, age, diabetes, serum albumin level, hemoglobulin level, body mass index(BMI), presence of heart failure, medication and etc. All the data including biochemical, weight and height were collected within a month of dialysis commencement.
Results; Among 203 patients enrolled, 18 patients were diagnosed with tuberculosis infection during the study period. The incidence was 28 per 1000 patient years. Upon univariate analysis, risk factors that seemed to be statistically significant for tuberculosis infection were reduced BMI, use of diuretics and the use of angiotensin receptor blockers. No other factors including diabetes mellitus, statin use, age, albumin, cholesterol level showed relevance. Logistic regression analysis revealed that low BMI ($P < 0.001$) was the significant predictor associated with the development of tuberculosis.
Conclusion; Our study revealed that low BMI at the start of dialysis is associated with tuberculosis. Since tuberculosis in chronic renal disease patients have potential progressive nature and dangerous outcome, we suggest that a more close observation made be needed for patients who have low BMI, initiating peritoneal dialysis.

R2500 Drug resistance of *Mycobacterium tuberculosis* in Adana, Turkey

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Objectives: Drug resistance of *Mycobacterium tuberculosis* has been widely reported in the world. We performed this study in order to evaluate the drug resistance to *M. tuberculosis* strains collected between 2001–2006, in Adana city, Turkey.
Methods: Isolation and antibiotic susceptibility tests were carried out using Mycobacterial Growth Indicator Tube (MGIT) system (Becton Dickinson). From 2001 January to 2006 December, a total of 910 strains of *M. tuberculosis* were analysed. Out of 910 strains isolated, 640 (70.3%) were from sputum, 45 (5%) from urine, 59 (6.4%) from bronchoalveolar lavage, 25 (2.8%) from abscesses, 37 (4.1%) from cerebrospinal fluid, 19 (2.1%) from gastric aspirate, 5 (0.5%) from peritoneal, 31 (3.4%) from pleural liquids, and 49 (5.4%) from others.
Results: The rate of the resistant *M. tuberculosis* strains were: 8.5% (78) to INH (Isoniazid), 0.9% (7) to RIF (Rifampin), 3.4% (31) to ETB (Ethambutol), 3.4% (31) to SM (Streptomycin), 3.5% (32) to INH+RIF, 1.2% (11) to INH+ETB, 1.3% (12) to INH+SM, 0.2% (2) to RIF+ETB, 0.1% (1) to ETB+SM, 3.9% (35) to INH+RIF+ETB, 1.1% (10) to INH+RIF+SM, 0.4% (4) to INH+ETB+SM and 1.2% (11) to INH+RIF+ETB+SM. The rate of the strains susceptible to all four drugs were 70.9% (645)

Table 1. Annual distribution (%) of drug resistant strains.

Years	2001	2002	2003	2004	2005	2006	2001–2006
Number	185	186	207	95	120	117	910
INH	13	8.7	6.8	7.4	5	9.5	8.5
RIF	1.6	0.5		–	1.7	0.8	0.9
ETB	2.1	2.1	7.7		5.9		3.4
SM	3.8		1.5	3.1	7.5	7.7	3.4
INH+RIF	1.1	3.2	3.3	8.5	2.5	5.1	3.5
INH+ETB		4.9	0.5		1.7	0.8	1.2
INH+SM	1.1	0.5	1.5	3.1		0.9	1.3
RIF+ETB	0.6	0.5	–				0.2
ETB+SM	0.6	–					0.1
INH+RIF+ETB	2.7	7.6	4.9	5.3		0.8	3.9
INH+RIF+SM	2.1		1.5	2.1		0.9	1.1
INH+ETB+SM	0.6	0.5	0.9				0.4
INH+RIF+ETB+SM	1.6	0.5	0.9	2.1	1.7	0.8	1.2
Susceptible to 4 drugs	69.1	71	70.5	68.4	74	72.	70.9
MDR (INH+RIF)	7.5	11.3	10.6	18	4.2	7.6	9.7

Conclusion: Tuberculosis is still an important public health problem in our district, particularly because of drug resistance. That is why, effective treatment and control programmes are necessary.

R2501 Genotyping of *Mycobacterium tuberculosis* MDR and XDR strains in Lima, Peru

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Objective: To characterise the genotypes of *Mycobacterium tuberculosis* both multidrugresistant (MDR), expensive drug resistant (XDR) and susceptible to drugs strains.
Methods: Forty-nine patients with TB were included in the study. The genotypes of the *M. tuberculosis* isolates were established by PCR using the primer Mtb2 (5′-CGG-CGG-CAA-CGG-CGG-CA-3′) in combination with primers sited at inverted repeats flanking IS6110.

The susceptibility to isoniazid, rifampicin, streptomycin, ethambutol, kanamycin, para-amin-salicylic acid, tioacetazon and pyrazinamide was studied.
Results: The 36.7% of the patients presents isolates susceptible to all the drugs tested. The 12.9%, 9.7%, 9.7%, 48.4% and 19.4% were resistant for one, two, three drugs, MDR and XDR, respectively. The resistance levels to isoniazid, streptomycin and rifampicin were the most relevant, ranged between between 47% and 49%.
The 16% of the patients were VIH+, three out of them presents MDR strains, while no XDR isolate was isolated from these patients.
When considered the patients with MDR strains the 80% reported previous TB.
Upon PCR-based genotyping analysis of 49 *M. tuberculosis* strains, the presence of 42 different fingerprints and 10 different clusters were established, the number of bands for each fingerprint ranged from 7 to 1 band(s). The MDR isolates were non-included in a specific cluster
Conclusions: An High number of MDR isolates were detected. Additionally, the recovered isolates presents a low level of clonality, showing a great variety of genotypes and a low level of clustering, this phenomena affects specially the MDR isolates.

R2502 Is there a place for the military in contributing to a global programme managing the MDR-TB challenge and beyond?

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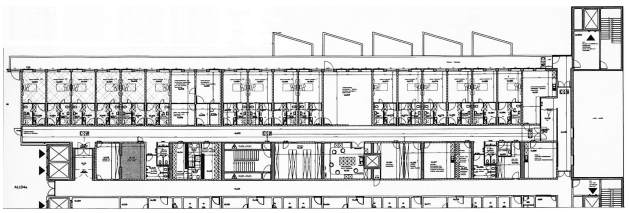
Background: In accordance with a reorganisation, the Central Military Hospital of the Belgian Defence is looking for new opportunities in cooperation with the civilian health services. Recently a partnership has started with a tertiary care university teaching hospital belonging to a hospital association specialised among others in the treatment of infectious diseases and accredited as a federal SARS reference hospital. The increasing mean annual incidence of MDR-TB in Belgium (0.1/100,000 inhabitants; 10 new cases annually) and the complex, prolonged and costly management of infected patients (on an average 14 country-wide) urge for a dedicated infrastructure and particular environmental control technologies.
Objective: To study the architectural feasibility of an MDR-TB isolation unit within the existing building of the military hospital.
Methods: A multidisciplinary design team was launched, consisting of critical care and infectious disease specialists, infection control experts and the Defence Operating Engineering staff.
Taking into account the hospital space programming and functional planning and according to the Hospital Law and the CDC guidelines for preventing the transmission of *Mycobacterium tuberculosis* in healthcare settings (2005), design recommendations and state-of-the-art requirements for an infectious airborne diseases isolation unit were identified. The most suited spatial implantation was subsequently picked out.
Results: Based on the modification of a pre-existing abandoned medium care burn centre, an optimum airborne disease isolation unit design is described. (Figure)



Emphasis is laid on the lay-out of 16 single-patient negative-pressure airborne infection isolation rooms, local exhaust and general ventilation, air cleaning (HEPA filtration), prolonged and socially warranted housing and recreational facilities as well as intensive care, surgery and laboratory capacities.
Conclusion: A dedicated MDR-TB isolation unit is justified.
The Belgian Defence can participate in a global national programme to treat MDR-TB by lending appropriate space and high technical infrastructure to achieve best practice environmental control.
The location in the country's capital permits future centralisation and nation-wide standardisation of protracted MDR-TB chemotherapy and diagnostic drug susceptibility testing.
This project entirely fits in the national contingency planning for the management of emerging airborne infectious diseases like SARS or avian flu.

R2503 Usefulness of MTBDRplus assay for rapid genotypic detection of *Mycobacterium tuberculosis* resistant to isoniazid and rifampicin directly in clinical specimens

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Objectives: To evaluate the usefulness of MTBDRplus assay for detecting resistance of *Mycobacterium tuberculosis* against isoniazid (INH) and rifampicin (RIF) in smear-negative and smear-positive clinical specimens.
Methods: Sixty-five clinical samples (53 sputa, 3 bronchoalveolar lavages, 5 bronchial aspirates, 3 pleural effusions and 1 lymph node) were recovered from 28 patients. All the samples were processed by conventional mycobacterial procedures and after strain isolation on solid culture, the antibiotic susceptibility was assessed by BACTEC460TB. According to the Ziehl-Neelsen staining 39 samples were smear-negative (19 INHs/RIFs, 4 INHr/RIFs and 3 INHr/RIFr) and 26 were smear-positive (12 INHs/RIFs, 1 INHr/RIFs and 26 INHr/RIFr). The assay, which consisted of DNA isolation, multiplex PCR with biotinylated primers and reverse hybridisation on nitrocellulose strips, was carried out following the manufacturer instructions. MTBDRplus combines control, wild type and mutation probes, which allow the detection of the most common resistance mediating mutations in rpoB, katG and inhA.
Results: Valid test results were obtained for 51 samples (78.4%). The 14 invalid results corresponded to Ziehl-Neelsen negative samples. The concordance between MTBDRplus susceptibility pattern and BACTEC460TB result for the samples with valid result was 98% (50/51) for RIF and 96.2% (49/51) for INH. In relation to RIF, the discordant sample was resistant according to the MTBDRplus, because of the absence of one of the wild-type bands, but susceptible according to BACTEC460TB. The two discordant samples for INH, belonging to the same patient, were RIFr/INHr according to BACTEC but the MTBDRplus INH pattern was susceptible. We detected 30 rpoB mutations for the 29 RIF resistant samples that consisted of: 17 D526Y (56.7%), 9 D516V (30%) and 4 S531L (13.3%). Mutations in katG and inhA for the 30 INH resistant samples were: katG S315T in 11 cases (39.3%) and both katG S315T and inhA-15 C-T in 17 cases (60.7%).
Conclusions: MTBDRplus assay is an easy to perform test that allows the rapid detection of RIF and INH resistant *M. tuberculosis* directly from clinical sample with a high sensitivity, being appropriated for the use in the routine work flow.

R2504 Genotypic detection of rifampicin resistance in *Mycobacterium tuberculosis*: analysis of mutations from high- and low-incidence areas using denaturing high-performance liquid chromatography

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Objectives: With the emergence of MDR- and XDR-TB, there is a need for a rapid method of detecting rifampicin resistance in *M. tuberculosis* that can be applied to all isolates in a geographic area. Denaturing high performance liquid chromatography (dHPLC) is a rapid screening method that can analyse PCR amplicons up to 700 bp in length. The aim of this study was to evaluate dHPLC analysis of the rpoB gene in *M. tuberculosis* using a extensive collection of 16 distinct mutations in

10 different codons from Hong Kong and the UK and a collection of 84 consecutive clinical isolates.
Methods: DNA from 52 rifampicin resistant *M. tuberculosis* isolates from the UK and Hong Kong identified from 1996–2005 was extracted using a sonication and heating method from positive liquid cultures incubated at 37C. Each mutation was defined by DNA sequence analysis using capillary electrophoresis. In order to evaluate clinical utility, 84 consecutive clinical isolates identified as *M. tuberculosis* by the Hain LifeScience GenoType MTBC assay were analysed by dHPLC. Phenotypic drug sensitivity testing was undertaken using the BD MGIT liquid culture system. A 400 bp product was amplified from each DNA extract, hybridised with a known sensitive control (H37Rv), and analysed on a WAVE system at 67.0C. Mutations were detected if two or more peaks were detected on a chromatogram with one peak indicating no mutation was detected. Sensitivity, specificity, PPV and NPV were calculated.
Results: 45/52 (88.2%) rifampicin resistant isolates with defined DNA mutations were detected by dHPLC at 67.0 ºC. 83/84 (98.8%) consecutive DNA extracts were amplified with the amplification failure bring phenotypically rifampicin sensitive. Two isolates were phenotypically rifampicin resistant and dHPLC detected a mutation in the rpoB amplicon for both these isolates (S531L and S531W). dHPLC detected a mutation in 1/82 phenotypically rifampicin sensitive isolates (M482T, a non-cluster I/II mutation). In a combined analysis of all isolates, detection of mutations in the rpoB gene by dHPLC analysis exhibited sensitivity of 88.7%, specificity of 98.8%, PPV of 97.9%, and NPV of 93.0%.
Conclusion: This study of dHPLC analysis of an expanded collection of mutations from high and low areas of incidence and clinical strains in an area of low incidence shows that dHPLC analysis is sensitive and specific and could be implemented in a routine clinical service alongside routine MIRU-VNTR DNA fingerprinting on a WAVE system.

R2505 Diagnosis of tuberculosis infection in HIV-infected patients: an interferon-gamma assay versus tuberculin skin test

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Objectives: To compare the tuberculin skin test (TST) with a whole-blood interferon-gamma (IFN-γ) assay in the diagnosis of tuberculosis infection (TBI) in HIV-infected patients.
Methods: Cross-sectional study of X HIV-infected patients evaluated at the HIV Unit of a third-level teaching hospital, between February and October 2007. Diagnosis of TBI was based on TB exposure, chest X-ray, two-step TST and IFN-γ assay (QuantiFERON® TB Gold-in Tube, QFT). According to the manufacturer's instruction, QFT was considered as positive when the TB antigen minus negative control IFN-g production $\geqslant$0.35 UI/mL; an indeterminate result was defined as either a positive control IFN-g response of $\leqslant$0.5 UI/mL or a negative control IFN-γ level of >8 UI/mL. A positive TST was defined as a $\geqslant$5 mm induration. Agreement between TST and QFT was assessed by the Cohen kappa (k) index.
Results: 39 patients were assessed for TBI, 69% men, with a mean age of 36 years. 49% were foreign-born. 54% had some TB risk factor and 39% were BCG vaccinated. 5% had a previous story of TBI. Mean CD4+ cell count was 376 (±279), 11 (28%) patients with CD4+ <200 cells/mL, and mean HIV-RNA was 118,569 copies/mL. 2 (5%) patients had a positive TST compared with 5 (13%) with QFT (p = 0.02). 3 patients had discordant results, all of them positive QFT/negative TST. 1/11 (6%) patients with CD4+ <200 showed a positive QFT but no one had positive TST. In the positive QFT group, some TB risk factor was presented in 60% (3/5) of cases but in no one in the positive TST group.)There were no indeterminate results due to low production of IFN-γ in positive control tube. Overall agreement between the 2 tests was 92% (33/36, k=0.53, CI 95% 0.24–0.82).
Conclusions: These results suggest that QFT has higher sensitivity than TST for the diagnosis of TBI in HIV infected adults, particularly among those more immunosuppressed. TST and QFT agreement was moderate. There were not any indeterminate results due to low production of IFN-γ even among patients with CD4+ <200.

Infection in the immunocompromised host & transplant recipients

R2506 Diagnostic yield of blood cultures from antibiotic-naive and antibiotically treated febrile neutropenic patients with acute leukaemias and patients after high dose therapy and autologous blood stem cell transplantation – a single centre experience

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Objectives: Infections are common complications during neutropenia after intensive cytoreductive therapy and a major cause of morbidity and mortality. Especially when drawn under empiric antibiotic therapy, the diagnostic significance of blood cultures concerning the detection of pathogens, the identification of pathogen shifts and resistance patterns in these patients is unclear.
Methods: To assess the yield of blood cultures, the spectrum of pathogenic organisms and the influence of blood culture results on the therapeutic management of febrile neutropenia, we retrospectively evaluated the results from 2520 blood culture samples obtained from consecutive febrile neutropenic patients with acute leukaemias (n = 90) and patients treated with high dose therapy schedules and autologous haematopoietic stem cell transplantation for other haematologic malignancies (n = 36) in our department.
Results: 2217 (88%) out of 2520 blood cultures remained sterile, 71 (2.8%) blood cultures yielded contaminating germs. In 232 (9.2%) of all blood cultures bacterial and/or fungal pathogens were detected, among them in 219 (94.4%) bacteria, differentiated as 78.5% Gram-positive und 21.5% Gram-negative bacteria. Fungi were found in 13 (5.6%) out of the positive blood cultures. Mainly coagulase negative staphylococci (n = 104/232), *Escherichia coli* (n = 2/232) and yeasts (n = 13/232) were detected. Pathogens were found in 14.3% out of the positive blood cultures drawn at the beginning of fever, the yield of repetitive blood cultures under empiric antibiotic treatment was 7.03%. The diagnostic yield in patients after PBSCT was distinctly lower than in patients with acute leukaemias. Yeast fungaemias occured later in the course of neutropenic episodes. The empiric antibiotic treatment was modified according to blood culture findings in 116/232 (50%) of positive blood cultures.
Conclusion: The diagnostic yield of repetitive blood culture sampling in risk patients populations with neutropenia and fever is significant, both in antibiotic-naive patients and in patients receiving antibiotic treatment, and provides important information for clinical decision making. The epidemiologic data obtained from blood cultures are helpful for selecting empiric antibiotic treatment regimens. A prospective multicentre trial is warranted to define benefits for patients and hospital economics of repetitive blood cultures under empiric antibiotic treatment in leukemic patients.

R2507 Registration of frequency of isolated *Candida* strains in immunocompromised and cancer patients and determination of their tolerance in the usual antimycotic treatment

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Objective: To investigate the frequency, the fungus type and their sensitivity towards the antimycotic treatment in patients with cancer or immunocompromised patients, because of their high risk in developing fungal infections.
Methods: We studied 182 patients in total (103 men and 79 women), mean age of 66.3 years who showed signs of fungal infection. From these patients 135 suffered of cancer (with histological confirmation of primary

or secondary neoplasia) and 47 patients suffered of immunosuppression. We isolated 49 *Candida* (*C*) strains, which came out of 25 sputum samples, 12 from stool cultures, 11 out of urine cultures, and one out of trauma culture. As for the identification we used the API 20C system and for the determination of sensitivity in antibiotics we used E-test and other classical methods.

Results: We found 25 cases (51%) of *C. albicans*, 9 cases (18.4%) of *C. parapsilosis*, 8 cases (16.3%) of *C. tropicalis*, and 7 cases (14.3%) of *C. lusitaniae*. All these strains were sensitive in Caspofungine and Amfotericine B, while we found that 32.7% (16/49) tolerance in Fluconazole, 24.5% (12/49) in Voriconazole. Nearly 50% of the non-*albicans* strains, presented equal tolerance in both antifungal drugs.

Conclusions: (1) *C. albicans* was the most common isolated strain. (2) Out of the non-*albicans* strains that we isolated, the most frequent was *C. parapsilosis*, then *C. tropicalis* and then *C. lusitaniae*. (3) All the isolated strains where sensitive in Amfotericine B and Caspofungine. (4) There was a high level of tolerance in Triazole (mostly by the non-*albicans* strains).

R2508 Oral infection with Prototheca zopfii (first human isolates in the Balkans)

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Objective: The genus Prototheca belongs to the family Chlorellaceae. It is an unicellular achlorophyllous alga that is frequently isolated from a variety of environmental sources. These colourless microalgae have been identified to be the only known plant causative agent of human and animal infections.

Prototheca wickerhamii and Prototheca zopfii have been associated with human disease. Cases of disseminated disease involved the blood, the peritoneum, the GI tract, the liver, and the meninges. Patients, who are severely immuno-compromised, can develop disseminated disease, which is often fatal. This yeast-like organism resembles the green alga Chlorella, and reproduces asexually by internal septation and cleavage to form sporangia containing sporangiospores.

This study illustrates the finding obtained in 53 years old female with palatum carcinoma in the upper left side. After ablation of the tumour intraoperationaly an acrylic plate was inserted to separate the newly postresectionally formed gap between the mouth and nose cavities. Eight months later a change was noticed in the soft tissues manifested as a whitish plaque. Swab was taken from the spot, as well as from the contact surface of the suprastructure of the postresection prosthesis

Methods: Swabs were inoculated onto the Sabouraud broth, Sabouraud dextrose agar and blood agar, and then subcultured on the same media. P. zopfii was identified by its cultural and biochemical properties and microscopic appearance, and confirmed using the RapID Yeast Plus system (Remel, Santa Fe, N.Mex., USA).

Results: After 72 hours of incubation, colonial growth was noticed in all media; colonies were most prominent on Sabouraud dextrose agar. In subcultures, the growth was noticed after 24 hours. Colonies were whitish, pasty and variable in diameter – a growth typical for yeasts. Under the light microscope (400×) surface of the colonies appeared granular. In smears stained with methylen blue, oval organisms, 10 μm in diameter, were observed. Inside the colonies round, rosette-like formations were apparent. Gram-staining revealed positively stained spores and negatively stained sporangia. All isolates were sensitive to amphotericin B and nystatin. RapID Yeast Plus system confirmed Prototheca zopfii by a code 730010.

Conclusion: Based upon the cultural, microscopic and biochemical properties, presence of P. zopfii in the oral cavity of the immune-compromised patient was confirmed.

R2509 Chronic granulomatous disease associated with *B. cepacia* complex infection in a developing country: a report of 2 cases and discussion

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Chronic granulomatous disease (CGD) is an inherited primary immunodeficiency syndrome characterised by a defective respiratory burst, with inadequate production of reactive oxygen molecules, resulting in defective killing of phagocytosed micro-organisms.

A variety of genetic defects in the NADPH oxidase enzyme complex exist: these include the commoner, more severe X-linked form due to abnormal gp91 gene, or other autosomal recessive forms due to defects in gp22, 27 and 67 genes.

Patients with CGD are particularly susceptible to bacterial and fungal infections. The organisms commonly responsible for infection include catalase-producing organisms, such as *S. aureus* and various Gram-negative bacilli, as well as fungi, especially *Aspergillus* species.

Burkholderia cepacia complex (Bcc), a group of environmental Gram-negative bacilli, may cause serious infections, especially pneumonia and septicaemia, in this group of patients. Bcc bacteraemia is associated with significant mortality, particularly in patients receiving inappropriate antibiotic therapy. Inappropriate antibiotic therapy is likely since antibiotics effective against Bcc are seldom included in empiric antibiotic regimes designed for immunocompetent patients. Increased awareness of both CGD and Bcc could limit this situation.

We present 2 cases of Bcc infection in patients with CGD: one in a patient known with CGD, who died of Bcc septicaemia, and one in a 10 year old boy, in whom persistent *B. cepacia* skin infection was the indicator condition which lead to the diagnosis of CGD.

CGD is under-diagnosed in sub-Saharan Africa where the majority of patients die of infection before their condition is recognised. There are currently no other CGD patients included in the SA Primary Immunodeficiency Register. In addition, little is known about the underlying genetic profiles of CGD in Africa, which may be diverse. Increased awareness of the disease is required since successful prophylaxis and treatment with available antibacterial and antifungal agents is possible in the local context.

R2510 Polymicrobial bacteraemia in cancer patients

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Objective: Most databases describing bacteraemias in cancer patients focus only on monomicrobical episodes. Our objective was to describe the microbiology of polymicrobial bacteraemia (PMB) in such patients.

Methods: Retrospective review of institutional microbiology records (Nov. 2005 – Dec. 2006) to identify patients with PMB, and chart review of patients with PMB.

Results: Eighty-one episodes of PMB were identified in 71 pts. (64 pts – 1 episode, 7 pts. – 2 or more episodes). There were 36 women and 35 men with a median age of 55 yrs (range 17–85). Forty-four (62%) had haematologic malignancies and 27 (38%) had solid tumours. Thirty-seven (46%) were neutropenic at the onset of PMB and 48 (59%) had received prior antibiotics, primarily quinolone prophylaxis. Thirty-eight (47%) had a definite catheter related PMB and 21 (31%) had a high organism load (500–1000 CFU/ml) on quantitative cultures. Multiple Gram-positive species were isolated in 20 (25%), multiple Gram-negative species in 20 (32%), both Gram-positive and Gram-negative species in 21 (26%), bacterial and fungal isolates in 11 (13%) and bacterial plus mycobacteria in 3 (4%). The most common Gram-positive isolates were GNS, *viridans* group streptococci, *Enterococcus* spp. and *Corynebacterium* spp. The most common Gram-negative isolates were *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., and *Pseudomonas* spp. and *Stenotrophomonas maltophilia*. The most common fungal isolates were *Candida* spp. The overall response to therapy was 84% (68/81). Sixty-six patients (82%) received initial concordant therapy which covered all isolated pathogens. No significant differences in response were noted in patients with haematologic

malignancies compared to those with solid tumours and in neutropenic patients compared to non-neutropenic patients.
Conclusions: PMB's are not uncommon in cancer patients. Approximately 70% of PMB have a Gram-negative component. Antibiotic therapy is associated with an 84% overall response rate.

R2511 *Pneumocystis jirovecii* pneumonia – An AIDS defining illness?

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Objective: While HIV patients were known as the most affected collective by *Pneumocystis jirovecii* (carinii) pneumonia (PcP) in the past, in recent years an increasing number of patients undergoing immunosuppressive therapy or chemotherapy are diagnosed with PcP. Details about the change in epidemiology, the clinical features and the clinical outcome of these patients are little-known.
Methods: Retrospective analysis of all patients diagnosed with PcP at the university medical centre of Regensburg during the years 2004–2007. All patients showed clinical characteristics of PcP and had either microscopically or molecular (PCR) proof of *Pneumocystis jirovecii* infection.
Results: 67 patients were diagnosed with PcP (49 men, 18 women, median age 52). Underlying disease was mostly malignancy (40%), HIV (27%) and rheumatic disease (15%). Treatment at the intensive care unit was necessary for 42 patients, respiratory therapy for 25 patients. Mortality of the whole group was 27%, considering the different underlying diseases 1 out of 18 HIV patients died while 16 of the 49 other patients did not survive.
Summary: PcP does not seem to be the disease of HIV infected persons any more, as most of the patients in this analysis had different underlying disease. Especially oncologic and rheumatologic cases seem to be a group at risk and PcP should be strongly considered as a differential diagnosis in these patients.
The total mortality of all PcP affected patients in this study was notably higher compared to average mortality rates of HIV patients diagnosed with PcP published in the literature and when comparing the mortality rate of HIV and non-HIV infected patients of our study. Further investigations are needed in order to identify risk factors leading to infection in non-HIV patients and to analyse differences in outcome.

R2512 Combination antifungal therapy for invasive aspergillosis in solid organ transplant recipients

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Background: Invasive aspergillosis (IA) in solid-organ transplant (SOT) recipients is still a major cause of infectious-related mortality. Combination antifungal therapy (CAT) represents a new challenge due to the availability of new antifungal drugs. We evaluate the clinical characteristics and outcomes of SOT patients with IA treated with CAT. We compared the outcomes of this cohort with an historical cohort treated with monotherapy.
Methods: All cases of probable or proven IA in SOT treated with double antifungal therapy were compared with those treated with antifungal monotherapy.
Results: From 1998 to August 2007, 25 cases of definite or probable IA in SOT were diagnosed of which 13 (52%) received combined antifungal therapy (11 liposomal amphotericin B [lAmB] + caspofungin, 1 lAmB + itraconazole and 1 voriconazole + caspofungin). Monotherapy was based in Amphotericin B in all cases but one (treated with itraconazole). Diagnosis of IA was: 9 proven and 16 probable. Late-onset IA (more than 180 days post-transplant) represented 24% of the cases (6 cases). By type of SOT: 6 kidney, 15 liver, 3 kidney-pancreas and 1 heart. *A. fumigatus* was isolated in 19 patients, *A. niger* in 2, *A. terreus* in 1, *Aspergillus* spp. in 1 and in 2 patients no microbiologic isolation was found (1 case biopsy proven without culture and 1 case with two positive galactomannan and suggestive radiological findings). There were no differences between both groups of antifungal treatment and type of SOT, age, gender, number of proven diagnosis and disseminated infection. Death occurred in 67% (8 cases) of patients treated with monotherapy vs 23% (3 cases) in those treated with CAT ($p = 0.028$).
Conclusions: Our results suggest a better outcome of SOT patients with IA treated with CAT. Randomized studies to evaluate the efficacy of CAT in IA are needed.

R2513 Two cases of fatal bacteraemia caused by unusual pathogens in patients with haematologic malignancy

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Objectives: The aim of this study was to introduce the 16S rDNA sequencing analysis for identification of unusual pathogens isolated from blood and to examine the aetiological background of these infections.
Methods: During the febrile period, blood was taken from patients into BACTEC bottles. In the case of positive fluorescence signal, blood was processed according to the standard clinical laboratory procedures. Using conventional methods, identification of the isolated strains was unsuccessful, thus nucleotide sequences of partial 16S rDNA of the isolated strains were determined and compared to all sequences in the GenBank database.
Results: In case 1, forty-one-year-old woman with bleeding disorders, thrombocytopenia and anaemia was admitted to haematology unit. On the basis of laboratory findings, the results of bone marrow biopsy and cytogenetic analysis, the diagnosis was acute promyelocitic leukaemia. After the chemotherapy, dyspnoea, abdominal pain, and interstitial pneumonia developed. Her blood cultures taken during the febrile period became positive for *Achromobacter xylosoxidans*. In spite of the adequate antibiotic therapy and intensive care, the patient died shortly after her admission to the ICU. In case 2, thirty-seven-year-old man with a 9-year history of large granular lymphocyte leukaemia was admitted to high fever, headache and confusion. CT scan of the brain revealed multiple hyperdense focal lesions surrounded by oedema. After 52 and 65 hours of incubation positive signals were generated in the BACTEC blood culture system, and two days later small colonies of *Nocardia farcinica* were isolated. The patient died shortly after taking these blood cultures.
Conclusion: In the above described cases, the identified species (*A. xylosoxidans* and *N. farcinica*) are rare causes of bloodstream infections, but nowadays these are increasingly recognised problems in immunocompromised patients. At the same time, because of the difficult identification of these pathogens and the special group of patients, the recognition of these species and the treatment of these infections are challenging to microbiologists and physicians as well.

R2514 Blood culture statistic study in febrile neutropenic haematology patients

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Objectives: The aim of study was to evaluate the frequency patterns of bacterial pathogens isolated from blood cultures in febrile neutropenic patients hospitalised in haematology unit over the three years period..
Methods: A total number 1322 of blood cultures were examined, using the (automated) Bact/Alert Blood Cultures System. All isolates from positive blood cultures, after classic subculture methods, were characterised at species level by the Vitek system. Antibiotic susceptibility testing was performed using automated Vitek system according to CLSI recommendations. All the investigated patients were neutropenic (WBC < 500) with fever (>38ºC) and were under antimicrobial and/or antifungal therapy at the time of blood culture.
Results: Over the three years period (2005–2007) 1322 blood cultures were performed on the basis of the physicians request from 321 patients (with febrile and neutropenic) hospitalised in the haematology ward of

our hospital. The pathogens were isolated from 363 blood cultures from 321 patients.
Among the 363 bacterial strains isolated from bloodstream infections 261 were Gram-positive strains (71.90%), 91 Gram-negative strains (25.07%) and 11 yeast (3.03%).
In the group of Gram-positive bacteria were: *S. epidermidis* 147, *E. faecium* 50, *S. aureus* 16, *S. hominis* 11, *S. haemolyticus* 9, *E. fecalis* 9, *S. mitis* 9, *C. jeikeium* 5, *S. salivarius* 2, *S. sanguis* 2, *S. intermedius* 2. In the group of Gram-negative strains were: *E. coli* 24, *P. aeruginosa* 13, *E. cloacae* 12, *K. pneumonia* 12, *S. maltophilia* 10, *A. baumannii* 7, *A. lwoffii* 5, *S. marcescens* 4, *C. freundii* 3, *S. enteritidis* 1.In the group of yeast were: *Candida albicans* 4, *C. krusei* 3, *C. glabrata* 2, *C. famata* 1, *C. parapsilosis* 1. In the *Enterococcus* isolates were 8/59 glycopeptide resistant strains (13.56%) and 2 isolates resistant to linezolid (3.39%). All the *Staphylococcus* isolates were susceptible to glycopeptide and linezolid. All the Gram-negative rods were susceptible to imipenem and meropenem.
Conclusion: Gram-positive bacteria are common pathogens in neutropenic haematology patients. Glycopeptides (teicoplanin and vancomycin) were effective for the treatment of infections caused by *Staphylococcus* spp. and *E. faecalis*. Linezolid is effective in the treatment of infections caused by Gram-positive pathogens including glycopeptide resistant *Enterococcus* spp. Imipenem and meropenem were effective for the treatment of infections caused by Gram-negative pathogens.

R2515 Antimicrobial prophylaxis in neutropenic patients undergoing peripheral blood stemcell or bone marrow transplantation

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Objectives: To assess antimicrobial prophylaxis in neutropenic patients undergoing bone marrow transplantation (BMT) or peripheral blood-stem cell transplantation (PBSCT) and analyse the data in relation with the incidence of nosocomial infections. Data were taken from ONKO-KISS (Hospital Infection Surveillance System for Patients with Hematologic/Oncologic Malignancies), an ongoing multicentre surveillance project established by the German National Reference Centre for Surveillance of Nosocomial Infections in 2000 [CID 2005; 40: 926].
Methods: Nosocomial Infections were identified using CDC definitions for laboratory-confirmed blood stream infection (BSI) and modified criteria for pneumonia in neutropenic patients [for detailed information and reference data see http://www.nrz-hygiene.de/surveillance/onko.htm]. Information on antimicrobial prophylaxis was obtained by a questionnaire sent to all ONKO-KISS participants.
Results: 10 out of 14 centres treating patients with allogeneic BMT or PBSCT returned the questionnaire. All routinely administered systemic antibiotics (9/10 cotrimoxazole, 6/10 ciprofloxacin, 4/10 levofloxacin and 2/10 other antibiotics), as well as antiviral agents. Systemic antifungal prophylaxis was applied by 9 of the 10 centres.
14 out of 19 centres treating patients with autologous BMT or PBSCT returned the questionnaire. 3 out of 14 did not routinely give systemic antibiotic prophylaxis. Only 5 centres applied systemic antifungals and half (7/14) applied antiviral agents. 3 participants had only recently joined the ONKO-KISS system and thus reported few or no data on nosocomial infections. 9 of the remaining 11 centres available for analysis administered systemic antibiotics. The incidence of BSI/1000 neutropenic days was 15.7 and of pneumonia 5.8 in this group (data from 7/2002–6/2007). In contrast, the 2 centres that did not give any antibiotic prophylaxis reported 19.4 cases of BSI and 8.1 cases of pneumonia per 1000 neutropenic days.
Conclusions: Our results offer very preliminary data suggesting that antibiotic prophylaxis is of benefit to these high risk patients in the prevention of nosocomial infections. However, the influence on the development of antimicrobial resistance must be closely monitored. It might be reasonable to consider routine administration of antibiotics also in autologous BMT/PBSCT in the case of above-average infection rates.

R2516 Mediastinitis after heart transplantation: clinical presentation and outcome

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Objective: To assess the incidence, predisposing factors and outcome of mediastinitis after heart transplantation.
Methods: From January 1997 to December 2006, a total of 774 heart transplants were performed in four tertiary care university centres in Spain. Those cases with possible mediastinitis were identified from the hospital infection register and discharge register. Patients' charts were reviewed and cases of mediastinitis confirmed based on criteria of the Centers for Disease Control and Prevention.
Results: Mediastinitis developed in 13 (1.8%) of 774 cardiac transplant patients. Patient's ages ranged from 37 to 64 years (mean 54.3 y, SD=8.1) and 9 (69.2%) patients were males. The incubation period for mediastinitis ranged from 2 to 40 days (mean 13.1d, SD=12.2). Ten (76.9%) patients were diagnosed in the first 2 weeks after transplantation and only 1 case developed longer than 1 month after this procedure. Seven (53, 8%) patients were diabetic, and 1 had a previous heart transplant. Fever (84.6%), purulent secretion through the wound (86%) and sternal instability on palpation (76.9%) were the main clinical findings. Bacteraemia developed in 8 (61. 5%) patients: *S. aureus* was the most frequent microorganism isolated (3 patients, 1 MRSA), followed by *S. epidermidis* (2 patients), *K. pneumoniae* (1 patient), *P. aeruginosa* (1 patient) and *S. viridans* (1 patient). In the wound secretion or in the mediastinum or both *S. aureus* was identified in 5 patients (2 MRSA), *S. epidermidis* in 2, and *K. pneumoniae*, *P. aeruginosa*, *Serratia marcescens* and *Candida glabrata* in 1 patient each. All patients received antibiotic treatment. Surgical treatment was performed in 12 (92.3%) cases, and the extension of the debridement varied with local conditions. In 7 (53%) patients, additional irrigation of the closed wound with povidone-iodine was performed. Two (15.4%) patients died in the first month after the diagnosis, and the causes of death were related to the infection.
Conclusion: Promptness in diagnosing mediastinitis and precocious surgical drainage has changed the natural evolution of this disease. Nevertheless, observance of the basic precepts of prophylaxis of infection is still the best way to treat mediastinitis. Gram-positive organisms were more commonly isolated than were Gram-negative bacteria.

R2517 Catheter-related bacteraemia with Methylobacterium species

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Objectives: Methylobacterium is an aerobic, slow-growing, Gram-negative bacillus rarely found in human, clinical specimens.
We present three cases of bacteraemia with Methylobacterium species and the results of phenotypic characterisation, antibiotic susceptibility testing and 16S rRNA gene sequencing.
Methods: Clinical records from three patients with Methylobacterium infections were obtained. The BacTec bloodculture system was used. Identification was made by standard methods, VITEK®2 GN card and direct sequencing of the 16S rRNA gene. Susceptibility testing was performed by direct agar diffusion with Rosco Neo-Sensitabs® and by E-test®.
Results: Three cases of bacteraemia with four isolates of Methylobacterium species in pure culture are presented. Two patients were immunocompromised and received chemotherapy for acute leukaemia; one patient had pancreatitis and multiorgan failure. All patients had a central venous catheter as the most likely focus of infection. All isolates of Methylobacterium were growing slowly with a time to detection of 3–5 days in the BacTec® system and two days incubation after

subculturing. Three of the four isolates were resistant to all betalactam antibiotics inclusive meropenem but susceptible to tetracycline and aminoglycosides. Comparison of the 16S rRNA gene sequences showed different patterns and a common source of infection therefore was unlikely.
Conclusion: Catheter related infections with Methylobacterium are seen in Denmark. The infection might be under-diagnosed as Methylobacterium is slow-growing and not always visible in the initial Gram-stain. The genus is often resistant to meropenem and other betalactam-antibiotics but susceptible to aminoglycosides and tetracyclines

Community-acquired infections including CAP, sepsis, STD, . . .

R2518 Piperacillin/tazobactam versus imipenem/cilastatin for severe diabetic foot infections: a prospective, randomised clinical trial in a university hospital

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Objectives: In this prospective, randomised clinical trial of comparing the effectiveness and side effects of two antibiotic regimens, we assessed the patients with diabetic foot infections whose infection grade is between Wagner grade 2 and 4.
Patients and Methods: Of the 62 patients enrolled the study, we assigned patients randomly into piperacillin/tazobactam (4.5 gr IV q8 h) or imipenem/cilastatin (0.5g IV q6 h) group.
Results: A total of 86 microorganisms were isolated from 62 patients. Infections were polymicrobial in 40% of patients while 21% had no bacterial growth in the culture. 61% of the isolated microorganisms were Gram-positive while 74% were Gram-negative. 66% of the patients had history of previous antibiotic therapy and 40% had a previous history of hospitalisation within 3 weeks of the study. The frequency of the microorganisms isolated from each therapy group was similar and the most commonly isolated pathogens were *Pseudomonas aeruginosa* (20%), Enterobacteriacea spp. (20%), *Staphylococcus* spp. (32%) and *Streptococcus* spp. (13%). The presence of ESBL among the Enterobacteriacea isolates was found in five isolates while the meticillin resistance in *Staphylococcus* spp. was found in four isolates. The amputation rate was 60% and 69% for piperacillin/tazobactam and imipenem group, respectively. Most (64%) of the amputations due to infection was observed in patients with Wagner grade 3. Clinical cure rates were similar between the two treatment groups. The adverse effects were more common in the piperacillin/tazobactam than the imipenem group (30% vs. 9%; $p = 0.055$) with nephrotoxicity (20% vs 3%), hepatotoxicity (17% vs 3%) and haematological side effects (7% vs 0) being most frequent.
Conclusion: Our study suggests that both piperacillin/tazobactam and imipenem/ cilastatin can be used for the empirical treatment of severe diabetic foot infections with polymicrobial and/or resistant microorganisms. Considering their relatively low frequency of side effects, both agents can be used with confidence

R2519 Meterological effects on the incidence of pneumococcal bacteraemia in Denmark

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Objectives: The seasonal nature of invasive pneumococcal disease with peak incidences during the winter months is well recognised. However, few detailed studies of the possible temporal relationship between actual climatic changes and subsequent pneumococcal disease are available.
Methods: We performed an 8-year longitudinal population-based ecological study in a Danish county to examine whether foregoing changes in climatic parameters, including temperature, relative humidity, precipitation, and wind velocity, predicted variations in pneumococcal bacteraemia (PB) incidence. We fitted harmonic sinusoidal models to estimate the exact phase difference in days between climatic changes and PB activity.
Results: There was a close inverse relationship between temperature changes and PB incidence, with an observed time lag of 16 days between temperature peaks and troughs and PB activity (Figure). Peaks in relative humidity preceded PB peaks by about 2 months. These relationships were seen independently of a strong seasonal pattern of PB.
Conclusion: This study suggests that changes in temperature closely predict PB incidence peaks, independently of seasonal patterns.

R2520 Study of community-acquired bacteraemias managed in a medical department

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Objectives: To analyse clinically and microbiologically the cases of community-acquired bacteraemias which were managed in a medical department of a 300 bed Hellenic hospital.
Methods: We studied prospectively all consecutive cases of community-acquired bacteraemias diagnosed (Bactec, Becton Dickinson) and managed in our 28-bed medical department.
Results: During a 32-month period, community-acquired bacteraemia was diagnosed in 90 patients (34 men, mean age 74.9 years), out of which 77 were evaluable for analysis. There were 89 different bacteria isolated, of which 68 were considered clinically significant. The pathogens were Gram(−) (51/68), Gram(+) (16/68) and fungi (1/68). Among Gram(−), *E. coli* (31/51) and *Klebsiella* spp. (8/51) were the most common pathogens, while *S. aureus* (7/24) and *Streptococcus* spp. (7/24) were the most common among Gram(+). There was also one case of *C. albicans* in an old patient with severe underlying diseases and immunosuppression. The most common diagnoses were UTI (35/77), biliary tract infection (15/77) and endocarditis (8/77). 71/77 patients received empiric antibiotic treatment and 6 according to blood culture results. The most common empiric treatment was monotherapy with ampicillin/amoxicillin+inhibitor (33/71), while combination treatment was given in 14/71 patients. According to pathogen sensitivity, empiric treatment proved appropriate in 56/71 cases. Modification of the initial treatment was done in 41/77 cases for various reasons. Mean duration of treatment was 12 days and the outcome was successful in 65/77 cases.
Conclusions: UTIs were the most common cause of bacteraemia in our patients. Gram-negatives and especially *E. coli* were the predominant pathogens. In most of the cases patients received empiric treatment, appropriate in the majority of cases, and the outcome was successful in 84% of cases.

R2521 Searching the Surviving Sepsis Campaign database for avoidable deaths

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Introduction: Recently it has been estimated that in the Netherlands yearly about 1700 deaths in hospitalised patients could be prevented by timely, accurate, diagnosis and adequate treatment. The surviving sepsis campaign is an international campaign aiming at a reduction in mortality with 25% in 5 years time. In March 2007 the SSC guidelines were introduced in the ICU, ER and the departments of Internal Medicine and Surgery of our hospital.
Aim of study: Analysis of cause of death in patients entered in the SSC database in terms of unnecessary and untimely deaths in order to detect possible flaws in the diagnostic and therapeutic process.
Methods: Retrospective analysis of prospectively obtained data from the SSC database a large non-academic teaching hospital.
Results: Ninety one patients (age 65 ± 1.6 mean$\pm$s.e.m., 70% male) were included. 46 (50%) were admitted to the ICU, 45 patients (50%) were treated in the wards. Nineteen patients (20.7%) did not survive, 10 of these patients were not admitted to the ICU because of limits on escalation of treatment (LOT). Nine patients were admitted to the

ICU. Six died in the ICU, cause of death multiorgan failure (n = 4) and withdrawal of futile treatment (n = 2). Three patients died after transfer from the ICU to the ward (all three with LOT).
In all patients blood cultures were taken, in 18 before intravenous empirical broad-spectrum antibiotics were started. Compliance to the resuscitation bundle was >80%. Blood cultures were positive in only two. Multiple sources of infection could be suspected in one patient. A pulmonary focus was suspected in 10 patients, however, in only one a suitable sputum specimen was obtained (*Streptococcus pneumoniae*, n = 1). Six patients were suspected to have a urinary tract infection, in five urinary cultures were taken (*E. coli*, n = 1; *Klebsiella pneumoniae*, n = 1). An abdominal focus was suspected in five patients, urine cultures were taken in four. In two patients the same microorganism was found in the blood and urinary culture (*E. coli*, *Klebsiella pneumoniae*). Microbiological data to narrow the antibiotic regimen were only available in a limited number of patients.
Conclusion: Compliance to the SSC bundles was excellent, but more microbiological samples of sputum should be obtained. Most patients who died in our SSC cohort had limits on escalation of treatment and cannot be counted as preventable deaths. Detailed analysis of cause of death improves the diagnostic and therapeutic process.

R2522 Two cases of Lactococcus endocarditis

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Lactococcus species are common in the environment and they are used in the food industry for food preservation. Although lactococcus species are generally considered as non pathogen for humans, case reports including serious infections such as endocarditis have been reported recently. We report two cases of subacute endocarditis due to Lactococcus spp. in native valves.
Case 1: A 75 years old man was admitted for fever, weight loss and sweeting lasting for a month. Three years ago he had cardiac by pass operation and had an ureterostomia due to urine bladder malignancy. On cardiac examination he has got 3/6 systolic murmur and a vegetation of 4×5 mm was reported on mitral valve by transeosophageal echocardiography. Three consecutive blood cultures grew Gram-positive cocci which were ovoid making pairs or short chains. With the suspicion of enterococcal endocarditis vancomycin 2 gr/day was begun. The bacteria was identified as Lactococcus lactis ssp. lactis. He was successfully treated medically for 6 weeks and no relaps was detected.
Case 2: A 63 years old man was admitted for fever of unknown origin. He was hospitalised in another hospital 3 months before and was given corticosteroids for the initial diagnosis of connective tissue disease. He was on prednisolone therapy (10 mg/day) for the last month but still had fever and malaise. On physical examination he had 3/6 pansystolic murmur and pretibial edema. Proteinuria and haematuria was detected. Serum creatinine was 1.5 mg/dl. Echocardiography showed 1×0.8cm vegetation on mitral valve and blood cultures grew Gram-positive coccus which was identified as *Lactococcus raffinolactis*. He was treated with ampicillin 12 gr/day. On follow up, echocardiography revealed mitral valve rupture and there was no regression in the size of vegetation. Mitral valve replacement was performed and medical therapy was continued for 8 weeks. Renal functions improved during therapy. He was in good health after surgery and no relapse was detected in a year period.
There are very rare cases of lactococcus endocarditis in the literature and to our knowledge *Lactococcus raffinolactis* endocarditis has not been reported before. We could not establish a clear source of infection but both patients were older in age, and one has got malignancy. No dental extraction history was noted.

R2523 Community-acquired urosepsis

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Objectives: Community acquired urinary tract infections (CAUTI) are among the most common bacterial infections. In our study we aimed to analyse retrospectively, the cases we followed in our clinic, diagnosed as CAUTI, and to share the risk factors, clinic, ethiologic and laboratory data in cases with urosepsis(US).
Methods: The cases followed in Eskişehir Yunus Emre State Hospital, Department of Infectious Diseases, during January 2005 and October 2007, diagnosed as CAUTI were analysed retrospectively. The Chi-squared test was used for qualitative variables and independent predictors of US were assessed using multiple logistic regression.
Results: 229 UTI and 71 (31%) US cases were determined during the study period. Between the US cases, 43 (60.6%) were women, 28 (39.4%) were men, with a mean age of 67.4+ 15.9 (20–92) years. The distribution of the US cases was: sepsis 65 (90.1%); severe sepsis 5 (7%); septic shock 1(1.4%), multiple organ disfunction 1 (1.4%). When the cases with and without US were compared, leucocytosis, thrombocytopenia, elevated sedimentation rate, elevated CRP, pyuria, haematuria, proteinuria and leucocyte esterase positiveness was significantly higher in the US cases. When the risk factors of the 229 CAUTI cases were compared according to the presence of US, age (year), diabetes mellitus (DM), neurologic disease, chronic obstructive pulmonary disease (COPD), steroid therapy, and immunosupression were statistically significantly higher in patients with US (table-1). The most frequent microorganism encountered by blood and urinary cultures, in patients followed for CAUTI, with or without US, was *E. coli*, *Klebsiella* sp. and *Enterococcus* sp. In one case it was polymicrobial. 6 of the 71 patients with US died, (mortality rate 8.5%). Of the 158 cases without US, one patient died (mortality rate 0.6%). The mortality in cases with US was statistically significantly higher (p = 0.001).
Conclusion: The CAUTI cases that are elderly, have other diseases such as DM, COPD, neurologic diseases and have received steroid therapy or immunosupression, have a higher risk of developing US. Although ratios may vary, the causative agents are not different in community acquired US cases and in the cases without.

Table 1. Comparison of the risk factors according to US

	OR	95% CI	*p*
Age (year)	1.03	1.01–1.06	0.004*
Male gender	0.78	0.36–1.71	0.547
Urolithiasis	0.48	0.15–1.52	0.216
Urinary catheter	0.82	0.23–2.28	0.768
Prostatic involvement**	0.67	0.10–4.30	0.678
Incontinence	1.36	0.56–3.33	0.492
Urological abnormalities	0.16	0.01–1.64	0.126
Diabetes mellitus	0.22	0.10–0.48	0.000*
Cardiovascular disease	1.01	0.46–2.22	0.962
Neurologic disease	0.13	0.03–0.49	0.002*
Chronic renal insufficiency	11.7	0.82–164.5	0.069
Chronic liver disease	1.95	0.33–11.4	0.456
COPD	0.22	0.06–0.71	0.012*
Steroid therapy	27.9	1.89–409.7	0.015*
Immunosuppression	0.11	0.01–0.88	0.038*
Neoplasia	2.35	0.24–22.28	0.455

*Significant. **Was evaluated in male patients.

R2524 Importance of pneumococcal bacteraemia for severity scoring in community-acquired pneumonia

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Objective: The severity rules pneumonia severity index (PSI) and CURB-65 are widely used for severity assessment in community-acquired pneumonia (CAP). We aimed to compare the performances of these rules in CAP patients with and without pneumococcal bacteraemia.
Methods: The records of all patients hospitalised at our 35-bed clinic with a discharge code of pneumonia during a 4-year period were retrospectively screened. Patients with chest X-ray infiltrates, at least

two symptoms indicating pneumonia, and no hospitalisation during the preceding month were considered to have CAP. The CAP patients who had blood cultures obtained on admission were included in the study. The PSI, CURB-65, and CRB-65 rules were calculated. Data on survival at day 30 from admission was collected from the national population register.

Results: Among 648 CAP patients, who fullfilled the study criteria, 46 patients (7.1%, mean age 65 years) had pneumococcal bacteraemia, while 602 patients (mean age 66 years) had blood cultures that were negative for *Streptococcus pneumoniae*. The frequencies of comorbid illnesses were similar in the two groups. Death within 30 days was noted in 15% (7/46) of those with and in 4.5% (27/602) of those without pneumococcal bacteraemia ($p = 0.002$). The table shows the 30-day mortality rates according to different severity scores in the CAP patients with and without pneumococcal bacteraemia.

Death within 30 days and/or admission to intensive care unit (ICU) was noted in 28% (13/46) of those with and in 12% (70/602) of those without pneumococcal bacteraemia ($p < 0.001$). Death and/or ICU admission among the CAP patients with high severity scores was noted as follows (pneumococcal bacteraemia vs. no pneumococcal bacteraemia): PSI V, 80% (8/10) vs. 35% (22/63, $p = 0.013$); CURB-65 3–5, 47% (7/15) vs. 23% (27/119, $p = 0.044$); and CRB-65 2–4, 50% (9/18) vs. 20% (35/176, $p = 0.004$).

Conclusion: The present study shows that pneumococcal bacteraemia is an important severity factor in CAP. In CAP patients with high severity scores, the outcome appears to be worse in those with than in those without pneumococcal bacteraemia. This knowledge should be borne in mind when severity scores are interpreted in CAP patients with known or suspected pneumococcal bacteraemia.

Severity rule	Score	30-day mortality rates [No. dead/total no. (%)]		p-value
		Pneumococcal bacteraemia	No pneumococcal bacteraemia	
PSI	I–III	0/27 (0)	1/340 (0.3)	1.0
	IV	1/9 (11)	12/199 (6.0)	0.45
	V	6/10 (60)	14/63 (22)	0.021
CURB-65	0–1	0/19 (0)	1/305 (0.3)	1.0
	2	2/12 (17)	8/178 (4.5)	0.12
	3–5	5/15 (33)	18/119 (15)	0.078
CRB-65	0	0/12 (0)	0/139 (0)	1.0
	1	1/16 (6.2)	7/287 (2.4)	0.36
	2–4	6/18 (33)	20/176 (11)	0.009

R2525 Usefulness of urinary *Streptococcus pneumoniae* test for early aetiologic diagnostic of community-acquired pneumonia admitted to a regional hospital (ATHENAS Study)

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Objective: *Streptococcus pneumoniae* is the main cause of community-acquired pneumonia (CAP). The cornerstone for ethiologic diagniostic, sputum culture, may persist negative up to 45% of cases. The aim of the present study was to assess the usefullness of urinary *Streptococcus pneumoniae* antigen test for early ethiologic diagnostic of CAP in patients who are admitted into a secondary-hospital.

Patients and Methods: A prospective observational study, including the whole 319 CAP diagnosed patients admitted into an Internal Medicine Department between Novemeber 1, 2005 to April 30, 2007, was designed. CAP diagnostic was performed when a new pulmonar infiltrate in Chest X-Ray was observed, along with at least two of the following criteria: fever >38ºC, cough, purulent sputum, crackles at chest exploration, peripheral blood leucocites >10,000 cel/mm^3). To diagnostic pneumococcal ethiology patients underwent these tests: sputum culture, peripheral blood culture (if patient had fever >38ºC) and urinary *Streptococcus pneumoniae* antigen test (BINAX-NOW pneumonia kit).

Results: Median age of patients analysed was 72 years. During the study were performed sputum culture in 183 patients (57.4%), blood culture in 143 (45%) y urinary *Streptococcus pneumoniae* antigen test in 287 (90%). 108 cases of pneumococcal-CAP were diagnosed, by means of urinary antigens (95 cases [88%]), sputum culture (23 [21.3%]) y blood culture (9 [8.33%]). *Streptococcus pneumoniae* was isolated from sputum in twelve patients (52.2%), although urinary test were negative. However, all nine patients who presented with pneumococcal bacteraemia showed a positive urinary antigen test. No statistically differences were found when compared the rate of positivity in urinary test after stratifying the population studied according to severity Fine criteria ($p > 0.05$; t de Student).

Conclusion: Inclusion of urinary *Streptococcus pneumoniae* antigen test as a part of early ethiologic study of patients with CAP at admission into hospital is useful. This test allows increase significantly the rate of diagnostics of pneumococcal pneumonia in our population, including high severity cases, such as those with bacteraemia.

R2526 C-reactive protein in predicting the need for reoperation in odontogenic maxillofacial infections requiring hospital care

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Objectives: Odontogenic infections may lead to hospitalisation and severe infection complications. Although management of these infections with antimicrobial therapy and surgical intervention has a good prognosis, reoperation is occasionally essential for resolution of the infection. Our aim was to find a marker that would identify those patients subsequently requiring reoperation.

Methods: In this retrospective study the medical records of 101 consecutive patients admitted to the Helsinki University Central Hospital due to an odontogenic infection were analysed. This patient series covers all cases of severe odontogenic infections in the Helsinki and Uusimaa Hospital District during 2004 (1.4 million inhabitants). Age, gender, occupational social class, body temperature, WBC counts, CRP levels, focus, fascial spaces involved, need for reoperation, need for intensive care, APACHE II score and length of stay were reviewed.

Results: CRP levels and WBC counts were higher on admission in patients later requiring reoperation ($P < 0.0001$ and $P = 0.0337$, respectively), and cut-off values of CRP level >120 mg/L and WBC count >14.0 $10^3/\mu L$ were significantly associated with a higher incidence of reoperation ($P = 0.0002$ and $P = 0.0019$, respectively). These patients also reached higher CRP levels and WBC counts than patients not requiring reoperation. Interestingly, they reached the maximal body temperature one day later, although admission body temperatures showed no difference. The length of stay was longer and the need for and duration of intensive care were higher in reoperated patients (all $P < 0.0001$). A multiple space involvement was present more often in patients requiring reoperation ($P = 0.0006$).

Conclusion: This study shows that C-reactive protein is a valuable diagnostic marker with a cut-off value of 120 mg/L on admission in identifying patients subsequently requiring reoperation due to severe odontogenic maxillofacial infection. A combination of CRP and WBC measurements showed to be more reliable than measurements of WBC only. Fever on admission was not found to be useful in identifying patients later requiring reoperation. These patients are more often in need of intensive care and require longer intensive care and hospital stay. Multiple space involvement was found to predispose to reoperation.

R2527 The incidence of SIADH in acute meningitis patients admitted to a hospital in Tehran, Iran, during a 2-year period

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Objectives: Meningitis is one of the dangerous & life threatening infections that delay in its diagnosis & management has great mortality

& morbidity. Syndrome of inappropriate secretion of Anti Diuretic Hormone (SIADH) is one of known complications of acute meningitis that can change the prognosis, duration of symptoms, mortality & morbidity of it. Hypotonic hyponatraemia is one of first manifestations of SIADH, but most of the patients do not have any symptoms, because clinical manifestations usually do not occur until serum sodium reaches below 120 meq/l.

Material and Methods: We enrolled 71 patients with diagnosis of acute meningitis (either Bacterial or Aseptic) that admitted during 2 years in Loghman Hakim General Hospital in our study. We checked serum sodium, Specific gravity of urine & calculate serum & urine osmolality of each of them in 1st & 3rd day of admission. We managed SIADH cases with water restriction & if it didn't work with hypertonic salin plus lasix. The patient's data was analysing by using SPSS Software. (Version 11.5).

Results: A total number of 71 patients were included in our study. 51 (72%) male & 20 (28%) female with an average age of 27.5±2.3 year. Their meningitis were bacterial in 45 (63%) & Aseptic in 26 (37%). Average length of stay of these patients in our hospital was 13.8±10.5 days. Only 5 patients (7%) had our criteria for SIADH (Serum Na <135, serum osmolality <275 & urine osmolality >100). Without peripheral edema or diuretic therapy. Two of them were died & the other two had generalised seizure that one of them had serum Na <120.

Conclusion: The incidence of SIADH in patients with diagnosis of meningitis in this study was (7%) & there was no statistical correlation between SIADH and patient's mortality, mental status in admission, seizure or length of admission.

R2528 Characteristics of patients having *Corynebacterium pseudodiphtheriticum* isolated in heavy growth from sputum specimens

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Introduction: *Corynebacterium pseudodiphtheriticum* (Cd) has been recognised as a respiratory tract pathogen in immunocompromised patients infrequently. We observed sputum specimens from inpatients that were excellent quality with numerous Gram-positive rods that cultured Cd in high numbers. We reviewed these patients to determine any possible clinical significance of Cd and to determine if further investigation of the role of Cd in respiratory infection was warranted.

Methods: A patient was included who was an in-patient in our institution between June 1 2002 and May 31 2004, and had an excellent quality sputum specimen showing heavy (growth in all 4 plate quadrants) and predominate growth of Cd, with the specimen Gram stain consistent with the culture result. Charts were reviewed retrospectively using a standard questionnaire to determine evidence of infection and possible predisposing factors to respiratory infection. The clinical significance of the isolate was assessed by author consensus.

Results: Eleven patients were identified, 9 male, aged 21–77. Seven had a history of smoking, 3 were receiving corticosteroids, but there were no other causes of immmunosuppression. Five had documented fever (⩾38ºC), 4 produced purulent sputum, 5 of 9 had cough (2 were intubated), 8 of 10 had elevated WBC (1 not done), 4 of 10 had radiological evidence consistant with pneumonia. Eight patients received antimicrobials. One patient died.

Conclusions: Two patients were identified as having Cd pneumonia, and 2 with Cd acute bronchitis. The constellation of symptoms, signs and the clinical course were not consistent with lower respiratory tract infection in the remaining 7. Further studies are warranted to define the role of Cd as a respiratory tract pathogen.

R2529 Aseptic meningitis in adults in a university hospital

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Objective: to determine the incidence and the epidemiological, clinical and laboratory characteristics of aseptic meningitis (AM) in adults.

Methods: Prospective evaluation of all adult patients who have undergone a lumbar puncture due to meningitis suspicion in a 600-bed university hospital, serving 800,000 inhabitants in north-eastern Spain, from February 2007 to November 2007. We assess all cases with cerebrospinal fluid (CSF) pleocytosis, and negative Gram stain and standard bacterial cultures.

Results: A total of 237 lumbar punctures (LP) were performed, confirming acute bacterial meningitis in 16 (7%). In 39 patients (16%) the final diagnose was aseptic meningitis, median age 40 years (range 19–80), 51% male. No seasonal distribution. 85% community-acquired. 23% didn't have underlying diseases, 26% had undergone neurosurgery in previous 3 months, 18% infected by HIV, 18% previous meningoencephalitis and 10% chronic ORL pathology. Clinical presentation: 65% headache, 50% fever, 44% nausea-vomiting, 23% decrease of consciousness, 46% neck stiffness and 13% neurological focal deficits. CSF was clear 66% of cases, haemorrhagic 23% and turbid 11%. Median CSF protein (g/L): 0.97 (82% above 0.5g/L), median CSF glucose: 3 mmol/L (38% below 2.5 mmol/L). CSF leucocytes count was less than 500/mm3 in 95%, lymphocytic predominance in 56%. CT scan previous to LP was performed in 87%, showing abnormalities in 47%. The final diagnose was: 8 (21%) infections (5 *Cryptococcus neoformans*, 2 *M. tuberculosis*, 1 Herpes zoster), 7 (18%) postoperative, 5 (13%) partially treated bacterial meningitis, 5 (13%) induced by non-steroid anti-inflamatory drug (NSAID), 2 (5%) carcinomatous meningitis, 2 (5%) migraine. Other (1): parameningeal infection, retroviral rebound syndrome and systemic lupus erithematosus. No cases of enteroviral AM were detected. 50% patients got antibacterial agents, 25% acyclovir, 19% antifungal and 6% tuberculostatic agents. Median stage was 12.5 days. 22% of patients required ICU, 14% endotracheal intubation and 5.4% died. Glasgow outcome scale at discharge was: II 3%, III 13%, IV 19%, V 60%.

Conclusion: AM is a prevalent disease that affects youngsters without previous pathology, patients infected by HIV and who have undergone neurosurgery. Non-enteroviral infections, postoperative and NSAID-related are the main causes of AM in our area. Usually it has a good outcome but sometimes requires ICU admission and has important sequels and mortality.

R2530 Local immune response in women infected with HPV and *Chlamydia trachomatis*

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Objectives: Progression of HPV-dependent dysplastic changes to cervical cancer is connected with other risk factors: immune dysfunction, presence of biological co-cancerogens. The aim of this study was to determine whether *C. trachomatis* can influence on locally produced cytokines (INF-g, IL-1a, IL-10, TNF-a, TGF-b and sTNFR)in women infected with high-oncogenic HPV types.

Methods: 183 women were studied: 39 with normal cytology results (mean age 39.8), 67 with ASCUS (mean 40.5), 49 with LSIL (mean 36.6), 23 with HSIL (mean 35.8) and 5 with SCC (mean 45.2). In each case 2 swabs: one to detect DNA of high-oncogenic HPV types and second to detect *C. trachomatis* DNA (Amplicor, Roche) were simultaneously taken. CVL samples were used for cytokine and anti-*C. trachomatis* IgG detection (VIRO-Immun Labor-Diagnostika GmbH). For statistical analysis U Mann-Whitney, Kruskal-Wallis-Anova and chi^2 tests were used.

Results and Conclusions: *C. trachomatis* DNA was found only in samples taken from 4 women in control group and anti-*C. trachomatis* IgG in 31 out of 183 (16.5%). No statistically significant differences were observed in occurrence of anti-*C. trachomatis* IgG in women from control group and groups with ASCUS, LSIL and HSIL.In women with SCC these abs were significantly more common (60%, $p = 0.0001$). DNA of high-oncogenic HPV was observed significantly more frequently in women with LSIL (46.94%; $p = 0087$) and HSIL (95.65%;$p = 0.0001$) compared with control group women (17.95%) and ASCUS (23.37%). No significant correlation was found between

presence of anti-*C. trachomatis* IgG and high-oncogenic HPV. We did not observe significant differences in concentration of INF-g, IL-1a, LI-10, TNF-a and TGF-b in women with or without anti-*C. trachomatis* abs. Significantly higher concentration of INF-g was observed in women infected with HPV($p = 0.01$) compared with women without HPV DNA. Significantly higher concentrations of TGF-b were demonstrated in HPV-positive women with ASCUS ($p = 0.004$) and LSIL ($p = 0.047$) compared with women with normal cytology infected with HPV. However IL-10 level was significantly lower in HPV DNA-positive women with ASCUS, LSIL and HSIL, compared with HPV-positive women without cytological changes. Positive correlation in concentration of IFN-g and TGF-b and negative correlation for IL-10 in HPV infected women with ASCUS, LSIL and HSIL compared with women without cytological abnormalities was detected.

R2531 Phagocyte Fcg receptors expression in bacteraemia

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Early markers of bacteraemia are useful for prognosis and, in decision making for i.v. antibiotic therapy.

Objectives: To assess the diagnostic power of the surface expression of Fc receptors for IgG (FcgRs) for the prediction of bacteraemia in febrile patients.

Methods: We performed a 5 year prospective case-control study on 193 consecutive patients (pts) with an episode of bacteraemia as compared to 198 randomly selected concurrent febrile pts with negative blood cultures (control). Demographic and clinical data were collected by chart review and/or questioning their attending physicians. Plasma levels of C-reactive protein (CRP), TNFg, IL-1a, IL-6, IL-8 and IL-10 were determined. The surface expression of Fc receptors for IgG (FcgRs): FcgRI, FcgRII and FcgRIII on peripheral blood monocytes (M) and granulocytes (G) was assessed by flow cytometry. These studies were done concomitantly with blood cultures.

Results: Both groups were not different for age, sex, previous administration of immunosupressants or antibiotics, clinical severity index or comorbid conditions. In univariate analysis, cases had significantly higher levels of CRP ($p < 0.001$), TNFg ($p < 0.001$), IL-1a ($p < 0.001$) and IL-6 ($p < 0.01$) than controls. The expression of FcgRIIA and FcgRIII by M and, that of FcgRI on G was significantly enhanced ($p < 0.001$) in bacteriemic patients as compared to culture-negative febrile pts; while the expression of FcgRIIB by either M or G was significantly decreased ($p < 0.03$). Setting a cut-off value =25% of the mean fluorescence intensity over controls for FcgRs surface expression and, assuming a prevalence of bacteraemia of 5–10% among hospitalised patients undergoing blood cultures, results in a sensitivity, specificity, positive and, negative predictive values of: 77%, 97%, 74%, and 98%, respectively for M-FcgRIIA, 73%, 96%, 74% and 97%, respectively for M-FcgRIII, 58%, 93%, 49% and 95%, respectively for G-FcgRI and, 71%, 91%, 57% and 83%, respectively for G-FcgRIIB.

Conclusions: Our results suggest that the surface expression of Fc receptors for IgG on peripheral blood monocytes and granulocytes may help clinicians to rule out bacteraemia in febrile patients.

Antimicrobial clinical trials

R2532 Clinical and bacteriological efficacy of sequential intravenous to oral moxifloxacin in hospitalised patients with community-acquired pneumonia and bacteraemia

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Objectives: To compare the clinical and microbiological efficacy of sequential IV/PO moxifloxacin (MXF) with comparator (COMP) therapy in patients with community acquired pneumonia (CAP) and bacteraemia.

Methods: Data were pooled from 6 trials of MXF 400 mg (IV/PO) in the treatment of CAP in hospitalised patients. COMP treatments were ceftriaxone + erythromycin, amoxicillin/clavulanate + clarithromycin, trovafloxacin, levofloxacin, ceftriaxone + azithromycin + metronidazole or ceftriaxone + levofloxacin. Blood cultures were performed prior to initiation of study drug therapy.

Results: The 6 trials randomised 3015 patients (1494 MXF, 1521 COMP) of which 2288 were valid per protocol (PP) (1141 MXF, 1147 COMP). Of the valid PP patients, 342 MXF and 361 COMP-treated subjects were also microbiologically valid. 68 (6.0%) patients in the MXF group and 83 (7.2%) in the COMP group had bacteraemia. Of these, 40 (58.8%) in the MXF group and 48 (57.8%) in the COMP group, were male, 20 (29.4%) and 17 (20.5%) were $\geqslant$75 years old, 12 (17.6%) and 14 (16.9%) had bilateral infiltrates and 28 (41.2%) and 39 (47.0%) had severe CAP (ATS 2001 criteria). *S. pneumoniae* was the most common pathogen (MXF 48, COMP 64), with a range of other pathogens occurring less often including *S. aureus* (5, 2), *H. influenzae* (4, 4), *E. coli* (2, 3). Efficacy data at test-of-cure for the bacteraemic population are shown in the table. Clinical success rates in the non-bacteraemic population were MXF 89.1% and COMP 87.7%.

	MXF, n/N (%)	COMP, n/N (%)
Overall clinical success	54/68 (79.4)	65/83 (78.3)
Clinical success in severe CAP	19/28 (67.8)	25/39 (64.1)
Overall bacteriological success[†]	56/68 (82.4)	67/83 (80.7)
Bacteriological success[†] *S. pneumoniae* bacteraemia	42/48 (87.5)	50/64 (78.1)

[†]Eradication + presumed eradication.

Conclusions: Monotherapy with MXF (IV/PO) resulted in clinical and overall bacteriological success rates similar to those of COMP therapy in patients with CAP and bacteraemia. The bacteriological success rate in bacteraemic patients with *S. pneumoniae* was numerically higher with MXF.

Paediatric infections

R2533 Clinical qualitative evaluation of the diagnosis of acute otitis media in general practice

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Objectives: Assess the quality of the diagnosis of Acute Otitis Media (AOM) given by General Practitioners (GPs) on a daily basis and compare it with the diagnosis of Ear, Nose and Throat specialist (ENTS) which is considered as the gold standard.

Methodology: Every GP had to include 6 children aged 1 to 4 years for whom he suspected or diagnosed that they were suffering from AOM. Parents had to accept to see the ENTS participating in the survey within 48 hours.

Results: 24 GPs took part in the survey and included at least one child, which amounts to a final 57% acceptability rate. 208 eardrums were included in the survey. 21.9% of assumptions or diagnosis's of AOM (30/137) were declared null by the ENTS. GPs diagnose AOM without any doubt only in 54% of all cases. The diagnosis and the assumption of AOM were respectively confirmed in 83.8% of all cases and 71.4% by the ENTS. The combination of redness and bulge, and isolated redness accounted for respectively 44.3% and 26.2% of the main otoscopical factors reminiscent of the AOM according to GPs. In the case of redness and bulge, the diagnosis was confirmed in 83% of all cases by the ENTS as opposed to 75% regarding the isolated redness.

An AOM was suspected in 57.1% of the eardrums barely or not visible or without any sign of infection and not confirmed in 25% of all cases.

Conclusion: The global over diagnosis is 21.9% and 25% when the otoscopy is hindered by the presence of cerumen or when the eardrums are only inflammatory. Even though the over diagnosis is inferior to the one mentioned in published writings, post-graduate teaching on the various cerumen removal techniques and the use of pneumatic otoscopy could contribute to improving the quality of diagnosing AOM.

R2534 To determine the efficacy and safety of different doses of faropenem medoxomil in acute otitis media as measured by double tympanocentesis

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Objectives: to determine the efficacy and saftey of 4 doses of FM in AOM and the impact of therapy on colonisation and antimicrobial resistance development in children aged 6 months – 7 years.
Methods: Subjects with AOM were enrolled in 2 sites, Costa Rica & Israel and randomised to receive one of following doses of FM in cohort 1: 7.5 and 15 mg/kg/dose and in cohort 2: 15, 30 or 40 mg/kg/dose bid for 10 days. Baseline tympanocentesis taken on all subjects and repeated on day 4–6 in those with positive baseline or those who had clinically failed. Test of cure clinical response was evaluated at d 12–14 and late follow up at d 28–32. Naso-oropharyngeal swabs were taken at each visit.
Results: Total of 328 subjects enrolled leading to 47(7.5); 77(15), 79(30) and 67(40) [total 270] who completed 10 days therapy (82%). 92% of patients were enrolled in Costa Rica, 56.9% <2 years old, 28.2% had received antibiotics in preceding 30 days. Pathogens isolated were *S. pneumoniae* 113 (44.0%), *H. influenzae* 103 (40.2%), *M. catarrhalis* 28 (10.9%) & *S. pyogenes* 12 (5.9%), total 256. Significant dose effect on bacteriologic eradication. However, higher eradication seen in cohort 2 of 15 mg/kg group (85% vs. 62.5%, p = 0.07), suggesting cohort effect may be confounding. Eradication of *H. influenzae* accounted for dose effect and significantly higher even within cohort 1 (15 mg vs. 7.5 mg, p = 0.01).

	7.5 mg/kg	15 mg/kg		30 mg/kg	40 mg/kg
		cohort 1	cohort 2		
Clinical response at TOC, %	84.6	72.5	75	88.4	82.7
Clin resp. at Late FU	78.8	65	70	77.9	73.3
Bact. erad mITT	16/29	25/40	17/20	37/43	46/49
	55.2%	62.5%	85%	86%	93.9%
S. pneumoniae erad., %	94.1	88.2	91.7	85.0	100
H. influenzae erad., %	0	45.5	83.3	81.0	84.2
M. catarrhalis erad., %	100	87.5	NA	83.3	100
S. pyogenes erad., %	NA	100	100	100	100
Subjects with 1 AE, %	36.8	43.3		48.9	41.4
Diarrhoea, %	12.3	17.8		25.6	12.6
Vomiting, %	3.5	7.8		6.7	13.8

No FM resistance emergence. Reduction in oral or nasal colonisation was observed for *S. pneumoniae* and *M. catarrhalis*.
Conclusions: FM was efficacious and well tolerated at doses of at least 15 mg/kg/dose given bid for 10 days in AOM.

R2535 Rothia dentocariosa bacteraemia in cardiosurgery patients in a children's hospital: report of two cases

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Introduction: Rothia dentocariosa is part of the normal oral flora, particularly in patients with periodontal disease and until recently it was not considered to be pathogenic to human. Lately, R.dentocariosa is evaluated when isolated from blood cultures of cardiosurgery patients and in cases of endocarditis, mainly when predisposing factors are present, such as heart valve disease and congenital heart defects. We present two cases of R. dentocariosa bacteraemia in children who were treated surgically for the congenital heart disease they were suffering from.
Case 1: A 14-month-old female infant was admitted to the Cardiosurgery Department of our Hospital because of Ventricular Septal Defect and Pulmonary Stenosis. She was successfully operated (Bidirectional Glenn shunt) and was discharged from the hospital in good general health. Ten days later, she was readmitted in the Cardiosurgery Department with chylothorax and pleuritis. Two of the blood cultures yielded R.dentocariosa, which was evaluated. Cefuroxime per os was administered to the child additionally to the prescribed cardiological medication and resulted to clinical improvement..
Case 2: A 2-month-old male infant was admitted to the cardiosurgery clinic for surgical repair of Coarctation of the aorta and Patent ductus arteriosus. Three days after the operation, R.dentocariosa was isolated from the blood culture. Cefuroxime per os was administered.
Methods: Blood specimens were inoculated into Bactec Ped Plus/F Medium (Becton-Dickinson) and were incubated in the automated blood culture system Bactec 9240 (B-D). The positive cultures yielded a pleomorphic Gram-positive rod which was identified as R.dentocariosa with the BBL Crystal GP System (Becton-Dickinson). Susceptibility test was performed with the Kirby-Bauer method. Penicillin, vancomycin, erythromycin, clindamycin, cefotaxime and chloramphenicol were tested. The evaluation of the results was based on the CLSI guidelines about streptococci. Only one of the two strains isolated was resistant to clindamycin.
Conclusions: Although R.dentocariosa bacteraemia is rarely observed, physicians should be alert to evaluate it correctly when R.dentocariosa is isolated from cardiosurgery patients.

R2536 Neonatal sepsis caused by *Streptococcus bovis* biotype II/2

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Objective: *Streptococcus bovis* is an uncommon cause of infection in neonates. However, little is known about the clinical manifestation and condition that predispose to *S. bovis* infection in this population.
Here we report a 5-day-old baby with bacteraemia and sepsis caused *Streptococcus bovis* biotype II/2.
Material and Methods: A 5-day-old baby boy presented with fever (39 C), irritability and physical examination revealed 200/min heart, 60/min respiration rate and hepatosplenomegaly.
The patient was born at term by spontaneous vaginal delivery after an uncomplicated pregnancy, weigth 3140 g, APGAR score was 9/10. Maternal prenatal culture was negative for group B streptococcus.
Laboratory studies showed the following: WBC: 20.9 G/l (neutrophils:62.8%, lymphocytes:23.8%, monocytes: 8.7%) platelets: 209 G/l, haemoglobin: 177 g/l, CRP: 55g/l. Antibiotic therapy was started empirically with ampicillin and aminoglycoside.
The blood culture demonstrated Gram-positive coccus after 16 hour of incubation. Subculture showed alpha-haemolytic colonies. Rapid latex agglutination tests for grouping of Lancefield were performed (Slidex Strepto plus, bioMerieux SA) and it appeared group D streptococcus. Subsequently, the isolate was identified as *Streptococcus bovis* biotype II/2 by the API 20 Strep system (bioMerieux SA). The strain was sensitive for penicillin, ceftriaxon, erythromycin, clindamycin and vancomycin. After continuing of single ampicillin administration the patient was rapidly recovered.
Conclusion: Among group D streptococci, enterococci are well recognised as causes of neonatal sepsis. In contrast, *Streptococcus bovis* is considered an uncommon pathogen in newborn. Accurate identification of *S. bovis* has important implication for treatment. *Streptococcus bovis* strains remain exquisitely sensitive for pencillin, whereas in case of enterococcus or *viridans* streptococcus infection addition of aminoglycoside or vancomycin are necessary for sufficient treatment.

R2537 Neonatal bacteraemia: bacteriological profile and antimicrobial susceptibility pattern

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Introduction: Neonatal septicaemia remains a significant cause of morbidity and mortality in the new born. This study was carried out

to determine the bacteriological profile, the antimicrobial susceptibility of the blood isolates and the change in the trends over the study period.
Methods and Materials: All blood isolates from cases of neonatal bacteraemia (single isolate per episode) were taken over a period of 5 years (January 2002–December 2006) at the Maternity hospital, Kuwait. Routine identification and susceptibility testing were performed mainly by using VITEK 2 system. E test may be used for some isolates.
Results: There was 1551 episode of bacteraemia during the study period. The most common isolated organism was coagulase-negative Staphylococci (45%). Gram-negative organisms were isolated in 464 (30%) of cases and the most common isolate was *Klebsiella pneumoniae* (37.1%). Other Gram-negative isolated were *Escherichia coli* (14.2%), *Enterobacter* spp. (14%) and *Acinetobacter* spp. (15.3%). All Gram-positive cocci were susceptible to vancomycin and most Gram-positive cocci other than Staphylococci were susceptible to ampicillin. No meticillin-resistant *Staphylococcus aureus* were isolated. Most of the Gram-negative were sensitive to amikacin (95.7%), ciprofloxacin (95.3%), tazobactam-piperacillin (95.3) and for meropenem (99.1%) while they were more resistant to cefotaxime (46.9%), ceftazidime (34.9%) and only 10.9% were resistant to gentamicin.
Conclusion: The bacteriological profile of organism causing neonatal bacteraemia is comparable to other reports in literature. Over the study period we observed a trend of increasing resistance to commonly used antibiotics among Gram-negative isolates.

R2538 ESBL-producing Enterobacteriaceae among neonates in an academic clinical centre, Gdansk, Poland

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Objectives: The aim of this study was to analyse occurrence of ESBL [Extended-Spectrum Beta-Lactamases] producing Enterobacteriaceae in patients who were hospitalised in the Depart. of Neonatology Medical University of Gdansk.
Methods: We analysed microbiological records obtained from 2003 to 2007. Strains were identified by classical method and VITEK, VITEK 2 (BioMerieux). Production of ESBL was detected by double disk method.
Results: In the studied period 8632 patients were hospitalised. ESBL+ isolates were recovered from 425 patients. In 2003 ESBL+ isolates were recovered from 216 patients, then 2004 – 48 patients, 2005 – 27 patients, 2006 – 110 patients and 2007 – 24 patients.
The most often isolated was *Enterobacter aerogenes* from 131 patients (47.1%), then *Klebsiella pneumoniae* 96 patients (34.5%) *Escherichia coli* 17 patients (6.1%); *Enterobacter cloacae* 16 patients (5.8%); *Klebsiella oxytoca* 11 patients (4%); *Citrobacter freundii* 6 patients (2.2%) and *Enterobacter intermedius* 1 patient.
Isolates were recovered from stools and rectal swabs (54%), respiratory tract (37.4%), urine (3.89%) and blood (1.73%).
All strains were susceptible to carbapenems, about 90% to fluoro-quinolones and nearly 80% urine isolates to nitrofurantoin, but about 90% isolates were resistant to aminoglicosides and co-trimoxazole.
Conclusions: High percentage of ESBL carriage among children complicates infection control measures and treatment of common infections like urinary tract infection.
Newborns should be screened for ESBL carriage in our setting.

R2539 Diagnosis of *Mycoplasma pneumoniae*: results of a prospective study in Tunisia

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Objectives: *Mycoplasma pneumoniae* is an important human respiratory tract pathogen; it causes 15 to 20% of community-acquired pneumonia in older children and adults and a variety of respiratory tract infection in younger children. The aim of our study was to diagnose and to determine the overall incidence of *M. pneumoniae* infection in young children.
Methods: a prospective study was conducted from November 2005 to October 2007. It concerned 273 neonates and infants hospitalised in the intensive care unit in children's hospital in Tunis and suffering from respiratory problems. We collected 285 different specimens, essentially from respiratory tract, placed into transport medium (2SP). Samples were tested by 16S rRNA gene- and P1 adhesin gene-based PCR and by inoculation in Hayflick modified broth medium supplemented with glucose. Two hundred and ninety-one sera obtained at the same time were used for detection of IgM and IgG antibodies using ELISA-PLATELIA *M. pneumoniae* TMB tests (BioRad®). Typing of *M. pneumoniae* clinical isolates was carried out by a PCR-RFLP method using HpaII restriction endonuclease.
Results: samples were distributed as follow: 174 nasopharyngeal aspirates, 42 throat swabs, 31 nose swabs, 25 tracheal aspirates, 7 bronchoalveolar lavages, 4 cerebrospinal fluids, 1 sputum, 1 articular fluid and 1 pleural fluid. We totally isolated 5 *M. pneumoniae* clinical isolates; all strains belonged to subtype I.
Fourteen patients (10 infants and 4 neonates) could be diagnosed with certain *M. pneumoniae* infection: 4 had positive culture, one had positive nasopharyngeal aspirate by PCR and culture, one had positive throat swab-PCR and serology (positive IgM and moderate IgG), one with positive IgM and high IgG titer, 4 had positive IgM associated with low or moderate or negative IgG and 3 with seroconversions.
Conclusion: our study showed a low incidence (5.12%) (14/273) of current *M. pneumoniae* infection. All *M. pneumoniae* clinical strains were of subtype I. Culture, PCR and serology tests are complementary for the confirmation of diagnosis.

R2540 Microbial analysis of breast milk as a tool to differentiate infectious mastitis and Raynaud's syndrome during lactation

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Objectives. Mastitis is an inflammation of the mammary gland that is frequent during the lactation period and that is usually associated with a bacterial infection. Raynaud's phenomenon consists in a vasospasm of peripherical blood vessels and may affect the nipples of breastfeeding mothers. Raynaud's syndrome is often misdiagnosed as infectious mastitis on the solely basis of a painful lactation. In this context, our objective was to elucidate if bacterial analysis of breast milk can be an useful tool to differentiate both conditions.
Methods. Samples of breast milk were collected from 5 women suffering infectious mastitis and from 5 showing symptoms of Raynaud's syndrome. The microbial composition of the samples was analysed by classical plate count techniques and, also, by molecular techniques as PCR-DGGE and real time quantitative PCR (RTi-Q-PCR).
Results. Using culture-based methods, significant differences were observed between the samples of mastitis-suffering women and those provided by women with Raynaud' phenomenon. Globally, staphylococci and streptococci were the predominant bacterial groups in mastitic milk while no bacteria could be detected after the plating and incubation of Raynaud' samples. The assessment of the bacterial composition of breast milk by PCR-DGGE also revealed significant differences between mastitic and Raynaud samples. In general, more complex profiles were observed in women with infectious mastitis where bands corresponding to staphylococcal and streptococcal species were often present. Interestingly, the intensity of the *Staphylococcus aureus* band was higher in Raynaud' samples. RTi-Q-PCR with different general or genus-specific primer couples confirmed the existence of significant differences in the bacterial composition of milk in both conditions. As an example, the milk of mastitis-suffering women showed higher levels of total bacteria ($p < 0.05$) than those from women with Raynaud' syndrome women.
Conclusions. Our results indicate that the two conditions studied (infectious mastitis/Raynaud' phenomenon) have a different impact on the microbial composition of breast milk. Bacteriological analysis of milk can be an useful tool in the differential diagnosis between infectious mastitis and Raynaud's phenomenon. The recognition of the exact condition responsible for breast pain will allow a more

appropiate treatment, avoiding the cost and side effects of unnecessary antibiotherapy when Raynaud's phenomenon is diagnosed.

Immunology, host defences, immunotherapy

R2541 Levels of anti-*Haemophilus influenzae* type B antibody in Turkey before routine vaccination

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Objectives: *Haemophilus influenzae* type b (Hib) vaccine was introduced into the routine vaccination schedule in Turkey on January 1st, 2007. This study investigated immunity to Hib before vaccination.
Methods: Serum samples collected from Samsun, Antalya and Diyarbakir provinces between 2000–2001 were tested in this study. A total of 1713 serum samples from patients aged 6 months to more than 60 years from 26 health centres were tested. A commercial ELISA (Immunozym Hib IgG, Progen Biotechnik GmhB, Heidelberg, Germany) test was used to measure antibody concentration of anti-Hib IgG. For quality control, 30 sera were selected and retested at the Health Protection Agency (HPA)(Salisbury, U.K) using an in-house ELISA. For evaluation of the concentration of anti-Hib antibody, $\leqslant 0.15\,\mu g/ml$ was accepted as non-protective, >0.15 to <1.0 μg/ml as short term protection and $\geqslant 1.0\,\mu g/ml$ as long term protective. Chi-square test and logistic regression were used for statistical analysis.
Results: Results of the commercial ELISA and the HPA in-house ELISA correlated well (correlation coeficient 0.87). The distribution of antibody concentrations according to age, gender and location by protection levels is shown in the Table. Between 38.1 to 79.3% of children under five years of age had antibody levels lower than those considered protective, and 15.2 to 61.9% had antibody levels cosistent with short term protection. These children should be considered at risk of Hib infection. Protection against Hib infection increased by age in all three provinces (Antalya: OR 1.028, 95.0% CI; 1.070–1.038, Diyarbakir: OR 1.027, 95.0% CI; 1.060–1.038, Samsun: OR 1.041, 95.0% CI; 1.030–1.052). There were no statistically significant differences between provinces when Diyarbakir was considered as the reference province (Samsun: OR 1.800, 95.0% CI; 0.617–1.036, Antalya: OR 1.212, 95.0% CI; 1.930–1.579).
Conclusion: Children under five years of age in Turkey were at high risk for Hib infection before the introduction of vaccination. The impact of the vaccination programme should be monitored at frequent intervals to ensure the maximum benefit is obtained from immunisation.

	Antalya				Diyarbakır				Samsun			
	n	⩽0.15*, %	>0.15–<1**, %	⩾1***, %	n	⩽0.15, %	>0.15–<1, %	⩾1, %	n	⩽0.15, %	>0.15–<1, %	⩾1, %
Age group												
0	19	42.1	52.6	5.3	26	46.2	46.2	7.7	29	79.3	17.2	3.4
1	32	53.1	34.4	12.5	16	68.8	18.8	12.5	31	67.7	19.4	12.9
2	30	50.0	36.7	13.3	21	47.6	52.4		33	75.8	15.2	9.1
3	31	48.4	41.9	9.7	21	38.1	61.9		25	72.0	28.0	
4	23	56.5	34.8	8.7	29	55.2	27.6	17.2	29	58.6	31.0	10.3
5–9	130	30.0	44.6	25.4	113	37.2	38.1	24.8	122	39.3	35.2	25.4
10–14	25	32.0	40.0	28.0	27	37.0	29.6	33.3	31	35.5	32.3	32.3
15–19	27	7.4	55.6	37.0	26	30.8	50.0	19.2	24	25.0	33.3	41.7
20–29	75	18.7	41.3	40.0	60	26.7	30.0	43.3	62	14.5	50.0	35.5
30–39	68	17.6	48.5	33.8	51	17.6	51.0	31.4	60	20.0	41.7	38.3
40–49	64	18.8	50.0	31.3	50	16.0	38.0	46.0	56	10.7	41.1	48.2
50+	85	17.6	48.2	34.1	84	19.0	44.0	36.9	78	25.6	53.8	20.5
Gender												
Male	279	26.5	45.9	27.6	254	34.3	39.0	26.8	283	36.0	40.3	23.7
Female	330	29.1	43.9	27.0	270	29.3	41.5	29.3	297	38.4	33.7	27.9
Location												
Rural	297	27.3	44.4	28.3	287	33.1	36.9	30.0	293	37.9	34.5	27.6
Urban	312	28.5	45.2	26.3	237	30.0	44.3	25.7	287	36.6	39.4	24.0
Total	609	27.9	44.8	27.3	524	31.7	40.3	28.1	580	37.2	36.9	25.9

R2542 Interrelation between typhoid fever process and monocytes with expression of C3bR receptor

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Objectives: To reveal interrelation between typhoid fever process (complicated and relapse flow) and monocytes with expression of C3bR receptor.
Methods: 60 patients in 16–67 of age (25 male and 35 female) with typhoid fever, caused chloramphenicol susceptible strain of *Salmonella enterica* serotype typhi, were tested for determination of C3bR receptor's expression of monocytes with test of rosette formation (Mendes N.F. et al., 1973). There was evaluated the percentage index of rosette formatting monocytes (RFM%) with C3b component of complement. Average rate of this index was 39.0±2.6% in the control group, consisted from 45 healthy people in 17–61 of age (21 male and 24 female).
Results: Among 60 patients relapse of typhoid fever occurred in 12 (20.0%) patients, complications in 7 (11.7%) patients [in 3 (5.0%) case of intestinal bleeding; intestinal perforation, nosebleed, meningoencephalitis and toxic shock in 1 (1.7%) cases]. RFM% index was in low level in 23 (38.3%) patients, in high level in 29 (48.3%) and in the limits of control group indexes in 8 (13.3%) cases. Among 23 patients with low RFM% indexes relapse of typhoid fever was registered in 12 (52.2%) patients, complications occurred in 5 (21.7%) patients. In 6 (26.1%) patients of this group complications and relapses did not registered. In the group of 29 patients with high level of RFM% indexes complications occurred only in 2 (6.9%) patients (intestinal bleeding and toxic shock), vice-versa in majority patients – 27 (93.1%) with high indexes of RFM% there was not any unfavourable outcome. All 8 (100%) patients with normal RFM% indexes unfavourable outcome was not registered as well.
Conclusion: Low indexes of monocyte with C3bR receptor expression in membrane associate with high risk of developing of typhoid fever relapses and complications. High indexes of C3bR expression associate with low risk of complication's developing and absence of relapse's risk in typhoid fever patients. The method of identification of monocytes expressed C3bR receptor can be used in patients with typhoid fever both as a revelation immunodeficiency persons and as a criterion of forecast of disease's flow.

R2543 Bronchial asthma and *Chlamydia pneumoniae*

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Introduction: *C. pneumoniae* is a known cause for respiratory diseases such as pneumonia, bronchitis and more but it has not yet been determined the role at the pathogenesis of bronchial asthma. The purpose of our study was to detect the presence of *C. pneumoniae* antibodies to the serum of patients of our hospital and correlate with the disease.
Material and Methods: We studied 750 patients from which 470 were female and 280 male. All patients were nursed at the pneumonological clinics of our hospital during the last year (2006–2007). 100 healthy controls were also used. 165 out of the 750 patients had symptoms of bronchial asthma following a respiratory infection. At those 165 patients as well as at the 100 controls (IFA) was used to detect C. Pneumoniae antibodies while the rest immunological tests were negative.
Results: All 165 patients had positive IgG antibodies for *C. pneumoniae* in titles ranging from 1/16 to 1/512 with a higher percentage at titles 1/32 and 1/64. The healthy controls had a high title antibodies at 1/16 while no one had a title of 1/128 or 1/512.
Conclusions: As shown on the table the title of IgG antibodies for *C. pneumoniae* is much higher to patients with bronchial asthma than it is to the healthy individuals. Despite the fact that high titles of IgG antibodies for *C. pneumoniae* can be found at countries with low morbidity for bronchial asthma (tropical countries) it is possible that chronic infection with *C. pneumoniae* can be a causing factor for the disease.

Positive IgG antibodies	165 Patients	100 Healthy controls
1/16	32 (19.5%)	78 (78%)
1/32	38 (23%)	16 (16%)
1/64	45 (27%)	5 (5%)
1/128	18 (11%)	0 (0%)
1/256	20 (12%)	1 (1%)
1/512	11 (6.5%)	0 (0%)

R2544 Relevance of A 2 phospholipases and matrix-metalloproteases in the assessment of the inflammatory process evolution in periodontal disease

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Introduction: In the oral cavity, indigenous bacteria are often associated with the aetiology of two major oral diseases, which are endemic in industrialised societies and are increasing in developing countries, i.e. caries and periodontal diseases. Periodontitis involves the destruction of the connective tissue attachment and the adjacent alveolar bone. The induction and progression of periodontal tissue destruction is a complex process involving plaque accumulation, release of bacterial substances, and host inflammatory response.
Purpose: to investigate the levels of proteolytic enzymes and phospholipases in inflammatory pocket exudates and saliva from patients with periodontopatic disease and their correlation with the severity of disease in order to assess their potential applications as biochemical markers in periodontitis clinical trials.
Material and Methods: A total number of 30 adult patients with chronic periodontal diseases of different severity degrees were analysed. Saliva and periodontal pocket content were prelevated after local hygenisation, before any stomatological treatment. All specimens were submitted to qualitative and quantitative microbiological analysis following standard oral microbiology protocols. All isolated microbial species were investigated for the expression of soluble, enzymatic factors. The total protein content and the level of matrix-metalloproteases was assessed by zymographic analysis. The sPLA2 type II was analysed using adapted NEFA C method. The samples were preserved at −200ºC until the analysis.
Results and Discussion: Our results demonstrated the presence of a constant high level of matrix-metalloproteases 2 and 9 (with gelatinase activity) in all periodontal pocket samples. The highest level of MMP-9 was correlated with the microbiological identification of viable, gelatinase producing *Proteus vulgaris* strains in the pocket specimens. The dynamic of sPLA2 type II levels varied with the patient, either succesively growing or remaining constant, or decreasing in consecutively prelevated specimens, but without no specific correlation with the clinical evolution of the disease.
Conclusion: These results are suggesting that despite its value as inflammation parameter, the sPLA2 type II cannot be used as a biochemical parameter of disease severity of evolution. In exchange, the level of MMP-9 could be an indicator of the release of bacterial substances with proinflammatory activity.

Vaccines

R2545 Varicella vaccination in HIV-positive individuals, a time to act

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Introduction: Varicella is a highly infectious disease with secondary attack rates of appriximately 90% for susceptible household contacts. HIV positive non-immune individuals represent such at risk contacts. A 16-year old HIV positive patient originally from Africa presented to A&E in June 2007 with primary varicella pneumonitis. Her brother, with whom she lived, was HIV negative and recently had primary varicella. She was known to be non-immune to varicella. She had received VZV IgG treatment but clinically progressed to require admission. CD4 count was 254 x 10^6. This case, along with recent guidelines, (MMWR 2007), prompted us to review the prevalence of varicella non-immune patients in our cohort. The department provides care for approximately 2400 HIV positive patients of whom 30% have migrated from COHP where rate of VZV non-immunity is known to be higher.
Methods: A retrospective analysis of all patients diagnosed with HIV between 2002 and 2007 was undertaken. Patient demographics including country of origin, VZV IgG status, CD4 count, HAART history and family history especially for children of school-going age were recorded. A phone survey was subsequently carried out to ascertain household membership of all at risk individuals, including children and other immunosuppressed household members.
Results: In the study period 594 HIV positive patients were tested for Varicella IgG, 36.8% from COHP. 15% patients from COHP were non-immune which compared with 4% of the European cohort, highlighting patients from COHP as an at risk group. 50(8.4%) of all patients were VZV IgG negative. Of the non immune patients 8(16%) had a CD4 count <200, 11(22%) had a CD4 count of 200 to 350 and 31(62%) had a CD4 count >350. The non immune patients with CD4 counts >200 were contacted and offered vaccination in-line with recent guidelines. The non immune patients with CD4 counts <200 were contacted and a detailed history of their cohabitants was recorded. Vaccination of non-immune household members was advised.
Conclusion: Primary varicella infection in the HIV positive population poses a credible threat, especially in patients from COHP. Guidelines published in June 2007 advise that non immune patients with CD4 counts >200 can be vaccinated, whereas those with CD4 counts <200 should have their household contacts vaccinated. In retrospect our individual case would have been identified and vaccinated.

R2546 Construction of a recombinant bacmid containing papillomavirus type16-L1 gene

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Objectives: Major capsid protein L1 of HPV-16 intrinsically of assembling and producing viral-like particle (VLP) in vitro.
Methods: HPV16-L1 gene extracted from paraffin embedded infected cervical tissues of patients who suffered from cervical cancer. This gene was inserted into pFast HTA plasmid. After the confirmation, the recombinant plasmid was transfected into DH10 E.Coli containing Bacmid and helper plasmid.
Results: It was observed recombinant Bacmid colonies turned to white colonies and non-recombinant colonies remained blue in absence of X-gal and IPTG. For confirming the recombinant Bacmid production, an in-house designed PCR was applied.
Conclusion: This research has lead to the production of recombinant Bacmid which can replicate in insect cells and produce L1 protein. This protein has the power of assemblage and construction of VLP.

R2547 Comparative study of serum bactericidal activity to serogroup C and serogroup A polysaccharide meningococcal vaccination

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Objective: The polysaccharide vaccine against meningococcus serogroups A and C was the prototype vaccine for this infection, which is safe, inexpensive and effective against the serotypes A and C. Serum bactericidal antibody (SBA) assay, an antibody-mediated complement-dependent method, is the gold standard surrogate of protection for *Neisseria meningitidis*. This study was performed to compare serum

bactericidal activity to serogroup C and serogroup A after polysaccharide meningococcal vaccination.
Methods: Sixteen subjects (36 patients with primary immunodeficiency diseases and 24 healthy controls) were vaccinated with meningococcal polysaccharide vaccine A + C. The SBA assay was performed according to the World Health Organization (WHO) expert Committee on Biological Standardization procedure. Bactericidal antibody response to *N. meningitidis* serogroups A and C in the sera of all subjects were compared before and 3 weeks after vaccination. The geometric mean titer (GMT) were calculated and compared.
Results: Following vaccination, the serum bactericidal antibody GMT against *N. meningitidis* serogroup A and serogroup C were significantly increased compared to the pre-vaccination level in both patient and control groups. The serum bactericidal GMT pre- and post-vaccination against serogroup C were 1.49 and 12.1 while against serogroup A were 1.32 and 16.25. Comparative studies of the serum bactericidal GMT per-vaccination was not revealed any significant difference between bactericidal activity to *N. meningitidis* serogroup A and serogroup C (P= 0.207), whereas the serum bactericidal GMT post-vaccination to serogroup A were significantly higher than the serogroup C (P= 0.006).
Conclusion: The high level of bactericidal antibody response to *N. meningitidis* serogroups A is seemed to be due to differences protective efficacy of serogroup A polysaccharide (89–100%) in comparison to serogroup C polysaccharide (85%) in clinical trials.

R2548 Factors associated with suboptimal compliance to vaccinations

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Objectives: We conducted a systematic review of the available evidence in order to investigate the factors associated with suboptimal compliance to vaccinations, focusing on children and adolescents in developed countries.
Methods: We searched the PubMed database to identify studies concerning suboptimal compliance to childhood vaccination. We categorised the studies in 2 groups with regard to the presence or not of an analytical statistical approach.
Results: We initially identified 553 potentially relevant articles and subsequently reviewed in detail 39 that provided original data regarding the possible association between various factors and suboptimal compliance to childhood immunisations. Factors influencing compliance to vaccinations related to parental-childhood characteristics and healthcare structure-professionals characteristics. Specifically, among the various parental-childhood characteristics studied, non-white race, low socioeconomic status, paying for immunisation, lack of health insurance, low parental education, older age of the child, younger maternal age, large family size, late birth order, lack of knowledge about disease and vaccination, negative beliefs/attitudes towards immunisation, fear of side effects/risks/contraindications, not remembering vaccination schedules and appointments, sick child delays, and delayed well child visits were statistically significantly associated with suboptimal compliance. Also, among healthcare structure-professionals characteristics, skepticism/doubts regarding provided medical information, inadequate support from healthcare providers, lack of available health structures, and problems concerning transportation and accessibility to immunisation clinics were statistically significant factors of suboptimal compliance to vaccination in children population.
Conclusion: By recognising and understanding the factors associated with suboptimal compliance to vaccinations we can better approach the risk populations and target our efforts at stressing and reinforcing the vital importance of immunisations. Methods to enhance compliance to vaccinations may include reminder calls and mail notification of parents by providers, initiation of health education programmes for parents and health professionals, and open communication and trust between care takers of children and physicians.