Abstracts accepted for publication only

Pathogenesis

R2126 The use of the PREVI™ Isola system in faecal and genital samples

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Introduction: This study is a follow up of the study with urine samples in which the value of the PREVI Isola (bioMérieux) system in the routine diagnostic laboratory was analyzed (ECCMID 2009 – Ab.Nr. 1459). PREVI Isola is a system for automated inoculation and streaking and is able to process any material from patients (liquid format). For urine samples we observed less sub culturing and earlier identification resulting in saving labor time and costs. The aim of this study was to explore the usefulness of the PREVI Isola for more difficult samples as feces and genital swabs.
Methods: Feces or genital swabs from 100 different patients were processed manual and by the PREVI Isola. Fecal samples were cultured for *Campylobacter* (*Campylobacter* agar), *Salmonella* and *Shigella* (XLD-agar) and *Yersinia* (*Yersinia* agar). Genital swabs were cultured for aerobic bacteria (blood agar), anaerobic bacteria (anaerobic blood agar), *Neisseria gonorrhoeae* (GO agar), *Gardnerella vaginalis* (*Gardnerella* agar) and for yeasts (Sabouraud agar). For the PREVI Isola both feces (20 μl) and the genital swabs were suspended in 2.5 ml NaCl.
Results for fecal samples: All samples could be evaluated. No *Salmonella* or *Shigella* was found. In 5 samples a *Campylobacter* was found but with the PREVI Isola individual, suspected colonies were better distinguished and were seen earlier (after 1 day). High counts of *Yersinia* were found in 1 sample but only with the PREVI Isola method. Results for genital swabs: In general counts of the different bacteria were somewhat higher (+) with the PREVI Isola method than after manual inoculation. With PREVI Isola individual colonies of the different bacteria were much better distinguished No difference in the isolation of gonococci (3 samples) was seen with both methods. *Gardnerella* was 1 day earlier seen and much easier distinguished from other bacteria with the PREVI Isola method.
Conclusions: As with urine samples PREVI Isola leads to better readable results for the more difficult cultures of feces and genital swabs: individual suspected colonies were better distinguished and counts were higher. Cultures were also often 1 day earlier positive for suspected colonies. Therefore PREVI Isola is very useful in the time consuming culture of especially genital swabs but also for fecal cultures in which identification can be done earlier.

R2127 Study of aetiologic agents in chronic osteomylitis

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Objectives: Osteomyelitis refers to an infection of the bone marrow which may spread to the bone cortex and periosteum via the haversian chanals. Osteomyelitis is an inflammation of bone caused by a payogenic organism. Osteomyelitis has been categorized as acute, sub acute or chronic, with the presentation of each type based on the time of disease onset. (Occurrence of infection or injury). Investigation of prevalent factors which produce infection, also, Estimation and comparison of disk diffusion agar method and E-test method in order to determination of antibiotic sensibility about isolated bacteria from osteomyelitis infections are the main purpose of this study.
Methods: 131patients with osteomyelitis infection disease were selected for this study during 2008–2009. Samples were collected from bone (14.6%), tissue (7.3%), wound (66.1%), secretions (11.3%) and body fluid (0.7%). In order to microbiologic studies all of samples were cultured according to standard procedure. Some disks with especial antibiotics were chosen for each bacterium during the study of antibiotic sensibility via disk diffusion agar (kirby bauer). Minimum inhibitory concentration via E-test method was used for especial antibiotic and finally compared two antibiogram methods together.
Results: Most isolated bacterium was *S. aureus* (33.6%), *Pseudomonas aeruginosa* (14.5%), and least isolated was Eikenella, *Morganella*, *Showenella* (0.8).
Conclusion: There are a number of possible pathogens but *Staphylococcus aureus* is by far the most common, that our study showed same result. Comparison of Antibiogram results that Treatment and evaluation of osteomyelitis infection according to E-test method was more successful than kirby bauer method. We found that *Eikenella corrodens* can be cause of Chronic Osteomylitis.

R2128 Seroprevalence and risk factors of *Helicobacter pylori* infection among schoolchildren in Algiers

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Objective: The aim of our study is to evaluate the seroprevalence and risk factors of *Helicobacter pylori* (Hp) infection in schoolchildren in Algiers.
Material and Methods: We investigated 647 children (aged from 5 to 18 years; mean age 11.5±3.3 years; sex ratio 0.85) from different public schools, located in different districts of Algiers, chosen by drawing of lots. IgG anti Hp antibodies were detected by ELISA (Platelia Hp IgG Biorad). Risk factors, collected from a questionnaire including: mother's and father's education levels, source water, presence or not of domestic animals, and the localisation of the school. For statistical analysis we used Epi Info.
Results: Over all 70% of children were Hp seropositive. There was not statically significant difference according to sex $P = 0.06$. The seroprevalence according to age was 42.85% among children 5 and 6 years aged, 60.57% among 5–11 years; 77% among 12–15 years and 85% among 16–18 years. There was not statically significant difference according to water source and presence of animals. The seroprevalence decreased when mother's or father's education level increased (respectively $P = 0.002$ and $P = 0.003$). The seroprevalence rate of Hp infection differed from a district to another one $P = 0.04$. Also we find again a statically significant difference according to the promiscuity's rate $P = 0.02$.
Conclusion: The seroprevalence rate in the school is high. Almost half of the children are infected at the age of schooling and rates increased with age. The infection is fond linked to poor socioeconomic conditions and to educational attainment of parents.

Animal models including experimental treatment

R2129 Clinical study on therapeutic effect of mycocidine in treatment of ringworm in cattle

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Ringworm or dermatophytosis is an infectious and zoonotic disease caused by different species of ringworm fungi. Many species of animals including humans are susceptible to ringworm infection. Cattle, horses, cats, dogs and domestic livestock are the most commonly affected animals. Lesions of ringworm is usually found on the head, muzzle,

ears, neck, trunck and particulary around the eyes of infected animals. These lesions are generally circular and oval in shape. Gray and dry to red and crusty hairless patches are typical of ringworm.
This study was conducted to determine the therapeutic effect of mycocidine(plant extracted antifungal drug)in treatment of ringworm in cattle. 150 infected cattle with skin lesions of ringworm were treated by mycocidine. Skin scales were collected by scraping of the lesion usig a strile scalpel in to apetri dish. Samples were used for direct microscopic examination and cultured on sabarouds dextrose agar for isolation of ringworm fungi. All animals in this study were divided in two age groups (under 2years old and over 2 years old). Infected animals were subjected for treatment with two times daily applications(locally and topical)of mycocidine. Our resuls showed that rapid and effective cure in the most affected animals occurred 4–5 weeks after use of drug. Morever there was no significant difference in therapeutic rate of two different age groups. Overall mycocidine was effective in both age groups.%78cattle in over 2 years age and %81 those under 2years age were cured completely after 5 weeks application of mycocidine. In conclusion the present study revealed that mycocidine is more effective in treatment of ringworm and its application should be recommended.

R2130 *Chlamydia* and *Burkholderia* pulmonary diseases of the *Mycobacterium tuberculosis*-susceptible I/St mice

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Objectives: To find out whether TB-susceptible I/St mice are susceptible to other pulmonary pathogens, we investigated manifestations of experimental infections caused by microorganisms taxonomically distant from mycobacteria: *Chlamydia pneumoniae* (intracellular pathogen) and *Burkholderia cepacia* (not intracellular bacteria). For challenge we used mutant *Burkholderia cepacia* with dramatically increased capacity of biofilm formation.
Methods: Real time PCR, flow cytometry, sandwich ELISA assays, histochemical staining. For intranasal inoculation we used I/St and TB-resistant A/Sn inbred mice (~10 g body weight).
Results: Comparison of TB-susceptible I/St and TB-resistant A/Sn mice demonstrated that the former are more susceptible to chlamydia, but not burkholderia infection, displaying a significantly shortened survival time following chlamydia challenge. *Chlamydia* infection: severe lung pathology rapidly developed in I/St, but not in A/Sn mice. In agreement with higher macrophage content in the lungs, significantly more macrophage-derived cytokines TNF-a and IL-6 were detected in I/St lung tissue. *Burkholderia* infection: the increasing virulence of mutant with super ability for biofilm formation was demonstrated both for I/St and A/Sn mice. Both I/St and A/Sn mice are more susceptible to mutant burkholderia infection, displaying a significantly shortened survival time following challenge. It was shown that the mutant strain with increased ability of biofilm formation more virulent for I/St and A/Sn mice than parent burkholderia strain. The equal mortality of I/St and A/Sn mice correlated to equal bacterial load in lungs and spleens in both mice strains.
Conclusion: Gained results probably indicates the common mechanism of control of different intercellular pathogens by I/St mice.

Biofilm

R2131 Biofilm formation of *Escherichia coli* O111 on food-contact stainless steel and high-density polyethylene surfaces

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Objectives: A biofilm can be defined as a sessile bacterial community of cells that live attached to each other and to surfaces. Attachment and biofilm formation by food-borne pathogens and spoilage microorganisms on food contact surfaces in processing plants are a public health and cross-contamination concern. Biofilm formation by *Escherichia coli* O111 on commonly used surfaces viz Stainless steel and high density polyethylene were studied.
Methods: For this study 12 stainless steel chips and 12 HDPE chips were used. *E. coli* strain was added to the beakers with TSB and the samples.
Results: *Escherichia coli* O111 formed biofilm with a mean cell density of 4.14 ± 0.80, 7.69 ± 0.19 log CFU/cm^2 on stainless steel and HDPE respectively. There was significant difference ($p < 0.05$) between bacterial counts of two type surfaces.
Conclusion: Based on the results, it can be concluded that *Escherichia coli* O111 can survive on milk contact surfaces e.g. stainless steel and HDPE surfaces forming biofilm. This is the first report, as far as we are aware, of biofilm formation by *Escherichia coli* O111 on food contact stainless steel and HDPE surfaces. We were unable to find reports in our search of the literature.

R2132 Evaluation of different mechanisms of biofilm formation in clinical and commensal isolates of *Staphylococcus epidermidis*

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Objectives: Currently, the two best-understood mechanisms of *S. epidermidis* biofilm formation are ica (intercellular adhesin)-dependent and ica-independent proteinaceous biofilm formation. Ica-dependent biofilm formation is characterized by ica-encoded polysaccharide synthesis; the second mechanism is characterized by surface proteins such as accumulation-associated protein (Aap), biofilm-associated protein (Bap) or Bap-homologous protein (Bhp). Glucose induces both mechanisms, whereas NaCl abolishes ica-independent biofilm formation completely. In this study, we examined the characteristics of biofilm formation of 39 *S. epidermidis* isolates.
Methods: Species identification was done by VITEK 2 (bioMérieux). Study isolates included 3 previously described *S. epidermidis* strains (10b, RP62A, and ATCC12228), 25 isolates from blood cultures of hospitalized patients and 11 isolates from skin of healthcare personnel. The presence of ica operon, aap and agr (accessory gene regulator) operon genes were investigated by PCR in all isolates.
Using a semi-quantitative microtiter plate method, biofilm formation of isolates in BHI, BHI supplemented with 4% NaCl or 1% glucose was quantified at OD595nm. Isolates with $OD595 < 0.4$ (negative control; non-biofilm-forming ATCC12228 strain), $0.4 \leqslant OD595 \leqslant 1$ (positive control; biofilm-forming RP62A strain), and $OD595 > 1$ (strong biofilm-forming 10b strain) were classified as non, moderate and strong biofilm-forming isolates, respectively.
Results: Four isolates were non-biofilm-forming. There was no significant difference in biofilm formation between clinical or commensal biofilm-forming isolates. All tested isolates were agr-positive. Prevalence of the ica operon was twice as high in clinical isolates than in commensal isolates. Mechanism of biofilm formation in different isolates was studied based on the effect of NaCl or glucose. It was ica-dependent for 9 isolates, and ica-independent for 20 isolates include 5 ica-positive. Six ica-negative isolates could produce biofilm in BHI whereas NaCl and glucose reduced their biofilm formation.
Conclusion: Our results show that presence of the ica operon doesn't always ensure the ability of the isolates to form biofilm via the ica-dependent mechanism. The presence of biofilm-forming isolates that are ica-negative, aap-positive or ica-negative, aap-negative and of which biofilm formation can't be affected by NaCl or glucose suggests a novel mechanism of biofilm formation.

R2133 Surface properties and biofilm formation in *Candida albicans* and *Candida parapsilosis* bloodstream isolates

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The yeasts of the genus *Candida* belong among important ethiological agents of the nosocomial bloodstream infections. Indwelling medical devices are considered one of the main predispositions to yeast infections. Therefore, the adhesion and the biofilm formation are important virulence factors in these microorganisms.

We assessed the biofilm formation in *Candida albicans* and *Candida parapsilosis* blood stream isolates by two commonly used methods – the polystyrene microtiter plate method (MTP) and the cultivation on PVC plastic discs. The biofilm layer structure was examined by the scanning electron microscopy (SEM) after processing of samples by freeze-drying technique. Simultaneously, hydrocarbon (xylene) adhesion assay was used for the hydrophobicity assessment of selected strains. The isoelectric points (pI) in these two yeast species were evaluated by capillary isoelectric focusing (CIEF).
The biofilm formation on the surface of the plastic discs was detected in all tested strains. On the other hand, we did not detect the biofilm formation in a number of strains by the microtiter plate method. Only 21.6% of *C. albicans* strains and 53.6% of *C. parapsilosis* strains were found as biofilm-positive by the microtiter plate assay. This is probably due to the differences in both culture surfaces and different manipulation with the samples. Our SEM observations showed that some strains, considered as biofilm-negative by MTP, formed a thin rudimental biofilm layer on the PVC discs. We found out that the biofilm-negative *C. parapsilosis* strains and all *C. albicans* strains are less hydrophobic in comparison with biofilm-positive *C. parapsilosis* strains. The isoelectric points were determined as 2.8 for all *C. albicans* strains. The clearly biofilm-negative *C. parapsilosis* strains focus near pI value of 3.8, while the pI value of the clearly biofilm-positive strains is near 3.6. The results of the CIEF correspond well with the cell surface hydrophobicity ($p < 0.001$).

R2134 The effect of sub-inhibitory concentrations of tigecycline and vancomycin upon *Staphylococcus epidermidis* intercellular adhesin and lipoteichoic acid gene expression in planktonic and biofilm cells

J. Rollason, A.C. Hilton, T. Worthington, A.B. Vernallis, T.S. Elliott, P.A. Lambert (Birmingham, UK)*

Objectives: The pathogenesis of *Staphylococcus epidermidis* (*S. epidermidis*) is enhanced by its ability to attach to the surface of biomaterials forming a multilayered structure with increased resistance to antimicrobials and the host immune response. Lipoteichoic acid (LTA) enables ionic binding to artificial surfaces and cell to cell adhesion is part mediated by a polysaccharide intercellular adhesin (PIA). This study investigates the effect of sub-inhibitory concentrations of tigecycline and vancomycin upon the expression of genes involved in the synthesis of PIA (icaA) and LTA (ltaS) in *S. epidermidis* RP62A biofilm and planktonic cells. Additionally the expression of icaA and ltaS in planktonic and biofilm cells was compared.
Methods: Planktonic *S. epidermidis* cells were grown for 8 hours at 37ºC in sub-inhibitory concentrations of tigecycline (0.003μg/ml; 0.125 x planktonic MIC), vancomycin (0.125 μg/ml; 0.125 x planktonic MIC) and a broth control. RNA was extracted using Trizol and converted to cDNA using a cDNA synthesis kit (Stratagene). Quantitative real-time PCR was carried out using gyrB as an internal control.
Results: Sub-inhibitory concentrations of tigecycline and vancomycin had no effect upon the expression of icaA (1.1 and 1.2 fold) and ltaS (1.0 and 1.0 fold) in planktonic cells. Sub-inhibitory concentrations of tigecycline and vancomycin had a weak effect upon the expression of icaA (1.9 and 2.2 fold) and ltaS (1.4 and 1.6 fold) in biofilm cells. When compared to planktonic cells grown in broth alone, biofilm cells demonstrated an 8.1 fold increase in the expression of icaA and no significant change in the expression of ltaS (1.1 fold).
Conclusion: Sub-inhibitory concentrations of tigecycline and vancomycin had a minimal effect upon the expression of icaA and ltaS in planktonic and biofilm cells providing re-assurance for the application of these antimicrobials in biofilm related therapy. The observed increase in icaA expression in biofilm cells when compared to planktonic cells provides further evidence for genotypic phase variation between the two cell states. In contrast when compared to planktonic cells, biofilm cells demonstrated no increase in ltaS indicating conserved expression regardless of cell state.

R2135 Sub-inhibitory concentrations of tigecycline reduce *Staphylococcus epidermidis* biofilm formation

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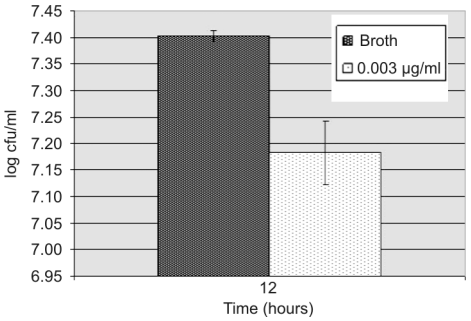
Objectives: *Staphylococcus epidermidis* (*S. epidermidis*) is an opportunistic pathogen and a lead cause of indwelling device and prosthetic infections. Tigecycline is a novel glycylcycline antibiotic with broad range antibacterial activity. With steadily increasing antibiotic resistance within the clinical environment, tigecycline may be considered as an alternative treatment option in prophylaxis and treatment of biofilm related infection. This study investigates the effect of sub-inhibitory concentrations of tigecycline and vancomycin upon biofilm formation.
Methods: A planktonic culture of *S. epidermidis* RP62A (5×10^4 cfu/ml) was exposed to sub-inhibitory concentrations of tigecycline (0.003μg/ml; 0.125 x planktonic MIC), vancomycin (0.125 μg/ml; 0.125 x planktonic MIC) and a Mueller-Hinton broth control. Cells were grown aerobically at 37ºC for 12 hours in a microtitre plate. Biofilm cells were washed three times. Following manual scraping and 30 minutes of mild sonication for cell dispersal, viable cell counts were determined by serial dilution and colony counting on Mueller Hinton agar.
Results: When compared to the broth control, sub-inhibitory concentrations of tigecycline reduced *S. epidermidis* biofilm formation by 39% at 12hrs ($p = 0.0135$). Sub-inhibitory concentrations of vancomycin had a minimal effect upon *S. epidermidis* biofilm formation (1% reduction at 12hrs).
Conclusion: Tigecycline may have a secondary effect upon *S. epidermidis* biofilm growth distinct from protein synthesis inhibition. Further work must now be employed to determine the genetic mechanisms by which low concentrations of tigecycline illicit this effect upon biofilm growth. Tigecycline is effective in reducing bacterial biofilm formation even at sub-inhibitory concentrations and its pronounced tissue retention makes it a potential candidate for use in prophylactic therapy.



The effect of sub-inhibitory concentrations of tigecycline upon *S. epidermidis* biofilm growth.

R2136 Investigation of slime production by *Candida* strains isolated from various clinical samples

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The aim of this study was to determine the slime production of different *C. albicans* and non-*albicans Candida* species isolated from various clinical samples, and to compare the intensity of biofilm layer produced by these yeast strains.
In the study, a total of 173 *Candida* species recovered from different clinical specimens were tested. Slime production of microorganisms was evaluated by modified tube adherence test. Briefly, the organisms were growth in Sabouraud broth supplemented with glucose (final concentration, 8%). After removal of liquid medium, the tubes were gently washed with distilled water and stained with 1% safranin. Then each tube was examined visually for the presence of the viscid slime layer on the internal wall. Slime production was scored as negative, weak positive (1+), moderate positive (2+ or 3+) or strong positive (4+) according to the intensity of biofilm layer.

Slime production was demonstrated in 114 (65.9%) of 173 *Candida* isolates tested. Thirty-eight (58.5%) of the 65 *C. albicans* strains and 76 (70.4%) of the 108 non-*albicans* candida strains were slime positive. Of the 65 *C. albicans* strains, slime production was weak in 18 strains (27.7%) whereas it was moderate in 20 strains (30.8%). Strongly slime production was not determined in *C. albicans* strains tested. In non-*albicans Candida* strains intensity of biofilm layer was weak, moderate and strong in 16 (14.8%), 32 (29.6%) and 28 (25.9%) respectively.
No significant difference was found between *C. albicans* and non-*albicans* candida species in terms of slime activity. Biofilm activity for non-*albicans* strains obtained from the bloodstream was significantly higher than those isolated from other sites ($p < 0.05$).

Antimicrobial pharmacokinetics, pharmacodynamics, pharmacogenomics, pharmacoeconomics and general pharmacology

R2137 Accumulation of sulphobutylether-β-cyclodextrin in critically ill patients with acute renal insufficiency undergoing extended daily dialysis and treatment with intravenous voriconazole

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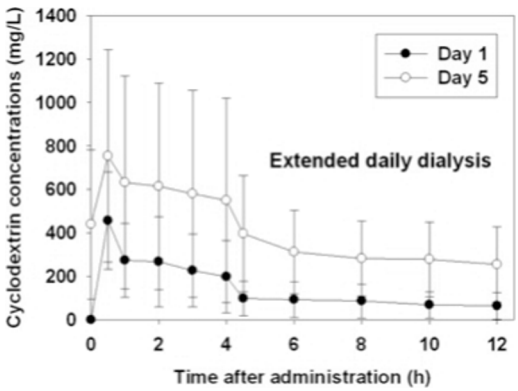
Objectives: Cyclodextrin derivates are used as solvent vehicle for poorly water-soluble drugs such as voriconazole or itraconazole. They are mainly cleared by the kidney with the consequence of accumulation in patients with renal insufficiency. Therefore, use of intravenous administration forms of voriconazole and itraconazole are not recommended for this patient population. In addition, there are very limited pharmacokinetic (PK) data regarding of cyclodextrin accumulation in patients receiving any form of hemodialysis.
Methods: In this investigation 4 critically ill patients (3 women, age 60–79 years) with anuric acute renal failure were treated empirically with 4 mg per kg body weight intravenous voriconazole twice a day for invasive fungal infections. Extended daily dialysis (EDD) over a period of 8 h was performed daily using the GENIUS® batch dialysis system (Fresenius Medical Care, Germany) with a polysulphone high-flux dialyzer (F60S, surface area 1.3 m^2; Fresenius Medical Care), a dialysate flow of 180 mL/min and a blood flow of 180 mL/min. On days 1 and 5 blood samples were collected before and at different time points up to 12 h after medication. sulphobutylether-β-cyclodextrin (SBECD) and voriconazole plasma concentrations were determined by a validated HPLC method.



Results: Tolerability of the treatment with intravenous voriconazole was good. No serious or dialysis related adverse events were observed. The SBECD plasma concentration–time curves of days 1 and 5 are shown in Figure 1. There was a clear accumulation of cyclodextrin on day 5 to see on higher peak and trough levels. The AUC0–12 (1598 vs. 4584 mg x h/L) and the terminal elimination half-life (8.7 vs. 15.1 h) were increased, too. Single and multiple dose PK parameters of voriconazole, however, were comparable with those from healthy control groups given in the literature.
Conclusions: Our data indicate an accumulation of SBECD in renal insufficient critically ill patients treated with intravenous voriconazole and EDD. Fortunately, no toxic effects were observed, although the accumulated dose was lower but comparable with those used in previous toxicity studies with animals. On the other hand EDD does not affect the pharmacokinetics of voriconazole.

Mechanisms of action and resistance

R2138 Clonal spread of carbapenem-resistant OXA-40 positive *Acinetobacter baumannii* in a Croatian university hospital

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Objectives: Carbapenems have a potent activity against and are often used as last resort for the treatment of infections due to multiresistant *Acinetobacter baumannii* (MDRAb). From July-October 2008, 43 *A. baumannii* isolates were involved in an outbreak at the Clinical Hospital Center, Zagreb. Thirty-four of these isolates were carbapenem resistant. The aim of the study was to characterize the mechanisms of carbapenem resistance and molecular epidemiology of these isolates.
Methods: Antibiotic susceptibilities were determined by broth microdilution. Oxacillinase genes were detected by multiplex PCR. Genotyping of the strains was performed by pulsed-field gel electrophoresis (PFGE), rep-PCR and determination of sequence groups by multiplex PCR.
Results: Thirty-three carbapenem resistant isolates were positive for blaOXA-40 and one unrelated isolate was positive for blaOXA-58. Nine carbapenem sensitive isolates possessed only the naturally ocurring OXA-51 β-lactamase which was associated with ISABaI insertion sequence in five isolates. No MBLs were found. Only OXA-58 β-lactamase was inhibited by sodium chloride. Chromosomal AmpC β-lactamases did not affect the susceptibility to carbapenems. ISAbaIII i was found upstream of blaOXA-58 gene. OXA-40 like producing isolates were uniformly resistant to ceftazidime, cefotaxime, ceftriaxone and piperacillin/tazobactam. Strains producing only OXA-51 β-lactamase were susceptible or intermediate to carbapenems but resistant to ciprofloxacin. These isolates showed variable degrees of susceptibility/resistance to ceftazidime and gentamicin. The blaOXA-40 positive isolates were shown to be clonally related by rep-PCR and PFGE and were part of the EU clonal lineage II. The single OXA-58 isolate and isolates possessing only OXA-51 type β-lactamase displayed distinct RAPD and PFGE fingerprints and were mainly EU clonal lineage I.
Conclusions: On the basis of susceptibility testing, β-lactamase characterization and genotyping of the isolates, we can conclude that clonal spread of endemic isolates was responsible for the high frequency of OXA-40-like positive MDRAb in this setting. Most of the isolates originated from the ICU indicating local dissemination within the hospital and pointing to the potential source of isolates.
Infection control measures should be introduced and restriction of meropenem use is recommended to reduce the spread of OXA-40-like positive *A. baumannii* isolates within the hospital.

R2139 Fluoroquinolone non-susceptibility in *Streptococcus pneumoniae* isolated in Turkey: should we change susceptibility testing methods to detect subtle mechanisms?

M. Bicmen, H. Sanli Avci, Z. Gulay (Izmir, TR)*

Objective: Although resistance rates of *S. pneumoniae* to various antibiotics have increased during the recent years in Turkey and in Izmir; fluoroquinolone (FQ) resistance rates seem to be low despite the wide usage. Therefore, in this study, our aim was to investigate whether the testing levofloxacin susceptibility solely is efficient in determining the susceptibility of *S. pneumoniae* to FQs.

Method: A total of 247 *S. pneumoniae* isolates recovered in the Bacteriology Laboratory of Dokuz Eylul University between 2003 and 2009 were analysed by levofloxacin (LVX) disk and by a modified disk diffusion test which was reported by Varon et al and in which susceptibility to norfloxacin (NOR), pefloxacin (PEF), ciprofloxacin (CIP) and sparfloxacin (SPX) were also determined. It was implicated that the modified method also shows the molecular mechanism of resistance. After the phenotypic test the results were also confirmed by sequence analysis of parC, E and gyrA and B genes of the resistant isolates. Presence of efflux pumps were also determined by the verapamil inhibition assay.
Results: Although the rate of LVX resistance was 0.8% among the isolates, when we analysed the susceptibilities of the additional fluoroquinolones, fluoroquinolone nonsusceptibility rate increased to 18.2% (15.8% for efflux type, 1.6% for single topoisomerase IV mutation type, 0.8% for topoisomerase IV and gyrase dual mutation type). Sequence analysis also confirmed our results. No mutation was detected in the QRDR region of the susceptible isolates and the isolates with efflux, whilst parC genes of isolates with topoisomerase IV type resistance contained mutations such as S79F and D83N. An additional S81F mutation in the gyrA gene was found in case of topoisomerase IV and gyrase A dual mutation type. In isolates with an efflux type of resistance, verapamil has reduced CIP MICs by 2–8 fold.
Conclusion: Although the clinical significance is not known, the FQ nonsusceptibility rate in *S. pneumoniae* isolates was higher than thought (0.8% versus 18.8%). LVX does not detect first step mutants and efflux type resistance in *S. pneumoniae*. Low level resistance usually facilitates the acquisition of higher resistance, so the potential risk of high prevalence of FQ resistance is shown by our study. In determining fluoroquinolone susceptibility in *S. pneumoniae* NOR disk (5 μg) should also be used in addition to LVX.

R2140 Characterization of macrolide resistance genes in *Staphylococcus saprophyticus*

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Objectives: From January 2005 to May 2009, 33 of 72 (45.8%) *Staphylococcus saprophyticus* clinical isolates, recovered from urine samples in our institution, were resistant to macrolides and/or related compounds (i.e. lincosamides, streptogramins). Mechanisms of macrolide resistance have been poorly investigated in this species. The aim of this study was to identify macrolide resistance genes as well as their genetic supports among the isolates of this collection.
Methods: For all 33 strains, MICs of erythromycin (ERY), spiramycin, lincomycin (LIN) clindamycin (CLI), dalfopristin (DAL), quinupristin (QUI), and dalfopristin-quinupristin were determined by the agar dilution method on Mueller-Hinton agar according to the recommendations of the Antibiogram Committee of the French Society for Microbiology. Induction of lincomycin resistance by erythromycin was checked by the D-zone test and clindamycin inactivation was tested by the Gots test. Resistance genes erm(A), erm(B), erm(C), msr(A), and lin(A) were amplified using PCR with specific primers. Plasmid analysis was carried out by using a modified Kieser technique.

Antibiotic	MIC (mg/L)		
	MIC_{50}	MIC_{90}	Range
Erythromycin	64	⩾256	0.12–⩾256
Spiramycin	4	8	0.5–128
Lincomycin	0.25	8	0.12–⩾256
Clindamycin	⩽0.03	0.06	⩽0.03–⩾256
Dalfopristin	2	4	0.5–16
Quinupristin	4	8	1–8
Quinupristin–Dalfopristin	0.25	0.5	0.12–0.5

Results: Out of 33 isolates tested, 32 (97.0%) were resistant to ERY including 3 (9.4%) also resistant to LIN but susceptible to CLI and one resistant to ERY, LIN and CLI. A single strain was only resistant to LIN. All isolates were susceptible to DAL and QUI. The MIC50 and MIC90 values are reported in the Table. The erm(A), erm(B), erm(C), msr(A), and lin(A) genes were detected among 0, 0, 5 (15.1%), 29 (87.9%), and 3 (9.1%) isolates, respectively. All erm(C)-positive isolates exhibited a positive induction test whereas all lin(A)-positive isolates had a positive Gots test. Preliminary results of plasmid analysis suggested that msr(A) genes were borne by small-size plasmids (<10 kb) whereas larger plasmids (>30 kb) harboured erm(C) and lin(A) genes.
Conclusion: Our study showed a high-level prevalence of resistant strains, especially those harbouring a msr(A) gene. This species might constitute a reservoir for macrolide efflux genes among coagulase-negative staphylococci.

R2141 V240H replacement, by site-directed mutagenesis, increases resistance toward carbapenems in TEM-149 ESBL producing *E. coli*

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Objectives: TEM-type B-lactamases represent the most prevalent ESBLs that are in ongoing evolution. Recently, TEM-149 ESBL has been well characterized in our laboratory. This enzyme showed the following amino acid substitutions E104K, R164S, M182T and an unusual valine residue at position 240 (E240V) never found in natural and mutagenized TEM variants. The goal of this study was to create a new mutant with a residue of histidine at position 240 in order to assess the contribution of this substitution on phenotypic resistance pattern in *E. coli* pTEM-149V240H.
Methods: The V240H mutation of the TEM-149 B-lactamase was generated by site-directed mutagenesis by use of the overlap extension method. The mutated primers were used in combination with the external primers TEM_for and TEM_rev to generate two partially overlapping DNA fragments, which were subsequently used in an overlap extension reaction coupled to amplification of the entire coding sequence with the external primers. Direct sequencing of the amplicons was performed on both strands derived from three independent PCRs according to the dideoxy chain termination method by using an ABI Prism 310 automatic sequencer to confirm the authenticity of the sequence. The resulting amplicon was cloned in pBC-SK vector and the recombinant plasmid pTEM-149V240M was inserted by transformation into *E. coli* HB101. The determination of MICs was performed by the conventional microdilution broth procedure, using a bacterial inoculum of 5 x 105 CFU/mL as recommended by CLSI.
Results: A mutant of TEM-149 enzyme, in which the valine at position 240 was replaced by histidine residue, was cloned into the pBC-SK vector and introduced in *E. coli* HB101 competent cells. The *in vitro* susceptibilities of *E. coli* HB101pTEM-149V240H was tested versus a large panel of β-lactams. Comparing these values to those obtained for *E. coli* HB101pTEM-149 wild type, we noticed a decreasing of MIC values of aztreonam (from >64 mg/L to 8 mg/L) and ceftazidime (from 32 mg/L to 16 mg/L). A MIC value of 8 mg/L was observed for meropenem.
Conclusion: In *E. coli*, the production of the TEM-149V240H, was surprisingly able to confer resistance to meropenem. The recombinant strain is susceptible to cefotaxime and aztreonam.

R2142 Extended-spectrum β-lactamase producers among non-typhoidal *Salmonella* isolated from clinical cases in Malaysia

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Objectives: To determine the presence of ESBL among non-typhoidal *Salmonella* sp from Malaysia.
Methods: A total of 200 non-typhoidal *Salmonella* isolates received from hospitals in Malaysia from year 2005 to 2009 isolates showing ceftriaxone zone sizes of ⩽25 mm were subjected to ESBL screening using CLSI recommended method and PCR amplifications of blaSHV, blaTEM, blaCTX-M and blaCMY-2 were performed using primers as described previously.

Results: From 200 isolates, 33 were phenotypically described as ESBL positive and 22 were AmpC positive. PCR results showed all were positive for at least one of the β-lactamase genes, except for 3 ESBL positive isolates. 19% of the isolates were positive for blaTEM, 12% were positive for blaCTX-M and 6% were positive for blaSHV. All except one isolate which were AmpC positive were also positive for the CMY-2 gene. Seven isolates which were negative for ESBL or AmpC phenotypically, were positive for either TEM or CTX-M gene.
Conclusion: ESBL producers are becoming increasingly common among our local isolates of *Salmonella* sp. Most of the resistance was caused by blaTEM but other resistance mechanisms are also not uncommon. Our study also showed that strains that were phenotypically negative for ESBL could also harbor the resistance genes. In such cases, treatment with β-lactam antibiotics may not be effective.

R2143 First description of Asp104Gly substitution in a SHV-type β-lactamase

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Objectives: In class A β-lactamases Ambler position 104 is not an especially conserved residue, however, it has been shown that amino acid substitutions at this position are associated with hydrolysis of 3rd generation cephalosporins. We biochemically characterized the *Klebsiella pneumoniae* SHV-99 enzyme, carrying the amino acid substitution Asp104Gly which, in our knowledge, appeared, in nature, in SHV β-lactamase for the first time.
Methods: SHV-99-producing *K. pneumoniae* KpARG220 strain was isolated from urine of a 35-year-old male in an intensive care unit of the Centre Hospitalo-Universitaire Mustapha Pacha, in Algeria. The blaSHV-99 gene was detected and identified by PCR and sequencing. PCR products were ligated in the SmaI site of the plasmid pBK-CMV, and the recombinant plasmids were electroporated in *Escherichia coli* DH5alfa. Purification was performed by ion exchange and gel filtration chromatography and kinetic constants were obtained by a computerized microacidimetric method.
Results: KpARG220 clinical strain was resistant to penicillins, cephalosporins and monobactams. Although SHV-99-producing transformant (EcDH5alfa-SHV-99) exhibited a β-lactam resistance phenotype similar to the clinical strain in respect to penicillins, it was susceptible to cephalosporins and monobactam. The kinetic parameters showed a lower catalytic efficiency for SHV-99 (kcat, 0.003 to 778 s^{-1}), when compared with SHV-1 (kcat, 220 to 1937 s^{-1}). Neither one showed the ability to hydrolyse oxyimino-β-lactams or aztreonam. The mutation Asp104Gly seems to be responsible for the higher Km value for oxyimino-β-lactams found in SHV-99 β-lactamase (Km, 136.0 to 196.0μM); in fact, SHV-99 presents catalytic activity (kcat, 0.5 s^{-1}) and catalytic efficiency (kcat/Km, 0.003 $\mu M\,s^{-1}\cdot s^{-1}$) for aztreonam, whose values were undeterminable for SHV-1.
Conclusion: The Asp104 residue is hydrogen bonded to Asn132, and it may therefore stabilize the catalytic Ser130 of the conserved SDN loop. The increase in oxyimino-β-lactams affinity, due to the substitution of an aspartate by glycine, seems to be the first step in the recognition of the side chain of those subtracts, which allows a better accommodation of these antibiotics in the active site of class A β-lactamases. This study showed that the Asp104Gly substitution alone is unable to generate an ESBL profile, however, it might be involved in the discrimination and recognition of antibiotics.

R2144 Phenotype identification of drug-resistance and inducible MLSB mechanism of *Brevibacterium* strains isolated from different clinical materials

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Introduction: The isolation of *Brevibacterium* strains from clinical materials is regarded as a contamination as the microorganisms constitute a normal flora of the skin. On the other hand, the case reports describing bacteriemias, peritonitis, ostemyelitis, call attention to these bacteria. The study of drug susceptibility and occurred drug-resistance mechanisms in isolated from clinical materials *Brevibacterium* strains are specifically important.
Objectives: The characteristics of drug-resistance and drug-resistance mechanisms by phenotypic methods in strains of *Brevibacterium* spp. isolated from clinical materials.
Methods: Twenty strains of *Brevibacterium* spp. isolated as pure cultures and in number indicating the etiologic agent of infection, isolated from different clinical materials (blood, urine, wounds, sputum) were included in the study. The identification was performed by APICoryne, APIZYM and supplementary biochemical tests. The drug susceptibility of the strains was determined on the basis of MIC values (Etests) accepted for *Corynebacterium* spp. and *Staphylococcus* spp. For MLSB resistance phenotype detection the disc diffusion method was used employed for *Staphylococcus* spp. and *Streptococcus* spp. β-lactamase production was detected with cephinase test.
Results: Among isolated strains, the following species were identified commonly: *B. casei* and *B. epidermidis.* The highest level of resistance was found to: Cotrimoxazole, Chloramphenicol, Fusidic acid. β-lactamases productions were also detected. In twelve strains, the inducible MLSB resistance mechanism was observed. All isolated strains were susceptible to Vancomycin, Teicoplanin.
Conclusion: On the basis of performed studies it was found that the antibiotics specifically recommended are Vancomycin and Teicoplanin with the highest effectives in the treatment of diseases caused by *Brevibacterium* species. The macrolides, lincosamids and streptogramins groups of antibiotics should by used with caution, because of the possibility of inducible MLSB resistance mechanism occurrence, as well as Cotrimoxazole, Fusidic acid, Chloramphenicol and β-lactams as studied strains were detected resistant to these drugs.

R2145 agr-deficiency and expression changes in regulatory and cell-wall genes responsible for hVISA and VISA phenotypes

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Objectives: Glycopeptides are still the gold standard to treat serious MRSA infections, but their widespread use has led to the emergence of glycopeptide-low-level resistant isolates (hVISA and VISA). The molecular basis of this reduced susceptibility is not clear, but different genetic loci encoding regulatory systems or proteins involved in autolysis and in cell-wall turnover have been implicated. We investigated the molecular mechanisms of glycopeptide intermediate resistance on prototype microorganisms, i.e. NRS149 (VSSA), Mu3 (h-VISA), Mu50 (VISA) of agr-II and on our Quasi-VISA clinical isolate of agr-II.
Methods: We analyzed delta-hemolysin production on 5% sheep-blood-agar. We investigated, with or without sub-inhibitory vancomycin concentrations, both the autolytic activity by TRITON X-100 induction and, by real-time RT-PCR, the expression levels of hld, graR/S (regulatory genes), atl, SAV2095 (sceD-like gene), mprF (cell-wall genes), all involved in h-VISA and VISA phenotypes.
Results: We observed the lack of delta-hemolysin (in sheep-blood-agar) in the VISA and its decreased production at 48 h in the hVISA. In both culture conditions, the VISA showed the lowest autolytic activity, the Q-VISA an intermediate level with respect to hVISA and VISA, whereas in hVISA an autolysis similar to VSSA was observed. These data correlate with the gene expression showing a gradual but substantial hld down-regulation as follows: VISA < Q-VISA < hVISA < VSSA. An atl down-regulation was found in VISA, whereas a low level of sceD transcripts was found in hVISA only with vancomycin. The regulator graR/S down-regulation, related to the atl down-regulation, was found in VISA. mprF up-regulation was found in all phenotypes towards VSSA.
Conclusions: Among agr-II, hVISA had a dysfunctional agr-locus and a regular autolytic activity, but, with vancomycin, this strain reduced its cell-wall turnover. In VISA, the lack of agr functionality and reduced autolysis were independent from the presence of vancomycin. The increased expression of mprF in hVISA and VISA could be related to a lower vancomycin binding than in VSSA.

R2146 The presence of GES enzymes in *Pseudomonas aeruginosa* strains isolated from patients in Warsaw hospitals

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Objectives: The GES-type enzymes have been found mainly in *P. aeruginosa*, but such enzymes have been also isolated from Enterobacteriaceae. Generally, genes coding GES β-lactamases are located on class 1 integrons. The aim of this study was to identify strains producing GES-type ESBLs, among *P. aeruginosa* isolates from two Warsaw hospitals.
Methods: The MDR *P. aeruginosa* strains (n = 332) were isolated from clinical specimens obtained from patients in two Warsaw hospitals. The MICs values of β-lactams were determined by agar dilution method, according to CLSI recommendation. The presence of ESBLs was detected by a double-discs synergy test (DDST) with inhibitors: clavulanic acid, sulbactam, tazobactam and imipenem. The presence of integrons and genes coding GES-type ESBLs was detected by PCR. Gene cassettes were identified by sequencing of the obtained amplicons. Standard plasmid analysis was performed.
Results: The ESBL-type enzymes were detected among 53 isolates by DDST. Eighteen out of all tested isolates were resistant to all β-lactams. These strains (n = 71) were screened for the presence of genes coding ESBL-type enzymes. In 8 isolates (seven from hospital A and one from hospital B) bla genes coding GES-type enzymes were identified. All found blaGES genes are located in class 1 integrons. Moreover, seven of them are present on plasmid. Among strains from hospital A enzymes as GES-1 (in 5 strains), GES-5 (in one) and the new ESBL-type β-lactamase GES-15 were detected. The complete nucleotide sequence of blaGES-15 was determined (NCBI GenBank Acc. No GU208678). Sequencing showed that GES-15 was identical to GES-5 except one amino acid. All of above-mentioned seven strains are resistant to ceftazidime and cefepime. The GES-15 carrying strain exhibited the most clear inhibitor-sensitive phenotype. Moreover, blaGES-1 gene in one strain from hospital B was identified, also in this case blaGES-1 gene is located in class 1 integron on plasmid.
Conclusions: The new extended-spectrum β-lactamase GES-15 encoded by class 1 integron-located gene was found in *P. aeruginosa* clinical isolate. Moreover, the GES-5 producing *P. aeruginosa* strain was identified in Poland for the first time. The presence of plasmid-located blaGES-1, blaGES-5 and blaGES-15 genes, within integrons in 8 clinical isolates from two hospitals suggests the possibility of spreading these ESBLs in Polish hospitals.

Resistance surveillance

R2148 Serotype distribution and antimicrobial resistance rates of human gastrointestinal *Salmonella enterica* isolates. A 3-year study from Crete, Greece

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Objective: Crete is the biggest Greek island attracting millions of tourists year-round. The aim of the present study was to determine the serotype distribution and the susceptibilities of *Salmonella enterica* strains isolated from patients with gastroenteritis.
Methods: All isolates of *Salmonella* spp. from fecal samples of patients with diarrhoea cared for at the University Hospital of Heraklion over the last three years (2006–2008) were included in the study. Identification and susceptibility testing were performed using the Vitek II (Biomerieux, France). Isolates with either intermediate or full resistance to an antibiotic were characterized as non-susceptible to this agent. Serotyping was performed using commercial antisera (Statens Serum Institut, Denmark) and the Kauffmann-White scheme.
Results: A total of 110 isolates were analyzed. *S. enteritidis* (63/110; 57.3%), *S. typhimurium* (20/110; 18.2%), *S. bardo* (5/110; 4.5%), *S. newport* (4/110; 3.6%), and *S. blockley* (3/110; 2.7%) were the five most common types encountered. Other types encountered were: *S. altona, S. anatum, S. arizonae, S. bredeney, S. bovis-morbificans, S. etterbeek, S. hadar, S. haardt, S. inpraw, S. kottbus, S. muenchen, S. pomona* and *S. uganda.* Among the 110 patients 18 (16.4%) were of foreign nationality. The non-susceptibility rates found were: cotrimoxazole, 5.5%; amoxicillin, 16.4%; amoxicillin+clavulanic acid, 6.4%; ticarcillin+clavulanic acid, 10.0% and piperacillin+tazobactam, 2.7%. All isolates were susceptible to third generation cephalospirins and carbapenems, whereas relatively high percentages of non-susceptibility were found for tetracycline (20.9%) and ciprofloxacin (30.0%).
Conclusion: In our geographical area *Salmonella enterica* serotype Enteritidis continuous to be the most common bacterial enteropathogen. Although we did not observe any resistance to third generation cephalosporins, the sizeable number of strains resistant to quinolones is worrisome and emphasizes the importance of continuous monitoring of antibiotic resistance of *Salmonella* isolates.

R2149 Resistance rates of *Campylobacter* spp. and *Yersinia enterocolitica* human isolates. A 3-year study from Crete, Greece

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Objective: Crete is the biggest Greek island attracting millions of tourists year-round. The aim of the present study was to determine the susceptibilities of *Campylobacter* spp. and *Yersinia enterocolitica*, which are two of the major causes of bacterial diarrhea.
Methods: All isolates of *Campylobacter* spp. and *Y. enterocolitica* from different patients in the University Hospital of Heraklion over the last three years (2006–2008) were included in the study. Cultures and identification of the isolates were done using standard microbiological methods. Susceptibility testing was performed with the disk diffusion method following the recommendations of the CLSI. Isolates with either intermediate or full resistance to an antibiotic were characterized as non-susceptible to this agent.
Results: A total of 109 *Campylobacter* and 12 *Yersinia* isolates were analyzed. *C. jejuni* (93/109; 85.3%), and *C. coli* (16/109; 14.7%) were the types encountered. Among the patients with *Campylobacter* and *Yersinia* 4.6% and 50% were of foreign nationality, respectively. The non-susceptibility rates found for *Campylobacter* were: amoxicillin, 33.9%; amoxicillin+clavulanic acid, 5.5%; tetracycline, 34.9%; gentamicin, 0.9%; ciprofloxacin, 54.1% and erythromycin, 19.3%. All isolates were susceptible to carbapenems. The non-susceptibility rates found for *Yersinia* were: amoxicillin, 100.0%; amoxicillin+clavulanic acid, 100.0%; ticarcillin+clavulanic acid, 75.0%; piperacillin+tazobactam, 33.3%; ceftazidime, 25%; imipenem, 25.0%; tetracycline, 25.0%; gentamicin, 0.0%; ciprofloxacin, 0.0% and cotrimoxazole, 50.0%.
Conclusion: The increased rates of resistance in *Campylobacter* and *Yersinia* isolated from humans in our region emphasizes the need of systematic surveillance of antibiotic resistance for determining appropriate therapeutic regimens.

R2150 Changes of multidrug-resistant *Pseudomonas aeruginosa* O serogroup dependence in a university hospital, 2003–2008

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Objectives: The aim of our study was to analyse prevalence of O serogroup dependence of multidrug resistant (MDR) *Pseudomonas aeruginosa* (*P. aeruginosa*) strains during 2003 and 2008 year periods in a tertiary intensive care unit (ICU).
Methods: *P. aeruginosa* strains isolated from respiratory tract patient's treated in Kaunas Medical University Hospital ICU were analysed. Antibiotic sussceptibility testing by disc diffusion method was performed accoding to the National committee for clinical laboratory standards (NCCLS, USA). Isolates resistant to three or more antipseudomonal antibiotics were considered as MDR. Serogroups of *P. aeruginosa* strains were established using serums, containing antibodies against O-group antigens of *P. aeruginosa* (Bio-Rad, USA).
Results: During the study 18 of 90 in 2003 year (20%) and 25 of 101 (24.76%) in 2008 year *P. aeruginosa* strains were determined as MDR. *P. aeruginosa* strains, belonging to O:11 serogroup were more often considered as MDR (38.9%, n = 7 compare with 2.8%, n = 2 in 2003 and 56%, n = 14 compare with 27.6%, n = 21 in 2008 year, $p < 0.05$). 16.7% (n = 15) in 2003 year and 28.7% (n = 29) in 2008

year *P. aeruginosa* strains were resistant to carbapenems. Carbapenem resistant *P. aeruginosa* strains more often were determined as O:11 serogroup dependency (33.3%, n = 5 in 2003 year and 51.7%, n = 15 in 2008 year). *P. aeruginosa* strains, belonging to the serogroups O:1, O:2 and O:3 were more often isolated in 2003 compare with 2008 year (23.3%, 27.8%, 12.2% and 9.9%, 10.9%, 4.0%, respectively, $p < 0.05$). *P. aeruginosa* strains, belonging to serogroups O:6 and O:11 were more often isolated in 2008 compare with 2003 year (27.6%, 34.7% and 4.4%, 10.0%, respectively, $p < 0.05$).

Conclusions:

1. *Pseudomonas aeruginosa* strains belonging to the serogroup O:11, were determined to be more resistant to the majority of antipseudomonal antibiotics, then other serogroups' dependency *Pseudomonas aeruginosa* strains.
2. Multidrug resistant *Pseudomonas aeruginosa* strains not increased in Kaunas Medical University Hospital during 5 years period, but increased multidrug resistant *Pseudomonas aeruginosa* strains belonging to O:11 serogroup.

R2151 Resistance to antimicrobials for veterinary or human use among *S. aureus* isolated from cows with clinical mastitis in central Italy

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Objectives: Acquired antimicrobial resistance in bacteria is an increasing threat in human as well as in veterinary medicine. *S. aureus* is one of the most causes of bovine mastitis. Although the main attention is directed to *S. aureus* resistant to methicillin (MRSA), the report of strains resistant to macrolide, lincosamide and streptogramin (MLS) antibiotics are increasing in humans. The aim of this work was to verify the antimicrobial susceptibility of *S. aureus* isolated from bovine clinical mastitis to antibiotics used in veterinary or human medicine.
Methods: 163 *S. aureus* isolated from milk of cows with clinical mastitis and identified by PCR were analyzed by the disc diffusion method for clindamycin (C), erythromycin (E), gentamycin (G), methicillin (M), oxytetracyclin (O), vancomycin (V) and quinupristin/dalfopristin (Q/D).
Results: The resistance rates were 42.4% for C, 69.9 for E, 69.9% for G, 11.6% for M, 47.2% for O, 8.0% for V, and 47.3% for Q/D. The susceptible strains were 14.1% for C, 1.3 for E, 14.2% for G, 88.4% for M, 13.5% for O, 92.0% for V, and 6.7% for Q/D. 12.9% strains were susceptible to all the antibiotics, while 54.0% was resistant to at least 3 antibiotics. All 19 MRSA were resistant to at least 4 antibiotics, 52.6% of which was resistant to Q/D. 62.3% Q/D resistant strains were also resistant to both E and C.
Conclusion: MRSA were detected in bovine with clinical mastitis. Many MRSA strains were multi-resistant and would be a problem for the antimicrobial treatment. The high resistance to E, G, O and C was probably related to the large use in veterinary medicine, while the finding of a high resistance to Q/D was surprising, because Q/D is for human use only. Genes conferring resistance to one of the MLS antibiotics may confer cross-resistance to others, because they have similar effects on bacterial protein synthesis. The high resistance to Q/D+E+C found in our strains is concordant with this hypothesis, and molecular tests are in progress. The chance that the large use of some macrolide (e.g. E) and lincosamide (e.g. C) antibiotics in animals increases the rate of *S. aureus* resistant to other MLS should be better evaluated in order to prevent the selection of multi-resistant strains. In particular, there is the risk that livestock becomes a reservoir of *S. aureus* resistant to some streptogramin, that are used very carefully in humans as the last chance for the treatment of infections not responding to other antibiotics.

Antibiotic name	Antibiotic class	Resistant		Intermediate		Susceptible		Total
		n	%	n	%	n	%	
Clindamycin	Lincosamide	69	42.3	71	43.6	23	14.1	163
Erythromycin	Macrolide	114	69.9	47	28.8	2	1.3	163
Gentamycin	Aminoglycoside	114	69.9	26	15.9	23	14.1	163
Methicillin	beta-Lactam	19	11.6	0	0	144	88.4	163
Vancomycin	Glycopeptide	13	8.0	0	0	150	92.0	163
Oxytetracyclin	Tetracycline	77	47.2	64	39.3	22	13.5	163
Quinupristin/dalfopristin	Streptogramin	77	47.2	75	46.0	11	6.7	163

R2152 Trends of resistance in methicillin-resistant *Staphylococcus aureus* (hospital-acquired MRSA versus community-acquired MRSA) in Southeast Austria, 2002–2008

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Objectives: Aim of this study was to determine any trends in resistance and in MIC-distribution of clinical isolates of Hospital acquired (HA) and Community-acquired (CA) Methicillin-resistant *Staphylococcus aureus* (MRSA) to non-β-lactam antibiotics within a 7 year period.
Methods: 156 non-duplicated clinical isolates of MRSA (84 HA-MRSA, 72 CA-MRSA) were analyzed, detected from January 2002 to December 2008 at the Institute of Hygiene Graz, Austria. HA-MRSA and CA-MRSA were defined applying the CDC criteria. Resistance testing and interpretation of the results were performed according to CLSI criteria. MICs of 12 (non-β-lactam) antibiotics were determined using the Etest method (AB Biodisk, Solna, Sweden).
Results: Within the 156 MRSA strains, no resistance was found for vancomycin, teicoplanin, quinopristin/dalfopristin, daptomycin and the newer antibiotics tigecycline and linezolid. Resistance to tetracycline was rare in HA-MRSA, whereas in CA-MRSA resistance raised from 0% in 2002 to 41.7% in 2008, reflecting the occurrence of MRSA ST398 (life-stock associated MRSA) in the local setting.
For ciprofloxacin resistance in HA-MRSA remained on a stable high level of 83.3% to 91.7%, by contrast resistance in CA-MRSA decreased from 25% in 2004 to 0% in 2008. Resistance to rifampicin was only found in rare cases of HA-MRSA (8.3% in 2005) and was not detected in CA-MRSA.
Inducible macrolide-lincosamide-streptogramin B (MLSB) and constitutive MLSB resistance were found to be more associated with HA-MRSA, for clindamycin and erythromycin resistance ranged from 83.3% in 2002 to 100% in 2008. In CA-MRSA MLSB resistance was found in 8.3% to 16.7%. Resistance to fusidic acid was found in 0% to 8.3% of HA-MRSA, and 25% to 33.3% of CA-MRSA, respectively. Focusing and analyzing the MIC50 and MIC90 values of the tested antibiotics, no statistically significant variances could be observed during observation period.
Conclusion: Resistance to non-β-lactam antibiotics varies between HA-MRSA and CA-MRSA. No resistance was found vancomycin, teicoplanin, quinopristin/dalfopristin and the newer antibiotics tigecycline and linezolid. Resistance was also rare for rifampicin. Resistance to ciprofloxacin, erythromycin and clindamycin were more common in HA-MRSA, in contrast resistance to fusidic acid and tetracycline was found more often in CA-MRSA. Concerning the MIC50 and MIC90 values of the tested antibiotics no MIC creeping could be observed during observation period.

R2153 Rapid molecular screening for methicillin-resistant *Staphylococcus aureus* among intensive care unit-admitted patients: preliminary results

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Objectives: Colonization with methicillin resistant *Staphylococcus aureus* (MRSA) is a risk factor associated to severe morbidity and mortality, excess length of stay as well as extra costs of care, especially in patients admitted to critical areas such as intensive care units (ICU). Nasal screening for MRSA, as current guidelines recommend (Calfee DP et al. Infect Control Hosp Epidemiol. 2008), is the most powerful strategy to prevent and limit the spread of infections if combined with major control measures such as isolation, decolonisation from carriage sites and hand hygiene programs. The aim of this study was to evaluate the MRSA colonization rate among patients admitted to ICU using a new rapid molecular method.
Methods: From April through October 2009, a total of 154 patients were screened for MRSA carriage at admittance to ICU. All nasal swabs were analyzed by molecular method (GeneXpert MRSA, Cepheid), able to

amplify by real-time PCR the target sequence for MRSA at the SCCmec-orfX junction. Samples were tested also by cultural analysis, plating swabs onto Columbia agar with 5% Sheep Blood and Mannitol Salt Agar (Kyma). Identification and oxacillin testing were performed on suspicious colonies using VITEK2 (bioMérieux).
Results: Our data with the GeneXpert MRSA showed high sensitivity and specificity of the new molecular method. Among the 154 patients examined for MRSA, 5 had a positive screening result by both, molecular and culture-based method, 19 were identified as MSSA and had a negative result by the molecular test. All samples negative by molecular method were negative also by cultures.
Conclusion: GeneXpert MRSA showed high efficiency and efficacy compared with the culture method. In fact it requires only seventy minutes to complete the analysis allowing a shorter turnaround time (TAT) and, by improving patient management, a better clinical and therapeutic outcome. This study allowed us to benchmark the colonization level among ICU patients at the admission. This is a start point for infection control strategies to prevent MRSA spread and for developing more focused therapeutic measures in all colonized patients.

R2154 Antibiotic susceptibility monitoring using a Microsoft Access® database

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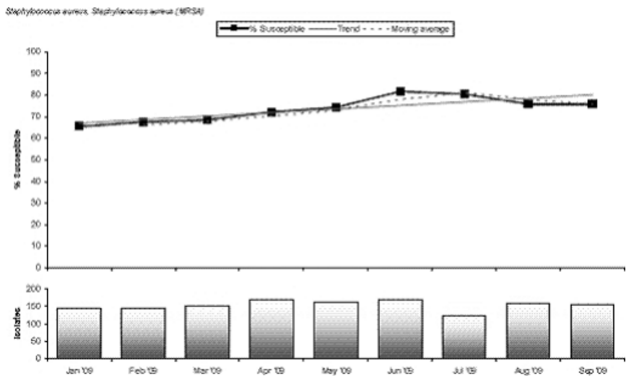
Objectives: The clinical effectiveness of antimicrobial therapy is constantly being undermined by the development of antimicrobial resistance. Appropriate antibiotic prescribing is critical to good patient outcomes and a significant factor in the battle against emerging resistance. As resistance is a constantly evolving problem active, real time surveillance of susceptibility rates is necessary to optimise empirical treatment and prophylactic regimens. We aimed to develop a local surveillance system for monitoring our susceptibility rates and present here the tool and some initial findings.
Methods: All microbiology sample results are retrieved from the laboratory computer system (Telepath, iSoft Laboratory Systems) using the list generation function for either hospital or community (general practice) locations. Microsoft Access databases are used to process these results and allow simple searching for antibiotic susceptibility rates and trends over time. De-duplication of results is achieved by identifying samples from the same hospital number with the same antibiogram to leave single episodes within the selected time frame (either monthly or quarterly). Twenty-three antibiotics and two antifungal agents are imported into the hospital database and cumulative results can be reported across the whole trust, by location (down to ward or speciality level). Eighteen antibiotics are imported into the community database, and cumulative results can be reported across the whole city or by Primary Care Trust. The databases are reported via a separate Access interface, allowing users to select locations and organisms and generate bespoke reports.



Results: Graphical results are obtained to display both the numbers of isolates tested within a time frame, the absolute susceptibility rate and the trend over the time period. An example of the output is shown in the Figure, which displays the susceptibility rate of *Staphylococcus aureus* from wound swabs to flucloxacillin.
Conclusions: The reporting of trends in antibiotic susceptibilities using Microsoft Access has shown to be both feasible and useful. A simple source for susceptibility rates has proved useful for the development of empirical prescribing guidelines and to allow proactive changes in local prescribing guidelines for both treatment and prophylaxis in response to observed trends.

R2155 Activity of common UTI agents against enteric urinary isolates from Europe and impact of patient population on activity profile

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Objective: A concern of empiric treatment of urinary tract infections (UTI) is resistance (R) among Enterobaceriaceae spp. (EN). R can vary by patient population. Furthermore, organisms harboring extended-spectrum β-lactamases [ESBL] or exhibiting multi-drug resistance [MDR] pose an additional threat. The GLOBAL Surveillance initiative monitors susceptibility of EN to agents commonly utilized to treat UTI. This study evaluates the current susceptibility of enteric UTI in EU to select agents and the impact of patient age and location on activity profile.
Methods: 2056 EN urinary isolates (906 *E. coli* [EC], 388 *Klebsiella pneumoniae* [KP], 448 *Proteus* spp., 314 other EN) were collected from 2005–2009 across six EU countries. Susceptibility of isolates was determined by broth microdilution (CLSI M7-A8; M100-S19) for a variety of agents (levofloxacin [LVX], ciprofloxacin [CIP], amoxicillin/clavulanate [AMC] and trimethoprim/sulfamethoxazole [SXT]). Multi-drug resistance (MDR) was defined as R to $\geqslant$3 separate classes of agents. Isolates were analyzed by patient location (outpatient [OP], inpatient [IP], and ICU), and age [pediatric (PED) patients $\leqslant$17; adult (ADT) 18–64; and elderly (ELD) $\geqslant$65)].
Results: Overall, % R among urinary EN were: LVX 13.9; CIP 17.2; AMC 13.5; SXT 34.6. Among EC/KP, %R was: LVX 22.5/9.8; CIP 24.8/14.4; AMC 5.4/7.5; SXT 38.1/19.8. ESBL rates among EC and KP were 4.5% and 11.9%, respectively, and 3.6% of EN were MDR. LVX, CIP, amoxicillin–clavulanate (AMC), and SXT MIC90s were all $\geqslant$16 mg/L for EC. There was a trend towards increased R with increasing age among EN (PED/ADT/ELD %R: LVX 9.4/11.5/16.6; CIP 9.4/14.2/20.8; AMC 6.3/12.9/14.1; SXT 18.8/33.8/35.2). R was generally highest among ICU patients relative to IP and OP (OP/IP/ICU %R: LVX 13.0/13.3/14.7; CIP 17.5/16.4/17.2; AMC 11.4/13.6/16.4; SXT 32.8/34.3/34.5). For EC/KP, ESBL were most common among ELD and IP (6.7%/12.3% and 4.8%/12.8%, respectively). MDR ranged between 2–4% across the subpopulations.
Conclusions: R to UTI agents among EN varied, with lower R to LVX/CIP/AMC relative to SXT. R among EN increased with patient age. ESBL rates were higher among KP compared with EC, and were more common among ELD and IP populations. MDR rates were relatively low (<4%) across the evaluated populations. As the activity profile of UTI agents is impacted by both patient age and patient location, it is important to consider these factors when treating UTI empirically.

R2156 First detection of VIM-1 producing *Pseudomonas aeruginosa* isolate in Slovenia

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Objective: Twenty years ago carbapenemases were described as species-specific, chromosomally encoded β-lactamases. However, the identification of plasmid encoded carbapenemases has changed our perception of the patterns of resistance genes dissemination predominantly through interspecies dispersion rather than clonal spread. The aim of our study was, therefore, to evaluate the prevalence of carbapenemase resistance

determinants among 37 ertapenem resistant or intermediate Gram-negative isolates.
Methods: A total of 33 *Klebsiella pneumoniae*, three *Escherichia coli* and one Pseudomans aeruginosa ertapenem resistant or intermediate isolates were tested for the presence of carbapenemases by phenotypic (Hodge test) and genotypic methods (PCR). Specific primers were used under standard PCR conditions to detect class A carbpenemases (NMC, SME, IMI, KPC and GES), class B metallo-β-lactamases (IMP-1, IMP-2, VIM-1, VIM-2, SPM-1, GIM-1 and SIM-1) and class D OXA β-lactamases (OXA-23, OXA-24, OXA-69, OXA-58, OXA-55, OXA-48, OXA-50 and OXA-60). PCR products were purified and sequenced.
Results: Among the 37 isolates tested by the Hodge method, 35 gave negative results, one was positive (*K. pneumoniae*) and one could not be clearly interpreted (*P. aeruginosa*). The analysed nucleotide sequences revealed the presence of blaVIM-1 gene in the *P. aeruginosa* isolate.
Conclusions: The presence of the screened carbapenemase genes could not be confirmed in *E. coli* and *K. pneumoniae* isolates, although one of the *K. pneumoniae* isolates gave a positive Hodge test. However, the first detected metallo-β-lactamase gene blaVIM-1 in a *P. aeruginosa* isolate indicates the need to perform further screenings to control the emerging carbapenemase resistance determinants, which are becoming a major public health issue.

R2157 Antimicrobial susceptibilities of Gram positive bacteria and antibiotic consumption in a Greek tertiary hospital, 2001–2008

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Objective: To examine differences in antibiotic susceptibility of Gram positive bacteria and in antibiotic consumption in a Tertiary Hospital, within 7 years of operation.
Methods: All clinical samples from inpatients. Identification and susceptibility testing, using the Wider® semi-automated system with CLSI breakpoints. Antibiotic consumption expressed as Defined Daily Doses (DDD) per patient-day.
Results: 841 and 413 isolates identified during 2008 and 2001, respectively.
Methicillin Resistant *S. aureus* (MRSA) rates were significantly higher in 2008 (56.5%, 74/131 isolates) than in 2001 (36.6%, 15/41; $p = 0.026$). The proportion of community-acquired strains among MRSA also increased in 2008 (32.4%, 24/74 vs. 26.3%, 4/15 in 2001). One MRSA in 2008 had reduced susceptibility to Vancomycin (MIC=8); and 1 to Linezolid (MIC > 4). No such strains were found in 2001.
S. aureus resistance increased for Penicillin (121/131 isolates, 92.4% in 2008 vs. 30/40, 75% in 2001; $p < 0.01$), Erythromycin (53.8% vs. 35.9%; $p < 0.05$) and Clindamycin (42% vs. 32.5%).
Among hospital strains (>48 hours from admission) Coagulase-Negative Staphylococci (CoNS) resistance to Methicillin increased in 2008 (81%, 222/274 isolates vs. 72.4%, 121/167 in 2001; $p < 0.04$). CoNS in 2008 had high rates of non-susceptibility to several antibiotics tested (ranging from 47.1% for Cotrimoxazole to 92.3% for Penicillin). 4 CoNS strains were non-susceptible to Vancomycin in 2008, and 1 in 2001. All CoNS were susceptible to Linezolid.
Non-susceptibility rates of *Enterococcus* sp. hospital strains increased for Amoxycillin/Clavulanate (37.4%; 49/131 isolates in 2008 vs. 22.2%; 14/63 in 2001, $p = 0.035$), Erythromycin (87.8% vs. 82.5%) and Rifampicin (70.3% vs. 64.8%). 2/192 Enterococci in 2008 were resistant to Vancomycin (VRE) and Teicoplanin (VanA phenotype). 9/192 Enterococci in 2008 had MIC $\geqslant$2 to Linezolid.
There was only 1 *S. pneumoniae* isolate (non-CNS) with Penicillin MIC=2 in 2008; all *S. pneumoniae* isolated in 2001 had MIC$\leqslant$;0.12.
Consumption of wide-spectrum antibiotics increased by 28.8% (17.9 DDD/100 hospital-days in 2008 vs. 13.9 in 2001). Consumption of anti-MRSA antibiotics also increased (1.94 in 2008 vs. 1.64 in 2001).
Conclusion: Within 7 years, there was a marked increase of non-susceptibility rates of Gram-positive bacteria, including MRSA. This paralleled the increased consumption of wide-spectrum antibiotics and of antibiotics with activity against multi-resistant Gram-positive bacteria.

Surveys of molecular epidemiology of resistance and resistance genes, strains or serotypes

R2158 Isolation of blaCTX-M group 8 extended-spectrum β-lactamase among clinical isolated Enterobacteriaceae in a 1,000-bed hospital in Thailand

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Objectives: The CTX-M-type encoding Extended-spectrum β-lactamases (ESBLs)-producing in Gram negative bacteria, particularly among members of Enterobacteriaceae family have become more recognized. Distribution of these genes varies in different countries. However, rare and uncommon CTX-M-type ESBLs such as CTX-M-8, 40 and 63 have been reported scattering in various parts of the world. Therefore, it came to our attention to investigate the existence of these rare genes in Thailand.
Method: Four hundred and seventy ESBL-phenotypic positive clinical isolates of Enterobacteriaceae members were detected for the presence of blaCTX-M groups including 1, 2, 8, 9 and 25 and upstream region using PCR method. In addition, ERIC-PCR analysis was performed.
Result: Among tested organisms, sixteen isolates (4%) were detected carrying an uncommon CTX-M group 8. These included *K. pneumoniae* (9 isolates), *M. morganii* (4 isolates), *E. coli* (2 isolates), and *P. mirabilis* (1 isolate). Interestingly, the sequence of blaCTX-M of all CTX-M group 8 positive *M. morganii, E. coli* and *P. mirabilis* showed a strict identity with the CTX-M-63, AF189721 (100% similarity). In contrast, only 2 isolates of *K. pneumoniae* were found containing CTX-M-63 while the other seven isolates revealed revealed a strict identity with the CTX-M-40, AB205197 (100% similarity) PCR and sequencing of the upstream region showed the presence of transposase gene (tnpA) of the ISEcp1-like element.
Conclusion: To our knowledge, this is the first report of clinical isolates producing CTX-M-40 and CTX-M-63 ESBL in Thailand. In order to gain clearer view of the distribution of these genes, further larger scale of investigation throughout the country may be required.

R2159 Detection of OXA carbapenemases in multidrug-resistant clinical isolates of *Acinetobacter baumannii* from Krakow, Poland

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Objectives: *Acinetobacter baumannii* is an increasingly important nosocomial pathogen often causing outbreaks in intensive care units. Many clinical isolates are resistant to almost all antibiotics including carbapenems. The most common mechanism responsible for carbapenem-resistance are carbapenem-hydrolysing β-lactamases belonging to molecular class D (OXA enzymes). Drug resistance of *A. baumannii* strains may also be associated with the presence of an insertion sequence (ISAba1). The aim of the study was detection of:1) OXA encoding genes; 2) presence of ISAba1.
Methods: The study included the total of thirty isolates of multidrug resistant *A. baumannii*. All strains were carbapenem-resistant. These isolates were obtained from patients hospitalized in ICU of Specialized Hospital in Krakow. *A. baumannii* identification and sensitivity test (VITEK-2 Compact, bioMérieux, Poland) were performed by standard criteria (CLSI). All strains were tested for MBL (metalo-β-lactamases) production by disk-diffusion synergy test. Multiplex PCR described by Woodford at al. (2006) was applied for detection of OXA carbapenemases encoding genes (blaoxa-51-like, blaoxa-24-like and blaoxa-23-like). All strains were tested for presence of 549-bp fragment containing a portion of ISAba1.

Results: Resistance rates to piperacillin, piperacillin–tazobactam, ceftazidime, cefepime, imipenem, meropenem, gentamicin, tobramycin, amikacin, ciprofloxacin were 100% (30), 90% (27), 90% (27), 100% (30), 97% (29), 100% (30), 93% (27), 20% (6), 57% (17), 100% (30) respectively. Phenotypic test for MBL production was negative for all strains. Multiplex-PCR analysis showed the presence of gene encoding a β-lactamase belonging to OXA-51-like group in all the isolates. Nine and 18 of 30 isolates carried a gene blaoxa-23-like and blaoxa-24-like respectively. Two of 30 isolates carried both of acquired OXA genes. All of the isolates contained the insertion sequence ISAba1.
Conclusions: Our results support the theses that detection of the blaoxa-51-like can be used as efficient method for identification of *A. baumannii* strains. Carbapenem resistance in tested isolates might be associated with: 1) expression of acquired oxacillinases belonging to OXA-23-like and OXA-24-like groups; 2) extended expression of intrinsic oxacillinases belonging to OXA-51-like group supported by the presence of insertion sequence ISAba1.

R2160 Distribution of capsular serotypes, clones and macrolide resistance mechanisms among macrolide-resistant *Streptococcus pneumoniae* clinical isolates

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Objectives: Macrolide resistance in *S. pneumoniae* has become a clinical problem in Turkey and other countries. The aim of this study was to analyze the distributions of capsular serotypes, clones, phenotypes and macrolide resistance genes among macrolide-resistant *S. pneumoniae* clinical isolates in our hospital.
Methods: A total of 89 *S. pneumoniae* clinical isolates were isolated from clinical samples between 2007 and 2009. Minimum inhibitory concentrations of erythromycin, clindamycin and penicillin were determined by agar dilution method according to the CLSI guidelines. Susceptibility of azithromycin, telithromycin, tetracycline, linezolid, vancomycin, and levofloxacin were determined by disc diffusion method. CLSI criteria were used for the interpretation of susceptibility testing results. Serotyping was performed using the capsular swelling procedure (quellung reaction). The double-disk method with erythromycin and clindamycin disc was used for determination of macrolide resistance phenotypes. Macrolide resistance genes (mefA/E, ermA, ermB, ermC, ermTR) were detected by PCR reaction. All erythromycin resistant strains were genotyped by pulsed-field gel electrophoresis (PFGE) after digestion with SmaI.
Results: Thirty five (40%) isolates were resistant to erythromycin. Penicillin, clindamycin, azithromycin tetracycline and levofloxacin resistance ratio were 13%, 30%, 40%, 36%, 9% respectively. All isolates were susceptible to linezolid, vancomycin and telithromycin. Serotype distribution among erythromycin-resistant isolates were 16 (45%) strains serotype 19, 4 (10%) strains serotype 23, 3 (9%) strains serotype 6, 2 (6%) strains serotype 14, 2 (6%) strains serotype 16, 1 (3%) strain serotype 18 and 1 (3%) strain serotype 33 and 3 (9%) strains nonvaccine serotype or not typied. Macrolide resistance phenotypes, erythromycin resistance genes and MIC 50/90 values among erythromycin-resistant *S. pneumoniae* isolates are presented in table 1. Twenty nine genotypes were detected among erythromycin-resistant *S. pneumoniae* isolates by PFGE. Clonal spreading of resistant strains could not be demostrated.

Table 1: Macrolide resistance phenotypes, erythromycin resistance genes and MIC50/90 values among 35 erythromycin-resistant *S. pneumoniae* isolates

		MIC range (mg/L)			MIC 50/90 (mg/L)			Resistance genes	
Phenotype	No. of strains (%)	Erythromycin	Clindamycin	Penicillin	Erythromycin	Clindamycin	Penicillin	ermB	mefA
$cMLS_B$	26 (73)	64–⩾128	⩾32	⩽0.03–4	⩾128/⩾128	⩾32/⩾32	1/2	26	7
$iMLS_B$	3 (9)	64–⩾128	⩽0.03–0.06	0.03–0.25	ND	ND	ND	3	0
M	6 (18)	1–4	⩽0.03	⩽0.03–4	ND	ND	ND	0	5*

*One strain in M phenotype was not include resistance genes. ND: not determined.

Conclusions: This study showed that the most common mechanism of erythromycin resistance in *S. pneumoniae* isolates were cMLSB (constutive) phenotype encoded by ermB gene (73%). Serotype 19 (45%) was predominant in erythromycin resistant *S. pneumoniae* isolates. However, clonal dissemination of resistant strains were not found.

R2161 First report of carbapenem resistance in *Klebsiella pneumoniae* due to porin loss from Croatia

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Objectives: To study the mechanisms of reduced susceptibility to carbapenems in an ESBL producing *K. pneumoniae* obtained from a patient at the Dubrava University Hospital, Zagreb, Croatia, that was isolated during meropenem therapy.
Methods: An ESBL-producing *K. pneumoniae* (as determined by a double disk assay) was isolated from a blood culture of patient with a cardiac transplant. The patient was treated with meropenem twice in six weeks. Two weeks later *K. pneumoniae* with reduced suscepeptibility to carbapenems was isolated from an anal swab. MICs were determined by broth microdilution according to CLSI. Modified Hodge Test(MHT) was used to screen for production of carbapenemases. MBL E-test was used to screen for production of metallo-β-lactamases. The presence of blaSHV, blaTEM, blaCTX-M, blaACT-1, blaMIR, blaCMY, blaDHA, blaKPC-1, blaOXA-48, blaIMP and blaVIM was determined by PCR and the obtained amplicons were sequenced. The genetic relatedness of the strains was investigated using PFGE. To study porin content of the strains, outer membrane proteins (OMPs) were extracted and separated by SDS-PAGE.
Results: The initial isolate was susceptible to ertapenem (MIC 0.125 mg/L), meropenem (MIC 0.032 mg/l) and imipenem (MIC 0.25 mg/l). MICs of carbapenems for the strain obtained after meropenem treatment were: ertapenem MIC 32 mg/l, meropenem MIC 16 mg/l, and imipenem MIC 8 mg/l. PFGE showed that the two strains were highly related. Both strains were shown to possess blaSHV-11 and blaCTX-M-15/28 genes. MHT was negative in both strains. The PCR reactions with primers specific for Amp-C, KPC and oxacillinase were negative. Both isolates produced an OMP-A like protein. The initial isolate expressed one single porin (OmpK36) while second isolate did not produce any of the two major porins of *K. pneumoniae* (OmpK35 or OmpK36).
Conclusion: *K. pneumoniae* with decreased carbapenem susceptibility was isolated from a surveillance culture (anal swab) after prolonged meropenem therapy. Since no carbapenemases were produced by the strains, carbapenem resistance is attributed to be due to hyperproduction of CTX-M β-lactamase combined with complete porin loss. This study highlights the need to establish an antimicrobial resistance survellance network for *K. pneumoniae* and to further monitor the trends and new types of resistance mechanisms.

R2162 Prevalence and molecular characteristics of faecal-colonizing plasmid-mediated quinolone-resistant Enterobacteriaceae in five tertiary care hospitals in Korea

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Introduction: The aims of this study were to investigate the prevalence and molecular characteristics of fecal Enterobacteriaceae bearing plasmid-mediated quinolone resistance that had colonized patients in five tertiary-care hospitals in Korea.
Methods: We collected 500 non-duplicated Enterobacteriaceae isolates from stool samples between March 2008 and May 2008 in five hospitals and screened for the qnr (qnrA, qnrB, qnrS), aminoglycoside acetyltransferase aac(6′)-Ib-cr, and plasmid-mediated qepA genes by PCR amplification. All positive results were confirmed by direct sequencing of the PCR products.

Results: The qnr gene was detected in 85 (17.0%) of the 500 isolates. Among 85 qnr-positive strains, *K. pneumoniae* (N = 39) was the most common, followed by *E. coli* (N = 18), *C. freundii* (N = 10), *C. braakii* (N = 9), and others (N = 9). These were finally identified as qnrA1 (N = 7), qnrB subtype (N = 70), and qnrS1 (N = 8). Nine strains revealed new variants, which were qnrB like with amino acid mutations. The aac(6′)-Ib-cr and plasmid-mediated qepA genes were detected in 58 (11.6%) and 3 (0.6%) strains, respectively. A total of 30 strains were positive in both qnr and aac(6′)-Ib-cr, and 1 strain showed a positive result for all three genes. The resistance rates of qnr-positive strains to ciprofloxacin, levofloxacin, norfloxacin, and nalidixic acid were 55.2%, 36.8%, 40.2%, and 49.4%, respectively. The resistance rates of aac(6′)-Ib-cr-positive strains were higher than those associated with qnr genes.
Conclusion: The qnr and aac(6′)-Ib-cr genes were highly prevalent in stool specimens. We presumed that the wide spread of plasmid-mediated quinolone resistance in clinical isolates was associated with high rates of fecal colonization in the hospital.

R2163 Characterization of β-lactamases and integrons in amoxicillin–clavulanic acid-resistant *Salmonella enterica* isolates of three Spanish hospitals

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Objective: To characterize β-lactamases and integrons in *S. enterica* isolates resistant or intermediately resistant to amoxicillin–clavulanic acid (AMCI/R) recovered during 2007–2009 from three Spanish hospitals of different geographical areas.
Methods: 90 AMCI/R *S. enterica* isolates [serovars Typhimurium, 80; Enteritidis, 6; Thompson, 1; Gnesta, 1; *Salmonella* spp, 2] were recovered from faecal (71), blood (4), urine (1) and other (14) origins. Susceptibility testing to 19 antibiotics was performed by disc-diffusion and microdilution methods (CLSI). ESBL phenotype was determined by double disc synergic method. The presence of blaCTX-M, blaTEM, blaCMY, blaSHV, blaPSE, blaOXA-1 genes; the genetic environment of blaCTX-M and the detection and characterization of class 1, 2 and 3 integrons were studied by PCR and sequencing.
Results: The percentages of resistance found in the 90 isolates were as follows: sulfamides (96%), tetracycline (89%), chloramphenicol (76%), streptomycin (64%), nalidixic acid (23%), co-trimoxazole (6%), ciprofloxacin (1%) and gentamicin, tobramycin, kanamycin, amikacin (range 1–3%). Two AMCI/R isolates also showed resistance to third generation cephalosporins, exhibited an ESBL phenotype and harboured blaCTX-M-15 plus blaTEM-1, and blaCTX-M-14a genes, respectively. The surrounding regions of these blaCTX-M genes were: ISEcp1-blaCTX-M-15-orf477 and ISEcp1-blaCTX-M-14a-IS903. The remaining 88 AMCI/R isolates harboured (no. of isolates): blaPSE-1 (44), blaOXA-1 (24), blaTEM-1 (13), blaPSE-1 plus blaOXA-1 (1) and blaPSE-1 plus blaTEM-1 (3). Class 1 integrase was detected in 73 AMCI/R isolates (81%). The blaPSE-1-positive isolates (n = 48) included this gene in one class 1 integron, and aadA2 gene in another integron, a structure related to *Salmonella* genomic island 1 (SGI1). The other gene cassette arrangements found in class 1 integrons were (no. of isolates): blaOXA-1+aadA1 (23), aac(6′)-Ib-cr+blaOXA-1+catB3+arr3 (1) and dfrA12+orfF+aadA2 (1).
Conclusions: Among clinical *S. enterica* isolates, AMC resistance is mainly due to the production of the PSE-1 β-lactamase. The blaPSE-1 gene was always found inside a class 1 integron related to SGI1. Other β-lactamases are also implicated in AMCI/R phenotype. ESBL is an emergent problem in *S. enterica,* and blaCTX-M-15 and blaCTX-M-14a were detected.

R2164 Prevalence of plasmid-mediated quinolone resistance and extended-spectrum β-lactamase determinants in *Escherichia coli* and *Salmonella* spp. isolated from processed food products and food-animals

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Objective: Esherichia coli and *Salmonella* spp. are important causes of gastrointestinal illness and are often found in production animal settings. Thus, the aim of the study was to investigate the presence of plasmid-mediated quinolone resistance (PMQR) and extended-spectrum β-lactamases (ESBL) determinants in *E. coli* and *Salmonella* spp. isolated from processed food products and food-animals.
Methods: From a total of 77 isolates collected between August 2008 and April 2009, from food producing animals, processed food and mud, 37 were *E. coli* and 40 *Salmonella* spp. strains. Antibiotic susceptibility tests were performed for β-lactams, quinolones and aminoglicosides, according to CLSI guidelines. Isolates were screened for PMQR (qnr, aac(6′)-Ib-variant and qepA) and ESBL (blaTEM, blaSHV and blaCTX-M) determinants using specific primers. qnr genes were identified by nucleotide sequencing.
Results: Eighty-five percent of *Salmonella* spp. strains were isolated from processed food, 10% from animals and 5% were isolated from mud. Forty percent of strains were resistant to nalidixic acid, while 8% and 5% showed resistance to 3rd generation cephalosporins, ceftazidime and cefotaxime, respectively. All strains were susceptible to netilmicin and imipenem. Five percent of strains were multirresistant. PMQR determinants were not detected among *Salmonella* spp. strains, however it was possible to detect four blaTEM genes. For *E. coli* strains, 95% were isolated from animals and 5% from processed food. Seventy-six percent of strains were resistant to nalidixic acid, 27% to ciprofloxacin and 8%, 3% and 5% were resistant to cefotaxime, ceftazidime and netilmicin, respectively. All strains were susceptible to imipenem. Fourteen percent of strains presented multirresistance. Two QnrB2 encoding genes were identified in strains from pigs, one of which presenting multidrugresistance phenotype. Two QnrS1 encoding genes were identified in strains recovered from poultry, which presented only resistance to quinolones. The determinants aac(6′)-Ib-variant and qepA were not detected. Forty-six percent of strains presented a blaTEM gene, 8% a blaSHV and 5% a blaCTX-M gene. Both strains expressing QnrB2 presented also a blaTEM gene and one of them a blaSHV.
Conclusion: This study demonstrates the increase in the dissemination of PMQR and ESBL determinants among foodborne zoonotic pathogens, constituting a major risk factor for public health.

R2165 Epidemiology of resistance to third-generation cephalosporins in Enterobacteriaceae from critically ill medical patients

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Objectives: To evaluate the epidemiology of resistance to third-generation cephalosporins (CEF3) in enteric Gram-negative bacilli (EGNB) from critically ill medical patients.
Methods: During 21 months, patients admitted to an 8-bed medical ICU were subjected to qualitative surveillance cultures of nares, pharynx, tracheal aspirates and rectum thrice weekly. Selective media were used to isolate CEF3-EGNB. ESBLs and plasmid-encoded cephamycinases were characterized by PCR. Clonality was determined by PFGE.
Results: 633 patients were included in the study. 78 isolates were recovered from 72 (11%) patients, almost all (n = 68, 94%) from rectal swabs. CEF3-resistant organisms included *E. coli* (n = 46, 76%), *K. pneumoniae* (n = 13, 17%), *E. cloacae* (n = 9, 12%), *E. aerogenes* (n = 2, 3%), *H. alvei* (n = 2, 3%) and others (1 *C. diversus,* 1 *C. freundii,* 1 *S. marcescens*, 1 *S. fonticola,* 1 *M. morgagnii,* 6%). 35 (76%) strains of *E. coli* possessed an ESBL (CTX-M group 9 in 28, CTX-M group 1 in 3 and SHV in 6) and 9 (20%) a cephamycinase (8 CIT group, 1 DHA), either alone (n = 5) or with a CTX-M group 9 enzyme. In

K. pneumoniae, 10 (77%) isolates had an ESBL (3 CTX-M group 9, 4 CTX-M group 1, and 3 SHV) and 5 (38%) a cefamycinase (2 EBC; 3 DHA, 2 with a SHV). In 40 (51%) patients, a CEF3-resistant EGNB was not detected on admission but apparently acquired thereafter. Acquisition was less frequent for *E. coli* (45%) than for *K. pneumoniae* (62%) or other species (76%) ($p = 0.08$). Molecular epidemiology revealed 2 clusters of 2 patients each among 46 *E. coli* isolates (9%), 1 cluster of 2 patients among *E. cloacae* (22%) and 1 cluster of 5 patients among *K. pneumoniae* (38%). However, clinical epidemiology was consistent with intra-ICU transmission in only one patient with *K. pneumoniae*.

Conclusions: In our setting, plasmid-encoded AmpC β-lactamases have become prevalent (⩾20%) among CEF3-resistant *E. coli* and. *K. pneumoniae* carried by critically ill medical patients. Apparent acquisition was unrelated to clonal transmission, which may indicate poor sensitivity of admission cultures. Conversely, clustering in *K. pneumoniae* should not be blindly attributed to intra-ICU horizontal transmission.

R2166 Prevalence of class 2 integrons and multidrug resistance among *Salmonella enterica* isolates from clinical cases in Iran

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Objective: The main objective of this study was to investigate the prevalence and diversity of class 2 integrons in *Salmonella enterica* isolated in Iran during 2007–2008.

Methods: *Salmonella* strains were isolated from several hospitals in Tehran, Iran. The isolates were identified by standard biochemical tests and agglutination using specific antisera. The strains were tested for susceptibility to the following antimicrobial drugs: ampicillin, streptomycin, gentamycin, kanamycin, tobramycin, chloramphenicol, tetracycline, ciprofloxacin, nalidixic acid and sulfamethoxazole-trimethoprim, by the disc diffusion method, according to CLSI (Clinical and Laboratory Standards Institute). Class 2 integrons were detected by PCR with specific primers for the int2 gene, and subsequently the cassette regions were amplified using primers hep74 and hep51 for the attI2-orfX region.

Results: In this research, 103 *Salmonella* strains were isolated. The most common resistance phenotypes detected were to nalidixic acid (64%), tetracycline (50%), streptomycin (42%), sulfamethoxazole-trimethoprim (29%), kanamycin (24%), ampicillin (16%) and chloramphenicol (13%). 75 (72.8%) of bacteria were resistant to two or more antibiotics that is considered as MDR. Twenty-one (20.3%) of the 103 isolates had a 2161 bp class 2 integron, containing four open reading frames, namely dhfI, satI, aadA and orfX.

Conclusions: Our findings showed that class 2 integrons are widely spread among *Salmonella enterica* isolated in Iran. Integron positive isolates were included into five different serotypes of *S. enterica:* Albany, Infantis, Muenchen, Reading and Typhimurium. Surveillance and monitoring of antimicrobial drug resistance, including screening for integrons as likely indicators of drug resistance and acquisition of new resistance traits, are necessary steps in planning effective strategies for containing this phenomenon within food-borne infection organisms.

R2167 Detection of carbapenemases and analysis of resistance in *Acinetobacter baumannii*

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Objective: To investigate carbapenemases and antimicrobial resistance in clinical isolates of *Acinetobacter baumannii* in our hospital, study the molecular epidemiology and resistance mechanisms.

Methods: 179 *Acinetobacter baumannii* clinical isolates were collected from January to June 2009 in our hospital. Modified Hodge test was used to screen the strains producing carbapenemases. Antimicrobial susceptibility test was performed by disk diffusion method. Carbapenemase genes were amplified using PCR and sequenced, plasmid conjugation experiments were done to study the transfer of carbapenemase genes, and the homology of these isolates was analyzed by ERIC-PCR, in order to explain the molecular mechanism of drug resistance.

Results: 74 of 179 strains were positive in modified Hodge test; 74 strains maintained highly sensitivity to cefoperazone/sulbactam and minocycline and their resistance rates were 16.2% and 13.5% respectively, and the resistance rates to other antimicrobial agents were more than 78.0%. OXA-23 gene was found in 71 strains but OXA-24, IMP-1, IMP-2, VIM-1 or VIM-2 genes were not found in 74 bacteria; Carbapenemase genes were unable to transfer via plasmid; 74 strains were identified as 4 predominant clones by ERIC-PCR and they had spread in many wards in our hospital widely.

Conclusions: OXA-23 gene was the popular carbapenemase genotype and clonal spread was the main reason for carbapenem resistance to *Acinetobacter baumannii* in our hospital.

R2168 Double resistance conferred by CTX-M and plasmid-mediated AmpC

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Objectives: Plasmid-mediated antibiotic resistance within Enterobacteriaceae is globally accelerating. Of great concern is *E. coli* and *Klebsiella pneumoniae* producing classical extended-spectrum β-lactamases (ESBL_A) or AmpC enzymes (ESBL_M) both which commonly are plasmid-mediated and confer resistance to 3th generation cephalosporins. ESBL-producing Enterobacteriaceae are since 2007 mandatorily notifiable by the laboratories according to the Swedish Communicable Diseases Act. In 2008 an incidence of 32 cases per 100 000 inhabitants was noted, which was a clear increase as compared to 2007. At the Swedish Institute for Infectious Disease Control we receive and further characterize clinical isolates with extended spectrum β-lactam resistance providing an overview of the Swedish expanding ESBL situation. Of notice is six isolates containing both blaCTX-M and blaAmpC, two different apparent plasmid-mediated resistance mechanisms towards extended spectrum β-lactams.

Methods: Our collection consists of extended spectrum cephalosporin resistant clinical isolates sent from different Swedish laboratories for further ESBL characterization. Susceptibility testing was performed by both Etest and disc-diffusion methodology. Breakpoints were applied according to EUCAST and SRGA, respectively. Phenotypic ESBL characterization was performed by double disc synergy tests. All isolates were further screened for the presence of bla_CTX-M and plasmid-mediated bla_AmpC using real-time PCR. PFGE was performed for assessment of genetic relatedness between the isolates.

Results: Between October 2007 and November 2009 six clinical isolates, five *E. coli* and one *K. pneumoniae*, with bla_CTX-M group 1 in combination with apparent plasmid-mediated bla_AmpC were detected. The isolates had clavulanic acid reversible resistance to cefotaxime and/or ceftazidime, indicating the presence of ESBL_A in combination with cloxacillin reversible resistance to cefoxitin, indicating the presence of an AmpC-enzyme. Genetic screening for CTX-M and apparent plasmid mediated AmpC demonstrated the presence of a bla_CTX-M gene of group 1 in all isolates and a gene coding for the bla_AmpC enzyme CIT in the five *E. coli* isolates and DHA in the *K. pneumoniae* isolate. All six isolates were multiresistant and the patients were of varying ethnicity, gender, medical record and their age range from 2 to 91 years. Analysis with PFGE and confirmation of the apparent plasmid origin of the bla_CTX-M and bla_AmpC genes are ongoing.

In vitro antibacterial susceptibility and drug interaction studies

R2169 *In vitro* efficacy of combination with various antimicrobial agents against trimethoprim/sulfamethoxazole-resistant *Stenotrophomonas maltophilia*

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Objectives: Although trimethoprim/sulfamethoxazole (TMP/STX) is considered the treatment of choice of serious infections caused by *Stenotrophomonas maltophilia*, TMP/STX-resistant isolates have emerged and became challenging in management of *S. maltophilia* infections. Thus we evaluated *in vitro* activity of the interactions with various antimicrobial agents on TMP/STX-resistant *S. maltophilia*.
Methods: The susceptibility tests of TMP/STX using microdilution method were performed with 73 clinical isolates of *S. maltophilia* collected from January 2006 to March 2009 at Samsung Medical Center. Among TMP/STX-resistant *S. maltophilia* isolates, 3 non-duplicated isolates were evaluated for *in vitro* activities of single and combination antibiotics of tigecycline (TGC), ticarcillin–clavulanic acid (T/C), levofloxacin (LEV), and colistin (CL), using time-kill assay. The time-kill assays of 0.25, 0.5 and 1XMIC at each antibiotic were performed for each isolate. *In vitro* activities of two-drug combinations were evaluated using 0.5XMIC.
Results: Among 3 isolates tested, 1 isolate showed *in vitro* resistance to TGC and T/C, 2 isolates showed resistance to LEV and all isolates showed resistance to CL. The interactions of antimicrobial agent combinations are shown in table.
Conclusion: The growth of TMP/STX-resistant *S. maltophilia* was significantly inhibited by the combination of colistin and levofloxacin, compared with other combinations. Given the limitation of therapeutic options against TMP/STX-resistant *S. maltophilia*, further investigation is warranted to ascertain the clinical relevance of our findings.

Isolates	TGC+T/C	TGC+LEV	TGC+CL	T/C+LEV	T/C+CL	LEV+CL
1.	Indifference	Antagonism	Antagonism	Antagonism	Indifference	Synergism
2.	Indifference	Antagonism	Antagonism	Indifference	Indifference	Synergism
3.	Indifference	Antagonism	Indifference	Indifference	Indifference	Synergism

R2170 *Acinetobacter*-associated infections in the Republic of Belarus: state of the problem

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Objectives: The purpose of our research was to evaluate *Acinetobacter baumannii* antibiotic resistance level in hospitals of the Republic of Belarus.
Methods: The subject of the study was a group of patients with clinical and laboratory documented nosocomial infections caused by *A. baumannii*. All patients were treated in 12 Minsk hospitals between December, 2008 and July, 2009. The total number of researched patients is 73 (65.7% males; middle age 51.7±4.4 years old). Pathogen identification and antibiotic resistance surveillance were performed at the microbiological laboratory of the Institute of Antimicrobial Chemotherapy (Russian Federation). Strains collected from different specimen sources of the same patient were excluded.
The isolates were identified by conventional methods. Antimicrobial resistance was evaluated by disk-diffusion method and determined according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS, USA). Intermediately susceptible isolates were recognized as resistant.
Results: None of the tested antibiotics achieved high activity (⩾80% susceptible) against nosocomial isolates of *A. baumannii*. The most of the examined strains were resistant to ceftazidime (91.7%), ciprofloxacin and amikacin (89% and 86.8% respectively). Susceptibility to gentamicin and carbapenems was relatively higher, but also under 50%. So 37% of the collected isolates were susceptible to gentamicin, 47.9% to imipenem and 46.6% to meropenem.
The difference in susceptibility to carbapenems was documented in three cases. Two strains were imipenem-susceptible and one strain was meropenem-susceptible.
It's interesting, that 9 of 73 collected isolates were susceptible only to gentamicin. It was also active against 14 carbapenem-resistant strains.
Conclusion: *A. baumannii* isolates from Minsk hospitals are characterized by high resistance to all studied antibiotics. We found out, that carbapenems have the highest activity against nosocomial strains of *A. baumannii*. It seems that gentamicin activity is relatively high, because it's not commonly used during the last ten years in our country. However, the resistance level to these antimicrobials was extremely high.

R2171 Antimicrobial activity of daptomycin against multidrug-resistant Gram-positive strains collected in Bulgaria

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Objectives: Daptomycin is a cyclic lipopeptide with potent activity and broad spectrum against Gram-positive bacteria currently used for the treatment of complicated skin and skin structure infections and bacteremia, including right sided endocarditis. We evaluated the *in vitro* activity of this new compound against clinical strains of staphylococci and enterococci collected from Bulgarian medical centers in the National Reference Laboratory for "Control and Monitoring of Antimicrobial Resistance" at the National Center of Infectious and Parasitic Diseases, Sofia.
Methods: A total of 100 fresh, non-duplicate clinical strains, including 60 *Staphylococcus aureus* (MRSA), 40 *Enterococcus* spp. (20 *Enterococcus faecium*, 15 *E. faecalis* and 5 *E. avium)* from different medical centers were tested for susceptibility by reference agar microdilution methods according to Clinical and Laboratory Standards Institute guidelines and interpretative criteria.
Results: All *S. aureus* strains were inhibited at a daptomycin MIC of ⩽1 mg/l. Among tested *E. faecium* strains the highest daptomycin MIC value was 2 mg/l (MIC50 under 0.5 mg/l), while among *E. faecalis* and *E. avium* the highest MIC value was 1 mg/l (Table 1).
Conclusion: Daptomycin showed excellent *in vitro* activity against staphylococci and enterococci collected in the National Reference Laboratory for "Control and Monitoring of Antimicrobial Resistance" and appears to be an excellent therapeutic option for serious infections caused by methicillin-resistant staphylococci and vancomycin-resistant enterococci. During this National study no resistant bacteria were found in Bulgaria and 100% of the tested strains were susceptible to Daptomycin.

Strain	Susceptible at concentration (μg/ml):												Positive control
	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	(without antibiotic)
Enterococcus spp. (40 strains)														
E. faecium (20 strains)		4	5	1	10									20
E. faecalis (15 strains)		1	2	12										15
E. avium (5 strains)				5										5
MRSA (60 strains)	9	31	18	2										60

R2172 Update on antimicrobial susceptibility rates among Gram-negative and Gram-positive organisms in Belgium and Luxembourg: results from the 2nd tigecycline evaluation & surveillance trial (2B-TEST)

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Background: Surveillance studies provide invaluable information in the tracking of antimicrobial susceptibility. The aim of this study was to measure *in vitro* activity of a panel of antimicrobial agents including Tygacil against Gram-positive and Gram-negative agents, collected in Belgium in 2008 and compare the results with a similar survey in 2006. Establishing the local prevalence of *in vitro* susceptibility is essential

in order to predict potential local efficacy, promote appropriate use and define a place amongst available antibiotics.
Methods: 12 major hospitals from Belgium (11) and Luxemburg (1) collected 2122 clinically relevant, consecutive isolates from the study list of Gram positive and Gram negative pathogens over a 6 month period (January 1st to November 10th 2008). Minimal Inhibitory Concentrations (MIC) were determined using a microdilution method (Sensititre®). Pathogens originated from all wards and all infections, with a maximum of 20 urinary isolates per hospital. *E. coli*, *Klebsiella* spp and *Enterobacter* spp. were screened for extended spectrum β-lactamase (ESBL) activity using local methodology.
Results: Tigecycline was the most active agent tested against Gram positive pathogens. Cumulative % with reference to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints were 100% for *E. faecalis* (n = 119), 97% for *E. faecium* (n = 75), for *S. aureus* – MSSA 98% (n = 121) & MRSA 95% (n = 120). Tigecycline was very active against Gram negative pathogens as well, with a cumulative % at EUCAST breakpoint of 95% for *A. baumannii* (n = 81), 100/98% for *E. coli* ESBL-/+(n = 215/82), 96/89 for *Klebsiella* spp ESBL-/+(n = 15/61). and 90/75% for *Enterobacter* spp ESBL-/+(n = 173/64).
Conclusion: Tigecycline continues to show excellent *in vitro* activity against Gram positive and more than reliable activity against Gram negative pathogens when collected consecutively in daily practice in Belgium and Luxemburg. These results will enable Belgian and Luxemburg physicians to use tigecycline appropriately in hospitalized patients.

R2173 Effects of human polymorphonuclear neutrophils alone or in combination with amikacin against *Pseudomonas aeruginosa* biofilms

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Objectives: Chronic *Pseudomonas aeruginosa* (PA) airway infections remain the primary cause of morbidity and mortality in the cystic fibrosis (CF) patients. The growth state of PA in CF airways consists of a bacterial biofilm (BF), which differs from that exhibited under conventional susceptibility testing conditions. Within the BF, PA cells are protected from polymorphonuclear neutrophils (PMNs) and exhibit a high level of resistance to antimicrobial agents. We examined the *in vitro* activity of PMNs alone and in combination with amikacin (AMK) against PA BF and compare them to the free-living planktonic (PL) counterparts.
Methods: Two clinical PA isolates, a resistant (AMKR, CLSI MIC: 64 mg/l) and a susceptible (AMKS, CLSI MIC: 8 mg/l), derived from two CF patients, were grown by incubation in cation-adjusted Mueller-Hinton broth in 96-well flat-bottomed plastic plates under constant shaking for 48 h at 37ºC in order to form BF. PMNs from healthy donors at an effectors to target (E:T) ratio of 1:10 or 1:20 were incubated further for 24 h alone or in combination with 2, 8 or 32 mg/l of AMK. Percent damage of BF or PL was assessed by metabolic XTT assay. Damage $\geqslant$50% indicated drug susceptibility. Synergy was concluded when the observed bacterial damage was significantly higher to the expected sum of damages, respectively; whereas, additivity was defined when the observed bacterial damage was significantly higher than each component but where synergy was not achieved. ANOVA (n = 6) with Dunnett's test was performed.
Results: PMNs induced lower percent damage to BF than to PL cells of both isolates (at 1:10 ratio, mean±SE: 20.0±3.3 vs 15.0±2.4, $p < 0.05$ for AMKR; 27.0±2.9 vs 13.0±1.8, $p < 0.001$ for AMKS). AMK at 8 mg/l for AMKS (54.0±3.2 vs 26.0±5.8) and at 32 mg/l for both isolates (42.0±2.5 vs 33.0±1.6 for AMKR and 57.0±4 vs 40.0±4.6 for AMKS) induced lower damage ($p < 0.001$) to BF than to PL cells. The combined effects of PMNs with various concentrations of AMK for both isolates on damage of BF was lower than that on the damage of PL cells ($p < 0.005$). Synergy was observed when PMNs (at 1:10) were combined with AMK (8 or 32 mg/l) for both AMKR and AMKS isolates in BF and PL cells.
Conclusions: BF of both resistant and susceptible isolates are significantly less susceptible to PMNs and to AMK than are PL cells. Both PL and BF growth forms of the resistant PA are less susceptible to AMK than that of the susceptible organism. Synergy is exhibited between PMNs and AMK in BF and PL cells.

R2174 Fosfomycin: the issue of emergence of antimicrobial resistance

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Objective: The reevaluation of older antimicrobial agents may provide at least temporary solutions to the challenge of advancing antimicrobial drug resistance among common bacterial pathogens. Fosfomycin is used rarely for the treatment of systemic infections, despite substantial antimicrobial activity against common pathogens. The emergence of mutational resistance to this agent is of concern. We aimed to evaluate the magnitude of the issue of emergence of bacterial resistance to fosfomycin.
Methods: We performed a systematic review of relevant published *in vitro* studies, clinical studies and studies examining trends of resistance over time were included.
Results: In 7 *in vitro* studies identified, development of bacterial resistance to fosfomycin after exposure to this agent was observed by various methods for 65/629 (10.3%) *Escherichia coli* isolates, 82/86 (95.3%) *Staphylococcus aureus* isolates, 32/33 (97.0%) *Serratia* spp. isolates, 15/17 (88.2%) *Pseudomonas aeruginosa* isolates, and all 3 *Klebsiella pneumoniae* isolates. In 5 clinical studies identified, failure of fosfomycin therapy associated with the emergence of resistance to this agent was observed in 31/1161 (2.6%) patients with various types of infections who received fosfomycin therapy. In 10 studies examining resistance trends over periods of 5–10 years, the absolute differences in the susceptibility rate to fosfomycin for most of the pathogens examined (mainly *E. coli*), were within 10 percentile units.
Conclusion: The development of mutational resistance to fosfomycin appears to be frequent *in vitro*, particularly for pathogens other than *E. coli*. However, the clinical relevance of this phenomenon appears to be of minor significance, supporting further research on fosfomycin use for systemic infections.

R2175 *In vitro* synergism of various antibiotics against *Stenotrophomonas maltophilia* clinical isolates

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Objective: *Stenotrophomonas maltophilia* is an emerging hospital pathogen. Susceptibility testing is difficult as it is affected by both temperatures and the medium. Though some antibiotics has been shown to be effective in treating patients, there is no good evidence to support a relationship between laboratory susceptibility testing and clinical outcome due to lack of clinical trial and standardised susceptibility testing. Our aim was to study the *in vitro* interaction among various antimicrobials against clinical isolates using an E-test method specifically modified for this project.
Methods: 30 *S. maltophilia* clinical isolates were collected over a period of 5 months and identity was confirmed with Vitek2. Isolates were refrigerated until use. A day prior to the experiments the isolates and a reference strain were subbed on blood agar to provide a fresh growth. 0.5 McFarland suspension was prepared from each isolate and they were inoculated on Muller Hinton agar plates marked and templated appropriately. co-trimoxazole (SXT) and ceftazidime (CZ) strips were applied to the designated template area using sterile technique. The plates were left at room temperature for 1 hour and then from all but SXT strips in template 1 and CZ in template 2 were removed. Then 4 strips [ciprofloxacin(CIP), moxifloxacin (MX), minocycline (MN), chloramphenicol (C)] were applied on the empty slots in template 1 and 4 strips [piperacillin/tazobactam(PT), ticarcillin/clavulinate (TIM), aztreonam (ATM), amoxicillin/clavulinate (AMC)] were applied in

template 2 after scale adjustment. The plates were then incubated for 24 hours at 27ºC and MICs were read (80% inhibition for SXT and complete inhibition for CZ). The experiment was standardised with a reference strain from National Collection of Type Cultures, Colindale.
Results: In the SXT group combination of SXT with MX was more effective than SXT alone and in CZ group combination of CZ with PT, TIM, ATM were more effective than CZ alone (Table 1).
Conclusions: The synergistic result yielded from this project appears to be active *in vitro* but there is no good evidence available to suggest a relationship with clinical outcome. This call for further investigation and clinical trials. Different antibiotics and combination could be tested to find out alternative and more effective treatment for a difficult to treat infection.

Table 1

Drugs	Synergy MIC ⩾2 dilution lower than MIC of SXT/CZ alone	Indifference MIC within ±1 dilution of MIC of SXT/CZ alone	Antagonism ⩾2 dilution than MIC of SXT/CZ alone
SXT + MX	71%	26%	3%
SXT + CIP	29%	52%	19%
SXT + MN	29%	52%	19%
SXT + C	23%	45%	32%
CZ + PT	68%	16%	16%
CZ + TIM	55%	19%	26%
CZ + ATM	55%	19%	26%
CZ + AMC	36%	19%	45%

R2176 Antimicrobial activity of herbal plants used in traditional medicine in Uzbekistan

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Objectives: We chose 23 promising and widely used medicinal plants of Uzbekistan used traditionally by local inhabitants for treating conditions likely to be associated with microorganisms, and evaluated them for potential antimicrobial activity, in order to confirm their popular use and to detect new sources of antibacterial agents.
Methods: Plant material (20 g) was extracted with 80% methanol and the extracts were dissolved in 10% (v/v) solution of DMSO to create a concentration of 10 mg/ml of stock solution. The preliminary antimicrobial activity of the extracts was carried out by disc diffusion test. The plant extracts which showed inhibitory effect were further investigated for MIC using the broth microdilution method. For comparative purposes standard antibiotic discs viz., tetracycline (20 μg/disc), ampicillin (10 μg/disc), were used as positive and DMSO (10%, v/v) as the negative controls.

Table 1. *In vitro* antimicrobial activity of plant extracts against human pathogenic bacteria (mg/ml)

Plant	*Ko*	*Kp*	*Ec*	*Pr*	*Cf*	*Ab*	*Pa*	*Sa*	*Ef*	*Efc*	*Se*	*Sar*	*Ml*	*Ca*	*Ka*	*Hp*
Achillea millefolium L.	na	na	5	5	na	na	5	1.2	2.5	5	na	5	na	na	na	na
Asparagus persicus Baker	na	na	na	na	na	na	na	na	5	2.5	5	5	5	5	na	na
Betula verrucosa Ehrh	na	na	na	na	na	2.5	1.2	1.2	1.2	2.5	na	2.5	2.5	1.2	na	na
Biden tripartite L.	na	na	na	5	na	na	5	2.5	na	5	5	na	na	5	2.5	na
Calendula officinalis L.	na	2.5	2.5	na	na	na	5	5	1.2	2.5	2.5	1.2	1.2	2.5	na	na
Centaurea belangeriana Stapt.	na	5	5	na	na	na	na	5	5	na	5	na	5	na	na	na
Dianthus tetralepis Nevski	na	5	5	na	na	na	na	5	2.5	2.5	5	5	na	na	na	na
Erodium hoeffitianum CAM	na	2.5	5	na	na	na	na	na	na	2.5	na	na	2.5	2.5	na	na
Helichrysum arenarium L.	na	na	na	na	na	na	na	na	na	1.2	5	na	na	na	1.2	na
Hypericum perforatum L.	na	na	na	na	na	5	5	2.5	2.5	1.2	5	5	2.5	5	na	5
Leonurus turkestanicus L.	na	na	na	5	2.5	na	5	2.5	2.5	2.5	5	5	5	na	5	na
Matricaria chamomilla L.	na	5	na	5	na	na	na	5	1.2	1.2	5	2.5	2.5	2.5	1.2	5
Melissa officinalis L.	5	na	na	5	5	na	na	na	5	5	na	5	na	na	na	5
Origanum tyttanthum L.	2.5	2.5	2.5	2.5	5	2.5	2.5	5	2.5	1.2	1.2	2.5	1.2	2.5	2.5	2.5
Peganum harmala L.	2.5	5	2.5	na	5	1.2	2.5	2.5	1.2	5	2.5	2.5	na	2.5	5	na
Plantago ovata L.	na	5	–	na	2.5	na	5	na	5	5	5	1.2	5	na	na	na
Scrophyllaria striata Boiss	na	5	5	na	na	na	na	na	na	2.5	5	2.5	na	2.5	5	na
Tanacetum vulgare L.	5	na	2.5	na	na	na	2.5	1.2	1.2	2.5	2.5	1.2	1.2	na	2.5	5
Thymus vulgaris L.	na	na	na	na	na	2.5	na	na	5	2.5	na	5	5	na	na	na
Trifolium pretense L.	na	na	na	5	2.5	na	2.5	2.5	2.5	1.2	1.2	2.5	5	na	2.5	na

na: not active; *Ko*: *K. oxytoca* 6653; *Kp*: *K. pneumoniae* 40602; *Ec*: *E. coli* NCTC9001; *Pr*: *P. rettgeri* NCIMB9570; *Cf*: *C. freundii* 82073; *Ab*: *A. baumannii* 60649; *Pa*: *P aeruginosa* NCTC6749; *Sa*: *S. aureus* MRSA16; *Ef*: *E. faecalis* NCTC775; *Efc*: *E. faecium* 8; *Se*: *S. epidermidis* NCTC7944; *Sar*: *S. aureus* MRSA462; *Ml*: *M. luteus*; *Ca*: *C. albicans* NCVC1467; *Ka*: *K. aerogenes* NCTC8172; *Hp*: *Helicobacter pylori* NCTC12823.

Results: Most of plant extracts were able to inhibit the growth of one or more of the tested strains at 2.5 mg/ml that corresponds to a concentration of 0.25% (Table 1). The plant species such Equisetum arvense, Polygonum aviculare and Limonium otolepis did not show any antibacterial activity against tested bacterial strains. The extract from Origanum tyttanthum showed the broadest spectrum of action against bacteria, inhibiting all of the strains tested with MICs ranging from 1.2 to 5 mg/ml, suggesting their potential as antimicrobial compounds. The extracts from aerial part of Betula verrucosa L. Calendula officinalis, Hypericum perforatum, Leonurus turkestanicus, Matricaria chamomilla, Tanacetum vulgare and Trifolium pretense were more active against Gram-positive, including *S. aureus* and MRSA (MICs ranging from 1.2 to 5 mg/ml) than against Gram-negative bacteria. They were also active against the fungus *Candida albicans*. Other plant species such Calendula officinalis, Hypericum perforatum, Melissa officinalis and Achillea millefolium have been reported and antimicrobial activities have been found. Among plant extracts only 5 species Matricaria chamomilla, Melissa officinalis, Hypericum perforatum, Tanacetum vulgare and Origanum tyttanthum inhibited the growth of *H. pylori* NCTC 12823.
Conclusion: The obtained results confirm the presence of antimicrobial principles in the examined herbal plants native habitats of Uzbekistan mainly against Gram-positive bacteria, which supports their traditional use as wound healing and skin infections in Central Asia.

R2177 *In vitro* susceptibility of bacterial blood isolates from patients with sepsis against often-used antimicrobial agents for empirical therapy

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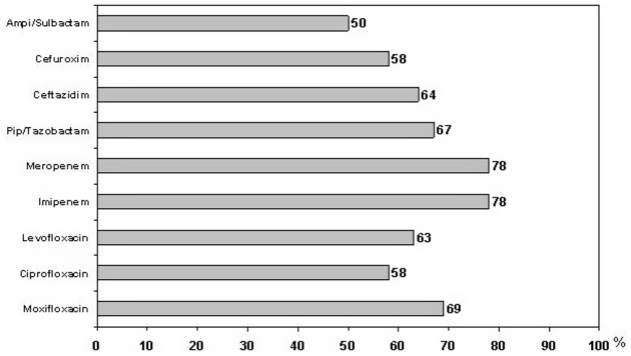
Background: Inadequate initial antibacterial therapy is associated with increased mortality in patients (pts) with serious infections, *in vitro* resistance being the major driver.
The aim of this study was to evaluate the susceptibility of blood culture isolates against antibiotics often used as empirical therapy for patients with clinical sepsis.
Methods: Susceptibility testing of blood culture isolates from pts with sepsis, severe sepsis or septic shock being hospitalized at the University Hospital Aachen, Germany, was performed against nine antimicrobials (BD Phoenix ™ expert system or by Etest®). Results were interpreted according to CLSI breakpoints.
Results: Out of 205 pts enrolled consecutively between March and September 2009, 127 pts (62%) had sepsis, 51 pts (25%) severe sepsis and 27 pts (13%) septic shock, respectively. Bacteraemia was hospital-acquired in 136 (66%) pts. Main infection sites were catheter-related (32%), urinary tract (17%), abdomen (15%) and lung (13%). Twenty pts (10%) had a polymicrobial infection; in total 228 clinically relevant pathogens were isolated: *S. aureus* (57/228, including 19 MRSA), *E. coli* (45/228), CNS (26/228), Enterococci (26/228) and *Klebsiella* spp. (22/228).
In 160/205 pts (78%) the blood culture isolates were susceptible to the Carbapenems (see Figure 1), followed by Moxifloxacin with 144/205 pts (70%). However, often used regimen like Ampicillin/Sulbactam or Cefuroxime demonstrated adequate *in vitro* activity in only 50/205 pts (50%) and 58/205 pts (58%), respectively. In community-acquired sepsis Moxifloxacin showed adequate activity in 99/116 pts. (85%).



Comparison of *in vitro* susceptibilities (n = 205 patients).

Conclusion: In this study population with pts with bacteraemia and different clinical stages of sepsis, antibiotic monotherapy showed *in vitro* activity in only 78% of the patients enrolled, thus leaving at least 22% pts. without adequate therapy. Thus a combination therapy e.g. a broad-spectrum β-lactam antibiotic and a fluoroquinolone seemed to be warranted. In patients with community-acquired sepsis, especially with pneumonia moxifloxacin is a potent therapeutic option.

R2178 Rapid detection of extended-spectrum β-lactamase producing bacteria by means of flow cytometry

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Objectives: The reliable detection of extended-spectrum B-lactamases (ESBLs) producing bacteria represents a major clinical challenge. Inappropriate treatment such as empiric administration of unspecific or wide spectrum antibiotics can lead, apart from therapeutic failures, to the promotion of multiresistent organisms. Phenotypic detection methods take at least 48 to 72 hours providing results that can be considered unspecific, thus demanding additional confirmatory tests. Molecular methods are expensive and time-consuming as several genes are involved in bacterial resistance. Flow cytometry allows a rapid analysis of a large number of individual cells using light scattering and fluorescence measurements. Our aim was to develop a flow cytometric protocol to provide an easy, fast and reliable detection of ESBL producers.
Methods: Forty clinical strains of *Escherichia coli* and *Klebsiella pneumoniae*, classified as positive (n = 20) and negative (n = 20) ESBL producers by phenotypic methods were tested. Strains genetically typed as belonging to the most common ESBLs genotypes (SHV, TEM and CTX-M) were used as controls. The bacterial strains were incubated in Muller-Hinton broth and exposed to cefotaxime and ceftazidime at several concentrations during one and two hours, with and without clavulanic acid (an ESBL-inhibitor). Bacterial suspensions were analysed by flow cytometry after staining during thirty minutes with Bis-(1,3-dibutylbarbituric acid) trimethine oxonol (DIBAC4), a fluorescent dye that penetrates the cell membrane as a result of membrane depolarization.
Results: ESBL positive strains showed an increase of the intensity of fluorescence only after incubation with clavulanic acid. The Flow Cytometry method was able to provide a fast (soon after one hour of treatment) and correct classification of the strains.
Conclusion: Flow Cytometry proved to be an excellent tool, being accurate and specific for the rapid detection of the most common ESBLs producing bacteria, avoiding the routine delays and high cost.

R2179 Clinical failure of daptomycin in the treatment of endocarditis due to susceptible *Enterococcus faecalis*

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Objective: To present a case of clinical failure of daptomycin in the treatment of endocarditis due to susceptible *Enterococcus faecalis.*
Case report: An HIV HCV co-infected 35 year old IV drug user presented with fever and general symptoms of fatigue two months after he had successfully completed treatment for right-sided MSSA endocarditis. He had been treated with vancomycin due to serious β-lactam allergic reaction. The patient had a CD4 >800 and undetectable viral load without antiretroviral treatment.
Upon admission a new episode of right-sided endocarditis was confirmed and the patient was started on daptomycin 8 mg/kg/day. *Enterococcus faecalis* was isolated from consecutive blood cultures susceptible to ampicillin, gentamicin, vancomycin and daptomycin (MIC < 0.5mcg/ml). Six days later phenotypic and genotypic (PFGE) identical isolate of *E. faecalis* continue to be isolated from his blood cultures. The patient was shifted to vancomycin (targeting trough levels >15 mg/L) and serum bactericidal activity of daptomycin and vancomycin were performed according to the Clinical and Laboratory Standards Institute guidelines. Our results suggested a higher serum bactericidal activity of vancomycin compared to daptomycin, a fact that was confirmed by the clearance of bacteraemia and the full clinical response of our patient.
Conclusions: Previous reports have shown the possibility of either emergence of resistance to daptomycin during treatment for enterococcal and MRSA infections or the limited reliability of present breakpoints for the detection of resistance. We believe that in our case the second possibility is the case. Clinicians and microbiologists should be aware of those issues and monitor closely the susceptibilities of isolates. Serum bactericidal activity appears to be a useful test in difficult clinical cases where the expected clinical response is delaying, besides *in vitro* susceptibility testing.

R2180 Antimicrobial susceptibility of streptococcal strains of mitis group isolated from respiratory tract infections in paediatric patients

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Objective: The mitis group belongs to the oral streptococci, which are commensal bacteria, but sometimes can be involved in the aetiology of serious infections. The aim of the present study was to investigate the antimicrobial susceptibility of 39 mitis group streptococcal strains belonging to: *Streptococcus oralis*, *S. mitis* and *S. sanguinis* species (the last one being included by R. R. Facklam in the sanguinis group), which were isolated from different respiratory tract infections (pneumonia, sepsis of pulmonary origin, peritonsillar abscess, rhinosinusitis, otitis media etc.) in paediatric patients from three hospitals in Bucharest, in 2009.
Methods: The susceptibility of the isolates was tested against: penicillin G (PG), ampicillin (AM), cefotaxime (CT), erythromycin (EM), clindamycin (CM), tetracycline (TC), levofloxacin (LE), linezolid (LZ) and vancomycin (VA), using the Etest (AB Biodisk, Sweden).
Results: The ranges of the minimum inhibitory concentrations were: 0.047–32 mg/l for PG, 0.032–32 mg/l for AM, 0.032–24 mg/l for CT, 0.016–256 mg/l for EM, 0.016–64 mg/l for CM, 0.25–48 mg/l for TC, 0.19–1 mg/l for LE, 0.064–0.75 mg/l for LZ and 0.5–1 mg/l for VA. About half of the isolates were resistant to the three β-lactam antibiotics. Only a quarter of the strains were susceptible to EM, while more than 90% and about 60% were sensitive to CM and TC, respectively. Resistance to: PG, AM, CT, EM and TC was found among the strains belonging to the three species, while all *S. mitis* isolates were susceptible to CM.
Conclusion: Since there is a great concern regarding the increased number of oral streptococci resistant to β-lactam antibiotics or multidrug-resistant, it is necessary to test the sensitivity of the isolates of clinical importance, especially to the commonly used antibiotics. LE, LZ and VA were the only antimicrobial agents fully active against the mitis group isolates investigated in the present work, which was a study supported by the project ID_2652 no. 1136/12.01.2009 from the Exploratory Research Projects of the National University Research Council and the Executive Agency for Higher Education and Research Funding from Romania.

New antimicrobials

R2181 New enzymatic principle for the synthesis of novel antimicrobials

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Objectives: Still today the treatment of choice for *Candida* infections are azoles. However, emerging resistance, decreasing susceptibility and a national cost of nosocomial candidemia of over $200 million per year [1] all increase the need for new antifungals. For this purpose we developed a new process for the synthesis of antimicrobials using fungal laccase as biocatalyst. Laccases [E.C. 1.10.3.2] are polyphenoloxidases which

oxidize hydroxylated aromatic compounds. The resulting radicals can undergo various non-enzymatic reactions. A heteromolecular coupling reaction was used successfully for the derivatization of morpholines [2] and some of the products had antimicrobial and cytotoxic activities which were higher than the activities of the reactants. We therefore then applied the process to the synthesis of new azoles.

Methods: The laccase was obtained from the ligninolytic fungus *Pycnoporus cinnabarinus* (Pcl). The reaction mixtures were analyzed using an HPLC system with DAD and a RP18 column. For mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy the products were isolated by solid phase extraction and dried by lyophilization. The antifungal susceptibility testing was performed with a modified method of the E.Dis. 7.1 [2].

Results: 1-Aminobenzotriazole (1) was used as model substance for the derivatization by Pcl. During laccase-catalyzed transformation of the para-dihydroxylated substrates 2,5-dihydroxybenzoic acid methyl ester (2a) and 2,5-dihydroxybenzoic acid ethyl ester (2b) into quinones a nucleophilic attack of the azole occurs, resulting in C-N-coupled heteromolecular products 3a and 3b respectively (yields up to 34%). Their structures were confirmed by MS and NMR analysis. The antifungal activity of 3a,b against *Candida* species exceeded that of the reactants (Table 1).

Table 1. Results of the antifungal screening of MIC determination by a modified method according to the EUCAST discussion document E.Dis. 7.1 (test concentration: maximum 1000 µM, test organism: *Candida maltosa*) for products **3a,b** and reactants **1**, **2a,b**

	MIC		MIC_{50}	
	[µg/ml]	[µM]	[µg/ml]	[µM]
1	>134.14[a]	>1000		
2a	>168.15	>1000		
2b	>182.17	>1000	182.17	1000
3a	>298.26	>1000	237.09	794.91
3b	>312.29	>1000	78.79	252.30

[a]The MIC was higher than the tested concentration of 1000 µM.

Conclusions: This novel synthesis principle makes it possible to produce compounds with antimicrobial activity. Our future aim is to amplify this effect by laccase-catalyzed derivatization of compounds which already show high biological activity to synthesize products with increased activity, improved bioavailability and reduced side effects. In particular the laccase-catalyzed synthesis of substances which are not available by conventional chemical methods permits the creation of completely new biological active agents.

Reference(s)

[1] Rentz AM et al. Clin Infect Dis 1998; 27: 781–788.
[2] Hahn V et al. Biotechnol Appl Biochem 2009; DOI: 10.1042/BA20090219.

R2182 *In vitro* antimicrobial activity of α-melanocyte stimulating hormone and its mechanism of action against potent human pathogen *Candida albicans*

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Objectives: To establish α-melanocyte stimulating hormone (α-MSH) as a novel agent for control of *Candida albicans* infection.

Methods: We examined *in vitro* antimicrobial activity of α-MSH by varying several parameters, viz., fungal cell densities, pH and ionic composition against *C. albicans* strain CAF2–1. Antifungal activity of α-MSH was also examined on its pathogenic (hyphal) form. To understand the mechanism of action of α-MSH on *C. albicans* cells we perform membrane permeabilization and ATP efflux assays.

Result: Our results shows that α-MSH possesses significant and rapid antifungal activity against both pathogenic (hyphal) and non-pathogenic (yeast) form of *C. albicans* (12µM α-MSH exhibits 80% killing in yeast and 68% in hyphal form for 2 hr). Antifungal activity of α-MSH was dependent on fungal cell density and killing of *C. albicans* is ion selective. pH change from 7.4 to 4 increased candidacidal activity of α-MSH by 19%. We also found that when α-MSH added to *C. albicans* under hyphal conditions, the hyphal formation was inhibited. From membrane permeabilization assays we found that there is no significant membrane permeabilization observed on incubation with α-MSH, suggesting that candidacidal activity was not through membrane damage. ATP efflux assay reveals that on incubation of *C. albicans* with lethal dose of α-MSH, there was considerable amount of ATP efflux. Furthermore, in presence of energy metabolism inhibitors such as azide and carbonyl cyanide m-chlorophenylhydrazone; there was substantial decrease in the ATP efflux.

Conclusion: These observations suggest that candidacidal activity of α-MSH may mediate through energy depletion and not through membrane damage. Thus we concluded that α-MSH emerges as excellent therapeutic agent against potent human pathogen *C. albicans*.

R2183 Antibiotic susceptibility including tigecycline and MALDI-TOF MS of *E. coli* and *S. aureus* isolates

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Objectives:

1. To test tigecycline against ESBL producing E coli and MRSA isolates and compare results to those of the E-test programme
2. To carry out antibiotic susceptibility to commonly used antibiotics using the Vitek 2 analyser
3. To carry out MALDI-TOF-MS on the isolates and evaluate its potential in discriminating organisms

Methods: A total of 50 MSSA, 50 MRSA, 50 ESBL-producing *E. coli* and 20 non-ESBL producing *E. coli* isolates were obtained from Northampton hospital and county, from various specimen types. Presumptive identification of isolates was on chromogenic agar (Oxoid). Confirmation of MRSA and *E. coli* isolates identification and assessment of antimicrobial susceptibility was by Vitek 2 analysis. Tigecycline E-test was carried out on confirmed ESBL *E. coli* and MRSA isolates according to manufacturer's instructions (AB Biodisk, Solna, Sweden). For MALDI analysis, organisms were grown on Columbia blood agar plates with 5% horse blood overnight. Direct inoculation technique was carried out by emulsifying a single isolated colony using a 5ml loop onto a MALDI target well. This was left to dry before addition of 0.5 ml of CHCA matrix. MALDI-TOF-MS analysis was carried out using 95% laser power, a maximum of 120 profiles per minute and 5 shots per profile.

Results: MSSA was sensitive to most of the antibiotics, with reduced susceptibility to penicillin. MRSA isolates showed increased resistance to antibiotics e.g. ciprofloxacin but were sensitive to vancomycin, teicoplanin and linezolid. ESBL producing-*E. coli* isolates were 100% sensitive to meropenem and ertapenem, and 97% sensitive to piperacillin/tazobactam. Tigecycline exhibited 100% potency against 50 MRSA and 50 ESBL producing *E. coli* isolates with mic values of less than 1 mg/l. Tigecycline results were comparable to those of the E-test programme, e.g. Spain and Canada.

The MALDI fingerprints derived from 120 isolates produced rich peaks with high reproducibility. Hierarchical cluster analysis categorized the MALDI spectra precisely into groups according to the antibiotic sensitivity patterns of *S. aureus* and *E. coli* isolates as well as β-lactamase production.

Conclusion: This study highlighted the problem of antibiotic resistance and potency of tigecycline against resistant isolates. Furthermore, MALDI-TOF-MS was shown as a rapid method for identification of bacteria at species and subspecies level and useful for characterization according to antibiotic susceptibility pattern.

Epidemiology of MRSA, VRE and other Gram-positives

R2184 Detection and characterization of methicillin-resistant *Staphylococcus pseudintermedius* in healthy dogs in Spain

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Objectives: To identify and characterize methicillin-resistant coagulase-positive staphylococci (CPS) recovered from healthy dogs in Spain.
Methods: 160 samples from external nares of healthy dogs were obtained in La Rioja (Spain) in March-August 2009. Following enrichment in nutrient broth with 6.5% NaCl, samples were cultured on ORSAB plates (OXOID) for methicillin-resistant CPS recovery (one/sample). Susceptibility for 17 antibiotics was tested by disk-diffusion agar method. Molecular identification of the isolates was performed by amplification of nuc gene of *S. intermedius*. Discrimination between *S. pseudintermedius* and *S. intermedius* was done by PCR-RFLP (pta gene digested with either MboI or AluI), being identification confirmed by sequencing of hsp60 or sodA genes. All methicillin-resistant isolates were tested for mecA gene by PCR. SCCmec typing was performed by PCR. The presence of 27 resistance genes was tested by PCR. Leukotoxin and exfoliative toxin genes for *S. intermedius* (lukS-I/lukF-I and siet, respectively), as well as lukS/lukF-PV, tsst1, eta, etb, hla, hlb, hld, hlg and hlg-2 genes were investigated by PCR.
Results: 9 methicillin-resistant *Staphylococcus pseudintermedius* (MRSP were isolated from the 160 studied samples (5.6%), being the only methicillin-resistant CPS species recovered. Comparison of restriction patterns using MboI versus AluI showed the same species identification in all tested isolates. They were typed as SCCmecV (1 isolate), SCCmec III (7 isolates) or non-typable (1 isolate, although the ccrC gene was detected). A multiresistant phenotype (at least 5 families of antibiotics) and the following resistant genes were detected (number of isolates): mecA (9), blaZ (9), ermB (9), tet(K) (7), tet(M) (2), aph(3′) (9), aac(6′)-aph(2′) (9), ant(6′) (9), str (9), dfrA (9), dfrD (2), dfrG (9), dfrK (9), cat(pC221) (1), being ermA, ermC, mph(C), mrsA, msrB, linA′, vga(O), tet(L), tet(O), ant(4′), ant(3'), fexA and cfr genes negative for all MRSP. All isolates harboured the exfoliative toxin gene siet and both leukocidin genes LukS-I and LukF-I, but not the Panton-Valentine leukocidin genes lukS/lukF. Toxin hlg-2 was detected in two MRSP. PCR for other toxin genes were negative.
Conclusions: MRSP is a relatively common colonizer of healthy dogs, frequently harbouring a high number of antibiotic resistance genes and some virulence genes what represents public health and treatment concerns. Siet toxic gene was presented in all the isolates and its role in disease requires further study.

R2185 The risk factors of methicillin-resistant *Staphylococcus aureus* infection in patients with bacterial spondylitis accompaning bacteraemia

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Background: Recently, the incidence of methicillin-resistant *Staphylococcus aureus* (MRSA) infection of spine has been reported to be increasing, but risk factors of MRSA spondylitis have not been studied well yet.
Methods: Medical records of patients with bacterial spondylitis accompaning bacteremia treated in tertiary care hospitals from 2004 to 2008 were reviewed retrospectively. All patients had radiological evidence of the spondylitis including vertebral osteomyelitis, spinal epidural abscess, paravertebral abscess, and discitis. Patients with spinal postoperative infection were excluded. Clinical and microbiological variables were analyzed as the predisposing factors of MRSA infection by univariate analysis with 95% confidence interval (P = 0.05).
Results: Of 463 patients with bacterial spondylitis, there were 46 patients (10%) found with bacteremia. The mean patient age was 64.8 (range, 40–87, male-to-female ratio, 2.3:1). 28 cases (61%) were caused by Staphylcoccus aureus, 11 of them were methicillin-resistant. Age, diabetes mellitus, hemodialysis, alcoholism, soft-tissue infection, previous antibiotics use, and a history of hospitalization were not correlated with MRSA infection. However, vertebral osteomyelitis was more frequently accompanied by MRSA bacteremia among the spondylitis entities with statistical significance (P = 0.037).
Conclusions: There was no significant risk factor related with MRSA spondylitis accompaning bacteremia. However, vertebral osteomyelitis was more frequent in spondylitis with MRSA bacteremia and this finding might contribute to appropriate empirical antibiotics selection in pyogenic spondylitis.

Table. Demographic characteristics of patients

Characteristic	Total (n = 46)	MRSA bacteremia (n = 11)	Other bacteremia (n = 35)	P-value
Mean age (mean±SD)	64.8±10.7	63.4±9.6	65.3±11.1	
Male:Female	32:14	8:3	24:11	
Comorbidities				
Diabetes mellitus	16	5	11	0.40
Chronic renal disease	8	1	7	0.38
Chronic lung disease	2	0	2	–
Cardiac disease	7	1	6	0.50
Chronic liver disease	6	0	6	–
Neurologic disease	2	0	2	–
Malignancy	4	0	4	–
Rheumatic disease	3	1	2	0.70
Charlson comorbidity index (mean±SD)	1.48±1.82			
Infected site of spine				0.49
Cervical	5	1	4	0.83
Thoracic	4	2	2	0.23
Lumbar	37	8	29	0.47
No. of levels involved in spine				0.89
1–2	37	9	28	
Above 2	9	2	7	
Classification of spondylitis				
Vertebral osteomyelitis	4	3	1	0.04
Paraspinal abscess	19	5	14	0.75
Spinal epidural abscess	12	3	9	0.92
discitis	36	8	28	0.62

R2186 Genetic characterization of methicillin-resistant *Staphylococcus aureus* isolated at a university hospital in Casablanca, Morocco: spread of a single multidrug-resistant clone

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Background: Methicillin resistant *Staphylococcus aureus* (MRSA) is often involved in the Hospital Aquirred infections in Morocco. At the Ibn Rochd University Hospital, the prevalence of MRSA is high (17.6%).
Objectives: To determine the genetic and toxin profiles and the antibiotic susceptibility of MRSA strains isolated at the Ibn Rochd University Hospital.
Materials and Methods: A prospective study was carried out between January and July 2007. Sixty three MRSA were isolated from various samples of inpatients at the university hospital. The antibiotic susceptibility of each isolate was determined by the disc diffusion method. The agr systems as well as the Stahylococcal Cassette Chromosome mecA (SCCmec, mediating-methicillin resistance), were typed by multiplex PCR. The isolates were also screened for toxins genes. The clonal relationship between the isolates was determined by Multilocus Sequence Typing (MLST).
Results: The MRSA strains were mainly isolated from nasal swabs (55.5%) and from infected skin and soft-tissue (34.9%). All MRSA isolates showed a multidrug resistance phenotype. The prevalence of antibiotic resistance was high: Pefloxacine (96.8%), Aminosides (Kanamycine, Tobramycine, Gentamycine) and Minocycline (93.6%), Rifampicine (92.1%), Cotrimoxazol (65.1%) and Erythromycine (61.9%).
The molecular typing revealed that 61 isolates had the agr 1 allele, and were SCCmec III or SCCmec III/mercury. All of them had either the Sequence Type 241 or 239 (ST241 being a single-locus variant of ST239), and showed the same profile than Hungarian and Brazilian clones. All MRSA isolates had the staphylococcal enterotoxin K (sek) and the staphylococcal enterotoxin like Q (selq). No PVL toxin was detected among these isolates. For 2 isolates the genetic profile was: agr

2, SCCmec IV and ST 5. These isolates harboured the gene encoding for PVL toxins.
Conclusions: The high prevalence of MRSA at the Ibn Rochd University Hospital is related to the spread of a multidrug resistant clone, which is genetically similar to the Hungarian/Brazilian clones. This multidrug resistance of MRSA clones has some consequences on the choice of an effective antibiotherapy for the treatment of MRSA infections.

R2187 Phenotypic and molecular characterization of methicillin-resistant *Staphylococcus aureus* isolates in a northern region of Italy

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Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a significant problem in healthcare settings around the world. The aim of this work was to study the molecular epidemiology of MRSA isolated from clinical samples in a region of northern Italy (Emilia Romagna).
Methods: We studied a total of 107 MRSA strains collected in a two month period (February-March 2009) from 13 hospital laboratories in Emilia Romagna. The sources of isolates were: blood (25 strains), pus/exudate (37 strains), tracheoaspirate (23 strains), bronchoalveolar lavage (15 strains), sputum (7 strains). Antibiotic susceptibility was determined by automated systems and E-test. Different PCR strategies were applied to confirm the species *S. aureus* and the methicillin-resistance status, to determine spa type, SCCmec type and to detect Panton Valentine Leukocidin (PVL) encoding genes. MLST was performed on selected strains.
Results: By spa typing, the majority of MRSA were assigned to t008 (34%) and t041 (25%). Among blood isolates, 9 strains were assigned to t008 and contained SCCmec type IV; 7 were characterized by t041 and SCCmec type I; 9 strains were assigned to 9 different spa types; no strains harboured PVL toxin genes. Of 82 strains isolated from pus/exudate and respiratory tract, 28 were assigned to t008 and harboured SCCmec type IV; 20 were characterized by t041 and contained SCCmec type I; 34 strains were assigned to 21 different spa type and harboured SCCmec types I, II and IV. One strain, isolated from an exudate, assigned to t044, harboured SCCmec type IV and was PVL positive. t008 isolates were more susceptible than those of t041; major differences between t008 and t041 regarded erythromycin (59% vs 89%), gentamycin (40% vs 89%) and rifampicin (3% vs 18%). Clindamycin resistance was inducible in t008 (57% of strains) while in t041 was constitutive (89% of strains).
Conclusions: In this study, t041 which was the most common and well-established "old" spa type in the Italian hospitals has been outnumbered by the "new" t008. t008 strains are less resistant to antibiotics than t041. Although t008 isolates resemble the prototype CA-MRSA USA300 they differ from the latter for the lack of the PVL genes and for being multi-drug resistant. Infact, these t008 isolates belong to a well-adapted hospital clone recently emerged in Europe. The only PVL positive strain of this study belonged to the CA-MRSA European clone ST80, confirming that CA-MRSA are infrequent in Italy.

R2188 Evaluation of the risk factors associated with community-acquired *Staphylococcus aureus* infections

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Objectives: The epidemiology of *S. aureus* infections has changed worldwide and MRSA is now frequently found as a community associated (CA) pathogen. The aim of this study was to assess the risks for CA-*S. aureus* infections.
Methods: 150 outpatient wound samples from skin and soft tissue infections were studied. The patients were classified as CA and hospital acquired (HA) for MRSA infection by CDC criteria. Methicillin resistance was determined by disk diffusion (DD) method with oxacillin and cefoxitin disks and confirmed with oxacillin salt screen agar test according to the CLSI guideline. The mecA gene was detected by PCR. Categorical data were compared by the chi-square test or Fisher's exact test, using SPSS 15.0. All hypotheses were two-tailed and were considered significant at the $P < 0.05$ level.
Results: 92 samples were identified as *S. aureus*. mecA gene was detected in 11 isolates. 13 isolates were identified as methicillin resistant with oxacillin, cefoxitin DD tests and oxacillin salt screen agar test. The most prevalent lesion and underlying disease was abscess and diabetes mellitus. 70 patients had risk factors associated with MRSA infection. According to CDC case description five isolates out of 11 were true CA-MRSA strains. As for the potential risk factors; variables associated with evidence of MRSA were hospitalization (4.69; 0.98–22.4), surgery (8.0; 0.46–138.1), hospitalization of close relatives (3.95; 0.328–47.593), recurrent skin infections (17.78; 1.463–216.017) ($p \leqslant 0.05$). The significant risk factor for the acquisition of CA-MRSA was (17.78; 1.46–216.01)($p = 0.037$) among hospital attendants.
Conclusion: This study showed that combination of both phenotypic and genotypic tests must be performed for methicillin resistance detection. Although CA-MRSA is not a critical problem in Turkey, it is crucial to screen outpatient clinics to prevent the distribution of antibiotic resistance.

R2189 Nosocomial bloodstream infections by methicillin-resistant (MRSA) and methicillin-sensitive *Staphylococcus aureus*: further exploring the role of prior antibiotic usage as a predictor of MRSA bacteraemic infection

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Objectives: Both total antimicrobial use as well as specific antimicrobial classes have been implicated as risk factors for nosocomially-acquired MRSA (NA-MRSA) infection and bacteremia. The aims of the study were: 1. to explore predictors of a new NA-MRSA bloodstream infections (BSI) in comparison with a new nosocomially-acquired methicillin-sensitive *Staphylococcus aureus* (MSSA) BSI, 2. to thoroughly assess recent antibiotic use (within the last 30 days of the positive culture date) qualitatively and quantitatively.
Methods: The time-period for our study was from October 1997 through September 2001. From the infection control records, we identified two groups of inpatients, one with a new positive MRSA blood culture and one with a new positive MSSA blood culture. We considered the BSI acquired in-hospital if 1) the culture was taken more than 48 hours after admission and 2) if it was taken less than 48 hours but the patient had been hospitalized within the last month. We recorded data pertinent to widely accepted risk factors for *Staphylococcus aureus* colonization and infection considering events up to 30 days before the positive culture date. We used the electronic pharmacy records to obtain detailed data on intravenous antibiotic use during the month before the culture date.
Results: We identified 28 patients with a new NA-MRSA BSI and 32 patients with a new NA-MSSA BSI eligible for further analysis. In univariate analysis, significant differences were noted in age, nursing home residency, presence of chronic wounds, rates of hemodialysis, intubation, enteral feeding receipt and presence of indwelling urinary catheter for more than 24 hours, receipt of at least 1, 2 or 3 antibiotics, qualitative and quantitative use of penicillins, β-lactams and antibiotics as a whole, qualitative use of aminoglycosides and fluoroquinolones, and quantitative use of cephalosporins, clindaycin and β-lactam/β-lactamase inhibitors. In 2 models of multivariate analysis, including either quantitative or qualitative use of total antibiotics or individual classes, the only independent predictor of NA-MRSA BSI was the prior receipt of at least 3 antibiotics. No siginificant differences in outcome were noted.
Conclusion: From the comparative analysis of these strictly defined patient groups deriving from a highly homogeneous population, we conclude that prior receipt of at least 3 antibiotics was the strongest predictor of a subsequent NA-MRSA BSI, more than individual antibiotic usage or other traditional risk factors for NA-MRSA infection.

Epidemiology of MDR-Gram-negatives

R2190 Colistin-resistant isolates of *Klebsiella pneumoniae*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* emerging in the ICU of a Greek hospital

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Objectives: The isolation of multidrug-resistant (MDR) Gram-negative pathogens has been increasingly described worldwide. The aim of the study is the retrospective analysis of colistin resistance of Gram-negative bacteria in our ICU, during 3 years.
Methods: Between January 2007 and October 2009, 326 strains of *Klebsiella pneumoniae*, 972 *Acinetobacter baumannii* and 650 *Pseudomonas aeruginosa* were recovered from an equal number of samples in our laboratory. Identification and MIC determination were performed by automated identification system (VITEK II). Colistin MICs were evaluated using the E-test methodology (AB Biodisk, Solna, Sweden). Resistance to colistin was defined as MIC > 2 mg/L for *Klebsiella pneumoniae* (EUCAST breakpoints) and *Pseudomonas aeruginosa*, *Acinetobacter baumannii* (CLSI breakpoints).
Results: Thirty-nine strains of *Klebsiella pneumoniae* (12%), 3 *Acinetobacter baumannii* (0.3%) and 10 (1.5%) *Pseudomonas aeruginosa* were found resistant to colistin. MDR Gram-negative pathogens were isolated from the following clinical specimens: bronchial secretions (25), blood cultures (12), catheter tips (18) and CSF(2). Colistin MICs for *Klebsiella pneumoniae* ranged between >2–16 mg/L, for *Acinetobacter baumannii* >2–>256 mg/L and for *Pseudomonas aeruginosa* >2–16 mg/L. All strains of *Klebsiella pneumoniae* were susceptible to tigecycline, while resistance to meropenem was 82%, gentamicin 18%, amikacin 64% and ciprofloxacin 84%. All strains of *Acinetobacter baumannii* were susceptible to doxycycline and minocycline. Resistance to meropenem was 92%, ampicillin/sulbactam 31%, gentamicin 7.5%, amikacin 7.5% and tigecycline 54%. Resistance of *Pseudomonas aeruginosa* to meropenem was 50%, piperacillin/tazobactam 50%, ceftazidime 80%, aztreonam 70%, amikacin 40% and gentamicin 100%.
Conclusions:
1. In our ICU setting MDR Gram-negative pathogens are increasingly isolated, especially *K. pneumoniae,* with 12% resistance to colistin which remained susceptible to tigecycline.
2. Therefore the development of new antimicrobials against MDRs, the appropriate use of colistin and the strict implementation of hand hygiene rules in ICU, are mandatory.

R2191 Emergence of KPC-2 carbapenemase-producing *Klebsiella pneumoniae* strains and spread of an isolate of sequence type 258 in the neuro-rehabilitation unit of an Italian hospital

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Objectives: To investigate the molecular nature of the first mini-outbreak caused by multidrug-resistant (MDR) *Klebsiella pneumoniae* producing class A carbapenemase, occurring in the neuro-rehabilitation unit of a tertiary care hospital in Liguria, a northern region of Italy.
Methods: Carbapenemase production was screened by a modified Hodge test together with an imipenem-EDTA disc synergy test and confirmed by PCR and sequencing. PFGE and MLST were used to study the genetic relatedness of the strains and epidemiological comparisons.
Results: Five cases of infections caused by MDR KPC-2 carbapenemase- and SHV-5 extended-spectrum β-lactamase-producing *K. pneumoniae* strains were identified. All isolates were intermediately susceptible to imipenem, resistant to all the other β-lactams antibiotics, ciprofloxacin, co-trimoxazole and were susceptible only to gentamicin, tigecycline and colistin. PFGE and MLST showed that four out of five isolates were clonally related and belonged to the international hyperepidemic clonal group of sequence type 258.
Conclusion: This is the first report on dissemination of KPC-2-producing *K. pneumoniae* clinical isolates in an Italian hospital.

Antibiotic usage

R2192 Reduction of bacterial resistance by 15-th month replacement of third-generation cephalosporins with fourth-generation cephalosporin

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The 3rd-generation cephalosporins have been used in the treatment of broad range of infections in 1000-bed hospital of Cancer Research Center of Russia for more than 10 years.
Aim: To evaluate the influence of replacement 3rd-generation cephalosporins (ceftazidime and cefotaxime) with cefepime on bacterial susceptibility.
Materials and Methods: We replaced ceftazidime (Cfz) and cefotaxime with cefepime (Cfp) from July 2008 till now (October 2009) and compared the susceptibility of *Escherichia coli* (209 strains), *Klebsiella pneumoniae* (123 strains) and *Pseudomonas aeruginosa* (222 strains) to Ctz and Cfp in the first half of 2008 (Jan-June 2008, the 1st period of time) and the second and third quarters of 2009 (April–September 2009, the 2nd period of time). Identification and susceptibility testing has been performed with VITEK-2 system (bioMérieux, France).
Results: The susceptibility of *E. coli* to Cfp and Ctz was the same: 32% of strains were susceptible to both antimicrobials (24/75 and 25/75 strains) in the 1st period of time and percentage of susceptible strains increased to 64 and 66% (86/134 and 88/134 strains) in the 2nd period of time, $p < 0.0001$. The same tendency was noted in *K. pneumoniae* and *P. aeruginosa*. The percentage of *K. pneumoniae* strains susceptible to Cfp and Ctz increased from 28% (18/65 strains for both) to 46% (27/58strains for both), $p < 0.002$. The percentage of *P. aeruginosa* strains susceptible to Cfp and Ctz increased from 40% and 49% (36/90 and 44/90 strains) to 77% and 66% (101/132 and 87/132 strains), $p < 0.005$. Between the 1st and the 2nd periods of time the susceptibility of all strains analysed was similar to that one in the 1st period of time.
Conclusion: Replacement of 3rd generation cephalosporins to 4th generation cephalosporin resumed susceptibility to this antibiotics in the most problematic pathogens – *E. coli*, *K. pneumoniae* and *P. aeruginosa* in 6–9 month after removal of 3rd generation cefalosporins (ceftazidime and cefotaxime) from hospital utilization, that can lead to reduction in carbapenem utilization and financial benefit.

R2193 A better documentation of the reassessment of antibiotic therapies does not improve the quality of prescription

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Objectives: To assess the impact of an intervention designed to improve the documentation of the reassessment of inpatient empiric antibiotic prescriptions on the quality of these prescriptions.
Methods: A prospective before and after intervention 7-month study on two wards (Internal Medicine and Medical Intensive Care Unit (ICU)) in one French University Hospital. The intervention consisted of a quality improvement project led by one doctor on each ward, aiming at improving the documentation of four process measures in the medical records: antibiotic plan, review of the diagnosis, adaptation to microbiological results and iv-po switch.
Results: 171 antibiotic prescriptions were assessed, 57 on the Internal Medicine ward and 114 in the ICU, 90 before and 81 after the intervention. The reassessment of antibiotic prescriptions was more often documented in the ICU after the intervention (58% vs 79%, $P = 0.03$), but not on the Internal Medicine ward (32% vs 45%, $P = 0.48$). The prevalence of appropriate antibiotic prescriptions was not statistically different on the two wards before and after the intervention (25% vs 31%, $P = 0.83$ and 44% vs 38%, $P = 0.72$ respectively).
Conclusions: A better documentation of the reassessment of antibiotic prescriptions was achieved on one ward, but it did not lead to a better quality of antibiotic prescriptions.

R2194 Inaccuracies in dosing drugs with teaspoons–tablespoons

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Objective: We aimed to evaluate the potential inaccuracies in administering the desired dose of drugs with teaspoons and tablespoons.
Methods: We collected all the different teaspoons/tablespoons that were available in 25 households in the area of Attica, Greece and measured their volume capacity (ml).
Results: A total of 71 teaspoons and 49 tablespoons were provided from the 25 female (mean age 48.0 years) study participants. When these utensils were filled with water, the volume capacity of the 71 teaspoons ranged from 2.5 to 7.3 ml; mean volume was 4.4 ml; median was 4.4 ml. When the standardized teaspoon was used, the volume ranged from 3.9 to 4.9 ml among the total of the 25 study participants. When a subset of 5 study participants filled this teaspoon with paracetamol syrup, mean volume was 4.8ml.
Conclusions: Teaspoons and tablespoons are unreliable dosing devices and thus their use should no longer be recommended.

R2195 A novel approach to antimicrobial stewardship programme: smart computerized decision support system

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Objectives: Our 1200-bed hospital has high rates of MRSA, ESBL *Escherichia coli* and *Klebsiella*, and multidrug-resistant *Acinetobacter*, with very high ceftriaxone and fluoroquinolone usage, and rising carbapenem and piperacillin–tazobactam usage. Daily antibiotic orders averaged 218 over 9 weeks, making manual review and feedback laborious.
Methods: Our hospital has comprehensive information technology (IT) systems including electronic inpatient medication record (eIMR). We designed ARUS_C integrated in doctors' work process to guide them in antibiotic use by adopting evidence-based medicine and real-time IT data.
Results: Doctors launch ARUS_C from eIMR to select empiric, definitive or prophylactic antibiotic, and infectious disease (ID) condition to treat. By entering data unavailable in IT systems, doctors can view antibiotic recommendation including renal dose adjustment, duration of therapy, allergy, antibiotic toxicity and monitoring, therapeutic duplication, and antibiotic and microbiological data summary. ARUS_C checks for healthcare-associated infection, prior antibiotic-resistant bacteria, and illness severity influencing antibiotic selection. It provides clues to diagnosis, investigation and referral for selected ID condition, and interpretation of positive microbiological cultures. From empiric to definitive antibiotic use, ARUS_C provides guidance to treat culture-positive infections using narrow-spectrum culture-guided antibiotic, step-down therapy in culture-negative infections with improvement, and recommend referral and further investigation in non-improving culture-negative infections. It advises antibiotic for multiple bacteria, including route and duration, based on ID condition, culture site and clinical response. Doctors are able to over-ride ARUS_C. Usage of ARUS_C rose from 76 episodes in week one to 216–295 from weeks 4–9, with over-rides ranging from 8–40 per week. Daily usage ranges from 30–36 for week days, and 24–25 for weekends. Screen shots of ARUS_C in action, and data on efficacy and safety will be presented.
Conclusions: Voluntary ARUS_C use was hampered by IT errors, and doctors' mindset. Mandatory ARUS_C use may be needed to achieve significant reduction in overall and broad-spectrum antibiotic use. Further evaluation of ARUS_C is under way.

R2196 Comparison of antibiotic prescription behaviour between general practitioners and specialists: data from the European Surveillance of Antimicrobial Consumption (ESAC) Nursing Homes subproject

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Objectives: To define in European nursing homes (NHs) who prescribes antibiotics (ABs), and to define differences between prescribers related to the indication, type, and route of administration.
Methods: A survey, including a point prevalence study, on AB use, characteristics of residents and characteristics of the NH was conducted in European NHs in 2009.
Results: Results from 270 NHs in 16 European countries, with 1740 NH residents receiving ABs, were available. Results showed that 76% of the ABs in NHs was prescribed by the general practitioner (GP), 19% by a specialist and 5% by another prescriber ($n = 1749$ substances). The majority of prescribed indications ($n = 1649$) for ABs by both GPs and specialists were empirical treatments (53% and 44%, respectively). Specialists prescribed significantly more frequently ABs for microbiologically documented infections (25%) than GPs (14%, $p < 0.0001$). Results on all AB treatments ($n = 1666$) showed that GPs prescribed significantly more frequently extended-spectrum β-lactam penicillins (J01CA, 11%) than specialists (6%, $p = 0.0027$). For GPs, 72% of the J01CA prescriptions were amoxicillin (J01CA04), compared to 53% of prescriptions by specialists. Combinations of penicillins and β-lactamase inhibitors (J01CR) were prescribed in similar amounts by GPs and specialists (14% and 15% respectively, $p = 0.68$). This was also the case for the prescription of quinolones (J01M) (14% by both, $p = 0.94$). Specialists prescribed cephalosporins (J01D) slightly more often than GPs (12% and 9%, respectively, $p = 0.063$). Finally, GPs and specialists prescribed other antimicrobials (J01X) in almost equal proportion (28% and 26%, respectively, $p = 0.45$). GPs opted in 94% of the cases for oral ABs. Oral administration was also the predominant route of administration (80%) for specialists, although they combined this with a substantial proportion of parenteral AB administration (20%) causing a significant difference between GPs and specialists ($p < 0.001$).
Conclusion: Compared to specialists, GPs prescribed comparable classes of ABs in NHs, with the exception of extended-spectrum β-lactam agents (e.g. amoxicillin), probably because GPs prescribed more empirical ABs. Specialists prescribed more frequently parenteral AB administration compared to GPs.

R2197 Detection of an antimicrobial residue in Irish hospital effluent using high-performance liquid chromatography with tandem mass spectrometry

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Background: The presence of antimicrobial compounds in the environment may exert selective pressure on bacteria and contribute to the emergence and dissemination of antimicrobial resistance. Antimicrobial agents are used extensively in hospitals and previous reports of both the parent drug and related metabolites in hospital effluent make it a significant point source of pharmaceuticals to the environment. The aim of this research was to examine hospital effluent for two such antimicrobials, ciprofloxacin and trimethoprim.
Methods: Effluent samples were collected from Hospital A sewers; municipal sewers at points upstream and downstream of hospital effluent discharge; intake effluent (untreated, stage 0) to secondary waste water treatment plant; primary treated effluent (stage 1); post return effluent (stage 2) and final treated effluent (stage 3). Effluent was filtered using glass-fiber filters, acidified to pH 2.5 and pre-concentrated using solid phase extraction on a mixed-mode reversed phase-strong cation exchange sorbent before analysis by liquid chromatography with both single and tandem mass spectrometry (using secondary product ion scan mode).

Results: Ciprofloxacin (parent m/z = 332; 2o ions m/z = 228 and 314) was not detected in any effluent sample indicating either rapid degradation, sorption to solid material ($\log P \approx 2.3$) or insufficient sensitivity of the method. Trimethoprim (parent m/z = 291; 2o ion m/z = 123) however was detected in effluent from Hospital A only, with both parent and fragment ions detected. The concentration of trimethoprim in Hospital A effluent was estimated at ~270 ng/L.
Conclusions: Trimethoprim can be detected at sub-inhibitory concentrations in hospital effluent samples in Ireland. The implications of this finding for the emergence and spread of antimicrobial resistance merit further investigation and it may be appropriate to consider measures to mitigate discharge of antimicrobial substances from hospitals.

R2198 Evaluation of culture results and empirical antimicrobial therapy in the Surviving Sepsis Campaign

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Objectives: The international Surviving Sepsis Campaign (SSC) is a triage system to identify patients with sepsis and ensure rapid initiation of empirical antimicrobial therapy. Besides diagnostic purposes, cultures for microbiology should be collected to enable de-escalation of broad spectrum therapy. We evaluated the culture collection and compliance to local antimicrobial guidelines after implementation of the SSC. *In vitro* susceptibility of isolated pathogens to recommended therapy and prescribed therapy was evaluated.
Methods: All adult patients diagnosed with sepsis at the emergency department (ED) of the Radboud University Nijmegen Medical Centre between November 1, 2006 and May 10, 2007 were included. Electronic medical charts were reviewed to collect the clinical diagnosis at the ED and the prescribed therapy. Culture collection, culture results and susceptibility results were retrieved from the laboratory information system. Culture results were evaluated for clinical significance. Empirical therapy was classified as according to guideline therapy (AGT) or not according to guideline therapy (NGT).

Table 1: Patient demographics, clinical diagnosis, numbers of positive cultures, AGT and microbiological diagnosis

	Meningitis (n = 7)	Biliary (n = 8)	Skin (n = 15)	Neutropenic sepsis (n = 15)	Miscellaneous (n = 29)	Urinary (n = 38)	SUO (n = 21)	Pulmonary (n = 139)	>1 susp. SOI (n = 46)
Mean age (y)	59	58	64	54	54	61	64	58	61
Sex (% male)	71	88	67	60	79	68	43	63	52
Mortality (%)	14	0	0	7	3	0	10	6	13
AGT (%)	57	38	40	80	52	97	62	38	30
Pos BC/BC obt	2/7	4/8	3/13	3/15	3/28	9/36	6/21	15/126	10/43
Pos UC/UC obt	0/3	0/6	0/6	1/8	2/14	28/33	4/17	1/62	13/39
Pos SC/SC obt	1/1			1/2	1/1		0/2	18/34	1/4
Pos LC/LC obt	4/6						0/4		0/1
Pos OC/OC obt			2/4	2/2	5/7				1/1
Microbiological diagnosis	57%	50%	33%	33%	34%	76%	38%	22%	41%

SUO: sepsis of unknown origin, SOI: source of infection, AGT: according to guideline therapy, BC: blood culture, UC: urine culture, SC: sputum culture, LC: liquor cerebrospinalis culture. OC: other culture, Pos: positive, Obt: obtained.

Results: There were 8570 visits to the ED. Of these, 400 patients (5%) presented with sepsis. In 43 patients no antimicrobial therapy was initiated: 8 went for immediate surgery, 20 were diagnosed with a viral infection and 15 with fever of unknown origin. The ED diagnosis of 21 patients was not defined in the guidelines. Insufficient information was available from another 18 patients.
ED diagnosis, culture collection and results of the 318 patients are shown in table 1. A microbiological diagnosis was established in 115 patients (36%). When blood cultures (BC) were obtained and the suspected site of infection was accessible and cultured, a pathogen was found in 59/81 patients (73%). BC were positive for a different pathogen or the only positive culture in 30 patients (9%). NGT was prescribed in 51% of the patients and was more broad spectrum than AGT in 74%. Isolated pathogens were equally susceptible to NGT (40/45; 89%) and AGT (39/45; 87%).
Conclusion: To establish a microbiological diagnosis in patients with sepsis it is important to collect cultures from the suspected site of infection. Adherence to local antimicrobial guidelines results in effective therapy. A multidisciplinary effort should be made to improve appropriate collection of cultures and compliance with local antimicrobial guidelines to reduce the use of broad spectrum antimicrobials.

R2199 Characteristics of outpatient antibacterial use in Hungary, 1996–2007

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Objectives: To analyse the changes in the amount and structure of antibacterial consumption in the ambulatory care sector in Hungary between 1996 and 2007.
Method: Crude consumption data on systemic antibacterial use (i.e. ATC group J01) were retrieved from a wholesaler database. Antibiotic use was standardized as Defined Daily Dose (DDD) per 1000 inhabitant-days. Trend analysis was used to investigate the trends in the national ambulatory antibiotic utilization through the study period. We also used the ESAC defined quality indicator (ratio of the consumption of broad spectrum penicillins, cephalosporins and macrolides to the consumption of narrow spectrum penicillins, cephalosporins and macrolides: B/N ratio). International comparison was made by the online available European Surveillance of Antimicrobial Consumption (ESAC) database (URL link: http://www.esac.ua.ac.be).
Results: During the study period only minor fluctuations in the national ambulatory antibiotic use could be observed (mean±standard deviation: 18.6±1.5 DDD per 1000 inhabitant-days). Macrolides, fluoroquinolones, penicillin plus β-lactamase combinations and third-generation cephalosporins showed increasing trend in use. The share of narrow spectrums antibiotics (N) decreased from 15.3% to 6.6% and parallel the Broad/Narrow ratio was increased from 2.2 in 1996 to 9.3 in 2007.
Hungarian aggregated national antibiotic use was in the middle range of European countries. The relative use of some antibiotic groups (e.g. second and third generation cephalosporins) was outstanding compared to other European countries.
Conclusion: The quantitative antibiotic use is quite reasonable in Hungary, while some trend of the pattern of use is unfavourable which should be reversed.

Molecular bacteriology

R2200 Multiplex real-time PCR: a practical approach for rapid diagnosis of tuberculous and brucellar vertebral osteomyelitis

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Objectives: To analyze the diagnostic yield of a multiplex real-time PCR in the differential diagnosis of tuberculous vertebral osteomyelitis (TVO) and brucellar vertebral osteomyelitis (BVO).
Methods: Fifteen vertebral samples from patients with TVO or BVO and nine from pyogenic and non-tuberculous mycobacteria VO were studied by multiplex PCR and conventional microbiological techniques. To identify *Brucella* DNA we used a fragment of 207 bp from the gene coding for an immunogenic membrane protein of 31 kDa of *B. abortus* (BCSP31) and for *M. tuberculosis* complex a fragment of 164 bp from SenX3-RegX3 genes. Amplification and melt curve analysis

were performed using a LightCycler 2.0 instrument. To guarantee the reliability of the results, all samples were processed in duplicate. Positive controls were included in all tests and comprised serial dilutions of *B. abortus* B-19 and *M. tuberculosis* DNA; negative controls were also included and contained all the elements of the reaction mixture except template DNA. Universal precautions and one-way flow of DNA extraction and amplification were used to prevent contamination. To avoid potential observer bias, the status of each patient for *Brucella* and *Mycobacterium* infection was unknown during the PCR assay.
Results: Of the 24 vertebral samples included, 5 (20.8%) were percutaneous biopsies and 19 (79.2%) were taken during surgery. The aetiological diagnosis of VO was established prior to vertebral biopsy in just 9 cases (39.1%); 6 from blood cultures (4 patients with PVO and 2 with BVO), one case of TVO detected by baciloscopy and sputum culture and another 2 cases of BVO detected by serological tests. In the other 14 cases (60.9%), the aetiological diagnosis required a bone biopsy. The histopathological findings were inconclusive in 4 of 14 cases (26.6%) with TVO or BVO and cultures were positive in 11 of 15 cases (73.3%). Multiplex PCR correctly identified 14 of the 15 samples from patients with TVO and BVO and was negative in all the control samples. Thus, the overall sensitivity and specificity of the multiplex PCR were 93.3% and 90%, respectively, with an accuracy of 92% (95% CI, 81.4%-100%).
Conclusions: Multiplex real-time PCR is far more sensitive than conventional cultures, and this, together with its speed, makes this technique a very practical approach for the rapid differential diagnosis between TVO and BVO.

R2201 Direct detection of *Mycobacterium tuberculosis* in paucibacillary forms of tuberculosis by real-time PCR

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Objective: The early confirmation of diagnosis in paucibacillary forms of tuberculosis, directly in clinical specimens, by Real Time PCR.
Methods: The results of the conventional methods (Ziehl–Neelsen smear microscopy, Lowenstein-Jensen and MB/BACT cultures, biochemical and cytological parameters) were correlated with detection and quantification of *Mycobacterium tuberculosis*/ MTB specific sequences, by Real Time PCR technique, directly from the clinical specimens. The MasterPure™ Complete DNA and RNA Purification Kit (Epicentre, Madison, Wisconsin) and Total Nucleic Acids Purification Protocol were used. The Primer Design™ Kit (Primer Design 2X Precision™ MasterMix) is designed for the *in vitro* quantification of Tb genomes. The RT PCR method used LightScanner 32 Instrument/LS32 (Idaho Technology, Salt Lake City, UT).
Results: 46 consecutive patients (17 HIV positive and 27 non-HIV) with clinical and imagistic diagnosis of tuberculosis were enrolled in this study, between 1st September and 15 November 2009. The same bacteriologic and molecular analyses were performed on 53 clinical samples: cerebrospinal fluid/23; pleural liquid/13; peritoneal liquid/2, lymphatic node/3; bronco-alveolar aspirate/6, gastric aspirate/2; urine/3; blood/1. As compared to 7.5% positively with demonstration of AFB (Ziehl–Neelsen smear microscopy) and 9.4% to 22.6% mycobacterium positive cultures (Lowenstein-Jensen, MB/BACT, respectively), RT PCR has been found to be positive in 62.2% of specimens from cases with typical features as well as biochemical, cytological, and imagistic evidence of tuberculosis. Using the RT PCR method, we obtained 62.2% true positive results (44 to 869 number of copies); 30.2% true negative results, and 7.5% false negative results. The latter required the repetition of the analyses to clarify the final results. There were no false positive results in the RT PCR. Detection of MTB specific sequences was founded in 13/33 MTB specimens from HIV-positive patients, and 18/33 MTB clinical samples from non-HIV patients.
Conclusions: Real Time PCR can usefully complement standard microbiologic methods for early confirmation of diagnosis in paucibacillary forms of tuberculosis, and contribute to specific management in patients with negative cultures.

R2202 Using real-time PCR to decrease time to appropriate management with staphylococcal blood cultures

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Objectives: Blood cultures (BCs) are key in directing antibiotic therapy, often affecting the sickest patients in hospital. Staphylococci are the most common organism seen in Gram stain. Data from University Hospital Coventry and Warwick, June-August 2008, showed roughly 80% of these are contaminants. At the time of phoning the clinician to discuss an unwell patient, it can be difficult to differentiate a contaminant, a possible methicillin sensitive *Staphylococcus aureus*(MSSA) or methicillin resistant *Staphylococcus aureus*(MRSA). A more rapid test could:
1. Reduce time spent by laboratory staff, microbiologists and clinicians working on and reviewing these results
2. Prevent unnecessary anti-staphylococcal antibiotics commencing
3. Allow earlier institution of optimal antibiotic therapy for MSSA/MRSA

All of this must be in conjunction with infection control teaching about correct technique for taking a BC to reduce the rates of contamination in the first instance.
Methods: A pre-study period for 1 month was observed before 100 sequential BCs were tested by GeneXpert Cepheid. This machine differentiates coagulase negative Staphylococci (CNS), MSSA and MRSA in 1 hour.
For each relevant Gram stain during these periods:
1. Standard questionnaire was filled in: last MRSA screen, current antibiotics
2. Advice given to clinicians based on clinical picture was documented, including changes made prior to PCR or culture results
3. Consultant on duty was blinded from the PCR result
4. PCR results reviewed and changes made to management as appropriate
5. Time to review a single patient, contact clinicians and input data was assessed

Results: See tables.
There were 10 failures with the PCR kit (10%). The cause is not known. 1 mismatch of PCR result to culture results occurred. Repeat PCR concurred with the culture results. Dealing with initial Gram stains took 20minutes/culture on average.
Conclusion: Throughout the study a substantial number of BC were misinterpreted by microbiologists and clinicians. Up to 55% of MSSAs were not identified until further testing was obtained, a 24hour delay to best treatment. Time spent phoning contaminated BCs equates to 5.15PAs/month=£884 (average consultant salary). If all 71 BCs a month were tested at £29/ test=£2059. A saving of 1.7 bed nights/month would make this test cost efficient (Bed stay=£691/night) Importantly reducing the rate of contamination is key for best practice, which would in turn reduce the number of BCs that would require testing.

	Pre-study period	Study period
Number of methicillin sensitive *Staphylococcus aureus* (MSSA)	9	9
Number of MSSA that were not thought to be significant initially	2 (22.2%)	5 (55.6%)
Number of methicillin resistant *Staphylococcus aureus* (MRSA)	1	2
Number of MRSAs not thought to be significant initially	0 (0%)	0 (%)
Number of GPC not MSSA or MRSA on PCR or culture	51	89
Number of above not thought to be significant initially	48 (94%)	80 (90%)
Total number of BC with likely staphylococci	71	100
Number where advice was changed after the culture result was available	8 (71%)	14 (14%)
Thought to be significant isolates but were not after culture	6 (8.4%)	9 (9%)
Thought not to be significant but were after culture	2 (2.8%)	5 (5%)

R2203 Significance of rapid detection of group B *Streptococcus*

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Introduction: *Streptococcus* group B (GBS) is a part of normal flora of the gut and harmlessly colonize the genital tract in about 20–40% of healthy women. The objective of our study was to evaluate the rapid and simple assay (SpeedOligo chromatography test, Vircell) for the detection of GBS in vaginal smears in pregnant women.

Material and Methods: In the study we involved 30 pregnant between 28th and 35th week of gestation. Double vaginal swabs from each pregnant women were analyzed. The first one was inoculated on Columbia blood agar and incubated for 24 h. Suspicious colonies were used for performing the bacitracin susceptibility test for identification of GBS. The second swab was used for proceeding SpeedOligo GBS assay. Bacterial DNA was extracted by elution the swabs in a sample solution provided in the kit followed by heating at 94ºC for 5 minutes and centrifuging on 14,000 rpm for 2 minutes. Only 10 microL of DNA template were used for PCR. For that purpose were used 15 microL of reconstituted PCR mix which contained all that was needed for amplification of specific cfb gene of GBS which was the princip of the test. PCR reactions were performed in a termocycler with the following temperature profile: 1 cycle at 92ºC for 1.5 min; 35 cycles consisted of three steps (92ºC for 20 s; 55ºC for 20 s; 72ºC for 20 s); 1 cycle at 72ºC for 2 min and 1 cycle at 95ºC for 1 min. The hole amplification time was less than 30 minutes. Amplicons were detected by a process of hybridization in a dipstick for a 5 minutes. Positive results were conformed according to the red lines on the sticks.
Results: 3 out of 30 (10%) swabs cultured for GBS revealed positive results. They were positive by SpeedOligo assay too. 5 out of 30 (16.6%) swabs used for SpeedOligo assay were positive for GBS. 3 of them were positive by culture too, but in two positive cases by SpeedOligo the results were negative by culture. Comparison of this rapid and simple assay by culture as a gold standard for detection of GBS revealed sensitivity of 100% and specificity of 92.6%.
Conclusion: SpeedOligo GBS proved to be a rapid, sensitive and specific assay for the detection of GBS based on nucleic acid amplification technique. This test could be very useful for detecting colonization and infection with GBS in pregnant women during the hole time of pregnancy. It would be especially important for pregnant women with pretermed delivery, rupture of membranes and high fever during the delivery.

R2204 Distribution of virulence protein secretion systems in a collection of *Pseudomonas aeruginosa* clinical strains

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Objectives: Protein secretion systems are virulence determinants that mediate interactions between bacteria and eukaryotic host cells and contribute to the severity of infections. Among the 8 secretion systems known to date, type I, II, III, IV, V and VI have been reported in *P. aeruginosa*. These secretion systems have been intensively studied in a small number of reference strains, but there is, with the exception of type III secretion system, a lack of data regarding their distribution and relevance in clinical strains. This study is aimed at identifying genes encoding components and effectors of protein secretion systems in *P. aeruginosa* isolated from acute infections.
Methods: *P. aeruginosa* strains were isolated from patients hospitalized in at high-risk departments such as intensive care unit and hematology. Genotyping was performed using SpeI-PFGE and rep-PCR with BOX and ERIC primers. A total of 8 multiplex PCR assays were designed to target from 1 to 3 genes encoding structural and secreted proteins of secretion systems I-VI.
Results: A total of 113 *P. aeruginosa* strains were isolated from 69 patients. Genotyping using rep-PCR and PFGE allowed to assign 78 strains to 13 clusters, while 37 strains showed an unrelated, unique profile. All strains possessed the genes encoding effectors associated with type V and VI secretion systems. Genes encoding proteins secreted by type II were found in all clones examined, except for gene lapA, which is located in a Region of Genomic Plasticity. The presence of genes encoding effector proteins ExoS, ExoY, and ExoU transported by type III secretion system was certified in the 58% of the strains, in the remaining ones there seems to be an incompatibility between exoS and exoU. Type I secretion system haspA, aprA and aprX genes were present in 100%, 84% and 91% of the strains respectively and for type IV secretion system the prevalence of genes D4 and B2 was 10% and 3% for Trb.
Conclusion: Rep-PCR results were confirmed by PFGE, supporting the reliability of the method. Genotyping revealed the presence of a clone isolated both from hematology and intensive care unit indicating that it has been cross-transmitted within the nosocomial environment. A remarkable heterogeneity of the distribution of secretion systems I-IV was observed, suggesting that genomic plasticity may contribute to the specificity of *P. aeruginosa* infections.

R2205 Frequency of *Helicobacter pylori* vacA genotypes in Iranian patients with gastric and duodenal ulcer

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Objective: *Helicobacter pylori* infection is a risk factor for developing chronic peptic ulcers and gastric cancer. The purpose of this study was to investigate the frequency of *Helicobacter pylori* vacA genotypes in patients with gastric and duodenal ulcer.
Methods: A total of 100 biopsy specimens of patients with gastric (n = 50) and duodenal (n = 50) ulcer were collected. The specimens were cultured on selective media and incubated in a microaerophilic atmosphere at 37ºC for 5–10 days. The isolates were characterized to species level by conventional biochemical tests. The extracted DNA from isolates was used to perform a polymerase chain reaction based, simultaneous analysis of the cagA status, allelic variation of the signal regions (s1, s2) and the middle regions (m1, m2) of the vacA gene.
Results: *Helicobacter pylori* isolated from 50 specimens of patients and the vacA gene was detected in all isolates. Among vacA genotypes the s1/m1 was the most common in *Helicobacter pylori* isolates from patients with gastric ulcer (56%) and duodenal ulcer (68%).
Conclusion: This study demonstrated that vacA slml is common genotype of *Helicobacter pylori* in patients with peptic ulcer and the vacA allele s1 of this bacterium is associated with ulcer.

R2206 Molecular analysis of faecal microbiota from breastfed Brazilian children

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The human intestinal microbiota plays an important role in body healthy and differences in its composition are related to different levels of environmental contamination and different endogenous factors. The establishment of this microbiota occurs in the first two years of life.
Objective: In this study, we analyze the establishment of the fecal microbiota in a group of 14 children during the first year of life, living in low socio-economic conditions in São Paulo, Brazil, based on 16S rDNA gene libraries analysis.
Methods: Fecal samples were collected from healthy and exclusively breastfed children at different time point: 2nd, 7th, 30th days and 3, 6 and 12 months of life. Bacterial DNA was extracted directly from samples, and 16S rDNA libraries were constructed using bacterial-specific primers 27F and 1492R. Randomly selected clones were partially sequenced. Real-time PCR for Bifidobaterium spp. was performed in the 30th day samples and 3 months.
Results: The main phylogenetic group identified in all time point samples was *E. coli*. *Clostridium* and *Streptococcus* were detected at high rates at 30th day and 3th month of life, respectively. At 6th and 12th months of age, high rates of OTUs classified as uncultured bacteria were detected, showing the increase of diversity microbiota. Bifidobacteria was not detected in 16S rDNA libraries but was present in all samples analyzed by Real Time PCR.
Conclusion: This molecular approach allowed the analysis of the fecal microbiota implantation in a group of children from Sao Paulo, Brazil. This bacterial profile may differ to what is described in developed countries, and it may be attributed to a highly contaminated environment, and neonate contamination may have been favored by hygiene habits.
Financial Support: FAPESP/Brazil.

Molecular virology

R2207 Phylogenetic study on matrix gene of H9N2 isolates in Iran

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Objectives: H9N2 AI outbreaks have been one of the major problems in Iranian poultry industry. The association of high mortality and case report of H5N1 and H9N2 influenza virus in wild birds in recent years raised the specter of a possible new genetic modified AI virus.
Methods: In this study, we do phylogenetic analysis on full-length Matrix (M) genes genes of 8 H9N2 isolates from Broilers in Iran, Tehran province during 1998–2008. Sample collection was performed according to the standard method from suspected clinical broiler specimens. Ten-day-old embryonated chicken eggs were inoculated and incubated at 37ºC for 48 h. Viral RNA was extracted from infected allantoic fluid. RT-PCR was done. Purified PCR products were cloned into plasmid for TA subjected to nucleotide sequencing for bioinformatic studies.
Results: The nucleotide sequences for all H9N2 influenza viruses used in this study are available GenBank under accession numbers GQ206302 through GQ206309. Comparison of nucleotide sequences of isolated viruses revealed a substantial number of silent mutations, which results in high degree of homology in amino acid sequences. In addition, the cluster of Iranian H9N2 isolates could be present into one subgroups. The high degree of similarity between the M genes of the Iranian H9N2isolates supports the hypothesis that these genes originated from a single predecessor.
Conclusion: Our result provides useful molecular epidemiological data to understand the dynamics of H9N2 evolution during years in Iran and support earlier phylogenetic observations.

R2208 Prevalence and type distribution of human papillomaviruses types in women with abnormal cytology results in Greece

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Objectives: Several epidemiological studies have been conducted worldwide in order to estimate the prevalence of human papillomaviruses (HPV) infection. The aim of this study was to evaluate the prevalence of HPV types and its possible association with the grade of histological lesions in a cohort of 148 women proceeding to gynaecology clinics of "Alexandra" Hospital for a 6 month period (03/2009–09/2009) with previous abnormal cytology findings, using a highly sensitive detection method based on nested PCR.
Methods: Cervical swabs were collected from all participants. HPV DNA detection was carried out by PCR and nested PCR using kit of AB Analitica. The detection limit of this method is 100 copies of viral genome/μL. HPV positive samples were genotyped for 32 different HPV types by reverse hybridization of specific probes with the amplified viral region L1 acquired from nested PCR (AB Analitica Padova, Italy).
Results: The mean age of the population was 37.3±11.6 years (range 20–76 years). Ninety six of 148 samples were graded as LGSIL, 15 as CIN I, 10 as CIN II-III and 27 belong to women which underwent loop excision for treatment. HPV was found in 67 out of 96 patients with LGSIL (69.8%), in 15 patients with CIN I (100%), in 10 patients with CIN II-III (100%) and in 19 out of 27 patients which underwent loop excision (70.3%). Twenty two (22) different genotypes were identified. The most prevalent types in LGSIL cases were HPV16 (26%), HPV31 (17.7%) and HPV54 (14.6%), in CIN I cases were HPV16 (40%), HPV58 (33.3%) and HPV31 (20%), in CIN II-III cases were HPV16 (50%), HPV31 (30%) and in patients with loop excision were HPV16 (37%) and HPV31 (7.4%). Multiple HPV infection was found in 30.2% of patients with LGSIL, in 20% of patients with CIN I, in 50% of patients with CIN II-III and in 29.6% of patients with loop excision. HPV16 and HPV31 were found in 58.7% and in 60% respectively of patients with multiple HPV infection. An association was indicated between multiple HPV infection and histological lesion ($p = 0.078$). The mean age was 36.7±12.4 years in the HPV infected group and 39.6±8.5 in the non HPV infected group ($p = 0.002$).
Conclusions: According to our findings HPV16 was the most frequent type followed by HPV31. HPV was present in most of the patients underwent a loop excision for treatment. Moreover, multiple HPV infection was a common finding among the participants.

R2209 Survey of viral gastroenteritis in childhood in Turkey using Multiplex RT PCR methods

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Objectives: Enteric viruses that have been reported as a cause of nonbacterial acute gastroenteritis include rotaviruses, noroviruses, sapoviruses, astroviruses and enteric adenoviruses. The purpose of this study is to determine the prevalence and the distribution of viruses responsible for gastroenteritis in children.
Methods: A molecular epidemiological study on common diarrheal viruses was conducted in Afyon City, Turkey between January and November 2008. One hundred and forty-four faecal samples from children under 6 years of age(mean age, 2.18±SD 3.64 years, range: 1–72 months)(negative for the presence of pathogenic bacteria by standard culture methods) were tested by novel multiplex RTPCR methods for detection of – Norovirus G1, G2, Rotavirus group A, Sapoviruses(SaV), Astroviruses(HAstV), Adenovirus Group F, Adenovirus genus and an internal control phage RNA (MS2). The assays were one-step RTPCR reactions based on detection of four different fluorophores in real-time on an ABi 7500. RTPCR negative samples were also examined by electron microscopy (EM).
Results: Among the diarrheal viruses detected, NoV GII was the most common, with a proportion of 27.7%(n:40), whereas group A rotavirus(28), adenovirus F(1), adenovirus GNS(8), SaV(5), and HAstV(2) were also found in 19.4, 6.25, 2.8, and 1.4%, respectively. Overall 82 (57%) samples were positive by RTPCR and of the 62 negative samples 53 had sufficient volume to be examined by EM. Of these 4 were positive for a virus of which 2 were group C Rotavirus(1.3%) and 2 were Reovirus (confirmed by specific PCR's) and no other additional positives were found.
Seventeen of 144 (11.8%) samples were found positive with more than one viral agent, in which 14 samples contained both group A rotavirus and NoV GII. By sequencing of 299 bp at the 5′ end of ORF2, of these 16 were Bristol GII.4, 13 were Toronto GII.3 and 1 was Virginia Beach-like GII.
Conclusion: These findings provide evidence that Noroviruses can be a leading cause of gastroenteritis, and highlight the need to implement norovirus and rotavirus ELISA detection assays in association with rapid EIA rotavirus and adenovirus EIA detection for the clinical diagnosis and the nosocomial prevention of gastroenteritis viral infections in paediatric departments. It is noteworthy that the group C rotavirus was first reported in Turkey, with a proportion of in this study.

R2211 Enhancing sensitivity of Clart® Entherpex for detection of human herpesvirus and enterovirus using DNA microarrays printed in strips

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Introduction: The Herpesviridae viruses are widely spread among human population and have the ability to establish lifelong latent infections. In immunocompetent individuals, the virus reactivation is usually harmless and undetectable. Human herpesviruses and enteroviruses are the major causative agents of the central nervous system (CNS) viral infections. The common clinical symptoms caused by these viruses make necessary the optimization of molecular methods that allow multiple, sensitive, and rapid identification of these viruses.
Objective: To improve sensitivity and automate a system for simultaneous detection of Human Herpesviruses (HSV-1, HSV-2, VZV, CMV,

EBV, HHV-6, HHV-7 and HHV-8) and Enteroviruses (Echoviruses, Poliovirus and Cosxackievirus), CLART® ENTHERPEX.
Method: Virus detection is performed by multiplex RT-PCR and improved PCR. We have developed an array detection system (ArrayStrip) for the simultaneous processing of multiple samples in microplate or individual strips. We have analyzed 40 samples and QCMD samples in order to determine the new diagnostic parameters of the kit: reproducibility, repeatability, sensitivity, and specificity. All the discrepancies were validated with sequencing, homemade PCR and nested-PCR. Furthermore, we have compared results from our kit, CLART® ENTHERPEX, with real-time qPCR results.
Results: We determined an analytical sensitivity of HSV1 and VZV of 10 copies. A 100% value of analytical sensitivity ranging from 10 to 100 copies was obtained in the detection of rest of viral specimens. Analyzing the diagnostic sensitivity and specificity, the behaviour of each virus after the validation of clinical specimens showed that most of viruses show sensitivity higher than 83%, specificity higher than 97%, reproducibility higher than 92% and repeatability higher than 95%.
Conclusion: CLART® ENTHERPEX is a useful tool for rapid screening and simultaneous detection of a Human Herpesviruses and Enteroviruses in clinical setting, being able to process up to 96 samples simultaneously in a working day. A new optimized version of the kit with improved amplification and detection makes this tool highly sensitive and useful for clinical diagnostic purposes.

R2212 Evaluation of the Roche cobas® 4800 HPV test for cervical HPV detection from SurePath liquid-based cytology specimens

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Objective: The cobas 4800® system is an automated platform that combines sample preparation with real-time PCR detection. The cobas® 4800 HPV Test is a multiplex assay that detects HPV 16, HPV 18, and 12 other high-risk (12-HR) carcinogenic HPV genotypes with separate reporter dyes. The purpose of this study was to investigate the correlation of the cobas 4800® HPV Test results obtained from direct testing of cervical cytology specimens collected in SurePath LBC medium (BD Diagnostics) to results obtained using the residual processed cell-pellet of the same SurePath sample.
Methods: A convenience sample of paired unprocessed and pelleted SurePath LBC cervical specimens were collected from a pool of cervical cancer screening specimens following cytological interpretation. Paired unprocessed and pelleted specimen fractions were tested by the cobas 4800 HPV® Test. Data analysis included percent agreement of paired HPV results and their association with cervical cytology. Discordant results and paired HPV 16 and HPV 18 positive results were validated using the Linear Array (LA) HPV genotyping assay (Roche Molecular Systems).
Results: A total of 300 paired specimens were tested and 274 were included for data analysis; the remaining 26 were deemed 'invalid' by the cobas 4800 due to specimen inadequacy. Overall, the percent total agreement between unprocessed and pelleted specimen pairs was 96.5%. The percent positive agreement for paired HPV 16, HPV 18, and HPV 12-HR tests was 92% (38 of 42), 75% (16 of 21), and 87% (98 of 105), respectively. The unprocessed SurePath specimen was HPV negative for thirteen of the 18 discordant HPV paired results. Cytological results showed an equal distribution of Normal, LSIL, and ASCUS findings for 92% of total specimens. Notably, 88% of HPV 12-HR positive paired specimens were associated with LSIL/ASCUS cytology. Operationally, we did not encounter any significant processing or technical difficulties during this evaluation of the cobas 4800 system.
Conclusion: This is the first report to describe the performance of the cobas® 4800 HPV Test using unprocessed SurePath LBC specimens, in comparison to results obtained following a routine concentration process performed to enhance cytological analysis. The excellent overall agreement between paired SurePath specimens in this study has identified an expanded utility of the cobas® 4800 HPV test that may, in time, accommodate a broader range of diagnostic algorithms.

R2213 Evaluation of the Artus Infl./H1 LC/RG RT-PCR kit, a reliable and sensitive method for detection of influenza virus using real-time PCR

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Objectives: Flu is an acute respiratory disease caused by Influenza viruses A, B and C, with symptoms ranging from mild to lethal. Influenza type A is usually the most common of the circulating influenza types. A number of different subtypes are classified within the Influenza A type based on two viral surface proteins: hemagglutinin (H) and neuraminidase (N). Yearly epidemics occur in part due to antigenic drift within these proteins. A novel Influenza A H1N1 subtype emerged in March 2009 and is the causative agent of the current influenza pandemic. The artus Infl./H1 LC/RG RT-PCR Kit is designed for the specific detection of all known Influenza A and B types, using primers and probes directed to regions of the matrix gene, as well as for the specific detection of a region of the hemagglutinin gene (H1) of the pandemic 2009 H1N1 Influenza virus. The presence or absence of the H1-signal provides the basis for differentiation between pandemic 2009 H1N1 Influenza virus and other influenza A strains.
Methods: We developed a real-time RT-PCR assay for the detection of Influenza RNA in clinical samples and differentiation of the pandemic 2009 H1N1 Influenza virus. RNA extraction, reverse transcription and amplification is controlled by use of an internal RNA control. Performance evaluation of the artus Infl./H1 LC/RG RT-PCR Kit covered determination of analytical sensitivity, reactivity, crossreactivity and precision for the LightCycler 2.0 instrument (Roche) and also for the RotorGene Q (QIAGEN). For these analyses virus and bacterial cultures of defined identity and concentration were used. Clinical performance was analyzed by retrospective testing of routine diagnostic specimen. Nucleic acid extraction was done using the EZ1 DSP Virus Kit and the QIAamp Viral RNA Mini Kit (both QIAGEN).
Results: Analytical sensitivity of the artus Infl./H1 LC/RG RT-PCR Kit is <250 TCID50/ml. Detection is highly specific for Influenza A and B and for pandemic 2009 H1N1, respectively. Intermediate precision is <3.5% based on Ct-values. Retrospective studies demonstrated high concordance between results obtained using the artus Infl./H1 LC/RG RT-PCR Kit and methods used for pre-testing, including virus culture and sequencing.
Conclusion: The artus Infl./H1 LC/RG RT-PCR Kit (RUO) is a reliable and sensitive method for detection of influenza virus RNA using real-time PCR.

R2214 Comparison of Linear Array HPV genotyping test and Hybrid Capture 2 assay for detection of high-risk human Papillomavirus genotypes in different clinical settings

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Objectives: To assess concordance and performance of two commercially available assays with different genotype coverage for detection of carcinogenic HPV in patients referred to colposcopy for abnormal Pap smear result (ASCUS or more) or for a follow-up after a cervical intraepithelial neoplasia (CIN) diagnosis.
Methods: 458 cervical samples (331 from women with abnormal Pap tests [AP group] and 127 in follow-up for CIN [FU group], respectively) were analyzed by Roche Linear Array HPV (LA-HPV) genotyping test, a qualitative PCR technique able to detect the 37 most prevalent low, intermediate and oncogenic high-risk (HR) HPV genotypes [6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73 (MM9), 81, 82 (MM4), 83 (MM7), 84 (MM8), IS39 e CP6108], and by Digene Hybrid Capture II assay (HC2)

detecting 13 oncogenic HR HPV (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) types. All tests were performed in the same cervical samples collected in PreservCyt® liquid media. External Quality Assessment was evaluated according to NEQAS and QCMD panels.
Results: 360 out of 458 samples examined (78.6%) showed positive (207 cases) and negative (153 cases) concordant results. In the remaining 98 cases we found discordant results: 93 LA-HPV positive/HR HC2 negative and 5 LA-HPV negative/HR HC2 positive. AP and FU groups concordance was 85.2% (282/331) and 61.4% (78/127) respectively. Overall concordance in detecting HR-HPV genotypes was 76.3% (184/241): 85.4% (164/192) in AP and 40.8% (20/49) in FU groups. Sensitivity for HR-HPV detection was 97.9% (LA-HPV) vs 87.5% (HC2) in AP group and 99.9% (LA-HPV) vs 42.8% (HC2) in FU group, respectively.
Conclusion: LA-HPV and HC2 showed a substantial agreement in the AP group, thus confirming that both methods can be used in the triage of abnormal pap-tests. LA-HPV was more sensitive than HC2 in the FU group.
Nevertheless, several future studies are needed to demonstrate the potential clinical impact of our results.

R2215 Differential detection of 19 respiratory viruses including the new influenza A strain H1N1 2009 with the ResPlex II Plus panel

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Objective: The ResPlex II Plus panel uses proprietary QIAplex technology for multiplex amplification of nucleic acids from 19 respiratory viruses and following differential detection by suspension bead hybridization on the LiquiChip 200 workstation. The ResPlex II Plus Panel was developed by implementation of an additional H1 target into ResPlex II v2.0 for differential detection of the newly identified Influenza A strain H1N1 primary described in Mexico, 2009.
The aim of this study was to evaluate the performance of the ResPlex II Plus Panel for differential detection of 19 respiratory viruses.
Method: Analytical specifications were addressed using pre-characterized real virus samples of all 19 respiratory viruses including the newly identified H1N1 strain. Eluted nucleic acids were analyzed for the presence of Influenza A/B virus, H1N1 2009, PIV-1/2/3/4, RSV-A/B, hMPV (A, B), Rhinovirus, Coxsackievirus/Echovirus, Adenoviruses (B, E), Coronaviruses 229E, HKU1, OC43, NL63 and Bocavirus. An internal control based on an unrelated RNA sequence is part of the panel to monitor the purification process and to proof enzymatic activity. The overall performance was directly compared to the ResPlex II Panel v2.0. In addition analytical sensitivity was evaluated for the new target of ResPlex II Plus, H1-Mexico. Dilution series of viral culture were generated in order to determine the limit of detection for new H1-Mexico target. In parallel different Influenza strains (Flu B, Flu A H3N2) were tested to prove specificity for the newly identified H1N1 strain showing no cross-reactivity to common Influenza strains.
Results: Both panel versions showed a high degree of concordant results. The ResPlex II Plus Panel provides improved performance by extended panel content for differential detection of the new identified H1N1 strain. After extraction of real virus specimens we found overall good concordance between the ResPlex II v2.0 results and the ResPlex II Plus results. Discordant results will be listed and discussed.
Conclusion: QIAplex technology combines user friendly handling with analytical performance needed for the detection of respiratory viruses.
Our work illustrates that the ResPlex II Plus Panel provides excellent options for parallel detection of 19 different viral nucleic acids including specific identification of the new H1N1 strain (2009) from respiratory samples.
*The ResPlex II Plus Panel is intended for research use only. Not for use in diagnostic procedures.

R2216 Minor genotypes of human papillomavirus can be recovered by hybrid capture technology according to phylogenetical affinity

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Mucosal or genital genotypes of Human Papillomavirus (HPV) can be classified as Alphapapillomavirus. In these genera several groups (A5–7, A9 and A11) are related with oncogenic potential whereas other (A1, A8 and A10) are associated to benign lesions. However, oncogenic and non-oncogenic groups can be closely related (A9 and A10) due to similarity in envelope genes. The molecular diagnostic is based in these genes and then show an analytical inaccuracy with cross-reactivity between groups. Hybrid capture 2 (hc2) (Qiagen) expresses the results of tested high-risk (HR) or low-risk (LR) HPV genotypes as positive or negative. In this work we show significant analytical inaccuracy, mainly due to cross-reactivity with several untargeted HPV genotypes.
We have included 699 cervical specimens obtained from women undergoing routine gynecological examination and recognized as HPV positive using the hc2 high- and low-risk probe cocktails. All the specimens were genotyped using INNO-LiPA HPV (Innogenetics) and Linear Array HPV (Roche Diagnostics) capable of recognizing 27 and 37 different α-HPV genotypes respectively.
Five-hundred-fourteen samples (77.9%) were correctly identified with HR and LR probes. Untargeted genotypes more frequently found were HPV53 (9.7%), HPV66 (9.4%), HPV61 (4.2%), HPV84 (4.0%) and HPV54 (3.8%). Other HPV genotypes detected by frequency order were 62, 73, 89, 55, 82, 40, 70, 67, 81, 71, 72, 74, 83, 26, 69, 85 and 64.
The genotypes untargeted more prevalent corresponding to A9 (HPV54), A8 (HPV40) and A10 (HPV55 y 74) were detected jointly by HR and LR probes. However A5 (HPV26, 69 and 82), A6 (HPV53 and 66), A7 (HPV70), A11 (HPV64 and 73) and HPV54 were detected only by HR probes, probably due to A7 (HPV18, 45 and 39), HPV 51 and 56 are present in the HR cocktail. Interestingly in A3 (not included in hybrid capture), HPV61, 62, and 89 were detected by HR probe. Also, A15 (low risk genera next to A5) was recognized by HR probes.
We concluded that the low specificity of hc2 probes, could be useful for to recover genotypes primarily untargeted. This property can be very interesting to detect minor genotypes but potentially important in vaccinated individuals.

R2217 Detection of HSV-1 and HSV-2 from cerebral spinal fluid and swab specimens using the 3M integrated cycler

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Objectives: Herpes simplex viruses (HSV) can cause severe disseminated infections in neonates and in immunosuppressed patients, with spread to the central nervous system resulting in potentially fatal cases of encephalitis and meningitis. Rapid detection of infections is critical since mortality and morbidity is significantly decreased when antiviral therapy is started early in an infection. In an effort to aid diagnosis, we developed a rapid molecular test (Simplexa™ HSV-1 and 2 assay), using the 3M integrated cycler, to detect HSV-1 and 2 in both CSF and swab specimens. In this study, we sought to determine the sensitivity and specificity of this newly developed assay.
Methods: Dilutions of quantified HSV-1 and 2 virus were used in analytical studies to determine the limit of detection of the assay. Analytical specificity was determined by testing a panel of related pathogens to determine whether any cross reactivity was observed. Clinical sensitivity was determined by comparison to an HSV-1 and 2 PCR assay developed in the Focus Diagnostics reference laboratory, with discordant samples being analyzed by DNA sequencing.
Results: Analytically, the assay was able to detect viruses down to 100 copies/ml, and no cross reactivity was observed with other pathogens. Method comparison studies showed that this assay is comparable to the

assay run on an alternate platform, although more rapid throughput could be achieved using the integrated cycler.
Conclusions:. The Simplexa™ HSV-1 and 2 assay showed excellent sensitivity and specificity and appears to be an effective tool for diagnosing HSV related disease. The small footprint of the 3M integrated cycler facilitates its placement in a laboratory, and its capacity for rapid cycling aids in providing expedited diagnostic results, which is beneficial to disease management.

Molecular typing

R2218 Characterization of endemic *Shigella boydii* strains in Iran by serotyping, antimicrobial resistance, plasmid profile, ribotyping and pulsed-field gel electrophoresis

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Objective: Shigellosis is one of the major causes of morbidity in children with diarrhea in Iran. The present study was undertaken to characterize apparently sporadic *Shigella boydii* strains isolated from pediatric patients in Iran and to compare different methods of subtyping.
Methods: Serotyping, antimicrobial susceptibility testing, plasmid profile analysis, ribotyping and PFGE. have been carried out for typing of *Shigella boydii* strains isolated from cases of gastroenteritis and acute diarrhea in Tehran occurring between December 2002 and November 2003.
Results: Ten out of 302 isolates of *Shigella* recovered from pediatric cases were identified as *S. boydii* and selected for the study. Seven isolates were attributed to serotype 2, whereas the remaining three belonged to serotypes 14, 18, 19, respectively. Six drug resistance phenotypes (R1 to R6) were defined with R4 – streptomycin, ampicillin, sulfamethoxazole-trimethoprim – being the most prevalent. Plasmid analysis resulted in seven different plasmid profiles with three to 10 DNA bands. All strains, but one, shared the same ribotype, but PFGE differentiated them in four groups.
Conclusion: The results indicated that PFGE patterns well corresponded to serotypes. Antibiotic resistance testing and plasmid profile analysis appeared to have a good discriminatory power for differentiation of Iranian strains of *S. boydii.*

R2219 Evaluation of a PCR-based approach to study the relatedness among *S. sonnei* strains isolated in Tehran

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Objectives: Infections caused by *Shigella* are a major cause of diarrhoeal disease in the developing and developed countries. The present study was conducted to apply and evaluate Arbitrarily primed PCR (AP-PCR) for investigation of genetic relatedness among the strains of *S. sonnei* isolated from cases of acute diarrhea occurred in Tehran during 2003.
Methods: A random sample of 60 *S. sonnei* strains isolated from enteritis cases in children at five hospitals in Tehran during 2003 and two sporadic isolates recovered in 1984 was selected for the investigation. Molecular typing was performed by AP-PCR. Depending on the number and size of amplified DNA bands, the strains were clustered into AP-PCR profiles.
Results: All strains of *S. sonnei* were typeable with this method. AP-PCR generated nine indistinguishable bands ranged from 0.35 to 2.5 kbp in all the strains under study. Only a single AP-PCR pattern was observed among the *S. sonnei* strains recovered in 2003. Two sporadic isolates recovered in 1984 showed different AP-PCR patterns compared to recent clinical isolates.
Conclusion: The results suggest that a very homogeneous AP-PCR cluster types might be responsible for shigellosis caused by *S. sonnei* in Tehran in 2003. Further molecular analysis conducted on a larger selection of isolates could confirm our findings.

R2220 Genotyping of *Chlamydia trachomatis* and human papillomavirus in clinical specimens from north-eastern Croatia

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Human papillomavirus (HPV) and *Chlamydia trachomatis* (Ct) are major causes of sexually transmitted diseases. Clinical consequences of high-risk HPV (HR HPV) infection, especially with HPV types HPV 16 and HPV 18, include abnormal Pap test results, low and high-grade cervical intraepithelial neoplasia and cervical cancer, for which Ct may sometimes be a cofactor. The aim of the study was to determine the prevalence of Ct and age-related profiles of HR HPV infections among cases of other sexually transmitted infections in our county.
Due to the chronic and "silent" infection of Ct, fast molecular diagnostics and adequate therapy of the infected individuals are the crucial steps in the Ct spread control. During three-year period we tested 2327 genitourinary samples for Ct and 945 gynaecological samples for HR HPV with normal and abnormal cervical cytological diagnosis. Subsequently all Ct positive samples were analysed by sequencing of the amplified omp1 fragments using Applied Biosystem 3130 Genetic Analyser. Genotyping and sequence mutation analysis were performed using ABI SEQSCAPE software and compared with the reference sequences of all known Ct serotypes. HR HPV positive samples with abnormal cervical cytological diagnosis were genotyped by Linear Array HPV Genotyping Test (Roche Diagnostics).
The determined serotype of Ct and HPV genotypes distribution were compared with the Ct and HPV distribution pattern in other regions of the world. The association between certain HPV genotype and cervical intraepithelial neoplasia was determined as well as mixed infections with four or more HPVs. The most prevalent Ct genotype in Osijek-Baranya County was serotype E (in concordance with Sweden and Taiwan data), followed by F, K, G, D, B and J (differs from Sweden and Taiwan data). The sequence of omp1 gene showed limited variation. HPV 16 and HPV 18 were the most common HR HPV genotypes in the cervical specimens with abnormal cervical cytological diagnosis. Obtained results also determined mixed infection of four HR HPV with one or more low risk HPV.
The obtained results for Ct and HPV and further analyses in this ongoing project could be useful tool for clinical and epidemiological characterization of circulating pathogens in our community.

R2221 pmpH real-time PCR assay using LightCycler 480 to diagnose and sequence Lymphogranuloma venereum

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Objective: The focus of this study is the identification of the Lymphogranuloma venereum (LGV), by means of LGV-specific pmpH real time PCR assay, using LightCycler 480 and sequencing that can easily identify LGV strains.
Material and Methods: Sixteen clinical samples from different anatomical sites (cells of cervical, urethral, penis, rectal, and ulcerative proctitis), sent to the Microbiology's laboratory of the Basurto Hospital, Bilbao Spain, were genotyped. The polymorphic membrane protein H gene (pmpH) was used as the PCR target for the real time PCR assay based on a unique 36-bp deletion region occurring only among LGV strain. Two sets of PCR primers were selected to amplify only LGV serovars: A set (pmpH) to amplify 168-pb DNA fragment and another (LGV) to amplify 130-pb. The PCR reaction was carried out in a volume of 20 μL and was performed in a LightCycler 480. The final product was purified and sequenced using BigDye Terminator V. 3.1 chemistry according to the kit instructions. Sequencing reactions were purified with AutoSeq G-50 and sequenced on an ABI 3130 Genetic Analyzer. DNA sequences obtained were aligned to obtain full-length sequence information of each sample and queried against the BLAST database.

Results: The design of the pmpH real time PCR target with the LightCycler 480 instrument detected LGV DNA of 3 specimens (3/16; 18.5%). The software checked the specificity of amplified products by melting curve analysis. And shows a dissociation curve for LGV. The product melts at 86.5. Sequencing showed that the sequences from the three LGV cases were identical and a Blast search revealed that there were of the L2b type.
Conclusion: Our results suggest that the pmpH real time PCR can be used for the sensitive detection of all LGV strains and could prove useful in the detection of LGV –L2b in clinical specimens. Sequence-based using LGV specific pmp H primers can discriminate between LGV serovars and less invasive *C. trachomatis* species can help detect cases and prevent further transmission of LGV.

R2222 Utilization of Raman spectroscopy for the rapid differentiation of clonal relationships among *Salmonella* spp. from human and animal sources

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Objectives: To evaluate the use of Raman spectra (RS) generated by the SpectraCell RA system (River Diagnostics BV, Rotterdam, The Netherlands) for subspecies evaluation of outbreak-associated *Salmonella* spp. (SS) compared with standard pulsed field gel electrophoresis (PFGE) and serotyping schema. RS reflect the overall molecular constituents of the bacterial sample being studied by laser interrogation. Rapid and technically simple typing of gastrointestinal pathogens with a high degree of interlaboratory comparability is needed to enhance outbreak investigations.
Methods: RS for 31 unique SS isolates from human, animal and food processing sources representing 16 PFGE clonal groups (defined using established guidelines) were determined in triplicate. Study isolates were harvested from overnight growth on tryptic-soy agar plates, suspended in sterile water, spun down and 3ul of a heavy suspension loaded onto 24-well test slides. Spectral fingerprints were recorded for each specimen with an average read time of 4–7 minutes. Analysis of RS was performed using a squared Pearson correlation coefficient of 0.9996 and compared with results for PFGE and traditional serotyping.
Results: RS of the tested outbreak isolates displayed 11 clusters compared with the 16 clusters identified by PFGE. Multiple PFGE clusters found within a smaller number of RS clusters in most all cases comprised the predominant PFGE type and related subtypes (A, A1; B, B2, B3; F, F1; C, C1, C2). Among the 16 PFGE clusters, 80 of 83 (96.4%) replicates were concordant within their respective RS clusters. Serotyping was less discriminatory than either RA or PFGE, with certain serotypes (e.g., *S. enterica* sv Montevideo, *S. enterica* sv Typhi Ty2, *S. typhimurium* LT2) occurring among multiple PFGE and RS clusters (2 and 3; 3 and 4; 5 and 4, respectively).
Conclusions: Epidemiological monitoring using RS as determined by the SpectraCell RA system was extremely rapid and technically unambiguous when testing SS outbreak specimens. Results were largely concordant with PFGE, highly reproducible and with the potential for >70 isolates to be processed in one working shift.

Molecular biology – others

R2223 Effect of culture medium and environmental factors in the patterns of outer membrane proteins expression of multi-resistant clinical isolates of *E. coli*

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Background: Current models of outer membrane proteins (OMP) expression and regulation in *E. coli* are mainly derived from the K12 strain. Preliminary information suggests that this may not apply to clinical isolates. This may difficult our understanding of the importance of OMP in antibiotic resistance and other relevant issues. The aim of this study was to compare OMP patterns of multiresistant *E. coli* and of *E. coli* K12 grown in different environmental conditions.
Methods: Clonally-unrelated (as defined by Rep-PCR/PFGE) multirresistant *E. coli* cultured from diferent patients, producing (n = 48) or not (n = 64) extended-sprectrum β-lactamases (ESBL) were studied. *E. coli* K12 (EcK12, strain MKW505) was used as a control. OMPs were obtained from sonicated cells treated with sarcosyl, concentrated by centrifugation and separated in SDS-PAGE (running gels with 12% poliacrilamide and 6M urea). All 112 isolates were grown overnight at 37ºC in Mueller-Hinton broth (MH) or in Nutrient broth (NB). Six isolates representative of the more frequent patterns were selected for additional studies, evaluating OMP expresion in different conditions of osmolarity (bacteria grown in NB alone or NB plus 20% sorbitol), temperature (cultures incubated in NB or MH at 30ºC, 25ºC or 41ºC) and pH (isolates grown in NB or MH adjusted to 8.5, 7.2, 5.5 or 5.0).
Results: SDS-PAGE showed 11 and 8 different OMP patterns in clinical isolates of multirresistant *E. coli* producing or not ESBLs, respectively. In most cases no differences were observed when isolates had grown in MH or in NB, in contrast to the results observed in EcK12, were OmpF expression was downregulated in MH. OMP patterns from clinical isolates grown in NB-20% sorbitol were not different of those from bacteria grown in NB alone, while OmpF downregulation was noted for EcK12 in the high osmolarity medium. Incubation at 41ºC caused decreased expression of OmpC in some (4/6) organisms (not including EcK12 when OmpF expression is affected) grown in MH, but no significant changes were observed at 30ºC or 25ºC. Variations in the expression of OmpF were also observed when organisms were grown in media with pH of 5.5 or 5.0.
Conclusions: OMP expression patterns in clinical isolates of multirresistant *E. coli* are usually different of the pattern observed in *E. coli* K12. In the clinical isolates we have studied, osmolarity changes do not significantly affect OMP expression, while high temperature and low pH affect OmpC and OmpF expression, respectively.

Diagnostic/laboratory methods (other than molecular)

R2224 A study of various commercially available transport swabs for the recovery of fastidious organisms

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Objectives: The Sigma Transwab ® is a liquid medium transport swab and uses 1ml of Liquid Amies Transport medium, which is based on the original formulation of Amies, but without the charcoal and is intended for processing by some of the new automated plating systems. The swab is foam tipped which allows the flow through of the liquid medium, reagents and microorganisms, thus increasing the sensitivity and recovery of organisms. The preliminary study used the principles of CLSI's M40-A standard, inoculating the swabs with specified dilutions of target organisms, and holding at either room temperature or 40C. For the preliminary study the organisms used were: *Staphylococcus aureus*, *Haemophilus influenzae*, β Haemolytic *Streptococcus*. Fresh overnight cultures were used in each case. The organisms were from Clinical specimens not NCTC or ATCC organisms.
Method: A McFarland standard 0.5 was made in sterile 0.85% saline from a fresh overnight culture of each of the organisms used.
Tenfold dilutions were made for the viability studies from the 0.5 McFarland's standard.100ul of these dilutions were lawned on to the appropriate agar plate to ensure the organism was viable from the saline dilution and used as the Growth Control. The 10^5, 10^6 and 10^7 cfu/ml dilutions were used to inoculate the swabs with 100ul (0.1 ml) using 3× 1/2 tubes. The swab was placed in the 100µl inoculum for 10 seconds and then returned to the transport media. Swabs were kept at room temperature and at 4 0C for the following holding times. 5–15 minutes (Zero Time), 4 hours, 24 hours, 48 hours, and 72 hours. Each set of dilutions were performed in duplicate. On completion of the holding time, the swab inoculated on to a plate appropriate

to the organism (Blood agar, Chocolate blood agar) in a lawn and incubated in the appropriate atmosphere – Aerobically at 370 C for *Staphylococcus aureus* and β haemolytic streptococcus, and CO2 at 370 C for *Haemophilus influenzae*. The plates read and colonies counted manually at 24 and 48 hours.
Results and Conclusion: The initial results showed that recovery was seen for all the organism/dilution/temperature combinations at 24 hours, and for most at 48 hours, and that Sigma Transwab ® would be a suitable transport device for these organisms. Further studies will be done and data presented for other organisms using more fastidious organisms, and a comparison of results obtained with standard transport swab devices (Eurotubo, Spain and Technical Service Consultants, UK).

R2225 Evaluation of frequency of anti-*Babesia microti* antibodies in serum of forest workers – a preliminary study

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Babesiosis is a tick-borne disease caused by *Babesia bovis*, *Babesia divergens*, *Babesia equi* and *Babesia microti*. Increase in babesiosis incidence in USA and Europe is considered to be correlated with increasing Lyme disease incidence. Pathogenesis and clinical symptoms of babesiosis are still not well known.
Objective: The objective of our study was to measure frequency of presence of anti-*Babesia microti* antibodies in serum of people exposed to tick bite.
Method: Study group consisted of 114 foresters (95 men, 19 women) working in area of Bialowieza parish in north-eastern Poland and in The Swietokrzyski National Park in central Poland, in whom IgM and IgG anti-*Babesia microti* antibodies were detected by immunofluorescent method. (*Babesia microti* IgG IFA kit Fuller Laboratories Fullerton Kalifornia USA). Results were analyzed statistically with Statistica 6.0 PL9 software ($p < 0.05$ was considered statistically significant). In the same group, antibodies against *Borrelia burgdorferi* were searched for with two-step procedure (ELISA and Western-blot) and found in 51 foresters (44.7%) (IgM in 28 and IgG in 33).
Results: Anti-*Babesia microti* IgG antibodies were found in only 4 forest workers (3.5%) in the analyzed group. In no case IgM class antibodies were found. Anti-*Babesia microti* antibodies were found only in patients with detectable anti-*Borrelia burgdorferi* antibodies.
Conclusion: Foresters, who are at high risk of repeated tick bites, may be asymptomatically infected with *Babesia microti*.

R2226 Technical and diagnostic performance of five commercial anti-diphtheria toxoid IgG ELISA kits

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Objectives: Accurate measurement of anti-diphtheria toxoid IgG levels is of immense value in (a) determining the rates of immunity within broad populations and therefore the immune status of individuals who may be at risk of infection especially as immunity can decrease over time in the absence of a booster; (b) in assessing immunisation schedule efficacy and; (c) in assessing the immune response to vaccination as part of the diagnostic protocol for primary immunodeficiency disorders. Five commercially available ELISA kits for the measurement of anti-diphtheria toxoid IgG antibodies were evaluated for performance.
Methods: ELISA kits, manufactured by Euroimmun, Scimedx, Serion, Binding Site and Virotech were evaluated for intra- and inter-precision, recovery of the NIBSC 00/496 international reference material and measurement of titres in pre- and post-vaccination samples.
Results: The imprecision of the five assays ranged from 0.7% to 27.4% for intra-assay and 4.9% to 26.6% for inter-assay. Recovery of the NIBSC international reference (00/496) across the kit specific calibration curves varied from 66.1% to 114.7% across the five assays. Evaluation of normal sera samples showed a significant difference existed between mean values obtained in the five assays. The accuracy and interpretation of the pre- and post-vaccination measurements differed among the five assays.
Conclusion: The data suggests that there are manufacture dependent characteristics which can affect the performance of the assays and may result in differing clinical and diagnostic interpretations.

R2227 Comparative studies for the serodiagnosis of *Chlamydophila* and *Mycoplasma pneumoniae* infections

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Mycoplasma pneumoniae and *Chlamydophila pneumoniae* have worldwide distribution and infect the upper and lower respiratory tract. Serology is still the most widely used method to diagnose both infections, even if its interpretation is difficult.
Objectives: *M.* and *C. pneumoniae* serology testing is currently carried out in our institute by microplate analyzer (ETI-Max® 3000, DiaSorin), used together with Virion *M. pneumoniae* antibodies assays using native antigens, as well as with Medac total *Chlamydia* and *C. pneumoniae* antibodies assays. The objectives of this study are to evaluate *Mycoplasma* antibody kits using recombinant antigens to improve specificity of results, and to review our current algorithm for *Chlamydia* serology.
Methods: One hundred and fifteen sera were tested with *M. pneumoniae* antibody kits from Virion, Medac, Savyon and AniLabsystems. Hemagglutination assay was used as reference method to confirm discordant results. Sixty-one sera from 52 patients, including 15 with documented respiratory infection, were tested with *C. pneumoniae* IgA and IgG kits from Medac, Savyon and Euroimmun. MIF was used as reference method to confirm discordant results.
Results: Agreement for at least 3 results or confirmation with hemagglutination assay established reliable diagnosis. Sensitivity was 100%, 100%, 90% and 90% and specificity was 92%, 96%, 100% and 96% for *M. pneumoniae* IgM from Virion, Medac, Savyon, and AniLabsystems kits respectively. Medac and Savyon *M. pneumoniae* IgG kits discriminated healthy from sick patients better than did Virion kit; agreement between Medac and Savyon was 89.5%, 91.4% and 81.9% for IgM, IgA and IgG respectively. Sensitivity of Medac, Savyon and Euroimmun *C. pneumoniae* IgA was 92%, 100%, 59%; accuracy, using MIF as reference test, was 90%, 94% and 67% respectively. Agreement between Medac and Savyon was 93.1%. Sensitivity of Medac, Savyon and Euroimmun for *C. pneumoniae* IgG was 100%, 100% and 80%; accuracy was 97%, 87% and 77% respectively.
Conclusion: Savyon *M. pneumoniae* kits show less or no threshold results compared with the 3 other methods. The IgG assay discriminates sick from healthy patients well, with good correlation with the Medac kit. To facilitate interpretation of the results, we decided to perform *C. pneumoniae* Savyon IgA kit, well correlated with MIF, while Medac *C. pneumoniae* IgG kit was preferred because of better specificity and quantitative determination.

R2228 Initial MIC and disc diffusion quality control ranges for BC-3781 using the CLSI Multi-Laboratory M23-A3 study design

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Objectives: To establish the disk diffusion (DD) and MIC quality control (QC) ranges for BC-3205, a novel semi-synthetic pleuromutilin derivative in the early stage clinical development for oral treatment of skin and skin structure infections (SSSI).
Methods: These QC studies for the 20-mcg BC-3205 disk and broth microdilution method follow the CLSI M02-A10 (2009), M07-A8 (2009) and M23-A3 (2008) document using eight laboratories, two lots of BC-3205 disks, three or more different medium lots. The results are presented as proposed QC ranges for four ATCC strains: *S. aureus* ATCC 25923, *H. influenzae* ATCC 49247, *S. pneumoniae* ATCC 49619 and *S. aureus* ATCC 29213 (MIC only). BC-3205 DD and MIC QC ranges were established per a CLSI M23-A3 study design. Ten replicates with each of 3 QC strains produced 1,440 zone diameters for two disk lots of

BC-3205 (20-mcg disk) provided by the Mast Group. Clindamycin (CL; 2-mcg disk), azithromycin (AZ; 15-mcg disk) and linezolid (LZ; 30-mcg disk) were utilized as control agents for DD, while retapamulin, AZ and levofloxacin were the control agents for MIC testing.
Results: Proposed QC ranges are listed in the Table. No significant differences (>1 mm) were noted between media or disk lots when testing either BC-3205 or control agents. *S. aureus* ATCC 25923 produced larger variations between laboratories with the most extreme laboratory modes having a 7 mm difference. One laboratory submitted significantly larger zone diameters for *H. influenzae* ATCC 49247 and was excluded from evaluation leaving seven laboratories for a valid CLSI QC study. MIC values for *H. influenzae* 49247 showed trailing endpoints of at least one dilution step. One laboratory was excluded from analysis due to outlier values. A 7 mm zone diameter is proposed for *S. pneumoniae* ATCC 49619 which includes 99.7% of all results. Excluding one aberrant laboratory from the MIC testing analysis produced all seven participant results within the proposed 0.06–0.25 mg/L range. All but one MIC result for the control agents were within expected ranges, when applicable. The control disks (LZ, AZ, CL) provided a valid internal control for the study, with 97.7 to 100.0% of zones within CLSI published QC ranges.
Conclusions: An acceptable QC range was established for the four QC organisms that will guide clinical and reference laboratories involved in clinical trials and facilitate the regulatory review process of BC-3205.

QC organism	MIC/Disk diffusion zone diameters for BC-3781:		
	Proposed range		% in range
	MIC (mg/L)	Zone diameter (mm)	
H. influenzae ATCC 49247	0.5–2	22–28	88.0 / 91.5 (94.3 / 99.8)[a]
S. aureus ATCC 25923	NA	25–33	– / 97.3
S. aureus ATCC 29213	0.06–0.25	NA	100.0 / –
S. pneumoniae ATCC 49619	0.06–0.5	20–26	98.6[a] /96.0

[a]One laboratory was excluded from analysis, *H. influenzae* QC range for MIC tests was not acceptable. NA = Not applicable.

R2229 Evaluation of four chromogenic media for the presumptive identification and differentiation of yeasts

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Objectives: Several commercial chromogenic media have been developed for species identification of clinical yeasts. In this comparative study, four chromogenic media, CHROMagar *Candida* (CAC) (CHROMagar, France), Albicans ID2 agar (AID) (bioMérieux, France), Candiselect medium (CS) (Biorad, France) and chromID *Candida* Agar (CAN2) (bioMérieux, France) were evaluated for the presumptive identification of yeasts.
Methods: Totally 125 clinical yeast isolates, 62 *Candida albicans*, 18 *C. tropicalis*, 17 *C. glabrata*, 13 *C. parapsilosis*, five *C. krusei,* five Trichosporon spp., three *C. kefyr,* one *C. guilliermondii* and one *Geotrichum candidum* were included in the study. The isolates were identified by germ tube test, morphological characteristics on corn meal tween 80 agar and API 20 C AUX system. The isolates were cultured to Sabouraud dextrose agar from stock cultures and after 48 hours streaked onto CAC, AID, CS and CAN2 plates. The plates were evaluated by considering the colour, texture of the colonies and the existence of halo around the colony by three different people after 24, 48 and 72 hours of incubation at 37ºC in the dark.
Results: All of the isolates grew well on the four media tested. The sensitivity and specificity values for *C. albicans* were detected as 100% and 100% for CAC; 98.4–100% and 96.8–100% for AID; 96.8–100% and 96.8% for CS; 98.4% and 96.8 for CAN2 at different incubation periods, respectively. These values for *C. tropicalis* were 94.4–100% and 100% for CAC; 83.3–94.4% and 96.3–98.1% for CS, respectively. CAC was found to be 11.8–88.2% sensitive and 100.0% specific for *C. glabrata*; 100% and 92.2% sensitive and specific for *C. krusei.* The sensitivity and specificity for *C. tropicalis* and *C. kefyr* were able to be calculated together as these species grow by forming the same colour on CAN2 and these values were found to be 90.5% and 99.9%, respectively.
Conclusion: In our study, CAC, AID, CS and CAN2 plates showed similar performances with respect to *C. albicans* identification and CAC and CS to *C. tropicalis*. As a result, CAC can be recommended as a reliable medium for the presumptive identification of *Candida* species as it can differentiate four species, *C. albicans*, *C. tropicalis*, *C. glabrata* and *C. krusei* successfully.

R2230 Incubation of fungal cultures: how long is long enough?

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Objective: Fungal cultures are traditionally incubated for 4 weeks or longer in order to maximize recovery of slowly growing fungi. However, the data in support of this is scarce. The purpose of this study was to determine the optimum incubation time for specimens in which moulds or yeasts are suspected.
Methods: 2216 dermatological and 820 non-dermatological specimens were prospectively analyzed. The day on which fungal growth was first noted, was recorded.
Results: Of a total of 1172 fungal isolates, 826 (70.5%) were detected by day 7, 1108 (94.5%) were detected by day 14, and 1165 (99.4%) were detected by day 21. Ten non-dermatological specimens were positive in the third week; all grew a fungus which was considered clinically non-relevant.
Conclusion: The results indicate that for specimens sent for detection of yeasts or moulds (except dermatophytes and systemic dimorphic fungi) an incubation period of 2 weeks is sufficient, whereas for dermatophytes 4 weeks are necessary. Based of these results and previous literature an algorithm for the incubation time of fungal cultures is proposed.

Table 1. Fungi isolated and time to detection

Species	No. isolated	No. detected by week			
		1	2	3	4
Candida spp.	424	392	25	7	–
Cryptococcus spp.	4	3	–	–	1
Yeasts, others	56	30	19	5	2
Aspergillus spp.	121	88	28	5	–
Moulds, others	348	217	104	26	1
Dermatophytes	216	94	105	14	3
Dimorphic fungi	3	2	1	–	–
Total	1172	826	282	57	7

R2231 Identification of *Streptococcus agalactiae* and investigation of intra-species variability using MALDI-TOF MS profiling

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Objectives: *Streptococcus agalactiae* is the main cause of neonatal infections and an increasingly frequent pathogen also in non-pregnant humans. A large number of different Sequence Types (STs) distributed over several major phylogenetic lineages or clonal complexes (CC) have been identified by multilocus sequence typing (MLST). STs 17 and 19 account for the majority of cases of *S. agalactiae* meningitis in infants while CCs 1, 12, 17, 19 and 23 are mostly associated with infections in adults. The presented study intends to investigate if MALDI-TOF mass spectrometry profiling (i) is suited for secure species identification of *S. agalactiae* and (ii) can also be used for analysis on the subspecies level.
Methods: 197 *S. agalactiae* strains characterized by MLST were analysed by MALDI-TOF mass spectrometry. Data processing was performed using the MALDI Biotyper 2.0 software (Bruker Daltonics, Germany). Mass spectra of each strain were compared with the mass spectra stored in the MALDI Biotyper database. To improve the database, new entries from different clonal complexes were created and introduced into the library. Further, to investigate subspecies variability, the spectra sets of isolates from different STs were investigated using the ClinProTools 2.0 and FlexAnalysis 3.0 (Bruker Daltonics, Germany) software to find characteristic markers for the different subtypes.

Results: MALDI-TOF MS correctly identified all the 110 *S. agalactiae* isolates of a first set at the species level with good [log(score) >2.0] to very good [log(score) >2.3] confidence. By introducing further references into the database characteristic for the major sequence types (ST1, ST10, ST17, ST19, and ST23), the identification results could be further improved [about 99% log(score) >2.3]. A second set of 87 *S. agalactiae* strains were identified at the species level with a very good log(score). Investigation of mass spectra from different STs revealed markers characteristic for two STs (ST1 and ST17, respectively) but also some minor variability inside the groups.
Conclusion: MALDI-TOF MS profile analysis is a very reliable tool for species identification of *S. agalactiae* but it also has a significant capability for subspecies identification. To evaluate the full potential of this, further studies will be necessary.

R2232 Seroprevalence and seasonal variation of *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* infection in Germany

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Objective: *Chlamydia pneumoniae* (CP) and *Mycoplasma pneumoniae* (MP) cause respiratory tract infections including community-acquired pneumonia in children and adults. Despite the availability of molecular diagnostic methods, detection of antibodies against CP and MP is a mainstay of diagnosis, especially in outpatients. We performed a laboratory-based multicenter surveillance in Germany to determine the prevalence of antibodies against CP and MP, detect seasonal variations, and age-dependent differences in seroprevalence.
Materials and Methods: Results of IgM, IgA, and IgG antibodies against CP and MP, determined by ELISA (medac, Wedel, Germany) were evaluated in 16,039 patients (55% female, 45% male, 0–96 years) in three medical laboratories located in North and South Germany. All serum samples submitted to the laboratories for CP and MP serology between June 2008 and May 2009 were included.
Results: The total seroprevalence indicating infection with CP and MP was 68% and 31%, respectively. The prevalence of CP infection increased with the age of patients (up to 85% above 90 years). Acute CP infection was determined in 9% of all patients respectively 13% of the seropositive patients and occurred predominantly in the age group of 1–10 years. Seroprevalence of acute infection showed peak rates between September and December. In contrast, past CP infection (52%) and current but inconspicuous findings (35%) in seropositive patients did not show seasonal variation. The prevalence of MP infection increased up to 50% between 16 and 30 years and declined with increasing age. Acute MP infection was determined in 4% of all patients respectively 12% of seropositive patients and occurred predominantly in the age group of 1–10 years. Seroprevalence of acute infection showed peak rates between November and December. Past MP infection was found in 75% and current findings in 13% of seropositive patients without seasonal variation. Serological evidence of acute coinfection with CP and MP occurred in less than 1% of patients.
Conclusion: According to our serological data, acute infection by CP and MP both occur all-season with a predilection in winter period. The prevalence of CP infections is increasing with age. In contrast MP infections decline in older population. The prevalence of acute CP and MP infections in our surveillance is comparable to results of international studies.

R2233 Detection of IgG antibodies to herpes simplex virus type 1 and 2 in various HIV-positive African populations by the BioPlex 2200 multiplexing immunoassay platform

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Objectives: The performances of commercial immunoassays in detecting herpes simplex virus type 1 (HSV-1) or type 2 (HSV-2) antibodies have been seemingly inconsistent for African and HIV-infected populations. We herein evaluated the new BioPlex 2200 immunoassay system (Bio-Rad Laboratories, CA, USA) to detect herpes-specific antibodies.
Methods: Sera were obtained after informed consent from HIV-1-positive patients followed in health care clinics of Bangui, the capital city of the Central African Republic, a country of high prevalences for HIV-1, HSV-1 and HSV-2 infections, including: (i) 51 adults clinically asymptomatic for herpes disease; (ii) 220 children born from HIV-infected mother clinically asymptomatic for herpes disease; (iii) 220 patients suffering from HSV-2 PCR-proven genital ulcer (recurrences); and (iv) 25 patients suffering from HSV-2 PCR-proven primary genital ulcer. All sera were tested for HSV-1 and HSV-2 specific antibodies by the fully automated bead-based multiplexing immunoassay BioPlex 2200 system.
Results: Among adults clinically asymptomatic for herpes disease, 90.2% and 45.1% were seropositive for HSV-1 and HSV-2, respectively. Among children clinically asymptomatic for herpes disease, 81.3% and only 5% were seropositive for HSV-1 and HSV-2, respectively. These observations are consistent with the natural history of HSV-1 and HSV-2 infections in sub-Saharan Africa, demonstrating early acquisition of HSV-1 in children, whereas HSV-2 infection appears largely acquired in later age of sexual life. Among patients suffering from HSV-2 genital recurrences, 95.9% were seropositive for HSV-2. Finally, among patients suffering from HSV-2 primary genital ulcer, 24.0% were seropositive for HSV-2 at inclusion. Thus, HSV-2 seropositivity appears highly sensitive to predict HSV-2 recurrency in patients with clinically symptomatic genital ulcer, and may also be found very early in seroconverting patients with HSV-2 primary genital ulcer. Comparisons of BioPlex 2200 results with those obtained by conventional HerpesSelect glycoprotein G2 immunoassay will be discussed.
Conclusion: The BioPlex 2200 immunoassay system provides a useful tool for HSV type-specific serology to be used in clinical care of African patients suffering from herpectic genital disorders, as well as in intervention studies in the field conceived to control genital HSV-2 infection representing the main modulatory co-factor of heterosexual HIV transmission in sub-Saharan Africa.

R2234 Evaluation of modular CMV IgG and IgM assay on challenging patient samples

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Objectives: Automated assays for cytomegalovirus (CMV) IgG and IgM determination in serum and/or plasma have been developed by several companies. The purpose of this study was to compare the performance of the Roche CMV IgG and IgM immunoassays with the Beckman Coulter, bioMérieux and Siemens assays using specifically selected patient samples. We focused on sample results in the intermediate range (grey zone) as determined by Vidas.
Methods: CMV IgG and IgM determination was performed on Access (Beckman coulter), Immulite (Siemens), Modular (Roche) and Vidas (bioMérieux) using 71 patient sera. We selected similar quantities of sera in the negative, the doubtful, the weakly positive (defined as positive <2× cut-off) and positive reference interval, determined by our routine method (Vidas). Relative agreement was calculated as the number of concordant samples/number of all tested samples (grey zone results considered as positive). The correlation between positive sera was calculated as number of concordant positive samples/number of samples tested positive by one method (grey zone results not taken into account).
Results: The relative agreement (%) for CMV IgG/IgM between Modular on one hand and Access or Immulite or Vidas on the other hand was respectively 94/83, 79/68 and 91/79. The correlation (%)between positive sera for IgG/IgM between Modular and Access or Immulite or Vidas was respectively 94/81, 76/61 and 88/87. For both assays, 79% of positive samples on Modular were positive with all methods. For IgG/IgM respectively 0/0, 3/5, 1/0 and 9/5 samples were exclusively reactive on Access, Immulite, Modular and Vidas. For IgG/IgM respectively 2/3, 7/4, 2/6 and 1/0 samples were exclusively negative on Access, Immulite, Modular and Vidas. From the selected 28 samples with doubtful or weakly positive result on Vidas, respectively

6, 3 and 5 remained doubtful or weakly positive for Access, Immulite and Modular (table 1).

Conclusion: Using selected patient samples, based on Vidas results, the relative agreement for both IgG and IgM assays is the greatest between Modular and Access. The amount of results in the grey zone is similar for Access, Immulite and Modular. The limited amount of sera that are exclusively reactive on Modular supports a good specificity, similar to the Access.

Table 1. Overview of results of CMV IgG and IgM determinations of 71 selected patient samples by Roche (Modular), Beckman Coulter (Access), Siemens (Immulite) and bioMérieux (Vidas)

CMV IgG (U/ml)

Modular	Access				Immulite				Vidas				
	<11	11–<15	15–<30	⩾30	<0.9	0.9–<1.0	⩾1.0–<2.0	⩾2.0	<4	⩾4–<6	⩾6–<12	⩾12	
<0.5	17	1	0	1	14	1	3	1	8	6	5	0	19
0.5–1.0	3	0	1	0	2	0	1	1	1	1	2	0	4
⩾1.0–<2.0	1	0	1	0	1	0	0	1	0	2	0	0	2
⩾2.0	1	2	3	40	7	1	6	32	1	2	10	33	46
	22	3	5	41	24	2	10	35	10	11	17	33	71

CMV IgM (cut-off index)

Modular	Access				Immulite				Vidas				
	<0.8	0.8–<1.0	1.0–<2.0	⩾2.0	<0.9	0.9–<1.0	⩾1.0–<2.0	⩾2.0	<0.7	⩾0.7–<0.9	⩾0.9–<1.8	⩾1.8	
<0.7	28	4	2	1	21	2	4	8	20	11	2	2	35
⩾0.7–<1.0	2	1	3	1	2	0	2	3	0	1	3	3	7
⩾1.0–<2.0	2	1	0	5	2	0	0	6	0	3	1	4	8
⩾2.0	1	0	3	17	2	0	3	16	0	0	7	14	21
	33	6	8	24	27	2	9	33	20	15	13	23	71

R2235 Comparative study between three automated immunoassays, the new Vidas, and Liaison tests for the detection of EBV antibodies

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Objectives: The aim of the study was to compare the new VIDAS tests for the detection of VCA / EA IgG, VCA IgM, and EBNA IgG antibodies with the IMMULITE and LIAISON tests on characterized samples, in terms of sensitivity, specificity and concordance with the patient clinical status.

We also evaluated the three techniques on 7 seroconversion panels.

Methods: 286 retrospective samples from outpatients were tested with the 3 methods. The sample status was determined according to the Bioplex 2200 reagent (Biorad) routinely used in our laboratory. The samples consisted of 95 acute infections (AI), 97 negative patients (Neg) and 94 past infections (PI). Seven seroconversion panels characterized by Enzygnost EBV IgG/IgM and BMD EBNA IgG reagents were tested with the 3 methods.

Results: The sensitivity obtained on AI for VIDAS VCA IgM, IMMULITE VCA IgM and LIAISON VCA IgM was 100%, 74.2% and 100% respectively; the specificity on Neg was 100% for all reagents, and the specificity on PI was 94.4%, 96.7% and 96.6% respectively. The sensitivity on PI for VIDAS VCA IgG, IMMULITE VCA IgG and LIAISON VCA IgG was 98.9%, 97.9%, 98.9%. Concerning primo-infections, among 95 samples, only 38 samples were detected VCA/EA IgG >0 with the Bioplex technique considered as the reference method. Therefore, reporting VCA IgG relative sensitivity results was not considered. The specificity was 100% on Neg for VIDAS and LIAISON and 93.75% for IMMULITE. The sensitivity on PI for VIDAS EBNA IgG, IMMULITE EBNA IgG and LIAISON EBNA IgG was 98.9%, 100%, 100% respectively, the specificity on Neg was 100%, 97.9% and 98.9% respectively, and the specificity on AI was 95.6%, 91.4% and 98.7% respectively.

Concordance to clinical status is as follows:

VIDAS EBV: 100% on Neg, 100% on AI and 98.8% on PI.

IMMULITE EBV: 94.7% on Neg, 75.9% on AI and 97.7% on PI.

LIAISON EBV: 100% on Neg, 100% on AI and 100% on PI.

The seroconversion panels did not show any delay in IgM detection with the 3 methods compared with the reference method.

Conclusion: VIDAS and LIAISON showed good performance in terms of sensitivity and specificity. Concordance with the reference status was highly acceptable.

IMMULITE showed poor VCA IgM sensitivity and EBNA IgG specificity results in the primary infections group, leading to poor concordance with the reference status. A high number of equivocal results were obtained with LIAISON EBNA IgG. In conclusion, VIDAS appears to be a very reliable automated method for EBV serology.

R2236 Comparison of two rapid assays used for the diagnosis of *Clostridium difficile* infections

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Introduction: The rapid detection of toxin-producing strains of *Clostridium difficile* is very important for management of infected patients. The rapid test that detect toxin A+B are a alternative to convencional culture. Recently, a rapid membrane enzyme immunoassay has been developed for the simultaneous detection of toxins A+B and glutamate dehydrogenase antigen (GDH), a *C. difficile* specific enzyme.

Objectives: The aim of this study was to compare a new rapid membrane enzyme immunoassay (method 1) and an immunochromatographic assay (method 2) for the diagnosis of *Clostridium difficile* infection from clinical specimens. The samples with discordant results between both assays were analyzed by a third method, a commercial real time PCR technique that detects toxin B gene.

Material and Methods: 92 stool samples from patients with diarrhea were analyzed between January 2008 and March 2008 for diagnosis of *Clostridium difficile* infections (CDI) in the routine laboratory practice. The samples were studied for TechLab C. Diff Quik Chek Complete™ test (method 1) and Remel X/pect Toxin A/B immunochromatographic assay (method 2) according to the manufacter's instructions. The Xpert™ *C. difficile* assay, performed on the Cepheid GeneXpert DX System is a multiplex real-time PCR assay for detection of genes for Toxin B (tcdB), Binary Toxin (cdt) and tcdC gene delection nt 117 in less than 1 hour. The system requires the use of single-use disposable cartridge that hosts the processes of DNA extraction and PCR.

Results: 76 samples were negative with both methods, 3 samples were positive with both methods and 12 samples were negative with method 2 and positive with method 1 (8 GDH(+) with tox AB(+) and 5 GDH(+) with tox AB(−)). In the 12 discordant results, the PCR assay detected Toxin B gene and the patients were diagnosed of *Clostridium difficile* infection in conjunction with the patient clinical history. The incidence of CDI using method 1 was 16% (15 of 92) and 3.2% (3 of 92) using the method 2. Comparison between the two methods was done using McNemar's test with the continuity correction ($p = 0.0015$). The difference was found to be very statistically significant.

Conclusions: The TechLab C. Diff Quik Chek Complete™ test detected more positive results for diagnosis of *C. difficile* infection than the X/pect Toxin A/B immunochromatographic assay. These results were confirmed by real time PCR.

R2237 Development and qualification of an immunodiagnostic assay for the detection of 13 *Streptococcus pneumoniae* serotype-specific polysaccharides in human urine

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Objectives: To improve detection rates of pneumococcal infection we developed a sensitive multiplex assay that can identify 13 serotype-specific S. pneumococcal polysaccharides (PnPs: 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F) in human urine.

Methods: Based on Luminex technology, this assay was developed using microspheres coated with PnP specific monoclonal antibodies (mAbs), to detect all 13 types in a single well of a urine sample. Positivity for a specific serotype was based on cutoff values established from a panel of 400 control urine samples which were calculated relative to a standard curve run on each assay plate. Although designed as a qualitative assay, this method is able to quantify the amount of PnPs in a sample and was qualified to address specificity, accuracy and precision.

Results: The assay was specific in that significant signals were detected only when each polysaccharide was paired with its homologous mAb-coated microsphere. The lower limit of linearity ranged from 0.10–8.5

pg/mL. Qualification experiments showed that the assay has acceptable accuracy (bias ratio: 76.5–<138%) and precision (%RSD: 6.8–<30%). Preliminary assessments of clinical samples obtained from CAP patients demonstrate that this assay is significantly more sensitive than blood culture in identifying S.Pn. serotypes.

Conclusions: Results demonstrate that this assay is a noninvasive, sensitive and reproducible method to detect the presence of S.Pn. polysaccharides in urine and has the potential to be a useful diagnostic test to support clinical as well as epidemiological evaluation of pneumococcal disease.

R2238 Performance of Vitek 2 for the detection of carbapenemase and extended-spectrum β-lactamase activity in selected *Klebsiella pneumoniae* isolates and evaluation of different methods

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Objectives: We have previously isolated a genetically related cluster of blaIMP-1 metallo-β-lactamase and extended spectrum-β-lactamase (ESBL) positive *Klebsiella pneumoniae* isolates (n: 12) which initially were detected as imipenem (IPM) and/or meropenem (MEM) resistant by Vitek 2 (bioMérieux). Manual tests like disc diffusion (DD) and Etest failed to confirm these results and to resolve the discrepancies we investigated these isolates in detail using different cards and versions of Vitek 2 along with various phenotypic methods.

Methods: The isolates were tested with different cards (AST-GN09, GN13, N091, N092) and versions of Vitek 2 (AIX 4.01, AIX 4.03, PC 3.01). ESBL activity was explored using double disc synergy test, CLSI's ESBL confirmatory test (ECT) using ceftazidime (CAZ) and cefotaxime (CTX) discs in combination with clavulanic acid (CLA) and also by Etest ESBL strips (AB Biodisk). Carbapenemase activity was investigated with modified Hodge test (MHT), EDTA-based combined disc (CD) assay with different concentrations of EDTA, and by IPM/IPM+EDTA Etest strips. MIC values for IPM and MEM were established with broth dilution, additionally IPM, MEM, ertapenem and doripenem MIC values were determined with Etest strips.

Table. Investigation of carbapenem susceptibility by different methods in blaIMP-1 metallo-beta-lactamase positive *Klebsiella pneumoniae* isolates

	Strains											
	Klb-21	Klb-62	Klb-73	Klb-85	Klb-200	Klb-201	Klb-202	Klb-223	Klb-232	Klb-234	Klb-266	Klb-275
MIC by broth dilution (ug/ml)												
Imipenem	4	4	4	4	4	4	4	4	2	4	4	4
Meropenem	8	16	16	16	16	16	16	16	16	8	16	16
MIC by Etest (ug/ml)												
Imipenem	1	0.5	0.5	0.5	0.38	0.75	0.38	0.75	0.5	1	0.75	1
Meropenem	2	0.5	0.5	0.5	0.75	1	0.5	0.75	1	2	1.5	1.5
Ertapenem	3	4	4	12	3	⩾32	0.19	2	6	3	6	2
Doripenem	0.5	0.5	0.75	2	0.5	4	0.016	0.75	0.75	0.5	1	1
Disc diffusion zone diameter (mm)												
Imipenem	18	19	20	17	21	19	22	19	20	18	19	18
Meropenem	19	18	19	19	18	19	20	19	18	18	17	19
Ertapenem	15	14	14	13	15	17	19	13	13	12	14	14
AST-GN09 card, Vitek 2 ver. AIX 4.01												
Imipenem	8	8	⩾16	⩾16	⩾16	⩾16	⩾16	⩾16	⩾16	⩾16	⩾16	8
Meropenem	1	1	2	2	2	2	⩾16	2	8	2	2	2
Carbapenemase*	No	No	No	No	No	No	No	No	No	No	No	No
AST-GN09 card, Vitek 2 ver. AIX 4.03												
Imipenem	8	4	2	8	4	4	2	8	8	4	4	8
Meropenem	2	2	1	4	2	1	1	4	4	4	2	2
Carbapenemase	No	Yes	No	Yes	Yes	No	No	Yes	Yes	No	Yes	Yes
AST-GN13 card, Vitek 2 ver. AIX 4.03												
Imipenem	8	4	2	8	4	4	2	4	4	4	4	4
Ertapenem	4	⩾8	⩾ 8	⩾8	⩾8	⩾8	4	⩾8	⩾8	⩾8	⩾8	⩾8
Carbapenemase	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
AST-GN09 card, Vitek 2 ver. PC 3.01												
Imipenem	8	8	4	4	2	4	2	4	4	4	8	8
Meropenem	1	2	1	2	1	1	1	1	1	1	1	1
Carbapenemase	No	Yes	No	Yes	No	No	No	No	No	No	No	Yes
AST N091 card, Vitek 2 ver. PC 3.01												
Imipenem	4	4	2	4	4	4	⩽1	4	8	2	4	8
Meropenem	1	1	2	2	1	1	1	2	4	1	1	2
Ertapenem	4	⩾8	⩾8	⩾8	⩾8	⩾8	4	⩾8	⩾8	⩾8	⩾8	⩾8
Carbapenemase	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
AST-N092 card, Vitek 2 ver. PC 3.01												
Imipenem	4	4	4	8	8	8	⩽1	8	⩾16	4	8	⩾16
Meropenem	1	1	2	1	2	1	1	1	4	1	1	2
Ertapenem	⩾8	⩾8	⩾8	⩾8	⩾8	⩾8	4	⩾8	⩾8	4	⩾8	⩾8
Carbapenemase	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

*Vitek 2 expert system warning for carbapenemase production as recognized by the phenotype.

Results: All Vitek 2 cards containing an ESBL well (AST-GN13, -N091, -N092) failed to detect any ESBL activity. Also, Etest ESBL strips (both CAZ/CAZ+CLA and CTX/CTX+CLA) yielded indeterminate results for all isolates and ECT failed in 9 isolates with CAZ/CAZ+CLA discs and in 10 isolates with CTX/CTX+CLA discs. However; synergy was observed between aztreonam and amoxicillin+CLA in all strains. DD and Etest methods failed to detect the carbapenem resistance in most instances whereas Vitek 2 showed overall a good performance with accompanying warnings for carbapenemase activity triggered by the expert system (Table). MHT revealed carbapenemase activity in all isolates. CD assay was most successful when 10 ul of 0.5 M EDTA was used. The IPM/IPM+EDTA Etest method remained futile because of the low IPM MIC values obtained.

Conclusion: CLSI's ECT, Etest ESBL strips and Vitek 2 ESBL wells all yielded unsatisfactory results for the challenge strains we tested. In the other hand, carbapenem resistance detected by Vitek 2 would remain undetected if only DD or Etest methods were used. An automated susceptibility system, with an expert system incorporated, seems very helpful in occasions where an unusual resistance mechanism is encountered.

R2239 Protein S100B feasibility in the diagnostics of central nervous system infection

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Objectives: The aim of the study is to evaluate the feasibility of S100B protein (S100B) as a marker of the central nervous system infection. The S100B is the serum marker used in rapid diagnostics of the traumatic or ischaemic damage of the central nervous system (CNS). The CNS marker would be great improve in the diagnostic before performing a lumbar puncture in ill-defined cases. The research tested whether the level of S100B is higher in serum and spinal fluid in patient with the CNS infection compared to those without.

Methods: The S100B was tested in 65 child patients in Child infectious diseases departement. 51 patiens were admitted due to suspected CNS infection. 14 were healthy controls. Patients underwent standard diagnostic procedures including blood sampling, lumbar puncture, serology, cultivation and PCR. All patients were tested for S100B from serum and spinal fluid with electroimunoassay method. The CNS infection was confirmed in 43 of 51 patients. The CNS infection was not proved by standard methods in 8 patients. These patiens were added to the control group. Values of S100B in the serum and in the spinal fluid were then compared for both groups.

Results: No significant difference of S100B values was found between the group of patients with the CNS infection and control group. From 43 patiens with confirmed CNS infection only 6 had level of S100B above cutt-off value for their age.

Conclusion: The results indicate that the protein S100B is not promising marker of the central nervous system infection.

R2240 Detection of anaerobic bacteria in patients with pericoronitis

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The oral cavity is one of the most complex and heterogeneous body parts inhabited predominantly anaerobic microorganisms, these may act synergistically to produce a series of purulent infections of mixed origin from which it is worth noting the pericoronitis. Has recently increased aid to dental patients pericoronitis therefore for the general dentist to the oral surgeon is important to continue therapy for the prevention and treatment of this entity, so the detection and identification of infectious agents that cause is critical because it guides us towards a correct antibiotic avoiding unnecessary use of antibiotics, thus avoiding the risk of bacterial resistance. Outside our borders have been made studies on the detection and identification of microorganisms involved in pericoronitis, being the most recent studies published in France and the United States of America which are highlighted in both the predominance of anaerobic

bacteria such as microorganisms producers of this condition, but not in our country where there are few studies performed. While it is true that there have been numerous studies related to detection and identification of microorganisms in other lesions of the oral cavity in dental caries, subprothesic stomatitis, periodontal disease, pulpal and periapical infections, has not been given importance to the microbiota associated with pericoronitis There is fully justifies this study as well as enabling the detection and identification of the causative agents of pericoronitis also serve to identify the associated microbial species, which help in selecting the most appropriate antimicrobial therapy for the treatment of this infection and serve as a starting point or background for other studies of the microflora of this entity. *Bifidobacterium* spp. (42%) B adolescentes (17%), Veillonellas spp (17%), *P. loeschii* (8%), *P. melaninogenica* (8%) y *P. oralis* (8) were detecte in this study.

R2241 Innovative Elecsys CMV IgM assay design using recombinant antigens reduces significantly the detection of mature anti-CMV IgM antibodies

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Objectives: Primary acute maternal CMV infection during pregnancy carries a high risk of intrauterine transmission which may result in severe fetal damage. In the absence of acute clinical manifestation, CMV infection is usually diagnosed by means of serology. A positive IgM result is normally caused by acute or recent primary infections, but also can occur due to recurrent CMV infections or due to unspecific polyclonal stimulation. Likewise, CMV IgM antibodies may persist for several months after acute infection. These multiple causes of IgM positivity in CMV serology hamper the reliable and straightforward diagnostics of acute/recent primary CMV infection and necessitate further confirmation analysis.
The performance of the Elecsys CMV IgM assay was evaluated with samples of pregnant women containing persistent or reactivation-induced CMV IgM antibodies.
Methods: Elecsys CMV IgM is an electrochemiluminescence immunoassay using recombinant antigens in a μ-capture format. A new concept of recombinant CMV antigen design reduces significantly the detection of mature anti-CMV IgM antibodies as induced by persistent infection or reactivation.
116 frozen serum samples without clinical evidence of a recent primary CMV infection, however, presenting with low CMV IgM values and high-avidity IgG in routine assays, were tested with Elecsys CMV IgM, DiaSorin Liaison CMV-IgM, Siemes Enzygnost A-CMV-IgM, Medac CMV-IgM-ELA and Abbott Architect Anti-CMV IgM.
Results: Elecsys CMV IgM yielded fewer positive results with samples of pregnant women with past CMV infection (characterized by low CMV IgM and high-avidity IgG in routine assays) when compared to comparison assays.
Conclusion: Elecsys CMV IgM uses highly innovative assay design based on recombinant antigens, which significantly reduces the detection of anti-CMV IgM in patients with past CMV infections. As a consequence, less confirmatory testing is required.

Methods for antibacterial susceptibility testing

R2242 Antibiotic susceptibility of *Clostridium difficile*

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Objectives: Reports of resistance to metronidazole and intermediate resistance to vancomycin emphasize the need for surveillance of antibiotic susceptibilities of *C. difficile*, CD027 and other hyper virulent or epidemic strains. The aim of the study was to evaluate the use of disk diffusion in detecting resistance against *C. difficile*.

Methods: Primary clinical isolates of *C. difficile* (n = 122) from Danish hospitalised patients with diarrhoea in 2008 were characterized by E-test and disk diffusion. The isolates were tested against vancomycin, metronidazole, clindamycin, erythromycin, and moxifloxacin as recommended in Denmark. Disk diffusion method was performed on both Anaerobe agar (Statens Serum Institut, Denmark) and Sensitivity agar with NAD (Biomérieux). MIC determination by E-test was performed on *Brucella* agar (Statens Serum Institut, Denmark). Disk diffusion was compared to E-test by linear regression and using interpretive criteria available from the CLSI.
Results: All strains were susceptible to metronidazole and vancomycin with MIC < 2 mg/L.
See Table.

Table. Antibiotic susceptibility of *C. difficile* and correlation between E-test and disk diffusion

Antimicrobial agent	MIC (mg/L)		Susceptible	r^2		VME[1] (%)		ME[2] (%)	
	MIC 50	Range	(%)	NAD[3]	AA[4]	NAD	AA	NAD	AA
Clindamycin	4	0.75–256	86	0.65	0.59	3	4	4	5
Erythromycin	0.38	0.094–256	89	0.80	0.81	0	0	0.8	0.8
Moxifloxacin	0.75	0.25–32	89	0.72	0.76	0	0	0	0

[1] VME: Very major error. Sensitive by disk diffusion. Resistant by E-test. [2] ME: Major error. Resistant by disk diffusion. Sensitive by E-test. [3] NAD: Sensitivity agar with NAD. [4] AA: Anaerobe agar.

There was an acceptable to good correlation between findings from disk diffusion and E-test for all antibiotics on both media, but for clindamycin we found an unacceptable high frequency of VME and ME.
Conclusions: Disk diffusion was a reliable method when evaluating susceptibility to moxifloxacin and erythromycin. However, disk diffusion was inadequate for the detection of clindamycin resistance and should therefore be supplemented with either E-test or broth dilution method.

R2243 Use of standardized method for testing the sporicidic activity of new hybrid materials with embedded silver nanoparticles

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Objectives: The use of standardized method for testing the sporocidic activity of new hybrid materials based on polyvinyl alcohol (PVA)/tetraethyl orthosilicate (TEOS) with embedded silver nanoparticles (AgNp's), synthesized by two different methods, will allow to compare the results obtained and the choice of the appropriate method of synthesis.
Methods: Hybrid materials on the basis of PVA and TEOS with embedded AgNp's with different silver concentrations (3.7 mg AgNO3/ml, 1.8 mg AgNO3/ml, 0.9 mg AgNO3/ml) were synthesized using sol-gel method by two strategies. The first strategy was based on in-situ formation of AgNp's via thermal annealing of the PVA/TEOS hybrid films. The second strategy consists of forming AgNp's in PVA/TEOS matrix without any thermal annealing of the films. The standard paper disks (d = 6±0.5 mm) were impregnated with 5μl of the solutions and allowing them to dry at room temperature. For the thermal treated materials, disks were annealed at 100ºC for 1 h. Thus prepared disks were used for testing the sporicidic activity of these hybrid PVA/AgNp's/TEOS materials by the pour agar method with test microorganism *Bacillus subtilis* ATCC 6633 *(B. subtilis)*.
Results: The results obtained by triple testing of the samples, showed that all tested hybrid materials with embedded AgNp's possessed sporicidic activity. The synthesized materials demonstrated sporicidic activity against *B. subtilis* in all used silver concentrations by the appearance of an inhibition zone (ranging from 10.5 to 11.5 mm). The difference in the zone at different silver concentrations was in the range of 0.5 to 1 mm regardless of the methods.
Conclusion: The most pronounced zones of inhibition were demonstrated at hybrid PVA/AgNp's/TEOS materials with highest silver concentration (3.7 mg AgNO3/ml) in the both methods. The proved sporicidic activity extends the possible applications of such hybrid materials as coatings in the clean rooms like surgery, industrial and laboratory places where the contaminations with spore cultures could affect negatively.

R2244 Evaluation of the VITEK® 2 system for the susceptibility testing of various *Streptococcus* sp. including *S. pneumoniae* 1

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Objective: The VITEK 2 System provides rapid, automated identification and susceptibility testing of bacterial isolates including *S. pneumoniae* (SPN). With the increasing prevalence of antimicrobial resistance, the ability to quickly and easily perform susceptibility testing on other species of streptococci is becoming more important. The purpose of this study was to determine whether susceptibility testing on VITEK 2 could be expanded to include *Streptococcus viridans* (VIR) group and β-hemolytic streptococci (BS) for 11 antimicrobials: ampicillin (AM), cefotaxime (CTX), ceftriaxone (CRO), linezolid (LNZ), penicillin (PEN), trimethoprim/sulfamethoxazole (SXT), clindamycin (CM), erythromycin (E), levofloxacin (LEV), tetracycline (TE), vancomycin (VA) and the detection of inducible clindamycin resistance (ICR).
Methods: Over 600 isolates representing 34 species were tested in VITEK 2 investigational use only (IUO) cards containing varying concentrations of the different antimicrobials. All strains were tested on both IUO cards and the CLSI broth microdilution reference method. Growth data were collected from the VITEK 2 cards and compared to the reference MIC results. Analyses were then developed using these data.
Results: Overall essential agreement from the development isolates for each group is shown in Table 1.
Conclusion: Essential agreement for all drugs with the three organism groups exceeded 93%. These development data indicate that the VITEK 2 can accurately determine susceptibility to the above-mentioned drugs for various *Streptococcus* sp.
[1] These new tests are not yet available for commercial use and the United States FDA has not cleared them for use with the VITEK 2.

Table 1

	%EA											
	AM	CTX	CRO	LNZ	PEN	SXT	CM	E	ICR	LEV	TE	VA
SPN	94.5	95.6	99.3	100	100	97.9	95.8	98.9	N/A	99.6	99.6	97.7
VIR	98.4	97.4	94.7	100	97.4	N/A	98.5	99.5	N/A	100	95.5	93.3
BS	100	99.4	100	100	100	98.5	97.6	98.2	100	98.8	95.1	97.6

R2245 Evaluation of a MicroScan Dried Overnight panel for detection of inducible clindamycin resistance in staphylococci

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Objectives: The CLSI recently added a broth microdilution reference method as an alternative to the disk approximation or D-Zone disk test to detect inducible clindamycin resistance in staphylococci. The accuracy of a test for detection of inducible clindamycin resistance (ICd) on MicroScan Dried Overnight Gram Positive panels was examined with a set of challenge staphylococci.
Methods: 75 erythromycin nonsusceptible, clindamycin susceptible challenge staphylococci [41 *S. aureus*, 17 *S. epidermidis*, and 17 other coagulase-negative staphylococci (CNS)] were tested and the MicroScan test results were compared to a pre-determined, expected D-Zone result. Panels were inoculated using both turbidity and the Prompt methods of inoculation, and the panels were read by the WalkAway System, the autoSCAN-4 instrument, and visually.
Results: Agreement for challenge isolates was 100% for all inoculum and read methods.
Conclusion: The ICd test on MicroScan Dried Overnight Gram Positive panel demonstrated excellent correlation with the CLSI D-Zone test for detection of inducible clindamycin resistance in staphylococci.

Read method	Inoculation method	No. tested	Categorical agreement	Sensitivity	Specificity
Manual	Turbidity	75	100	100	100
WalkAway	Turbidity	75	100	100	100
autoSCAN 4	Turbidity	75	100	100	100
Manual	Prompt	75	100	100	100
WalkAway	Prompt	75	100	100	100
autoSCAN 4	Prompt	75	100	100	100

R2246 Comparison of MicroScan LabPro AlertEX System software rules to EUCAST expert rules for Enterobacteriaceae and β-lactam antimicrobial agents

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Objectives: The EUCAST Expert rules provide assistance to clinical microbiologists in the interpretation of antimicrobial susceptibility testing (AST). Automated microbiology AST systems such as MicroScan likewise have software to assist in interpretation of results, and can also be customized by the user. It is important for the user of the software to know the concordance.
Methods: Rules listed in EUCAST Expert rules, version 1, April 2008 for Enterobacteriaceae and β-lactam drugs were compared to those in MicroScan LabPro AlertEX System multiregional software, v3.01 (France v3.03).
Results: EUCAST Expert Rule Table 1 lists intrinsic resistance present in 16 species of Enterobacteriaceae with 8 β-lactams. There is complete concordance with the AlertEX software including separate alerts and expert rules for 7 drugs and for 15 of the organisms. Concordant rules exist for the 8th drug, cefamandole, but the drug is not available for testing. MicroScan additional rules are listed for antimicrobial agents not detailed by EUCAST, and correspond to antimicrobial class. Additional rules are also listed for ampicillin/sulbactam. Some differences exist for *C. freundii, Enterobacter* spp. and *Providencia* spp. with cefuroxime, and *S. marcescens* with cefoxitin. There are no AlertEX system rules for Escherichia hermannii.
EUCAST Expert Rule Table 5 only lists 1 exceptional phenotype that applies to Enterobacteriaceae: resistance to ertapenem and meropenem, and resistance to imipenem for Enterobacteriaceae other than *Proteus* spp. The AlertEX system has both a general alert for carbapenem resistance, as well as specific rules based on MIC and not interpretation that correspond to the current CLSI-recommended detection of KPC enzymes based on MIC. The AlertEX system excludes *Morganella* as well as *Proteus* spp. from the imipenem rule.
EUCAST Expert Rule Table 9 lists interpretive rules. ESBL-positive Enterobacteriaceae susceptible to cephalosporins and aztreonam are to be reported as Intermediate, and intermediate results reported as Resistant. The AlertEX system reports 16 of the more common Enterobacteriaceae, including *K. oxytoca* and *C. koseri,* as resistant, and includes penicillins. EUCAST indicates ESBL-negative organisms are to be reported as found, and the AlertEX system follows that guideline.
Conclusion: System-provided MicroScan AlertEX rules correspond to those recommended by EUCAST Expert rules with some minor noted differences.

R2247 Vancomycin MICs for methicillin-resistant *Staphylococcus aureus*

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Objectives: In a recent letter (Prakash V, et al. AAC, 2008) and more recently in an article by Leonard et al. (Leonard SN, et al. JAC, 2009) the significant differences found between microdilution and Etest have clearly been highlighted in the detection of vancomycin MIC for *Staphylococcus aureus*, with an overestimate of the results obtained with Etest compared to those obtained by microdilution.

Given that the increase in vancomycin MICs below the breakpoint level seems by now to be extended inexorably everywhere (Rybak MJ, et al. Am J Health-Syst Pharm. 2009), it is fundamental that the microbiologist makes the best diagnostic options available to the clinician in order to correctly detect the phenomenon. As a result of the kinetic/dynamic studies that have clearly shown a greater probability of reaching an optimal AUC/MIC ratio when vancomycin MIC is 1 mg/L than when the MIC is >1–2 mg/L, whilst being aware that the difference of even just one dilution in the MIC can lead to a potential failure of therapy especially in case of respiratory or systemic infections (Pea F, et al. CID. 2006; Soriano A, et al. CID. 2008), we have recently decided to compare three different methods to assess concordance/discordance in the measurement of the level of vancomycin MIC below the sensitivity breakpoint.

Methods: Sensititre, GPALL1F panel (Trek Diagnostic System), Vitek 2, card AST-P580 (bioMérieux), and Etest, vancomycin strip on Mueller-Hinton agar plates (bioMérieux), using a 0.5 McFarland standard to prepare inoculum. For the evaluation we used 80 nonduplicate MRSA clinical isolates collected in 2008, kept at −80ºC and subcultured twice prior to being tested. Quality control of each antimicrobial method used was performed, as stated by the manufacturer, using *Staphylococcus aureus* ATCC 29213.

Results: The comparison of the three methods, whose results are highlighted in Table 1, confirms the overestimate of Etest compared to broth microdilution, according to the literature (Prakash V, et al. AAC, 2008) and of Rybak group (Leonard SN, et al. JAC, 2009), whilst it also shows that Vitek 2 cannot correctly detect MIC below the sensitivity breakpoint.

Conclusion: Microdilution, in this case undertaken with Sensititre, a commercial semi-automated microtitre broth dilution method that can be easily used in all microbiology laboratories, actually represents the most suitable method correctly measuring the level of vancomycin MIC below the sensitivity breakpoint.

Vancomycin MIC (mg/L)	Etest (%)	Sensititre (%)	Vitek 2 (%)
0.5	0 (0)	12 (15)	48 (60)
1	7 (9)	64 (80)	31 (39)
1.5	44 (55)		
2	29 (36)	4 (5)	1 (1)

R2248 Detection of resistance to mecillinam in *E. coli* using the BD Phoenix™ automated microbiology system

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Objective: Mecillinam (MEC), a semisynthetic β-lactam antibiotic with activity against many Gram-negative bacilli, provides an effective therapeutic option for uncomplicated urinary tract infections. Though resistance to MEC is unusual in most wildtype *Escherichia coli*, the ability to detect true resistance is imperative. The unique, comparatively slow bactericidal activity of MEC can result in elevated false resistance rates *in vitro* in broth-based AST methods. In this study, MEC, an antibiotic under development for use in the BD Phoenix™ Automated Microbiology System (BD Diagnostics, Sparks, Maryland), was evaluated against *E. coli* isolates and the recommended agar dilution reference method.

Methods: A total of 203 clinical and challenge set *E. coli* isolates, including 61 MEC non-susceptible strains, were included in the evaluation. Among these, 29 were previously confirmed as ESBL and AmpC-producing strains. Phoenix MEC reproducibility was also evaluated with 7 isolates, and daily QC was monitored. Each strain was tested in parallel in both the Phoenix System and the CLSI-recommended agar dilution reference method (ADM). Bacterial suspensions were adjusted to the equivalent of a 0.5 McFarland standard, then inoculated simultaneously into the BD Phoenix System and the ADM. Phoenix panels containing MEC were placed into the BD Phoenix instrument for incubation and automated reading to completion. ADM plates were incubated at 35ºC for 18–20 hours in ambient air and read manually for MIC endpoint determination. Mecillinam breakpoints and QC strain ranges were based on those recommended in the current CLSI standard (M100-S19).

Results: The Phoenix MEC yielded essential (EA) and categorical (CA) agreements of 94.1% and 93.1%, respectively, when compared to the ADM. Very major error (VME) and major (ME) rates were 2.0% (1/49) and 1.4% (2/142), respectively. Both MEs were identified as ESBL-producing strains, while the single VME was confirmed as a plasmid-mediated AmpC producer (CMY-2). Reproducibility and daily QC rates were both ⩾99.5%.

Conclusions: The BD Phoenix System provides an acceptable level of agreement for mecillinam to the reference agar dilution method results, and is able to detect resistance to the antibiotic in *Escherichia coli*.

Public health and community-acquired infections

R2249 Seroepidemiology of hepatitis A virus in Iranian children

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Objective: Hepatitis A is one of the most frequently reported vaccine-preventable diseases worldwide and remains endemic in many areas of the world. Geographical areas can be characterized by high, intermediate, low and very low levels of prevalence of HAV infection. Studies in various communities have shown that Hepatitis A virus (HAV) prevalence rises with age. It may cause significant morbidity and mortality among both adolescents and adults. Now, the current available data regarding hepatitis A epidemiology in Iran are limited. The aim of this study was to determine the seroepidemiology of hepatitis A in children of different age groups in Tehran, Iran.

Methods: Plasma samples of 1065 children between ages of 6 months and 20 years were tested for the presence of total anti-HAV. The children were separated to four age groups: Group 1 (6 months–5.9 years; n = 276), Group 2 (6.0–10.9 years; n = 344), Group 3 (11.0–15.9 years; n = 279) and Group 4 (16.0–20.9 years; n = 166).

Results: The overall prevalence of total anti-HAV was 61.6% (95% CI: 63.25–68.94%). HAV prevalence rates according to age groups were as follows: Group 1, 55.1%; Group 2, 52.9%; Group 3, 65.2% and Group 4, 85%. The HAV seroprevalence among 6 months to 10 years old children was 52.9–55.1%, reaching 65.2% in the 11–15 year age group and 85% in the 16–20 year age group. Except the 6–10 year age group, older age was associated with higher seroprevalence of HAV. Total anti-HAV positivity in terms of age groups was significantly different from each other ($P < 0.001$). For all age groups, there was no statistically significant difference between genders regarding to anti-HAV positivity.

Conclusion: Our study findings indicate that hepatitis A is prevalent in children of Tehran, Iran and hepatitis A infection is an important public health problem in this region. Our survey also showed that Tehran is a region with moderate endemicity for hepatitis A infection. So for HAV prevention, vaccination of children will be beneficial.

R2250 The association between *Helicobacter pylori* seropositivity and the frequency of cardiac adverse events in patients with coronary artery diseases in a 1-year follow-up: a cohort study

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Objectives: Coronary artery disease (CAD) is the major cause of death worldwide. Infection and the following inflammation in coronary arteries, as one of leading basis of atherosclerosis, is a matter of ponder in recent studies. Here we evaluate antibody titer against helicobacter pylori (HP) in patients with CAD compared with normal coronary artery subjects (NCAS) and its association with adverse cardiac events in CAD after a 1 year follow up.

Methods: In this study 117 CAD patients and 61 NCAS were evaluated for IgG antibody (ELISA) against HP. Both groups were angiographically assessed. NCAS were selected from those who had undergone angiography for evaluating chest pain but was reported normal. Those who needed HP treatment or had previously received eradication therapy were excluded. Angina [Seattle angina questionnaire (SAQ)] and CAD stenosis [Modified Gensini score (MGS)] severity were recorded. CAD patients were followed for acute coronary syndrome (ACS), coronary revascularization and cardiovascular death during one year of follow up.
Results: HP titer was comparable in CAD and NCAS. There was no significant correlation between HP titer and angina severity (SAQ) in CAD patients. Furthermore, CAD severity (MGS) and HP titer were not significantly correlated. During one year of follow-up, 18 (15.4%) CAD patients were admitted to hospital with acute coronary syndrome (ACS). HP titer was comparable in those with ACS and the other CAD patients.
Conclusion: HP infection may doesn't play an important role in CAD pathogenesis in endemic regions. Moreover no correlation between HP infection and CAD stenosis severity, angina severity or adverse cardiac events in CAD patients was found.

R2251 Outpatient parenteral antibiotic therapy for cellulitis. A 10-year single-centre experience

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Objectives: Outpatient parenteral antibiotic therapy (OPAT) for a wide range of infections is an alternative that can avoid or reduce hospitalization, while improving the quality of life of the patient and family. Cellulitis refers to a frequent inflammatory process caused by bacterial infection of the dermis and underlying subcutaneous tissues of the skin, which in uncomplicated forms and by now availability of long half-life antibiotics may benefit from antibiotic treatment outside hospital.
Methods: We conducted a retrospective analysis of clinical and microbiologic data for cases of cellulitis treated in our 439-bed general hospital in outpatient setting during the period 1999 to 2009.
Results: From september 1999 to march 2009 were treated in outpatient settings 171 patients (M = 108) with a mean age of 57.3 years (±16.9, median 60 range 17–87) with uncomplicated cellulitis. The median distance of the patient's home from the infusional center was 10km (range 5–60). One or more comorbidities were ascertained in 63% of patients (diabetes mellitus in 27%). The most common site of infection were lower limbs (42.7%) and feet (21.8%); in 23% of cases OPAT was preceded by a period of hospitalization and 9.6% of the cases have required hospitalization after the start of outpatient treatment. The drugs most frequently administered were ceftriaxone (59.8% of cases) and teicoplanin (22.2%); in monotherapy in 38% of cases for the first and in 23.8% of the cases for the second. The average duration of treatment was 13 days (±9, median 10, range 1–47). A culture result was obtained in 49.8% of cases and the major pathogens isolated were *S. aureus* (34.2% (3.4% MRSA)), *Streptococcus* spp (18.1%) and CNS (17.2%). A clinical cure was achieved in 93.7% of cases with a total saving of 2186 days of hospitalization.
Conclusion: The use of OPAT programs in the treatment of uncomplicated cellulitis is likely to increase due to ongoing efforts to shorten hospital stays and reduce health-care costs. Selection criteria and careful medical monitoring of patients are critical in determining the success of any OPAT program and also in case of cellulitis. Our experience suggests that OPAT programs can be promoted in uncomplicated forms of cellulitis with advantage.

R2252 Three cases of Crimean-Congo haemorrhagic fever with renal impairment

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Objectives: Crimean–Congo hemorrhagic fever (CCHF) is a fatal disease caused by a tick-borne virus. In this report, we present three cases with renal impairment, relatively uncommon clinical presentation of the disease.
Case 1: A 69-years-old male patient living in endemic region for CCHF admitted with fever, fatigue, anorexia and nausea. His physical examination was normal except fever. Laboratory investigations revealed severe thrombocytopenia, prolonged aPTT, elevated blood urea and creatinine. CCHF PCR was positive. Thrombocytopenia and aPTT prolongation didn't improve despite platelet and fresh frozen plasma infusions. His blood urea and creatinine levels were increased day by day. On fourth day, acute pulmonary edema and metabolic acidosis were developed. His urea level detected as 236 mg/dL and creatinine 4.8 mg/dL. He couldn't be treated with hemodialysis because of his worsened clinical state and he died on fifth day.
Case 2: A 54-years-old female patient admitted with fever, fatigue and headache. She was febrile on physical examination. Laboratory investigations revealed thrombocytopenia and prolonged aPTT. Blood urea and creatinine were within normal limits. CCHF PCR was positive. Platelet and fresh frozen plasma infusions were started. On sixth day, a hemorrhage was developed in the biceps muscle. On sixth day, renal impairment was occured. On tenth day, blood urea level increased to 177 mg/dL and creatinine to 5.6 mg/dL and continuous ambulatory peritoneal dialysis (CAPD) was started. All the clinical and laboratory abnormalities of the patient were improved except renal impairment. CAPD still goes on.
Case 3: A 30-years-old female patient admitted with fever, nausea and headache. Her physical examination was normal except fever. Laboratory investigations revealed thrombocytopenia and prolonged aPTT. Blood urea and creatinine were normal on admission. CCHF PCR was positive. Platelet and fresh frozen plasma infusions were given. On the second day, purpura occurred on the trunk. On the fourth day, hemorrhagic bullous lesions were developed on the trunk and spread to the whole body. On the same day renal impairment was started. Patient's clinical state was worsened rapidly. After the increase of creatinine level to 2.9 mg/dL, CAPD was started on. She died during the dialyses due to cardiac arrest.
Conclusion: Renal impairment is one of the uncommon clinical presentations of the CCHF. It can be mortal despite supportive therapy and dialyses.

R2253 Lethal community-acquired *Streptococcus agalactiae* meningitis in an adult with systemic lupus erythematosous

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Objectives: Group B Streptococcal (GBS) or *Streptococcus agalactiae* meningitis in adults is an uncommon manifestation of invasive GBS disease found especially among elderly and those with significant underlying disease. We report an unusual case of fatal community-acquired meningitis due to *Streptococcus agalactiae* in an adult with systemic lupus erythematosous (SLE).
Methods: A 41-year-old man who quoted a 20-years history of SLE under corticosteroid treatment and without any recent hospitalization was transferred to the emergency department of our hospital. Seven days prior to his admission he developed atypical gastrointestinal disorders, fever >39.5ºC and ciprofloxacin was subscribed. The patient showed clinical improvement but soon after, he developed diarrhea and another febrile episode with drowsiness, cephalalgia and cervical stiffness. He was transferred to the closest hospital where he was immediately intubated with GCS: 6/15. The patient was transferred to our hospital for admission to the Intensive Care Unit (ICU) and a brain computed tomography scan (CT) revealed cerebral edema and signs of meningitis. Because of high cerebral pressure no lumpar puncture was performed. Laboratory blood evaluation revealed neutrophilic pleocytosis and increased C-reactive protein (22.2 mg/dl). Urine, blood and bronchoalveolar cultures were all found negative for *Streptococcus agalactiae*. The patient showed severe vital organs' dysfunction and despite highly invasive treatment he died out of multiple organ dysfunction syndrome 24 hours upon his admission. Post mortem lumpar cerebrospinal fluid (CSF) was cultured following routine methods. Gram staining of CSF showed many WBCs

with Gram-positive cocci. Bacterial identification and antimicrobial susceptibility testing with VITEK2-automated system (bioMérieux, France) identified *Streptococcus agalactiae* susceptible to penicillin and ceftriaxon.
Conclusion: To our knowledge, for the first time *Streptococcus agalactiae* as an aetiologic agent of lethal meningitis in SLE is being reported. From a clinical point of view, acute bacterial meningitis caused by GBS is indistinguishable from meningitis caused by other pyogenic bacteria and for this we recommend that *Streptococcus agalactiae* should be included in the differential diagnosis of acute bacterial meningitis in patients with underlying disease like SLE.

R2254 Measles: did we prevent the hazard or is it more hazardous now? Measles, spread and prevention in children of an Afghan migrant family in Istanbul

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Objective: In Istanbul no proven measles case was detected since 2006 with the vaccination campaigns started in 2003. For the reason of measles detection in 2 children (9 months and 3 years old) of a family living in Istanbul, Mop-up studies performed to prevent the spread of these cases were explicated.
Cases: In Zeytinburnu district which has a low socioeconomic level and harbours most of the immigrants, people who has residential permit can get health care by 13 health posts without charge.
Since 5 different vaccination campaigns performed with success, local people supported this campaign also. Measles vaccination rates were over 95% in last 4 years.
2 consecutive cases were detected in this immigrant family which has a travel history to Iran in august 2009. When a secondary case was detected a 3 years old cousin in the same family who didn't travel, brought up the necessity to find out the unvaccinated children in the district.
As part of social mobilization studies, local administrative chiefs were informed. Field work lasted for 48 days was completed in 4 subdistricts where immigrant families lived densely. Health officials visited 22484 house and left invitations when the resident were away. In this study we found out that; 44 children were unvaccinated, 10 of them didn't get health care at all, 17 came in the last 3 months and 17 were 12–18 months old and delayed MMR vaccine.
Measles IgM was found negative in 51 children with rash in measles surveillance until today, 2 families rejected to get measles vaccine, 9 cases showed mild reactions after vaccination and 1 case was detected as an application mistake.
Conclusion: Some difficulties were lived to diagnose measles clinically in the first 2 cases can cause secondary cases and an outbreak. We took the physician's attention when some diseases are off the agenda they shouldn't be behind in differential diagnosis.

	Vaccination in		Total
	Field	Health Post	
6–12 months (Measles)	93	596	689
1–5 year-old (MMR)	1930	4626	6556
6–14 year-old (MMR)	610	125	735

R2255 Botulismus epidemic caused by home-made canned food and 8 members affected in a family

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Objective: *Clostridium* botulinum spors are heat-resistant and can survive in inaccurately processed food and can cause a neuroparalytic disease with its neurotoxins. Disease processes of 8 family members were explicated after got sick of consumption of the same food.
Methods: On February 2009 patients applied to different hospital emergency departments with complaints of dysphagia, dyspne, dyplopia, blurred vision and weakness. Concerning botulism infection the patients were hospitalized and Zeytinburnu Health Group Directory sent a health team to investigate the epidemics to visit the houses. They took samples from the food they had eaten in the last two days. Food samples were sent to the reference laboratory. Patients' relatives were informed about the disease spread and preventive measures. The food left were destructed as a precaution.
Results: Home-canned purslane was thought as the cause was prepared in Yukari Ulupinar village in Malatya city and kept in deep freeze. Data of 8 patients between 16–45 years old can be seen on table. National poison information center (NPIC) sent antitoxic serum containing antitoxin to *C. botulinum* type A, B and E and they were slowly perfused to the patients. Reference laboratory detected Gram positive bacilli in purslane with yoghurt, anaerobic culture yielded *Clostridium* botulinum and animal experiment showed toxin.
Conclusion: Home-canned food preparation and consumption stil exist in our country. This tradition carries the risk of botulism like diseases. Early diagnosis, antitoxin infusion and follow in intensive care unit are critically important to decrase the mortality rate. But the principal is to lessen the consumption of home-canned food or to provide appropriate conditions.

	Patient							
	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8
Gender	male	female	female	male	female	male	female	male
Age	35	36	27	32	36	45	16	20
Clinical findings								
dysphonia			+				+	
phytosis		+						
diplopia	+	+	+		+		+	
dysartri				+	+			
dispne	+	+	+		+		+	
emesis-nouse	+	+	+		+		+	
dysphagia		+	+		+		+	
weakness		+	+		+		+	
ICU necessary	+	+	+	+	+		+	
Outcome	recovered	recovered	recovered	recovered	recovered	recovered	recovered	recovered

R2256 Prepared pandemic plan for H1N1 in Istanbul metropolis, April-June 2009: procedures and first results

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Objective: The aim of this report is to evaluate the patients with H1N1 influenza that first reported in Istanbul/Turkey between 29th April and 22nd June 2009; to take attention to the infection control measures to prevent transmission of the disease and to support surveillance activities for H1N1 influenza in Istanbul metropolis. Prepared pandemic plan was applied and consequences of it was controlled via Istanbul Provincial Health Directorate.
Methods: Between 29th April and 22nd June 2009, probable and confirmed cases defined by CDC and WHO were evaluated in terms of age, gender, symptoms, airline agencies and nationalities, laboratory findings.
Results: One hundred and sixty probable and 16 confirmed cases were followed up between 29th April and 22nd June 2009 in Istanbul. The most seen age group was 20–39 years (81 cases) and male/female ratio was 1:1. Fever was a universal symptom in patients with H1N1 virus infection (70%); other symptoms included sore throat (40%), myalgia or arthralgia (26%), and cough (22%). All of our patients had an identifiable epidemiologic link to another confirmed patient. The largest cluster of cases of H1N1 virus infection occurred between 15–20 May and 30 May-4 June 2009. KLM was the most used one among airline agencies in all cases. The most of patients came from USA. Only 5% of patients were detected in thermal cameras at the airport. Airport staffs composed 10% of all cases. Probable and confirmed cases were isolated in seperate rooms and treated in referrance tertiary hospitals. Antiviral prophylaxis was administered to the close contact persons of these cases (2000 persons).
Conclusion: In this pandemic which declared as alarm level 5 by WHO, the spread of the disease was prevented in our city till production of H1N1 vaccine by infection control measures and an convenient pandemic

plan. Preventing an infection to enter a country is as important as to prevent and slow the spread of epidemic in the global world.

R2257 Evaluation of epidemiological, clinical and laboratory characteristics of pandemic influenza A (H1N1) cases in a tertiary care hospital in Turkey

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Objectives: In April 2009, a novel H1N1 influenza A virus, so-called pandemic H1N1/09 virus was identified in Mexico. The virus has since spread throughout the world and caused an influenza pandemic. In this report, we present epidemiological, clinical and laboratory data of hospitalized patients in our clinic between 25 October and 18 November 2009.
Methods: Totally 41 patients hospitalized due to probable pandemic influenza H1N1 were evaluated. Nasal and/or nasopharingeal samples were taken from all patients. These samples were tested for Influenza A (H1N1) in Refik Saydam National Public Health Agency, National Influenza Reference Laboratory with the real-time RT-PCR. Epidemiologic, clinical and laboratory data were recorded.
Results: Of the patients, 33 were female (80.5%), 8 male (19.5%), mean age was 39±16.5 years and mean hospital stay was 4.8±1.6 days. Most of the patients were homemaker (65.9%). Only one patient was vaccinated with the seasonal flu vaccine. One patient had travel history and eight patients had history of close contact with persons having symptoms of respiratory infection. Of patients, 15 (36.6%) hadn't any underlying condition. Ten patients (24.4%) were pregnant. Other 16 patients (39%) had one or more underlying conditions including DM, chronic obstructive pulmonary diseases, coronary artery diseases, FMF, multiple myeloma, multiple sclerosis and Behcet's disease. On admission, most common symptoms were cough, fever, myalgia, headache and sore throat (92.7%, 90.2%, 85.4%, 68.3% and 65.9%, respectively). Twelve patients (29.3%) had dyspnea on the admission but none of them required mechanical ventilation. On the physical examination, 31 patients (75.6%) were febrile; the mean degree of the fever was 38.1±0.9ºC (36.5–40ºC). Thirteen patients (31.7%) had crepitation on the lung. In the laboratory investigations, mean hemoglobin, WBC, platelet, AST, ALT, ALP, blood urea, creatinine, LDH and ferritine levels were within normal limits. Mean GGT and CK levels were detected high and mean zinc level was detected low than normal limits. Of patients, 32 (78%) were found PCR positive for pandemic Influenza. Thirty-seven patients (90.2%) received oseltamivir therapy (75 mg twice daily) and 19 (46.3%) received antibiotics. No patient dead.
Conclusion: Most of our patients had underlying conditions but they recovered without complication. We think that pregnant women and patients with other underlying conditions must closely follow-up.

Emerging infectious diseases

R2258 The first confirmed case of a human infected with *Mycoplasma suis* in Serbia

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Objective: The pig infection with *Mycoplasma suis* is very well-known and described in veterinary medicine. According to the new classification, the cause can be found in the group of haemotrophic mycoplasmas, and previously it used to be classified as belonging to the genus of Eperythozoon. During the last decade more and more reports have confirmed the presence of *Mycoplasma suis* among people as a cause of a new zoonosis. Many issues are unfamiliar in connection with epidemiology, clinical picture, diagnostics and therapy of this infection among people. It is considered to be an opportunistic infection that occurs among immunocompromised patients. The aim of this research was to confirm possible presence of this infection among human population in Serbia.
Methods: In order to determine the presence of *Mycoplasma suis* in EDTA human blood samples, we used a classical PCR test with primers specific for a genetic sequence that codes MSG1 protein of *Mycoplasma suis* (MSG1 is an immunodominant protein (p40) localised on the surface of the cause with adhesive function).
Results: By applying the described PCR test in EDTA blood sample of the patient who underwent hemodyalisis, we have confirmed the presence of *Mycoplasma suis*.
Conclusions: This is the first confirmed case of human infection with *Mycoplasma suis* registered in Serbia. Further epidemiological and clinical research is necessary, considering that many data on this infection of humans are not familiar.

R2259 Clinical features of patients with confirmed infection from A/H1N1 virus

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Objective: To describe the clinical presentation of patients with confirmed A/H1N1 infection and to compare them with other patients who were negative for A/H1N1 virus.
Methods: More than 800 patients presented to our hospital with flu-like symptoms since the beginning of the A/H1N1 pandemic. In 156 of them a pharyngeal sample for PCR test was obtained. Most of the samples were obtained during the surveillance phase, in order to identify and isolate patients and thus to prevent the spread of the disease.
Results: From the 156 samples, 103 were positive and 53 were negative. The table summarizes the symptoms of both the patients groups.
Conclusions: Among patients with positive PCR the prominent symptoms were malaise and fever. Generally, the more typical clinical presentation was more likely to lead to a positive test. It should not be ignored though, the fact that there were cases with low fever and very mild disease that were proved to be positive, while others with more typical clinical presentation were negative. Patients who had not strong possibility (based on their clinical condition) to be positive but were tested for safety reasons (e.g. people with immunocomprimised members in their families) were most likely to be negative.

Symptoms	Positive PCR	Negative PCR
Fever	90 (87.3%)	41 (78.6%)
Cough	81 (78.6%)	32 (60.1%)
Sore throat	67 (64.9%)	35 (65.8%)
Myalgia	80 (77.6%)	44 (82.7%)
Malaise-weakness	96 (93.1%)	48 (90.2%)
Headache	75 (72.7%)	27 (50.7%)
Rhinorrhea	47 (45.5%)	22 (41.3%)
Vomit	9 (8.7%)	4 (7.5%)
Diarrhea	14 (13.5%)	7 (13.1%)

R2260 Cases of Waterhouse-Friderichsen syndrome over a 3-year period

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Introduction: Waterhouse-Friderichsen syndrome (WFS), known in the Anglo-Saxon literature as "hemorrhagic adrenalitis", is a disease of the adrenal glands, caused most often by *Neisseria meningitidis*. Syndrome bears the names of the English doctor Rupert Waterhouse (1873–1958) and Danish pediatrician Carl Friderichsen (1886–1979), who first described it.
Material and Methods: For three-years period 2007–2009, we discuss four cases of Waterhouse-Friderichsen syndrome. These are children,

respectively, 14 year, 2 year, 6 year and 3 months old, who were treated in the intensive care ward of the Infectious Diseases Clinic – Plovdiv and in Infectious Disease unite, Pazardjik. Hospitalization time is during 1 hour 40 min to 10 hour 10 min and the outcome is fatal for four cases.
Results and Discussion: 1. T.Z.Z. – 14 year. Suddenly fell ill with high fever, repeated vomiting, fast-growing hemorrhagic rash. After a generalized tonic–clonic convulsion – asistoliya and exitus letalis. CSF: cells – 8.106, protein – 0.97 g/l, glucose – 4.2 mlmol/l. 2. K.S.K. – 6 year old. Admission – shock, hypothermia – 35ºC, hemorrhagic rash – petechiae, ecchymoses covering the entire surface of the body. PLT – 34.1012, fibrinogen – 1.39 g/l. 3. H.A.H. – 3 months old baby. 4. I.F.A. – 2 years old – the illness begins with repeated vomiting.
Culture: CSF on BACTEC - *Neisseria meningitidis*.
Conclusion: In the four cases there are irresistible bacterial sepsis, disseminated intravascular coagulation (DIC), shock and adrenal insufficiency.

R2261 First detection of Lymphogranuloma venereum L2b serovar in Madrid, Spain

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Objective: The reported number of *Chlamydia trachomatis* (CT) associated with lymphogranuloma venereum (LGV) has increased in the last decade (around 10%/year) with small outbreaks since 2003 described in Europe. However in Spain only sporadic cases have been reported in North and Northeast areas. We explore the presence of serovars associated to LGV among high risk population in Madrid, Spain, and the usefulness of molecular methods to detect and genotype CT-LGV associated serovars.
Methods: Two high risk population groups from our city were included in this study: i) sentinel population and ii) patients attended in the 2 Units of Sexually Transmitted Infections (STI) in two public hospitals. 496 urethral, cervix and rectal swabs from symptomatic patients were recovered during six months (May-October, 2009). Two commercial PCR-based methods (Abbott Molecular and Beckton Dickinson) were performed for routine diagnostic of CT infection. An in-house TaqMan real time-PCR (qPCR) based in a deletion on the pmpH gene was used to specifically detect LGV related serovars (L1, L2 and L3). To identify specific serovars, nested-PCR and sequencing of ompA gene were applied.
Results: 34 samples from sentinel population and 20 from group-ii yielded a positive CT amplification, which represent 10% of analysed samples. Among these positive samples, 6 showed also specific amplification for L-serovars (11%), one urethral and five rectal swabs. These results were obtained in 6 men who have sex with men, with multiple sexual partners during last year. Interestingly, four patients were Spanish, indicating spread of LGV among native population. Four of them were HIV positive and all had concomitant STI: three had gonococcal proctitis, two infections by high risk papilomavirus and one herpes simplex infection. BLAST analysis of the sequence of the ompA gene shows concordance with the L2b serovar in all cases, as has been previously described in recent outbreaks in Europe.
Conclusion: To our knowledge, the percentage of L2b serovar found in this work (11%) is the highest value published in Spain, confirming the spread of this serovar across Europe. The high correlation between CT-positive and L2b serovar in native population suggests that this serovar is already established among Spanish population. From the public health perspective, routine surveillance of LGV may be important to assess its real prevalence in high risk population.

R2262 Isolation of *Clostridium difficile* from marine coastal environment (Gulf of Naples, southern Italy)

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Objectives: The aim of this study was to evaluate the occurence of *Clostridium difficile* in a coastal environment of the Gulf of Naples (Southern Italy) exposed to different degrees of anthropic pressure. Samples of seawater, sediments, mussels and zooplankton were investigated for the presence of *C. difficile* in order to provide a first ecological insight to the environmental behaviour of this bacteria.
Methods: Samples of water, sediment, mussels (Mytilus galloprovincialis) and zooplankton were taken at 5 sampling stations in the Gulf of Naples, on September 2009. The samples were enriched in *Clostridium difficile* Enrichment Broth supplemented with 0.1% sodium taurocholate and then incubated for 10 days at 37ºC in anaerobic conditions. After incubation, alcohol shock and centrifugation were performed for spore selection and the resulting pellet was streaked onto a *C. difficile* Agar Base supplemented with Moxalactam Norfloxacin and 5% horse blood and incubated for 48 h in the same conditions. Rhizoid colonies of spore-forming Gram positive bacilli, non-hemolytic and positive to proline aminopeptidase test (Oxoid) were identified by miniaturized system Id Rapid 32A (bioMeriux). The identified *C. difficile* strains were then challenged for the detection of esotoxins by Xpect *Clostridium difficile* Toxin A/B Test (Oxoid).
Results: The results of this work shown a widespread presence of *C. difficile* in the investigated marine environment. A total of 17 samples was taken yielding 6 isolates with a positivity rate of 40%. *C. difficile* has been isolated from 2 samples of water, 3 of zooplankton and 1 of mussels, respectively. All the 4 isolates founded in water and zooplankton samples collected in a very faecal polluted area were found able to produce *C. difficile* Toxin A/B. The isolates found in an area with low faecal pollution were non toxigenic.
Conclusions: The presence of *C. difficile* in mussels is certainly related to the presence of this bacterium in water, as mussels are filter feeders. It is to clarify wheter *C. difficile* was associated to the particulate organic matter or it can also be member of the planktonic microbial community. The isolation of *C. difficile* as epizootic contaminant of zooplankton exoskeleton was never reported before. Further studies are needed to understand how the zooplankton enables the spreading of this bacterium in the environment, especially in areas with low or absent faecal pollution.

Infection control

R2263 Pharmaceutical equivalence of some commercial samples of artesunate and amodiaquine tablets in south-western Nigeria

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Purpose: To study the physical properties and dissolution profiles of commercial samples of artesunate and amodiaquine tablets.
Method: Fifteen generic brands of artesunate and five generic brands of amodiaquine tablets were obtained from drug retail outlets in Oyo and Ogun States of Nigeria. The tablets were subjected to various compendia tests including identification, weight uniformity, uniformity of content, assay of active ingredient and uniformity of diameter. Additional tests used as a basis of the assessment of the pharmaceutical equivalence of the products include hardness, disintegration time and dissolution rate. Data obtained were analysed by correlation analysis, Chi-square and ANOVA.
Results: Thirteen generic brands of artesunate (87%) and four amodiaquine brands (80%) investigated were imported. Two brands of the imported artesunate brands were found to contain undetectable amount of artesunate while another 8 samples contained overages. All the amodiaquine brands passed the assay test as stipulated by the USP for amodiaquine tablets while tablet disintegration time of amodiaquine products ranged from 5.8–20.7 min. All but one artesunate sample (B2)

passed the disintegration test too. Majority of the artesunate brands tested had significantly different dissolution profiles ($P < 0.05$). Four (80%) of the amodiaquine tablet brands tested had similar dissolution profiles and percent drug released within 30 min ($p > 0.05$). One amodiaquine brand CC demonstrated poor dissolution profile as it did not meet the essential minimum of content dissolution within 30min.

Conclusion: The detection of substandard artesunate tablets and a poorly formulated amodiaquine tablet amongst the few sample brands studied highlights the need for increased drug surveillance and monitoring of the qualities of antimalarial medicines currently in use in order to prevent widespread treatment failure.

R2264 Nosocomial infections after colonic surgery

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Objective: The aim of this study is to know the rate of Surgical Site Infection (SSI) and other nosocomial acquired infections (NI) after colonic surgery in our hospital and the evolution of SSI after changes in antibiotic prophylaxis schedule.

Patients and Methods: The Infection control team prospectively studied all patients operated on colon surgery from October 2002 to October 2009. Variables under surveillance are age, sex, underlying illnesses, predisposing conditions, ASA physical status classification, NNIS risk index, antibiotic prophylaxis, nosocomial infections, microorganisms, length of hospital stay, treatment and outcome. Antibiotic prophylaxis schedule in our hospital is neomycin + erythromycin po and amoxicillin + clavulanate IV (AMC). We have introduced the administration of a second dose of AMC if the length of surgery is >6 hours or hemodilution >15 ml/kg or blood loss >1.5 l. Case definitions: CDC definitions for Nosocomial infections. Surveillance after discharge: 1 month.

Results: 1541 patients were studied, 1000 of them were males, mean age 67.6 y (SD 12.8). 345 patients (22.38%) had 485 NI: 281 surgical site, 92 UTI, 48 bacteremia, 35 local catheter insertion site infection, 20 respiratory, and 9 other locations. Cumulated incidence of infected patients 22.39%. Antibiotic prophylaxis was administered in 99.5% of the cases. The dosage, time, drug and duration of the prophylaxis were appropriated (99.9%, 99.7%, 97.3% and 93.3% respectively). Surgical site infections (SSIs) 287 cases: superficial incisional SSIs (109), deep incisional SSIs (76) and organ/space SSIs (96). Cumulated incidence patients with SSIs 16.87%, it decreased from 18.69% in 2003 to 9.69% in 2009. NNIS Score 0: 560 patients, 10.5% SSIs; Score 1: 516 patients, 21.3% SSIs; Score 2: 197 patients, 31% SSIs; Score 3: 46 patients, 32.6% SSIs; score M 222 patients, 6.8% SSIs. Microorganisms in SSI: *E. coli* 92, *Enterococcus* spp. 33, *Bacteroides* 39. Mean length of hospital stay 30.1 days (SD 26) in patients with NI and 11.9 days (SD 8.6) in patients without NI. Crude mortality 2.8%: 7.8% in patients with NI and 1.3% without NI.

Conclusions: Cumulative incidence of SSIs in our hospital decreased from 18.69% in 2003 to 9.69% in 2009. The only change introduced was the timing of antibiotic prophylaxis administration: 5 minutes before surgery and the second dose of antibiotic if the length of surgery is >6 hours or hemodilution >15 ml/kg or blood loss >1.5 l.

R2265 "To pronto or not to pronto . . . ": MRSA topical bio-burden reducing regime failures – a clinico-economic study

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Background: *Staphylococcus aureus* carriage increases the potential for post-op infections, mortality and associated costs. Since Mar 09 NHS hospitals in UK are committed to screen all patients for elective surgery. Cancelled/delayed surgery upon MRSA detection or failure to clear MRSA carriage after repeated courses of topical bioburden reducing regime(TBR) enhances patient anxiety, depression and affects quality of life.

Since Mar 09 conventional TBR regime [mupirocin & chlorhexidine]was replaced by Prontoderm® (BRAUN) pack[nasal gel & body/hair foam] for elective patients. MRSA screen protocol for electives includes nasal & perineal swab at PAC; MRSA+ve patients are offered 5-days TBR followed by repeat screens on days 3, 7 & 14 [3-negative MRSA screen [NMS] before booking for surgery.

MRSA TBR Failure Clinic [MTFC]: PAC orthopaedics and cardiac surgery nurse specialists & microbiologist set this clinic following concerns regarding TBR failure, delayed surgeries, patient safety and experience.

MTFC protocol: Individualised plan is offered to patients failing TBR based on the urgency of surgery. MRSA recolonisation study and source exploration [gut carriage, partner carriage, etc]. The three 5-day regimes used: Regime A [Prontoderm® pack]; Regime B [mupirocin nasal plus chlorhexidine body wash/shampoo]; Regime C [Regime B + doxycycline and rifampicin].

Method: Data collected from MTFC and the pathology database.

Results: Since Mar09, 19 patients [>1000 screened] benefitted from the clinic.

Ortho (15 pts]:27%[4/15] had 3NMS after regA; 36%[4/11]had 3NMS following 2nd course regA; 29%[2/7] had 3 NMS after regB; 20% [1/5] had 3NMS after regC. Remaining data pending.

Cardiac(4pts):100% failed NMS with regA; 25%[1/4] had 3 NMS after 2nd course regA; remaining 100%[3/3] failed regB. 33.3%[1/3]each had 3 NMS after regC; 2NMS then positive; data pending.

Conclusion: Ortho & cardiac PAC attendees have <3% MRSA carriage. 21%[4/19] had 3 NMS following regime A (prontoderm) whilst 79% required individualised management plans (based on urgency of surgery, risk of SSI and MRSA recolonisation studies). The clinic has been immensely successful (several patients requiring urgent cardiac surgery were operated on the day after completion of regC using Teicoplanin prophylaxis. Prontoderm® pack requires no prescription, supplied in PAC, user friendly info leaflets & 38% cheaper. Patients find chlorhexidine shower more cleansing than prontoderm foam application after shower on dry skin.

R2266 Stethoscope audit: do not forget to clean your third hand!

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Objective: The importance of immediate hand decontamination before and after direct patient contact has been well accepted and practised for many years. However comparitavely little research looks into risk of spreading infection via stethoscopes; a medical equipment that has direct contact with all patients examined. The aim of this audit was to assess bacterial contamination on stethoscopes and to assess the effectiveness of the proposed cleaning method using an alcohol wipe.

Method: Forty stethoscopes were randomly selected from a wide range of medical personnel in the Intensive Care Unit (ICU), Medical High Dependency Unit (MHDU), Accident & Emergency (A&E) and Medical Department (MD). Stethoscopes were swabbed before and after cleaning with an alcohol wipe (70% Isopropyl Alcohol). Samples were sent for culture and results expressed as counts of colony forming units (CFU) per 5 ml ringer solution used to prepare each swab.

Results: Overall results showed 82% of stethoscopes before cleaning were contaminated with skin flora. Average contamination was 921.25 CFU. All contamination was reported as skin flora which includes potentially pathogenic *Staphylococcus* Aureus and *Pseudomonas* Aerginosa.

A&E had the lowest average of contamination per stethoscope with 115 CFU and 2/10 clean (0 CFU). A third (3/9) of stethoscopes in ITU/HDU were clean. The average contamination was 411 CFU, however 1 stethoscope grew >2000 CFU. The MD had the highest number of contaminated stethoscopes and an average of 1523.8 CFU, only 2/21 were clean.

Following cleaning the results showed 39/40 (97.5%) stethoscopes grew 0 CFU (Fig.5). The remaining stethoscope accounting for the last 2.5%, grew 50 CFU prior and after cleaning; possibly there was an error in the cleaning process.

Conclusion: This audit demonstrates the importance of cleaning stethoscopes before and after patient contact as this is a plausible vector for transmitting potentially pathogenic infections. It proves

the effectiveness of the cleaning method which erradicates bacterial contamination to 0 CFU in 97.5% of cases. Whilst focus must be maintained on hand washing, we also advise cleaning stethoscopes. Improving staff knowledge is vital and could be achieved by ward posters, easy access to alcohol wipes and presenting the result of this audit. Further research is required to see if conditions improve with proposed interventions and looking specifically at transmission of hospital acquired infections via stethoscopes.

R2267 An integrated approach to control ICU-associated infections focusing on antimicrobial consumption and resistance rates

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Objectives: The emergence and spread of antimicrobial resistance has become a major public health threat and antimicrobial usage is a key factor for resistance because it allows selection or emergence of resistant pathogens. The main objective of our study was to provide Intensive Care Unit-specific and national benchmark data both on antimicrobial consumption and on resistance rates (RR).
Methods: Intensive Care Units (ICUs) already participating in the first edition of the Italian Nosocomial Infections Surveillance in Intensive Care Units (Sorveglianza Prospettica delle Infezioni Nosocomiali nelle Unità di Terapia Intensiva – SPIN-UTI), established by the Italian Study Group of Hospital Hygiene (GISIO) of the Italian Society of Hygiene (SItI) were invited to take part in the study. The project used an integrated approach: a unit-based approach for the surveillance of antimicrobial use and a patient-based approach for the surveillance of antimicrobial resistance in Italian ICUs.
Results: The study was conducted between November 2006 and April 2007, and included 21 ICUs, 1685 patients with length of stay longer than two days and 18,694 patient-days, producing a total of 79,423 defined daily doses (DDDs). During the study period, the antimicrobial usage density (AD = DDD/1000 patient days) was 4,248.47 DDD units per 1000 patient-days. The three most used drug groups were penicillins/β-lactamase inhibitors (AD 13,921), quinolones (AD 12,806) and glycopeptides (AD 7,453). The single most frequently prescribed antimicrobial agent was ampicillin/sulbactam (DDDs 12,220; AD 653.7), followed by levofloxacin (DDDs 9,940; AD 531.7) and fluconazole (DDDs 8,836; AD 472.7). Susceptibility data were reported on 353 isolates. The most frequent infection-associated pathogen was "*Pseudomonas aeruginosa*" followed by "*Acinetobacter baumannii*" and "*Staphylococcus aureus*"; "*Candida*" spp accounted for 4.3%. RR were 95.3% and 48.0% for ceftazidim-resistant "*A. baumannii*" and "*P. aeruginosa*" respectively; 83.3% and 35.2% for imipenem-resistant "*A. baumannii*" and "*P. aeruginosa*" respectively, and 47.6% for oxacillin-resistant "*S. aureus*".
Conclusion: Comparison of resistance patterns and prescribing practices in different ICUs underlines the need for locally adapted guidelines on empiric antimicrobial therapy, based on the evidence of the link between antimicrobial resistance and consumption and on international benchmarking, in order to address effective control measures.

R2268 Utilization state of analysed data in Japan Nosocomial Infections Surveillance (JANIS)

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Objectives: Nosocomial infections with drug-resistant bacteria have become a great concern in medical facilities. The Japan Nosocomial Infections Surveillance (JANIS) has been conducted by the Ministry of Health, Labour and Welfare since 2000, and the system was renewed in July 2007. JANIS consists of five divisions; the Clinical Laboratory Division (CL), Antimicrobial-Resistant Bacterial Infection Division (ARBI), Surgical Site Infection Division (SSI), Intensive Care Unit Division (ICU), and Neonatal Intensive Care Unit Division (NICU). @All analyzed data reported to each participating hospital are displayed as gbox-plot h manner and this lets each hospital notice its position in the distribution of participating facilities in Japan. Several data such as isolation frequency of each drug-resistant bacterium and each infection rate with specific antimicrobial resistant microbe are reported periodically to each participating hospitals, together with the trends. However, there is no data about the utilization rates of analyzed data in participating hospitals, despite all the participants can download their own analyzed data by PDF file through their personal website constructed in the JANIS homepage.
Methods: We investigated the download frequencies of analyzed data in all the participating hospitals in 2008 to estimate the utilization rate of analyzed data in individual participating hospital.
Results: A total of 817 hospitals were participating in JANIS as of October 2009. The number of hospitals of each division were 564 in CL, 427 in ARI, 301 in SS, 141 in ICU, and 111 in NICU. Download ratios of PDF file through the own homepage were 59.6% in CL, 47.4% in ARBI, 82.9% in SSI, 75.4% in ICU, and 63.8% in NICU. In ARBI, 18.2% of participating hospitals did not download the analyzed data at all.
Conclusion: We become aware of the fact that the JANIS participating hospitals do not necessarily well utilize analyzed data for their infection control measures. Our next step is to investigate the reason why several hospitals hardly use the analyzed data. To encourage all participating hospital to improve their infection control measures, we intend to present good samples of efficacious and prudent use of analyzed data especially to those hospitals where analyzed data have been hardly utilized to date.

R2269 Survival of *Acinetobacter baumannii* with *Acanthamoeba* sp.

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Objective: *Acinetobacter baumannii*, potentially found in water sources, is an important emerging hospital-acquired pathogen, affecting patients in intensive care units. It is also known that protozoa can influence the growth of microorganisms as bacteria or yeasts, but little is known about the influence of free-living amobae on *A. baumannii*. We therefore explore in this study the relationships during a coculture of two strains of *Acanthamoeba* (*A. castellanii* or *A. culbertsoni)* and one strain of *A. baumannii*.
Methods:
- The first experiment was a coculture of *A. castellanii* (ATCC 30234) or *A. culbertsoni* (ATCC 30171) trophozoites and *A. baumannii* in PBS at 27ºC. After 24, 48 or 72 h, the cocultures were plated on Mueller Hinton medium to count CFU. Controls were realized by incubating bacteria in the same conditions without amoebae.
- The same experiment was then conducted, but after 24 h, the cocultures were transferred in encystment medium, and CFU of *A. baumannii* were counted after 1, 3, 5, 7, 14, 21, 30 and 60 days of incubation at 27ºC. Moreover, at various times, samples of the suspensions were examined in electron microscopy.

Results:
- In the coculture experiments realized in PBS at 27ºC, the presence of *A. castellanii* or *A. culbertsoni* induced a major increase in *A. baumannii* growth, compared to the control.
- Concerning the incubation in encystment medium after 24 h of coculture, the results showed a marked persistance of the viability of *A. baumannii* in the presence of *Acanthamoeba*. The electron microscopy showed internalized bacteria in trophozoites after 24 h of coculture. After 10 days in encystment medium, bacteria were found within cyst wall.

Conclusion: Under certain conditions, the survival and growth of *A. baumannii* is favored by *Acanthamoeba* strains. So, in hospital water systems, a special attention should be paid to the presence of free living amoebae, which can promote *A. baumannii* growth.

R2270 Laboratory-based surveillance system of sexually transmitted infections in Italy

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Objective: To assess the prevalence of *Chlamydia trachomatis* (Ct), *Neisseria gonorrhoeae* (Ng) and *Trichomonas vaginalis* (Tv) infections among Italian general population using a Laboratory-based Surveillance System.
To evaluate the role of this system to add knowledge on the circulation of Sexually Transmitted Infections (STIs), already monitored by a National Surveillance System based on STI clinics.
Methods: In 2009, the Istituto Superiore di Sanità, in collaboration with the Associazione Microbiologi Clinici Italiani, launched a programme for the surveillance of new case of Ct infection, as well as Ng and Tv.
The data are provided by 13 large clinical microbiology laboratories, with high clinical-diagnostic standards, located in the main areas of Italy (Northern, Central and Southern), which collect data on all individuals who undergo testing for Ct, Ng and Tv infections. Socio-demographic, clinical and behavioural informations are also collected. For each individual, laboratories may report the possible identification of more than one pathogen.
Results: From 1 April to 30 September 2009, 9,570 individuals have been tested, 90.0% of these were female and 11.6% were non-nationals. The median age of individuals was 34 years (IQR=29–40 years). A total of 4,275 individuals (50.0%) were asymptomatic and 29.8% of the women were pregnant. Of the individuals, 76.4% had no used any contraceptive and 90.1% reported having had one sexual partner, in the previous six months.
In total, 8,490 (88.7%) individuals were tested for Tv infection, 7,333 (76.6%) for Ct and 5,178 (54.1%) for Ng. The prevalence of Tv, Ct and Ng was 0.7%, 3.5% and 0.5%, respectively. The highest prevalence was observed among symptomatic and asymptomatic males, for Ct (15.5% and 6.4%, respectively) and Ng (4.4% and 0.5%, respectively), and among symptomatic and asymptomatic women for Tv (0.9% and 0.5%, respectively).
The positivity for Ct was associated (p value for $\chi^2 < 0.001$) with younger age (14–24 years vs. >24 years of age, 9.0% vs. 2.7%), having had two or more partners in the previous six months ($\geqslant$2 vs. 0–1 partners, 12.5% vs. 2.6%) and having used oral contraceptive in the previous six months (oral contraceptive vs. other, 5.4% vs. 2.8%).
Conclusions: Preliminary results from the Laboratory-based Surveillance System of STIs seem to suggest important data on the circulation of these infections among individuals more like to general population than those consulted in STI clinics.

R2271 Employing ATP detection technology (3M Clean-Trace) to improve cleaning standards and practices in different clinical areas

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Background: To control hospital infections new ways to improve cleaning processes are needed. The 3M Clean-Trace Clinical Hygiene Monitoring System, which employs ATP detection technology, has been validated by the rapid review panel to provide a rapid quantifiable assessment of the quality of the cleaning process as part of a structured approach to hygiene monitoring. Our hospital has recently introduced generic ward and clinic based cleaning schedules, designed by the Infection Prevention & Control Team, to focus all staff on cleaning the patient equipment and on being able to demonstrate that they had done so through documentation.
Objectives: To use Clear-Trace technology as a tool to look at the value of implementing ward based cleaning schedules with the aim of improving the cleaning standards of medical equipment.
Methods: Two clinical areas were selected: a minor surgery treatment room and a surgical ward. Ten sites in each clinical area were identified. A written plan was produced to enable the swabs to be taken at the exact same sites and at the same time every day for 2 weeks prior to the schedules being implemented. Following the introduction of the schedule, swabs were collected daily for 5 days per week for 4 weeks. No feedback was given to staff for the first two weeks post-schedule. For the last 2 weeks post-schedule daily feedback was given to the senior nurse and the manager of the cleaning staff.
Results: The background swabbing of sites identified the areas where cleaning needed to be improved upon. The commencement of the cleaning schedules did focus staff on cleaning, which led to improved documentation of the cleaning process, as expected. However, some sites continued to give high signal in the ATP swabs. When feedback was given to staff cleaning improved on all sites, with low ATP signals. Ease of access for cleaning of particular pieces of equipment was found to affect the likelihood of cleaning taking place. An example of this was the x-ray light box which was sited too high for most staff. The box was removed following discussion with staff.
Conclusions: The study identified the benefit of introducing the cleaning schedule as it refocused staff on the need to be vigilant in cleaning equipment between patients and on the value of documenting equipment cleaning practices. The Clean-Trace system has been useful as it provided a tool to audit the cleaning quality.

R2272 Mupirocin resistance in methicillin-resistant *Staphylococcus aureus* and use of intranasal mupirocin

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Objectives: To determine the rates of mupirocin resistance in methicillin-resistant *Staphylococcus aureus* (MRSA) and the efficacy of intranasal mupirocin (3 days versus 5 days) in reducing nasal colonization with MRSA.
Methods: Hospitalized patients admitted at a university hospital (650 beds) were screened for MRSA nasal colonization according to established hospital guidelines. Isolation and identification of MRSA were based upon standard microbiological procedures. All isolates were tested for resistance to mupirocin (Mup) with a 5 μg disk (zone of inhibition $\leqslant$13 mm). Mup resistance organisms underwent MIC analysis by the Etest (high-level resistance was defined as a MIC of $\geqslant$512 mg/L and low-level resistance was defined as a MIC of 8 to 256 mg/L). MRSA nasal carriers received mupirocin ointment applied to the anterior nares 3 times daily for 3 days (April 2000 to January 2003) or for 5 days (February 2003 to December 2004). Follow-up nasal samples for culture were obtained two days after completing treatment and successful decolonization was considered to have been achieved if results were negative.
Results: Between April 2000 and December 2004 we detected 326 nasal carrier of MRSA. Overall, 84.36% were susceptible to mupirocin (93%, 90%, 87%, 76% and 82% in 2000, 2001, 2002, 2003 and 2004 respectively), 4.29% had low-level resistance and 11.35% had high-level resistance. A total of 209 nasal carriers of MRSA Mup susceptible were treated with mupirocin and follow-up samples were obtained (from April 2000 to January 2003, 118 patients were treated for 3 days and from February 2003 to December 2004, 91 patients were treated for 5 days). After treatment for 3-days, successful decolonization occurred in 82% of patients and 92% of patients who received 5-days course ($p = 0.03$). The other 117 patients did not received Mup treatment or were not available for follow-up because they were lost (63 had discharged or had died), Mup resistance (51) or they had multiple skin lesions (3).
Conclusions: Treatment with topical mupirocin for 5 days was more effective in eradicating MRSA nasal colonization but the rate of Mup resistance increased. In order to control increase of Mupirocin resistance, use among patients identified as MRSA nasal carriers and continue screening for resistance to mupirocin.

R2273 Germinate to exterminate: the role of the tetracyclic region of sodium taurocholate in the germination of spores of *Clostridium difficile* ribotype 027

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Objectives: To investigate the germinating efficacy of sodium taurocholate on dormant spores of *C. difficile* ribotype 027 at room temperature following the partial acetylation of the compound's tetracyclic region.
Methods: Sodium taurocholate (ST) was partially acetylated by means of acetyl chloride in dimethylformamide. The derivatives were then used to prepare a germination solution comprising 2% (w/v) ST in double strength thioglycollate medium. To assess if acetylation of the tetracyclic region of ST affects its germination potential, a suspension-germination test system was designed which consisted of inoculating the derivatized germination solution with *C. difficile* ribotype 027 (2.6×10^6 cfu/mL). Control suspension-germination assays comprised 2% (w/v) non-derivatized ST in double strength thioglycollate germination solution. After 1 hour incubation at room temperature the samples were re-suspended in Wilkins-Chalgren anaerobic broth, and heated to 70ºC for 10 minutes to eliminate spores which had germinated. Control suspensions were kept on ice. Suspensions were then inoculated onto fastidious anaerobic agar supplemented with 5% defibrinated horse blood agar and 0.1% ST and incubated at 37ºC in anaerobic conditions. Total viable counts from the samples comprising derivatized sodium taurocholate and non-derivatized sodium taurocholate were then determined.
Results: Partial acetylation of ST significantly reduced its germination potential compared to non-acetylation of the compound ($P < 0.05$). Following exposure to the germination solutions and subsequent heat shock, 94.7% of *C. difficile* O27 spores remained viable in the presence of partially acetylated ST, whilst 99.1% of spores were eliminated in the presence of the non-derivatized formulation.
Conclusion: Germination of *C. difficile* spores remains poorly understood, however, a clear comprehension of the underlying process may pave the way for novel prevention and treatment strategies. The results from this investigation offer an insight into the germination of *C. difficile* spores and clearly indicate that the tetracyclic region (and/or hydroxyl groups) of ST play a major role in the germination process. Further studies are clearly warranted.

R2274 How much do healthcare workers pay attention to hand hygiene in hospital?

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Objective: Optimal hand hygiene behavior is considered the cornerstone of healthcare-associated infection prevention, but healthcare worker (HCW) compliance with hand hygiene practices remains low for the most part in most settings. The aim of this study was to determine hand hygiene compliance rates of HCWs in various intensive care units in our hospital.
Methods: This prospective study was performed in Ankara Numune Research and Traning Hospital between March to August 2009. Hand hygiene behavior of health care workers working at one of Medical Intensive Care Unit (M-ICU), Surgical Intensive Care Unit (S-ICU) or Medical/Surgical Intensive Care Units (M/S-ICU), were observed during their patient care activities. Hand hygiene practice before and after patient contact were observed individually.
Results: A total number of 1252 occasions for hand hygiene were observed. The overall compliance was 30.5%, whereas this rate was 11.8% before patient contact and 29% after patient contact. Most of the patient contacts were done by nurses (65%), followed by doctors (23.2%) and housekeeping staff (7.2%). Compliance to hand hygiene before versus after contact was 2.8% versus 20.7% for physicians, 13.5% versus 31.6% for nurses and 21.9% versus 32.8% for the other HCWs respectively. The same rates for procedures such as preparing pharmaceuticals, patient care and invasive procedures were as follows; 12.6% vs 31%, 17.7% vs 28.5% and 9.4% vs 32.1%, respectively. When comparing hand hygiene compliance before patient contact with the type of procedures (invasive versus noninvasive), there was statistically significant difference when performing non invasive procedures ($p < 0.05$). Nurses were more compatible group about hand hygiene when compared to the other HCWs ($p < 0.05$). Compliance to hand hygiene was higher after patient care in all of the groups of HCWs. Hand hygiene compliance rates were nearly the same in all of the ICUs.
Conclusion: The results of this observational study showed us that our hand hygiene compliance rate was low especially before patient contact among all of HCWs. Effective strategies such as continuing educational programs on improving hand hygiene should be developed especially HCWs other than nurses.

R2275 Infection control procedures in European facilities designed to deal with HIDs: EuroNHID data from a survey of 44 isolation facilities in 14 European countries

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Objective: Highly Infectious Diseases (HIDs, e.g. Viral Haemorrhagic Fevers and SARS) are life-threatening, human-to-human transmissible diseases that may cause Public Health emergencies, requiring special procedures for their containment. To review hospital infection control procedures, the European Network for Highly Infectious Diseases project conducted, through a specifically developed checklist, a survey in the facilities designed to deal with HIDs. Data from 44 facilities in 14 European Countries are described.
Methods: The checklist, including 22 items and 62 questions, was developed through a "networking strategy": a project partner with specific expertise sent drafts for comments and amendments. Final agreement had been reached during a meeting involving all partners. Facilities to be surveyed were selected by national authorities, and are those planned for giving care to patients affected by HIDs. In site surveys were conducted from March to November 2009.
Results: All facilities refer to have specific procedures for hand-hygiene, but availability of adequate devices (non-hand operated sinks, distributors of alcohol solution) differs among countries. About Personal Protective Equipments, almost all facilities refer protocols for their selection and supply, and procedures for donning and removal. Procedures for the prevention of needle-stick injuries are in place in all facilities, but the use of specific devices differs strongly among countries. About 70% of facilities have protocols for the transport of HID patients, but special vehicles are used in 5 countries only. Solid waste are autoclaved in 14 facilities, while in the others are transported in secure containers to incineration without prior decontamination. Liquid waste are treated with chemical or physical processes or jellified in 34 facilities, and disposed without decontamination in the remaining. Procedures for the management of corpses are available in 85% of facilities, and protocols for autopsies are present in 50%. Eight facilities have a special equipped autopsy room.
Conclusion: Infection control procedures are generally available in European isolation facilities planned to give care to HID patients. The main critical point remains the availability of adequate structural and technical issues, that strongly differs among participating countries. Further efforts should be done also for the implementation and the monitoring of the compliance to these procedures.

R2276 The impact of Xpert™ MRSA in the prevalence and incidence of methicillin-resistant *Staphylococcus aureus* in a Portuguese hospital

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In our hospital Meticillin-Resistant *Staphylococcus aureus* (MRSA) is the most frequent agent of healthcare-associated infections (HAI).
Screening for MRSA colonization in individuals admitted to hospitals is very important to adopt infection control methods and in the prevention of its cross-transmission.
The main aim of this study is to evaluate the impact of this new methodology in the diminution of the tax of MRSA in Hospital Pedro Hispano (431 beds) during the last year.
In 2007, was initiated a protocol of control of colonisation for MRSA in the hospital admission. Screening nasal (inguinal in the impossibility of the nasal) was effectuated in all high-risk patients (patients that came from nursing homes or other health care institutions where they had remained more than 48 hours).
Preemptive isolation was used. Patients who tested positive for MRSA keep contact precautions until hospital discharge or documental eradication of MRSA. Contact precautions were discontinued if the results were negative. The method used was culture in chromogenic agar ID MRSA (bioMérieux). These procedure take a minimum of 48 h before the results are known. The prevalence rate of MRSA was of 66%.
With the goal of lowering this tax and the isolation days, with infection control measures remained constant (hand hygienic and contact precautions), a new rapid diagnostic test was adopted. The research of MRSA was performed by real time polymerase chain reaction (PCR) in GeneXpert (Cepheid) 24 h/7 days a week and gives a result within 2 h.
In 1674 screenings, 433 (26%) were positive, 1187 (71%) were negative e 54 (3%) were invalid. The invalid results were repeated by culture.
The incidence of healthcare-associated MRSA colonization or infection was compared before and after the introduction of real time PCR (table 1).
In our hospital the use of Xpert™ MRSA reduce significantly both the rate of MRSA and the number of patient isolation-days.

Table 1

	2007	2008
No. of *Staphylococcus aureus*	356	285
No. of MRSA	235	177
Prevalence	66%	62%
Incidence (per 1000 patient-days)	1.8‰	1.2‰
Cumulative incidence (per 100 patients)	1.4%	1.0%

R2277 Factors associated with the detection of *Mycobacterium tuberculosis* in sputum among isolated inpatients with suspected pulmonary tuberculosis and validation of a clinical prediction rule

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Objectives: Guidelines for preventing the transmission of *Mycobacterium tuberculosis* in health-care settings are successful when properly implemented but may also result in unnecessary isolation of many patients without tuberculosis with a significant increase in hospital costs. We wished to assess the prevalence and identify predictive clinical factors of culture-proven tuberculosis among inpatients isolated for suspected pulmonary tuberculosis (PTB) in our department. We also wished to validate a previously published clinical decision rule (CDR) to predict the need for respiratory isolation of these patients.
Methods: From August 1, 2005 to January 31, 2007, patients isolated on admission to the infectious diseases ward for suspicion of PTB were prospectively enrolled in this study. The presence of tuberculosis risk factors, clinical symptoms, and findings form physical examination and chest radiography were recorded on admission. The decision to isolate patients was not based on the CDR, but made by the admitting team which ranked the likelihood of PTB as high, low or intermediate.
Results: During the study period, 1207 patients were admitted to the ward and 134 (11.1%) were isolated for suspected PTB. Only 1 patient was diagnosed with PTB among those not isolated upon admission to the ward. Of the 134 isolated patients enrolled in the study, 26 were found to have PTB (prevalence: 19.4%, 95% confidence interval (CI): 13.6–26.7). Multivariate analysis revealed that PTB among isolated patients was significantly associated with cavitary lesions on chest X-ray (adjusted OR: 32.9 (95% CI: 6.4–171), $p < 0.0001$), and weight loss of at least 10% of body weight (OR: 5.15, 95% CI: 1.5–17.5, $p = 0.008$). A high or intermediate index of suspicion of PTB by the admitting team was also significantly associated with PTB ($p < 0.0001$) with a sensitivity of 96.2% and a negative predictive value of 98.5%. The CDR had a sensitivity of 96.2%, a specificity of 21.3%, a positive predictive value of 22.7% and a negative predictive value of 95.8% for the diagnosis of PTB. Use of the CDR would have correctly identified all but one patient with PTB, and avoided 23 isolations (17.2%).
Conclusion: The prevalence of PTB was 19.4% among isolated inpatients in our ward, and PTB was associated with cavitary pulmonary lesions and weight loss. Use of a CDR in addition to clinical judgment might avoid unnecessary isolations.

R2278 MRSA screening by real-time PCR to release patients from preventive isolation on hospital admission

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Objectives: To evaluate a rapid screening test enabling hospital wards to manage potential MRSA carriers on hospital admission.
Methods: Patients presenting MRSA carriage risks were screened using double swabs each for nasal and inguinal samples for improved sensitivity. One of each nasal and inguinal swabs were tested pooled on a GeneXpert® (Cepheid) system. The second swabs were incubated individually in enrichment saline broth (MSB) overnight at 35ºC before subculturing on chromogenic plates (MRSA, bioMérieux) which were incubated again overnight at 35ºC. On the next day, observation of green colonies confirmed the presence of MRSA.
Results: From October 2008 to October 2009, 365 samples were tested by PCR. We obtained 49 (13.4%) positive PCR results of which 33 (67.3%) could be confirmed by culture which gives a low positive predictive value (PPV= 69.4%). Out of the 16 negative cultures, the presence of MRSA could be detected in 7 (43.7%) samples. The molecular technique proved to be more sensitive than routine primary culture. Neither subsequent subcultures nor PCR on MSB succeeded in obtaining any MRSA positivity in the 9 other initially PCR-positive samples; suggesting culture failure or a GenXpert specificity problem originating from amplification or detection targets determination. In some rare *S. aureus* strains, an absence of the mecA gene in their integral chromosomal cassette mec may give false positive results. One equivocal PCR gave negative cultures. Four invalid results (1.1%) were controlled with the two remaining swabs to obtain finally 3 negative results and 1 (0.3%) repeatedly invalid result.
During the first 6 months, all 186 valid PCR samples were cultured. Among the 161 negative PCR results, only 2 samples were culture positive. We obtained another 18 positive cultures from the 25 PCR positive samples, giving a sensitivity of 90% (18/20)for PCR versus culture. Specificity was higher at 95.8% and the most significant result was obtained for the negative predictive value (98.7%). These characteristics enable us to propose this PCR for screening in order to rapidly release suspected MRSA carriers from isolation on hospital admission.
Conclusion: The GenXpert rapid molecular test presents a high negative predictive value which can be used efficiently to improve patients' comfort and hospital wards management without loss of security in infection control. However, the low PPV obtained suggests that culture remains necessary to assess strain viability.

R2279 Comparison of a fully automated electro-chemiluminescent immunoassay with haemagglutination inhibition for determination of Rubella virus antibody: evaluation of immune status with commercial reagents in a clinical laboratory

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Objectives: Rubella is a common communicable disease of childhood which is ordinarily benign in children and adults. However, for the developing fetus the infection may be very serious. It is still important to accurately determine the immune status of women of reproductive age, because still a women are not vaccinated against rubella virus, and to diagnose and confirm recent infections related to congenital syndromes. Hemagglutination inhibition (HAI) is still the most commonly used technique for the laboratory diagnosis of rubella in some countries (e. g. Germany or Austria). However this test is lengthy, labor intensive, and poorly adaptable to automation. In addition, there my be considerable variation from one laboratory to another.
The use of a fully automated electrochemiluminescent immunoassay (ECLIA) is an improved alternative to HAI. This study describes a comparative laboratory analysis of indirect hemagglutination and the Elecsys Rubella IgG assay on the Roche Modular Immunoassay platform.
Methods: A total of 599 serum specimens were studied retrospectively. All sera were tested with a commercial HAI assay (Siemens Medical Diagnostics, Marburg, Germany) and a fully automated Elecsys Rubella IgG assay on Modular Analytics (Roche Diacnostics, Mannhein Germany). Discrepant samples were additionally tested with a rubella IgM immunoassay (Roche Diagnostics, Mannheim, Germany) and resolved by virus neutralization assay to determine the immune status.
Results: The relative sensitivity for the detection of immunity was 100% for both assay types. The amount of indeterminate results of the HAI was 5.9% compared with only 1.8% of the Elecsys Rubella IgG. The relative specificity of both assay for the detection of immunity to rubella virus was 94.6% for the HAI and 98.3% for the Elecsys Rubella IgG assay. The overall correlation between both assays was 96.3%.
Conclusions: IHA is still a reliable method for screening sera for immunity to rubella virus. But today fully automated quantitative antibody immunoassays like the Elecsys rubella IgG assay are superior referring to sensitivity and specificity. The grade of standardization and stability of these assays is even higher resulting in a better comparability between different labs. In our point of view the IHA should be replaced by quantitative IgG assays for the screening of prenatal sera for immunity to rubella virus.

R2280 Evaluation of National Infection Prevention Week on NRIC www.nric.org.uk: a comparative study of success of the event during 2007/08/09

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Objectives: National Infection Prevention Week (IPW) takes place in October annually and provides an opportunity to promote infection prevention and control as a key element of safe care for both patients and those receiving care in the community keeping them safe and free from healthcare associated infections. NRIC participates in this event in line with its key aims – to provide best available evidence on management, prevention and treatment of HAIs. After two successful IPWs in 2007 and 2008 NRIC in partnership with the Royal College of Nursing (RCN) focused during 2009's IPW on the contribution of two important groups of healthcare workers, nursing students and healthcare support workers, providing links to information on infection prevention and control knowledge to meet their specific needs. IPS and IFH endorsed this initiative.
Methods: The IPW 2009 was run on NRIC during October 19–23rd 2009 (http://www.nric.org.uk/IntegratedCRD.nsf/ICWeek2009?OpenForm) previously the IPW 2008 and 2007 weeks taking place on October of these years. The dedicated online resource included links to best available evidence, up-to-date resources and activities for raising awareness in hospitals and community. A qualitative methodology was used to analyse the traffic on the NRIC web server.
Results: In October 2009, the interest in the IPWs pages was the highest so far: 1079 visitors and 3833 page views, 100% increase in comparison to the months leading to it; 499 visitors and 1795 page views in Sep 2009, 378 visitors and 1151 page views in Aug 2009. The traffic during the actual IPW week in 2009 was the highest since the event began three years ago; 405 visitors and 692 page views in 2009, which is 39% more than in 2008 (291) although the number of page these visitors looked at decreased by 37% (1096 in 2008). In 2007, we received 264 visitors viewing a total of 1138 ICW pages. The total traffic on the NRIC portal as a whole during the IPW in 2009 was 27% above Oct average (1525 visitors, 6588 page views). The full result set will be presented at the conference.
Conclusion: The NRIC IPWs success in 2007/09 demonstrates a clear need for provision of these resources and raised awareness of this important week – 27% increase visitors above NRIC average and the number of visitors during IPW 2007 almost doubled at IPW 2009. In 2010, NRIC IPW will aim to make use of media that appeals to today's audiences – Twitter or FaceBook and focus on international evidence (IFIC support).

R2281 Reducing paediatric blood culture contaminants

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Objective: Reducing blood culture contaminant rates and subsequent long term maintenance of this reduction can prevent instigation of unnecessary antimicrobial therapy. Achieving this reduction in the paediatric setting requires different infection control interventions to adult policies. This report describes a retrospective observational study of 685 consecutive paediatric bacteraemias in patients attending a tertiary referral paediatric directorate both before and after intensive infection control measures were introduced to reduce contamination rates.
Methods: The study period covers two years from November 2007 to October 2009 with infection control interventions introduced following a high rate of blood culture contamination rates in October 2008. At that point intensive training and education was carried out (induction) and regular real time epidemiological feedback to medical and nursing staff was implemented (maintenance). Demographic, clinical and laboratory data was reviewed for each positive blood culture during the study period.
Results: Following the induction and maintenance phases of the infection control interventions a reduction in blood culture contaminants with coagulase negative staphylococci was achieved (mean 18 per month pre-intervention to 11 per month post intervention; p-value 0.01). There was no statistically significant drop in overall blood cultures taken pre- and post intervention (273 and 263 per month respectively; p-value 0.43) nor in the isolation of any other pathogen with the exception of yeasts (pre intervention 36 isolates in a year; post intervention 7 isolates in a year; p-value 0.01) There was also a statistically significant decrease in coagulase negative staphylococcal bacteraemias from those patients with indwelling long lines (56.1% pre-intervention to 47.6% post intervention; p-value 0.03) – despite gross total parenteral administration within the unit actually increasing during this period.
Conclusions: We find infection control interventions focusing on education and training can reduce blood culture contamination rates and this reduction can be maintained with regular real-time epidemiological feedback to clinical staff.

R2282 Do limited resources affect the hand hygiene performance, beliefs and perceptions of healthcare workers?

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Objectives: To identify the beliefs and perceptions associated with hand hygiene performance in two different institutions with limited resources and started infection control programme later than developed institutions.

Methods: The study was conducted in two different hospitals – University Hospital (U-hospital) and Community Hospital (C-hospital) in the same city by a self-administered questionnaire. Most questions were drawn from questionnaires used previously in other studiesfrom "industrialized" countries based on "The Theory of Planned Behavior". All nurses, nurse students, physicians and medical students in the U-hospital, and all nurses in the C-hospital were included into the study.
Results: Of 1764 questionnaires, 941 (41%) were returned. The return rate was highest for nurses in C-hospital(63.8% [303 of 475]) and lowest for senior physicians in U-hospital (7.5% [16 of 212]). Respondents provided demographic information and data about various behavioral, normative, and control beliefs that determined their intentions with respect to performing hand hygiene. Among individuals from the other professional categories, a greater percentage of U-hospital nurses (57.6% vs.53.9%, respectively) believed that healthcare-associated infections to be greater than 20%, and mortality rate among infected patients to be greater than 5%.However, all professional categories believed that good hand hygiene effectively prevents infections (98%). In univariate analysis, receipt of structured training in hand hygiene, perceived colleagues adherence's as good, adherence models good practices for others, having been observed for their adherence (normative beliefs), the perception that hand hygiene is relatively easy to perform (control beliefs) was associated with good hand hygiene. However, in multivariate analysis, high self reported adherence to hand hygiene was independently associated with receipt of structured training in hand hygiene, perceived good adherence by colleagues, the perception that hand hygiene is relatively easy to perform and having been observed for their adherence.
Conclusions: In a country with limited resources, intention to comply was associated with training and strong normative and control beliefs. Also, in two different kinds of institution with the similar hand hygiene promotion campaign in the same city, the believes of nurses were different. In developing countries, more resources have to be saved for training of HCWs and easy access for hand hygiene products.

R2283 Prospective study of surgical site infections

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Objective: To examine and evaluate incidence, risk factors and microbiology patterns of Surgical Site Infections (SSIs), in a Greek hospital.
Methods: This prospective study included 290 patients (66% males and 34% females) who had undergone a total of 311 general-surgery operations during the period February 2009 to October 2009. An SSI was defined on the basis of clinical and laboratory findings. All isolates were identified and tested for antibiotic susceptibility with Vitek-2 automated system (bioMérieux, France). Confirmation of MICs was determined by E-test (AB Biodisk, Sweden). Descriptive and logistic regression analyses were performed to determine risk factors for SSIs. A p value $\leqslant 0.05$ was regarded as significant.
Results: SSI occurred in 23 (7.4%) of the 331 operations. Of the pathogens isolated, seventeen were Gram-negative rods, five Gram-positive cocci, and one was *Candida albicans*. The infection was polymicrobial in fourteen patients, while infection coexisted in another site. Of all the isolates, we had 10 *K. pneumoniae,* 9 *P. aeruginosa*, 7 *E. coli*, 4 *Enterococcus* spp., 3 coagulase-negative staphylococci and 2 isolates of *Staphylococcus aureus*. Six strains of *K. pneumoniae* were KPC-producers and five strains of *P. aeruginosa* were multi-drug resistant. All Gram-positive cocci were methicillin-resistant but glycopeptides-sensitive. The risk factors of significance are summarized in the table.

Risk factor	P
Pre-operative anaemia	<0.001
Malnutrition	<0.001
High score of ASA (American Society of Anesthesiologists)	0.029
Prolonged pre-operative hospitalization	0.001
Prolonged operation time	0.001
Blood transfusion	<0.001
ICU hospitalization	<0.001
High National Nosocomial Infections Surveillance (NNIS) risk index	<0.001

Conclusions: Despite the use of precautionary antimicrobial treatment and the antiseptic measures, the rate of SSI is high. The efforts to control SSIs, should be focused on hospital infections surveillance programs and modification where this is feasible, of the factors that affect significantly the development of SSIs. Awareness of risk factors before certain surgical procedures allows for targeted prevention measures.

R2284 Is there any new evidence on the efficacy of rapid screening tests on hospital-acquired MRSA acquisition rate?

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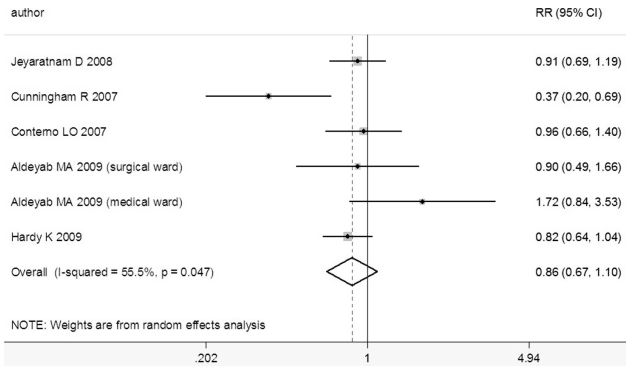
Objective: In a previous systematic review of the literature and meta-analysis we documented that, compared with culture screening, use of rapid screening tests was not associated with a significant decrease in MRSA acquisition rate. Recently, new important evidence supported the screening by molecular method as associated with a significant reduction in MRSA acquisition rate. The objective of the current study was to verify our previous results and conclusions, according to the most recent evidence.
Methods: The computerized search was updated until July 2009. We judged as eligible those studies that compared hospitals and wards in which active screening for the detection of MRSA carriers was done at hospital admission by use of a rapid molecular test to those in which active screening was done with enrichment culture. To account for statistical heterogeneity between studies, random-effects models were used. Case reports, reviews and letters were excluded. Primary outcome was defined as MRSA acquisition rate per 1000 patient-days.
Results: The updated search revealed additional 193 relevant articles. One new study including 13,952 patients was eligible for inclusion. Overall 5 studies (3 interventional studies and two crossover trial) were reviewed. All studies were performed between 2000 and 2007. Four studies were done in Europe (UK) and 1 study in Canada. Only one study was a cluster-randomised, unblinded, crossover trial. All studies used the same commercial assay. Nasal samples were tested in all studies. In 4 studies, MRSA was associated with at least another site (eg, axillae, perineum, groin, and skin breaks). Compared with culture screening, use of rapid screening tests was not associated with a significant decrease in MRSA acquisition rate (risk ratio 0.86, 95% CI 0.67–1.10). Heterogeneity among studies was reduced by the inclusion of the new study.
Conclusion: Cumulative meta-analysis did not demonstrate any substantial variation in the point estimates with the addition of the recently published study. We confirm that rapid screening tests do not seem to be effective in significantly reducing hospital-acquired MRSA acquisition rate when compared to culture screening.



R2285 How long could an educational intervention improve hand hygiene practices?

A. Erbay, Y. Tezer Tekce, H. Cabadak, S. Sen (Ankara, TR)*

Objectives: Hand hygiene is essential for the prevention of nosocomial infections, but compliance in clinical practice is often low. This study was planned to determine the compliance rates of hand hygiene practices, the impact of educational programs on hand hygiene compliance rates and the duration of the effect of the educational programs.
Methods: This prospective and observational study was performed in Turkiye Yuksek Ihtisas Education and Research Hospital in Turkey. Hand hygiene compliance, the effect of educational program and duration of this effect on hand hygiene compliance were investigated. Observations were carried out on weekdays during the working hours. Hand hygiene practices were evaluated in following procedures; before the patient contact, after the patient contact, after environmental contact, before high risk contacts and after contact with blood and body fluids. Compliance of medical doctors and nursing staff were evaluated.
Results: Hand hygiene compliance was evaluated in 8893 contacts during 6 months period. At the initial phase of the study a total of 1460 contacts were observed and in 38.2% of these contacts hand hygiene practices were proper. After the educational program overall compliance rates improved to 54.6% ($p < 0.05$). Following months compliance rates were detected as; 47.6% and 43.3%. Four months after intervention compliance rates decreased to 38.0%. After a repeated educational intervention, compliance rates increased to 49.8%. Nursing staff had better compliance rates than medical doctors overall, 57.3% versus 32.4% ($p < 0.05$). Overall hand hygiene compliance after the patient contact was greater than before the patient contact, 60.4% vs. 34.5% ($p < 0.05$). Hand hygiene compliance before high risk contacts were detected 36.6% before the intervention and 51.1% after the first intervention ($p < 0.05$).
Conclusion: Continuous educational programs are needed in order to maintain higher compliance rates of hand hygiene practices. We suggest that educational interventions should be repeated in every 3 months.

R2286 The effect of an educational intervention to nosocomial catheter-associated urinary tract infection rates

A. Erbay, H. Cabadak, Y. Tezer Tekçe, S. Sen (Ankara, TR)*

Objectives: To obtain the incidence of nosocomial catheter-associated urinary tract infections (CAUTIs), microbiological profiles and bacterial resistance in intensive care units (ICUs) and observe the effect of an educational intervention to the CAUTIs rates.
Methods: Prospective cohort surveillance of CAUTIs was conducted in five ICUs with 96 beds in Turkiye Yuksek Ihtisas Education and Research Hospital in Turkey, by applying the definitions of the Centers for Disease Control during 2 years period. Rates of CAUTIs per 100 patients and per 1000 device days were determined. In January 2008 urinary catheter implementations were reviewed and an educational program was carried out.
Results: From January 2007 to December 2008 CAUTIs were followed up. The mean age was 65.2 and 57% of the patients were male. In 2007, 4886 patients followed up in ICUs for an aggregate of 18013 ICU days. Of these, 86 CAUTIs were detected in 73 (1.49%) patients. The rate for CAUTIs was 7 cases per 1000 catheter-days. Urinary catheter use rate was 0.71. In 2008, 5591 patients followed up in ICUs for an aggregate of 21055 ICU days. Of these, 51 CAUTIs were detected in 45 (0.8%) patients. The rate for CAUTIs was 2.6 cases per 1000 catheter-days. Urinary catheter use rate was 0.79. The period between ICU admission and CAUTIs ranged 3–126 days. The most commonly isolated migroorganisms were as follows; *Escherichia coli*, *Enterococcus* spp., *Klebsiella* spp., *Acinetobacter baumannii*, *Pseudomonas aeruginosa*. *E. coli* isolates were resistant to cefotaxime in 64%, were resistant to ciprofloxacin in 86%, and were resistant to piperacillin–tazobactam in 26%. Seventy percent of *Klebsiella* spp. isolates were resistant to ciprofloxacin, 62% were resistant to cefotaxime and 54% were resistant to piperacillin–tazobactam. All of the *A. baumannii* isolates were resistant to ciprofloxacin, and 40% were resistant to imipenem. Fifty percent of *P. aeruginosa* isolates were resistant to ciprofloxacin, all of them were resistant to ceftazidime and piperacillin–tazobactam, and 30% were resistant to imipenem. Ampicilin resistance was detected in 28% of the *Enterococcus* spp. isolates and vancomycin resistance was not observed.
Conclusions: The CAUTIs rates in our hospital decreased after educational intervention. However antimicrobial resistance rates were high in this study.

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

R2287 Bacteraemia without a focus: to what extent is the focus really unobserved or just unreported?

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Objectives: The absence of a focus of bacteraemia is associated with a poor outcome. However, the term is ambiguous and the aim of this study was to evaluate the positive predictive value of 'focus unknown' in a bacteraemia research database.
Method: This retrospective cohort study included bacteraemias diagnosed in 2003 at Aalborg Hospital, a Danish university hospital. The clinical significance of blood culture isolates and the likely focus of infection were determined jointly by medical doctors in clinical microbiology and attending physicians. Episodes were recorded concurrently with the clinical episode. The focus was classified as unknown if unsupported by evidence at the time of registration or if the likelihood of one focus was not superior to other foci. For all episodes with unknown focus we re-evaluated available information with special attention to time of death, neutropenia, procedures, foreign bodies and results of imaging. For each episode we concluded whether one focus or two (or more) equally plausible foci or no focus was present. The two latter categories were regarded as 'focus unknown'.
Results: In 184 (29%) of 645 bacteraemias the focus was recorded as unknown in the database; the number of patients was 162, including 11 children, with 1 to 4 episodes each (median age 68 years, male/female ratio 85/77). The re-evaluation disclosed a focus in 39 of the 184 episodes (abdomen 15, thorax and respir. tract 10, IV cath. 5, skin and connective tissue 5, urinary tract 3, bone 1); there were two or more plausible foci in 9 and no focus in 136. Hence, the proportion of bacteraemias with a focus increased from 71% (461/645) to 78% (500/645) (Chi-square, $p = 0.015$). The positive predictive value of 'focus unknown' was 79% (145/184).
In only 2 episodes a decision had been made to decline from searching for a focus. A search was prevented by precipitous death in 31 of the 184 episodes (17%), and localizing signs were missing in 37 (20%) due to neutropenia. Together these two groups accounted for 65 cases (35%). Three infants 10–16 months old had primary pneumococcal bacteraemia.
Conclusion: Using retrospective chart review as reference we found a positive predictive value close to 80% for the category 'focus unknown' in the database. A high proportion of patients with an unknown focus either died rapidly or had severe neutropenia. This contributes to the negative prognostic impact of an absent focus and perhaps even more than the hidden focus itself.

R2288 Epidemiological and microbiological survey of infections in rehabilitation units of the Lombardy region, Italy

M. Tinelli (Sant'Angelo Lodigiano, IT)*

Objectives: This report describes an epidemiological and microbiological survey of infections occurred in Rehabilitations Units (RUs) of the Lombardy Region during the years 2005 and 2006.
Methods: 123 RUs for 7,830 beds, 184,916 hospitalizations, 149,471 patients and 14,201 ascertained cases were considered in this study.

The eligibility criteria for the epidemiological analysis included the ICD-9-CM codes belonging to ten main groups of infections: UTIs, low respiratory tract, intestinal, bone, sepsis, candidiasis, bacterial not specified, SSTIs, iatrogenic, cardiovascular. The validation of ICD-9-CM diagnosis, by review of 3,028 medical charts in 28 out of 123 RU, was performed in order to assess the sensitivity of the multiple regression method.

Results: 3,028 admissions were analyzed, 662 of whom had a diagnosis of infection validated. Multivariate analysis showed that the presence of "at least an infection", during hospitalization in Rus, is positively associated with: age 65–74 and over 85 years old (baseline under 45: respectively OR 1.5, 95% CI 0.97–1.37 and OR 2.4, 95% CI 1.5–3.9), general geriatric RUs and neurorehabilitation RUs vs. cardiorespiratory RUs (respectively OR 1.35, 95% CI 0.97–1.86 and OR 2.7, 95% CI 1.2–5.8), discharge from hospital "with" or "without" intervention surgery/invasive procedure within the week prior to admission to RU vs. "non-patient" (respectively, OR 1.47, 95% CI 1.02–2.11 and OR 1.38, 95% CI 1.09–1.75); discharge within the week prior to admission with a diagnosis of infection vs. "non-diagnosis of infection or hospitalization" (OR 2.8, 95% CI 2.0–3.8). In the adopted model, the single settings were included as "random effects". A logistic regression model, similar to the incident, highlighted associations comparable to those estimated for the prevalence. The UTIs were the most frequent (14.8%, 95% CI = 13.4–16.2). The distribution of isolated microrganisms showed respectively: *E. coli* 48%, *P. mirabilis* 13%, *K. pneumoniae* 5.4%, *E. faecalis* 13.8%, *S. aureus* 3.1%, *P. aeruginosa* 9.5%, *A. baumannii* 0.45%. The prevalence rates of MDR showed that uropathogens were the most frequently isolated.

Conclusion: The frequency of nosocomial infections in RUs is positively associated with host characteristics, settings and health care, including: age, ward, recent discharge from hospital and with diagnosis of infection (surgery/invasive procedures). UTIs and uropathogens are significant in RUs.

R2289 Changing epidemiological features of nosocomial candidaemia at an Italian tertiary care hospital

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Objectives: To evaluate incidence, demographic features, causative species, risk factors, treatment and outcome of nosocomial candidemia.
Methods: Retrospective study of nosocomial candidemia (at least one blood culture positive for *Candida* spp) in 2008 at an Italian tertiary care hospital (Trieste, Italy) with no pediatric and transplantation departments.
Results: Fifty-two cases of candidemia occurred in 52 patients (48% males) with median age 79 years (range 33–95 years). At diagnosis 82.7% of patients were hospitalized in medical wards, 9.6% in surgical wards, 7.7% in intensive care units. Overall hospital incidence was 1.88 episodes per 10,000 patient-days. The incidence was higher in intensive care units (3.26 episodes per 10,000 patient-days)and medical wards (2.35 episodes per 10,000 patient-days) than in surgical wards (0.61 episodes per 10,000 patient-days). The most frequent *Candida* species was *Candida albicans* (51.9%), followed by *Candida parapsilosis* (32.7%) and *Candida glabrata* (9.6%). No patient was neutropenic. Underlying diseases were the following: solid cancer (37.2%), diabetes mellitus (13.7%), surgery (5.8%), HIV (2%). Twenty-eight (54.9%)patients had a central venous catheter (CVC); of them, 63% had proven catheter-related candidemia. 85.2% of patients with CVCs underwent line removal with median removal time of 1.5 days (range 0–19 days) after diagnosis. During three previous weeks, potential risk factors were: antibiotic therapy (92.3%), urinary catheter (80.8%), total parenteral nutrition (43.3%), one or more surgical operations (13.5%), mechanical ventilation (7.7%). The overall crude mortality at discharge was 46.2% and was higher for *Candida glabrata* (60%) than for *Candida albicans* (48.1%) and *Candida parapsilosis* (47.1%). Mortality was not significantly associated with isolated species ($p = 0.76$) and CVC removal time ($p = 0.81$). 66.7% of patients received adequate antifungal therapy, and treated patients had significantly lower mortality than untreated patients ($p = 0.0029$).
Conclusions: This study showed very high incidence of nosocomial candidemia, especially in medical wards. Candidemia was found to involve non neutropenic and very elderly patients, which were receiving antibiotic therapy. Only one half of our population had CVC and severe underlying diseases. A case–control study is warranted to define risk factors for candidemia in this population, in order to get effective preventive strategies.

R2290 Diversity of *Pseudomonas aeruginosa* isolates in sputum samples from chronically infected patients at a university cystic fibrosis centre, Innsbruck

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Introduction: *Pseudomonas aeruginosa* (PA) remains an important pathogen in the cystic fibrosis (CF) lung. Chronic PA infection is associated with an increased morbidity and mortality. Infection control measures and early eradication regimen are very effective for preventing cross infection among patients.
Objective: The aim of our study was to investigate a diversity of PA isolates in sputum of single long-term chronically infected patients and a change in genotypes over time. Also a determination of a possible cross infection of PA between patients was a term of the present study.
Methods: The study was carried out prospectively on a random of sputum isolates ($n = 55$) collected from chronically infected patients. During the study period of 6 months (10/2008–5/2009) 26 of 130 CF patients, categorized as chronically infected, with PA according to modified Leeds criteria and/or anti-pseudomonas antibodies were included. Twenty of 26 were outpatients, 6 – inpatients. Antibiotic therapies were recorded in our CF database and were available. *Pseudomonas aeruginosa* isolates were cultured from sputum samples and identified by standard methods. Strains were characterised by antibiotic resistance testing using disc diffusion and E-test. All isolates were analysed of clonal diversity using random amplified polymorphic DNA (RAPD) – PCR with 2 different primers (P 272 and P15) as a molecular typing method.
Results: During the study period 9 of 26 patients (34.6%) carried multiple PA isolates. Four patients carried 2 isolates each, 2 patients – 3 isolates each, 2 patients – 4 isolates each, and one patient – 6 isolates each. Molecular typing revealed no clonality among isolates tested. We found no transmission of PA isolates with the same RAPD genotype among patient collective. Only in one patient we found two genotypic different PA in one sputum sample. A great genotypic diversity was found in repeated sputum samples from the same patients.
Conclusion: Due to a strict patient segregation/infection control policy in in/outpatient care and continuous education there was no transmission of PA between the patients at the Innsbruck CF Centre. The intensive antibiotic use seems to have a remarkable impact on the preeminent organisms in multiple strains carriers and may induce adaptive mechanisms such as genome rearrangement with phenotype alteration in a subset of chronically infected PA patients.

R2291 Clinical and epidemiological characteristics of *Clostridium difficile*-associated diarrhoea in a tertiary hospital in Korea

J. Kim, H. Pai, J. Kang, T. Choi (Seoul, KR)*

Objectives: In order to find the epidemiological and clinical characteristics of *Clostridium difficile*-associated diarrhoea (CDAD), prospective study was performed in a tertiary hospital in Korea.
Materials and Methods: From September 2008 to August 2009, the patients with diarrhea (stool passage $\geqslant$3 days for $\geqslant$2 days or $\geqslant$6 diarrhea during 36 hours) after or during antibiotic usage were included. Stool culture, toxin A&B assay and direct stool toxin PCR were performed for those patients, and CDAD was defined when more than one of three tests was positive. The clinical characteristics of CDAD cases were studied.

Using 98 strains cultured, multiplex PCR for tcdA, tcdB and binary toxin and PCR ribotyping were performed.
Results: Incidence of CDAD was 54/100,000 patient-days in our hospital. Among cases with positive CDAD-tests, 71% was hospital-acquired CDAD (HA-CDAD), 19% was heath care-associated CDAD (HCA-CDAD) and 10% was toxigenic carrier. Mean hospital days of admission before HA-CDAD occurrence was 39.5 days (2–256). 39.7% of HA-CDAD showed severe disease (severity score$\geqslant$2 by Zar et al, CID 2007;45:302). Comparing the characteristics of HA-CDAD and HCA-CDAD by univariate analysis, severity score was higher in HCA-CDAD ($P = 0.043$), and more H2 blocker was used in HA-CDAD ($P = 0.005$). Among 121 cases of HA- and HCA-CDAD, 17% (20/121) improved without treatment, 47% (57/121) improved with treatment, 28% (34/121) relapsed after treatment, 5% (6/121) died and 3% (4/121) were lost. However, among 5 fatal cases, CDAD-attributed mortality was not identified.
On multiplex-PCR using 98 cultured organisms, all isolates were tcdA and tcdB-positive, and 3 isolates produced binary toxin. PCR ribotyping of 93 isolates showed diverse ribotypes with 13 isolates for the most common ribotype. Comparing ribotypes of 10 paired strains of relapsed cases, the same ribotypes were observed in 5 cases and different ribotypes were found in 5 cases, and the interval of relapse was 37.2 days (13–62) and 58.2 days (12–148), respectively. Three B1/NAP/027 strains were identified among 98 isolates; 1 was from toxigenic carrier and 2 were from HA-CDAD cases. Severity score of the two cases was higher than mean value of other cases; 4.5 (4–5) vs 1.63.
Conclusion: Incidence, morbidity and mortality of CDAD were not high in our hospital. However, B1/NAP/027 strains appeared, thus, further observation for incidence, disease severity and spread of B1/NAP/027 to community or hospital is necessary.

R2292 Incidence and aetiology of ventilator-associated pneumonia in the intensive care units at a university hospital

E. Azak, A. Willke, N. Altindag (Kocaeli, TR)*

Objectives: The aim of this study is to determine the incidence, etiology and antibiotic resistance patterns of ventilator-associated pneumonia (VAP) in intensive care units (ICU) of anesthesiology and cardiothoracic surgery.
Methods: The patients in intensive care units were applied active prospective surveillance between January 2007 to December 2008 and VAP were defined according to Centers for Disease Control and Prevention (CDC) criteria. Ventilator utilization ratio, VAP rate were calculated and compared using the National Nosocomial Surveillance (NNIS) definitions.
Results: A total of 2074 patients from ICUs of cardiothoracic surgery and anesthesiology were included in the study. 6367 patient-days and 3863 ventilator-days were recorded. 80 cases of VAP occured in 63 of 2074 patients (3.03%, 1.26 episodes of pneumonia per patient). Ventilator utilization ratios were determined as 0.69 and 0.30, VAP rate/1,000 ventilator-days were determined as 19.9 and 26.2 in the anesthesiology and cardiothoracic surgery of ICUs, respectively. *S. aureus* (34/101, 34%), *P. aeruginosa* (30/101, 30%) and *A. baumannii* (15/101, 15%) were the most commonly isolated microorganisms. Methicillin resistance were 76.4% in *S. aureus* isolates. Resistance patterns of *P. aeruginosa* and *A. baumannii* strains to ceftazidime, imipenem, meropenem, ciprofloxacin, piperacillin–tazobactam, sefoperazon-sulbactam and gentamicin were 52–100, 43–94, 38–87, 30–100, 50–100, 42–77, 27–93 percent respectively.
Conclusion: VAP rates and ventilator utilization ratios were determined as high among ICU patients in our hospital. *S. aureus* and *P. aeruginosa* were observed main microorganisms at the VAP patients and they were determined as quite resistant to mainly used anthibiotics. These results emphasizes the importance of preventive measures against hospital infections including VAP.

R2293 Nosocomial infection surveillance data of a burn centre, 2005–2009: what we have learnt

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Objective: Survival has improved due to the supportive treatment quality and the main cause of death amongst these patients is infections. Our aim in this study is to determine the types of infections, causative microorganisms in order to guide the antimicrobial therapy an infection control.
Methods: Hospital infection control committee data was reviewed retrospectively between years 2005 and 2009 fist nine months. CDC case definitions for nosocomial infections were used. Antimicrobial susceptibilities were determined by VITEK 2 system. Microbiological data, the site of isolation were extracted.
Results: Totally 381 patients were followed in 10807 patient days(pd). General hospital infection rates and their distribution were summarized in table 1. Infection rates in 2007 were seen to be higher with 27.03 infections/1000pd. The burn unit was temporarily closed and beds were decreased in order to plan a revision because of the high infection rates in 2007 and infection rates decreased dramatically. Burn infections were increased in 2009(66.66 infections/1000pd). This increase was linked to transferring to a temporary clinic with inappropriate conditions. Urinary infection rates were highest in 2006(31.66 infections/1000pd) decreased from that time. This was thought to be the result of urinary tract infection control education all over the hospital. Blood stream infections were at most in 2008 with a rate of 34.28%. Gram-negative microorganisms tended to decrease until 2007 and than increased. The most prevalent microorganism was *Pseudomonas aeruginosa* from 2005 to 2007(38%, 30.4% and 26.6% respectively) but *Acinetobacter baumannii* took place in 2008 and 2009 with rates of 25.9%, 59.1% respectively. Extended spectrum β lactamase rate was high in all years (60%, 70%, 59%, 45.6%, 63.2% in *Escherichia coli* and 78.1%, 40%, 53.5%, 56% and 68% in *Klebsiella pneumoniae* respectively) and all *Staphylococcus aureus* isolates were meticilline resistant. Antimicrobial resistance was also remarkable in Gram-negatives especially in *Acinetobacter baumannii* spp.
Conclusion: As a conclusion high overall infection and resistance rates were seen. Hospital infection rates, causative microorganisms tended to be influenced from physical conditions, infection control measures and compliance and also from overall hospital implementations. Unit based and general standards should be established with the support and reinforcement of hospital administration in order to decrease hospital infections.

Table 1: General hospital infection rates and their distribution according to years 2000–2009*

	n/rate*				
	2005	2006	2007	2008	2009**
Number of patients	114	104	72	53	38
Patient days	2920	2912	1961	1920	1094
Nosocomial infection	74/25.3	60/20.6	53/27.03	35/18.23	18/16.45
Burn infection	35/47.29	25/41.7	27/50.9	17/48.57	12/66.66
Bloodstream infection	13/17.56	14/23.33	13/24.52	12/34.28	5/27.77
Urinary infection	17/23	19/31.66	13/24.52	6/17.14	1/5.55
Respiratory infection	9/12.2	2/3.3	–	–	–

*Infection rate per 1000 patient days.
**For 2009, first nine months.

R2294 Mortality and prognosis factors for MRSA bloodstream infections in the intensive care unit: a retrospective cohort study

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Objectives: To evaluate mortality and prognosis factors in a multicenter cohort of patients with MRSA bloodstream infection in Intensive care units (ICU) in Bogota (Colombia).

Methods: we perform a retrospective cohort study in 16 high complexity hospitals in the city. We include 374 patients with bloodstream infection (BI) in ICU, identified from an antimicrobial resistance surveillance system. A systematic chart review was performed, severity of illness, comorbidity, type of infection, therapy and demographic variables were collected. Attributable mortality was analyzed in a post hoc committee. Time to event date was modeled.
Results: 187 Methicillin-Resistant *Staphylococcus aureus* (MRSA) and 187 Methicillin-susceptible (MSSA) in ICU were documented. Gross mortality 51.6%, attributable mortality to *S. aureus* bacteremia 25.13%. MRSA-BI attributable mortality 31% vs. 19% in MSSA-BI. Log rank test ($p < 0.05$) between survival functions for MRSA and MSSA (Figure 1). When multivariable adjusted in a proportional hazard model, independent predictors for attributable mortality are Charlson's comorbidity index >3, septic shock, no adequate clinical response in day 3 and early change in antimicrobial therapy. Meticillin resistance and inappropriate initial therapy were not observed as significant predictors of attributable mortality.
Conclusion: MRSA-BI show a different survival function compared with MSSA-BI, high gross and attributable mortality for patients with BI by *S. aureus* in ICU. Some mortality predictors were comorbidity, severity, early treatment and early response.

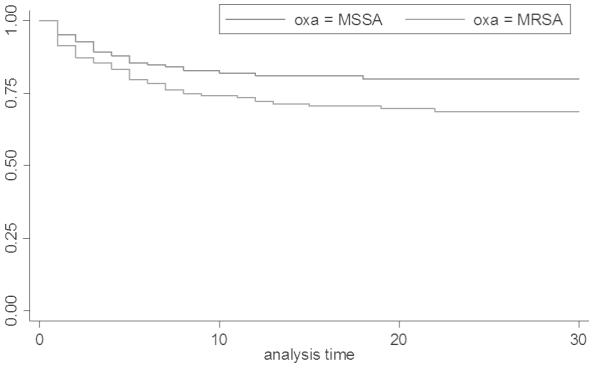

Figure 1. Kaplan–Meier survival estimates.

Travel medicine, tropical and parasitic diseases

R2295 Comparison of four methods for the detection of *Trichomonas vaginalis* infection in symptomatic and asymptomatic women in Athens, Greece

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Objectives: To assess the prevalence of *T. vaginalis* infection in symptomatic and asymptomatic women, attending a major gynecological hospital in Athens, Greece and to evaluate four methods for the diagnosis of *T. vaginalis* infection.
Methods: Specimens were collected consecutively, from 502 women attending the outpatient clinic of Alexandra Hospital, during the period 2006–2007. Two hundred fifty-five of them were symptomatic and 247 asymptomatic. Three hundred fifty-eight were Greek and 126 were immigrants. All women completed a questionnaire including demographic data, medical history and behavioral/sexual information. The presence of *T. vaginalis* in vaginal samples was assessed using wet mount, culture in modified Diamond's medium, antigen detection and two PCR assays, targeting different regions of *T. vaginalis* genome. Specimens were considered positive for *T. vaginalis,* when found positive either by culture or by both PCRs. Kappa test for agreement between diagnostic tests was also determined.
Results: Twenty-three women (4.6%) were found positive for *T. vaginalis.* Infection was more prevalent in symptomatic women (6.7%) than in asymptomatic ones (2.4%). *T. vaginalis* was more frequently detected in immigrants (7.9%) than in Greek women (3.3%). *Gardnerella vaginalis* infection was significantly more frequent in women infected with *T. vaginalis.* PCR was the most sensitive method (100%), followed by culture (69.6%), wet mount (69.6%) and latex agglutination (54.6%). The kappa index was 0.94 between culture and wet mount, 0.81 between culture and latex agglutination and 0.79 between culture and PCR.
Conclusions: The present study indicates a relatively low percentage of trichomoniasis in the female population living in Athens. The infection was more prevalent among immigrants and the majority of infected women was asymptomatic. PCR was found to considerably improve the diagnostic yield when compared to conventional diagnostic methods.

R2296 Autochthonous taeniasis associated with intake of wild boar meat in Asturias, Spain

A. Rodriguez-Guardado, F. Perez, P. Capon, N. Moran, G. Martin, J. Carton (Oviedo, ES)*

Introduction: Taeniasis is the infection of humans with the adult tapeworm of *Taenia saginata* or *Taenia solium*. Human Taeniasis is to public health problem that affects not only endemic areas. We described the clinical and epidemiological characteristics of several episodes of taeniasis associated with the consumption of wild boar meat in Asturias, Spain.
Methods: We studied the clinical-epidemiological characteristics of all autochtnous taeniasis diagnosed on the Tropical Medicine Unit of Hospital Universitario Central de Asturias, a region in Northern Spain from 2008 to 2009. The parasitological diagnostic was based on examination of three formalin-ether concentrated stool samples and by examination of proglottids or body segments if were availables. The patients were following during one year before the diagnostic with parasitological screening every 3 months. The disease was cured if two consecutive tests were negative.
Results: We studied 8 patients that presented *Taenia* spp eggs in stools samples (56% women, mean age 49 years (range 17–72). All patients reported having eaten undercooked meat from wild boar with an average of 187 days prior to the onset of symptoms. Two patient had urticarial clinic, three had abdominal pain and the rest were asymptomatic except for the broadcasting of tapeworms in stools. Not patient had eosinophilia (mean 330 cells/mm^3, limits 30–144). All patients were treated with praziquantel 5–10 mg/kg orally spaced 2 weeks apart. The parasitological controls were negative in all patients.
Conclusions: Taeniasis is a major public health problem. In Asturias had been considered erradicated but recently it is resurgence associated with consumption of wild boar meat. It need epidemiological controls to prevent the emergent aparition of this disease.

R2297 Prevention of benznidazole-associated adverse effects using slow dose escalation and histamine H1 antagonist (dexchlorpheniramine)

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Introduction: The appearance of rash, haematologic or hepatic toxicity is one of the most frequent and limiting side-effects of treatment with benznidazole (BNZ). The slowly escalating dose has been used in other tropical disease treatment like loiasis. We explored the efficacy and safety of a strategie for reducing the incidence of this complications.
Methods: Twelve patients diagnosed to Chagas' disease on Tropical Medicine Unit of Hospital Universitario Central de Asturias were treated with BNZ in a slowly escalating dose, beginning with 100 mg daily the firs 3 days and increasing the dose by 50 mg/ 3 days up to the full daily dose of 300 mg or 5 mg/Kg/day; and combining the addition of dexchlorpheniramine with the slowly escalating dose. The patients were revised every 15 days. In all patients we realized a exhaustive questionnaire about the apparition of any adverse effects. At each revision we made a determination of blood count and liver function tests. The treatment was continued during 60 days.

Results: No patients discontinued treatment. No patients described the apparition of rahs or other cutaneous disease during all the treatment. The blood count and the liver function test did not changed until the end of treatment. Two patients reported having nausea in the first 15 days of treatment as the only adverse effect.
Conclusion: The incidence of rash complicating the first few weeks of treatment with BNZ can be diminished by adding histamine H1 antagonist for 2 weeks to the standard recommendation, or by using a slowly escalating dose. The incidence of other adverse effects (haematological or hepatic) decreases using slowly scalating dose too. It is necessary most studies tos proven if this slowly dose is pharmacokinetically safe.

R2298 Epidemiological and clinical features of 111 patients with imported chronic Chagas' disease in Valencia, Spain

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Background: Chagas' disease is caused by *Trypanosoma cruzi*, endemic in Latin America. 16 million people are affected by chronic disease. The disease can develop into a cardiac form (arrhythmia, heart failure, sudden death, thromboembolism) or a digestive form (megaesophagus, megacolon).
Objectives: To analyze epidemiological and clinical features of patients with imported Chagas Disease in Valencia, Spain.
Methods: Prospective study of patients with Chagas' disease diagnosed between January 2005 and December 2008 in Hospital General de Valencia Tropical Medicine Division. Subjects of the study: Blood donors or people born in Latin America, children born from chagasic mothers, and travelers with epidemiological risk for *Trypanosoma cruzi* infection. Immunological diagnosis was made using commercially available serological tests: Recombinant ELISA (BioElisa-Chagas, Biokit S.A.), that was the test used for serological screening, and IFI (MarDx Diagnostic) used in addition in case of ELISA positivity. Case definition: Any patient with epidemiological risk factors and two or more different serological test positives. Clinical and epidemiological review, physical examination, chest radiography, and electrocardiography (EKG) were performed in all cases. Radiographic contrast study of esophagus and colon and echocardiography only were performed if patient had any symptom or EKG abnormalities.
Results: 111 cases of Chagas' disease have been identified, all of them Latin American immigrants. Countries of origin were: Bolivia: 95, Argentina, 2, Chile 2, Ecuador 2, N.A.10. Mean age: 38.21 years. Gender: 66.6% females, 33.3% males. Polymerase chain reaction was available in 65 cases, and was positive in 26, negative in 39. Chest radiographies were normal in 96% of patients. We found abnormal EKG in 19%, mainly branch blocks and 3 patients needed a pacemaker. Echocardiography was abnormal in 20.3% of patients. One patient died due to terminal cardiomyopathy. Colonic barium enema was performed in 48 patients founded megacolon in 5 of them (10.41). Contrast radiography of esophagus was done in 59 patients with abnormalities in 4 (6.7%).
Conclusions: Chagas Disease is an emergent disease in Europe because migratory movements and causes important morbidity in young population. This situation requires improvement in clinical and diagnostic knowledge and determine priorities on preventive and assistencial needs.

R2299 African trypanosomiasis in the south-eastern Uganda, Buikwe South Health sub-district, 1989–2008

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Background: In central and south-eastern Uganda, where the Buikwe South Health subdistrict (HSD) is located, the acute form of sleeping sickness, caused by *Trypanosoma brucei rhodesiense*, is predominant. Here we report the incidence of *T. b. rhodesiense* sleeping sickness in the last functioning treatment centre in Buikwe south HSD in south-eastern Uganda, for a 19-year period (1989–2008), the treatment outcome, structure of population affected, and functioning of sleeping sickness control programme in this area.
Methods: Sleeping sickness data from 1989 to 2008 were collected retrospectively in 2009 at Buikwe Sleeping Sickness Treatment Centre from available sleeping sickness registers. Case definition was based on the clinical and laboratory diagnosis, all recorded cases were included. Data have been analysed by EpiInfo programme.
Results: In the period from 1989 to 2008, 372 cases of sleeping sickness were diagnosed and treated. Number of patients in age 0–5 years was 12 (3.22%), from 6 to 15 years 51 (13.7%), and above 15 years 309 (56.18%). 158 (42.5%) patients have been diagnosed in the early stage of disease. After excluding the presence of microfilaria in BS was treatment with suramin initiated and all of them recovered, except 4 men, who escaped from the hospital. 214 patients (57.5%) had been diagnosed in the late stage of disease. After therapy with melarsoprol 170 (79.4%) were treated, 30 (14%) died and 14 (6.5%) were referred to other health unit. Case fatality rate was 8%, in the late stage of the disease was case fatality rate 14%. Median interval between the diagnosis and death was 14.4 days.
The highest incidence was in early nineties, in 1991 (83 cases). Until 2004 the incidence was decreasing, but due to closure of treatment centers, lack of supervision and motivation of health-care workers, the disease started to re-emerge. In 2009 5 new cases have been diagnosed.
Conclusion: Sleeping sickness still remains serious public health problem. Since the preventive and educational activities for the control of this neglected disease are not functioning appropriately, it can re-emerge very easily.

R2300 Development and application of a real-time PCR assay to detect blastocystis in human faeces

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Objectives: The protist Blastocystis can be found in the digestive tract of humans with a worldwide distribution, but its clinical significance remains unresolved. Nine major distinct subtypes (ST) of Blastocystis have been identified based on rRNA18S gene sequence. Recent studies suggest an association between some subtypes of Blastocystis and acute diarrhea or irritable bowel syndrome. However, few data are available regarding the prevalence and the genetic diversity of Blastocytis in France. This is probably due to the difficulty to point out Blastocystis in human feces. The anaerobic culture of fresh stool sample remains the gold standard method, but is heavy to set up for epidemiological studies. Direct microscopy of fecal smears is frequently used but exhibits a poor sensibility compared to the culture. The aim of this study was to develop a sensitive real time PCR assay to detect Blastocystis directly from stools. The PCR was used to estimate the prevalence of Blastocystis in patients of the Clermont Ferrand hospital (France), compared to microscopy and culture.
Methods: A couple of primers was designed to amplify a 330 bp length fragment of the rRNA18S gene of Blastocystis. Real time PCR assays were performed using a Rotorgen-6000® with Sybr Green detection. DNA extract from genotypes 1 to 9 were tested. Repeatability, reproducibility and the limit of detection were determined on fecal samples. Finally 100 stool samples from hospitalized patients were tested for Blastocystis using direct microscopy of fecal smears, culture in Jone's medium and the real time PCR assay we developed.
Results: We successfully amplified DNA extract from subtypes 1 to 9 and the lower limit of detection was 10^2 Blastocystis per gram of stool. During the prospective clinical study, among 100 patients, 4 positive samples were detected using microscopic analysis, 7 using culture and 10 using our real time PCR assay. Three samples were positive only by PCR, indicating a better sensitivity of this method. Specificity and ST determination were checked by sequencing of the PCR products.
Conclusion: In general population of industrialized countries, the prevalence of Blastocystis is often considered to be about 5%. With our sensitive quantitative PCR assay, Blastocystis was detected in 10% of

stool samples. Our results show the usefulness of molecular PCR-based diagnostic approaches to obtain relevant data about epidemiological features of Blastocystis.

R2301 Dengue fever: clinical and laboratory profile

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Objectives:
1. To study the clinical profile of patients with confirmed Dengue fever.
2. To study the Laboratory profile in patients with Dengue fever.
3. To study the mortality in these patients.

Methods: We enrolled all patients above the age of 15 years in our study. They included confirmed cases of Dengue fever by way of positive Dengue IgM antibody and/or Dengue NS1 antigen. A total of 78 patients were enrolled in the study over a period of two months and their complete clinical and biochemical profile was recorded as per a preset performa.

Results: The study showed 50 males and 38 females with a mean age of 35±9 years. All the patients who were admitted had an initial platelet count of less than 50000/cumm. All patients had fever for a mean duration of 5±1 days. The mean age of the patients was 45±15 years. 70 out of 78 patients had complaints of nausea, body ache and or vomiting. 45 out of 78 patients were recorded to have either itching or a skin rash. 2 out of 78 patients had severe bleeding in the form that required urgent intervention in the form of blood or platelet transfusion. 15 out of 78 patients had minor bleeding in the form of gum bleed, petechial rash, bleeding form nose or one episode of malena. 1 out of 78 patients died due to bleeding, aspiration and hypotension. One of the patients had concomitant vivax malaria. 72 out of 78 patients had raised transaminases ranging more >2 times to 5 times the upper limit. 68 out of 78 patients had features of hepatomegaly, gall bladder wall oedema, pleural effusion and or ascites. All patients responded to therapy in form of IV fluids, Platelet transfusion in case of bleeding or platelet count <20,000/ul, acetaminophen and other symptomatic care. 25 of 78 patients required platelet transfusions. A total of 15 patients required multiple platelet transfusions. All patients recovered within a mean duration of 6±2 days.

Conclusions:
1. Dengue fever has no specific age or sex preponderance.
2. Most patients presented with fever, body ache and nausea.
3. Majority of the patients present with an elevated liver enzymes which is a self limiting phenomenon.
4. Dengue fever in our study had very low mortality and showing that this particular strain is self limiting disease in majority of cases if supportive treatment is given appropriately.
5. Platelet transfusion is required in approximately 1/3rd of patients and major bleeding is not seen in majority of cases with adequate supportive treatment.

R2302 *In vitro* susceptibility test for *Acanthamoeba* sp. isolated from clinical specimens against chlorhexidine, propamidine isethionate, gentamicin and chloramphenicol

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Objective: Acanthamoeba keratitis is one of the most severe and potentially sight-threatening ocular parasitic infectious diseases and is recognized as the most challenging among ocular infections. *In vitro* susceptibility testing of *Acanthamoeba* isolates may prove beneficial for application of early treatment regimens. This study was conducted to determine the effectiveness of the drugs in therapeutic dose and the minimum cysticidal concentrations (MCCs) of the drugs.

Methods: Serial doubling dilutions of chlorhexidine digluconate from 200 μg/ml to 0.097 μg/ml, propamidine isethionate (Brolene) from 1000 μg/ml to 0.488 μg/ml and gentamicin from 40000 μg/ml to 19.531 μg/ml were performed in microtiter plate and tested against 3 *Acanthamoeba* isolates which were isolated from keratitis cases. After the exposure of the cysts to the drugs for 24 hours, the cysts were washed free of drugs by centrifugation. The deposit (cysts) was cultured onto nonnutrient agar plates overlaid with heat-killed *Escherichia coli*. The replication and growth of the trophozoites from cysts exposed to each of the dilutions were observed and recorded microscopically for 14 days to determine the MCC of each drug. The effectiveness of the drugs in therapeutic dose against the cysts was tested directly without any doubling dilutions.

Results: Chlorhexidine digluconate and propamidine isethionate (Brolene) successfully exhibited their cysticidal activities in therapeutic dose but not for gentamicin and chloramphenicol. The minimum cysticidal concentration (MCC) of chlorhexidine ranged from 25 μg/ml to 50 μg/ml, propamidine isethionate ranged from 500 μg/ml to 1000 μg/ml and gentamicin ranged from 10000 μg/ml to 20000 μg/ml. The mean MCC of chlorhexidine, propamidine isethionate and gentamicin on *Acanthamoeba* isolates was 33.33 μg/ml, 666.66 μg/ml and 13333.33 μg/ml respectively.

Conclusion: The *in vitro* sensitivity test enables the determination of MCC of drugs on *Acanthamoeba* isolates and can be used for the screening of new anti-acanthamoebal therapeutic agents. Chlorhexidine digluconate and propamidine isethionate (Brolene) have proven to be very effective anti acanthamoebal agents.

R2303 Cryptosporidiosis in Iranian farm workers and their household members: possible zoonotic transmission

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Objectives: The prevalence of cryptosporidium and the risk factors of zoonotic transmission on Najafabad, Isfahan, Iran dairy farms were examined.

Materials and Methods: *Sampling and specimen processing*: One fecal sample collected from all calves less than 6 months old on 8 dairy farms around Najafabad (Isfahan province, central Iran) as well as individuals working in these farms and their household members. During September to March 2008, 218 and 422 fecal samples collected from calves and humans respectively. Each specimen placed in a plastic vial, brought immediately to laboratory and stored at 4ºC until analysis. All of the samples were stained by the modified Ziehl–Neelsen method and examined under bright field microscopy.

DNA extraction: Fecal samples were subjected to six cycles of freeze-thaw in liquid nitrogen and a 95ºC water bath to rupture the oocysts. DNA was isolated from aliquots of frozen stool. 18S rRNA gene amplification and sequencing. A two-step nested PCR protocol was used to amplify the 18S rRNAgene (830 bp). PCR products were analyzed on 1% agarose gel and visualized by ethidium bromide staining.

Results: *Cryptosporidium* was identified in the stool of 36 (prevalence 8.5%) of 96 farm workers and 326 household members. Furthermore, 31 (14.2%) of 218 calf samples were positive. Based on 18s rRNA gene amplification and sequencing, cryptosporidium parvum was identified in 72% of the positive farm workers and 65% of the positive household members. Of the positive calves, 64.5% were infected with *C. parvum,* indicating possible zoonotic transmission on these farms. Univariate analysis of potential risk factors revealed that contact with calves ($P < 0.0001$) was the most significant risk factor of *C. parvum* infection. A considerable negative association was observed between *C. parvum* infection and cleaning of shoes/boots after daily work ($P < 0.004$), hand washing ($P < 0.013$) and use of piped water ($P < 0.006$). In the multivariate analysis with logistic regression, only contact with calves was significant.

Conclusion: Zoonotic transmission of *C. parvum* due to contact with calves is predominant among farm workers and their household members of this region and appropriate health measures must be applied to control the infection and decrease of zoonotic transmission of this parasite.

R2304 Fever in hospitalized travellers and migrants over 11-year period at a teaching hospital in Italy

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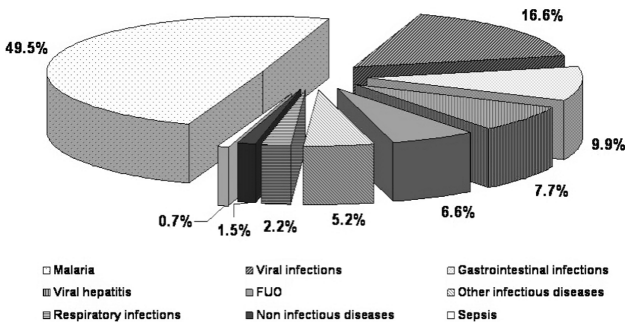
Objectives: To describe prevalence of hospitalization and clinical spectrum of fever in returning travellers and migrants in Italy.
Methods: Retrospective charts review of all febrile illnesses developing within 3 months after a stay in the tropics and hospitalised between 1998 and 2008 at the Infectious Diseases Clinic of Milan, Italy.
Results: Near 4% (270/6827) of all hospital admissions in the study period were due to fever in travellers and migrants returning from the tropics. 188 (69.6%) were men, the median age was 32 years (range 16–75 years). 173 were Italian citizens, 97 (36%) were extra-European migrants (52% classified as visiting friends and relatives). As shown in figure 1 malaria was the most common specific etiologic diagnosis, found in 49.5% of ill returned travellers with fever. Fifty-three percent of all malaria cases were diagnosed in migrants. Causes of fever varied by region visited (76% of malaria were acquired in sub-Saharan Africa, OR 47.6, 95% CI 13.8–163.4; dengue fever was acquired in Latin America Indian subcontinent and south-central Asia in 86% of cases) and by time of presentation after travel (dengue accounted for the early presentation). 9.2% of travellers with fever had a vaccine preventable infection. Sixteen percent of patients had an acute viral infections excluding viral hepatitis that accounted for 7.7% of all causes of fever.
Conclusions: During a 11-year period, the number of patients returning from tropical areas who were admitted with fever to a university hospital in northern Italy remained stable. Malaria remains the most frequent diagnosis accounting for near 50% of all hospitalization. Dengue fever was the most frequent tropical infection besides malaria. The time of presentation after travel and region visited provides important clues toward establishing a correct diagnosis..



R2305 Unusual case report of larva migrans of *Toxocara canis* with hepatic involvement, severe lumboishialgia, lymphadenopathy and fever

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Toxocara canis infections are uncommon in developed countries. However sporadic cases and possibility of systemic involvement, require considering this diagnostic possibility.
This is a case report of a 25-year old previously healthy female from urban surroundings, who presented at our clinic with prolonged low-grade fever, abdominal pain, severe lumboishialgia, paraesthesiae, pruritic rash, generalized lymphadenopathy and liver damage. There were no anamnestic data concerning recent travel or contact with animals.
Complete laboratory examination revealed hypereosinophilia (12.5%), elevated liver enzymes (AST 246, ALT 306), hyperbilirubinemia, histological non-specific lymph node infiltration, without any radiological findings of focal infection.
Toxocara canis infection was confirmed with positive serology tests, and as other tests including virology (hepatitis A, B, C, HIV, herpesviridae, Coxsackie), bacterial cultures (including serology for *B. burgdorferi,* Brucellae, tests for Tuberculosis), stool tests and available immunoserology for other parasites were negative. After a 10 day treatment with albendazole (400 mg twice a day), patient was afebrile, without any physical complaints including normal levels of liver enzymes and leukocyte formula.
Most cases of visceral larva migrans are mild, self-limiting and may mimic many different conditions, but as the available treatment options are effective, it should be suspected in patients with hypereosinophilia and liver damage.

R2306 Prevalence of *Cryptosporidium* in immunocompetent children with acute diarrhoea in Upper Normandy, north-western France

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Objective: The intestinal protozoa *Cryptosporidium* is increasingly recognized as a major cause of diarrheal disease, however, the incidence of cryptosporidiosis in children is unknown in France. This study was conducted to assess the significance of *Cryptosporidium* as causing agent for gastro-enteritis in immunocompetent children in Upper-Normandy, France.
Patients and Methods: The study was conducted between January 2007 and November 2009. During the 34-months period study, stool specimens from 2,041 children (aged <16 years), all immunocompetent, with acute diarrhea were prospectively screened for the presence of *Cryptosporidium* oocysts. 85% (1,746) were patients attending the Pediatric department of Rouen University Hospital, Upper-Normandy (North-western France) whose stools were examined at the hospital Parasitology laboratory. 15% (299) were patients attending general practitioners whose stools were examined at a private clinical laboratory in Saint Valéry en Caux, 60 kms from Rouen. Presence of *Cryptosporidium* was assessed by microscopy with semi-quantitative results obtained after Heine staining of faecal smears. *Cryptosporidium* species and genotype determination were based on polymerase chain reaction with PCR targeting the Hsp70 and the 18S rRNA genes followed by 18S rRNA gene fragment sequencing.
Results: Twenty three (1.3%) out of 1746 and 3 (1%) out of 299 children seen at Rouen University Hospital and at Saint Valéry en Caux, respectively, were reported to excrete oocysts. Maximum of cases (81%) were reported in children at ages between 0.5 and 6 years. Genotyping revealed 6 and 17 positive stools for *C. hominis* and *C. parvum,* respectively. More than 75% of children had vomiting and 44% were dehydrated. Other symptoms included fever and abdominal pain. 62% of cases were reported between July and October. Four out of the six *C. hominis* cases have reported travel outside France prior to illness compared with 2 out of 17 for *C. parvum.* Conclusion: Few reports are available on frequency of cryptosporidiosis in immunocompetent children in France and this study has demonstrated the public health importance of this parasite in Upper-Normandy. Cryptosporidiosis is likely to be unrecognized and underdiagnosed. Diagnostic testing for *Cryptosporidium* is rarely ordered, even when patients have symptoms consistent with cryptosporidiosis, leading to a lack of specific preventive initiatives to limit the overall health impact of cryptosporidiosis.

Resistance and mechanisms of action of antifungals

R2307 New antifungal microbial strains effective against *Candida* strains isolated from infections

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It is estimated that *Candida* species account for more than 90% of fungal infections. Selecting microbial strains with large antifungal activity, could represent important alternative treatment.

Objectives: Antifungal activity studies on lactic acid bacteria (LAB) and Metschnikowia pulcherrima yeast strains against *Candida albicans* (Cc, C3), *Candida parapsilosis* (M6) and *Candida tropicalis* (OT4) strains isolated from vaginal and oral infection.
Enhancement of antifungal activity of *M. pulcherrima* strains using sodium bicarbonate (NaHCO3) and calcium chloride (CaCl2).
Methods: For this study 114 strains of lactic acid bacteria were isolated from sourdough, newborn faeces, fermented milk and plants. Selection of LAB strains was made by cultivation on MRS CaCO3, Gram stain and catalase test. All strains were screened for *Candida* growth inhibiting capacity by using spot agar method.
Antifungal and killer activity were tested by spoting three *M. pulcherrima* strains (SG1, SG2, CPM1) on plates flooded with the *Candida* isolates. For further tests cell suspensions were mixed with: NaHCO3 0.1%, 0.5%, 1%, 2%, and CaCl2 1%, 2%, 3%.
Results: Nine LAB strains were selected for high antifungal activity against our *Candida* isolates. API, BIOLOG and REP-PCR analysis allowed us to place LAB strains in *Lactobacillus*, Pediococcus, Weissela and *Enterococcus* genera. The antifungal activity was correlated with the biosynthesis of organic acids.
The three *M. pulcherrima* strains showed high killer activity against OT4 and Cc. The weakest action was recorded against M6. The best results for antifungal activity were obtained for all three strains on OT4, with wide halos and growth inhibition. Significant results were observed for SG1 and SG2 against Cc. In the case of M6, only SG1 formed a shallow halo. OT4 strain was sensible at SG1 and SG2 with 2% NaHCO3 or CaCl2 1%, while CPM1 was active only in 0.1% NaHCO3 mixture. Clear halos were obtained on Cc plates for all three *M. pulcherrima* strains with 2% NaHCO3 or 2% CaCl2. The weeker results were recorded against C3 strain.
Conclusions: The antifungal activity of the nine LAB selected strains was correlated with the biosynthesis of organic acids.
The most important killer and antifungal activity of the three *M. pulcherrima* strains were observed against OT4 and Cc, and was enhanced in mixture with 2% NaHCO3 or 2% CaCl2. SG1 and SG2 showed the higher antagonistic potential against all the *Candida* isolates tested.

R2308 Sensitivity of *Candida albicans* during fluconazole prophylaxis

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Objectives: Fluconazole prophylaxis in adult neutropenic leukemia patients was introduced in our hospital in 2000 and continued until 2006, with the exception of a period of about 12 months. We studied the sensitivity of *Candida albicans* to fluconazole in adult haematologic patients from 1998 to 2007 retrospectively. The species distribution of yeast isolates in all samples from the haematology ward was recorded for the study period.
Methods: 75 yeast isolates from adult haematologic patients were tested, 25 before start of prophylaxis, 25 in 2003 and 25 in 2007. Available *C. albicans* isolates from any material were selected in chronologic order from our archive. The strains were cultured on Sabouraud glucose agar and on RPMI agar for resistance testing by Etest® (AB-Biodisk and bioMérieux), according to the producer's recommendation. The plates were incubated for 24 hours, the MIC was read at 80% inhibition and the result was controlled after 48 hours of incubation. Simultaneous culture on CandidaCHROMagar® (BD Diagnostics) was made to exclude non-*albicans* and mixed infections. A search in our database for yeast isolates and species distribution in all patients admitted to the adult haematological ward was made for the years 1998 through 2007.
Results: *C. albicans* was the most prevalent yeast found. The number of samples and patients tested in the time period was increasing, in total were 404 samples and 226 patients found. *C. albicans* was isolated in 84–100% of the samples and non-*albicans* strains in 0.14–32%. The number of patients with non-*albicans* strains, mainly *C. glabrata* but also *Saccharomyces cerevisiae* was increasing. The small amount of samples may have masked a preexisting prevalence of these strains.
The MIC results demonstrated low values for all years studied. In 2007, we observed a rise in the MIC values and a higher frequency of double inhibition zones for fluconazole.
Conclusion: Fluconazole prophylaxis in haematologic patients may induce subpopulations of *C. albicans* with an elevated MIC. The prevalence of *C. albicans* was high in samples from the haematologic ward. Non-albicans species were found in addition in increasing numbers, suggesting a selection pressure induced by fluconazole prophylaxis.

R2309 *In vitro* susceptibilities of invasive samples isolates of non-*albicans Candida* sp. to antifungal agents

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Objectives: To study the susceptibility to azoles, echinocandins and amphotericin B for *Candida* species other than *C. albicans* isolates obtained from invasive samples over a period of four years in the Microbiology Service, University Hospital Virgen de las Nieves, Granada (Spain).
Methods: The susceptibility to antifungal agents for non-*albicans Candida* species isolated has been studied in invasive samples obtained between October 2005 and October 2009, generally there was a single isolated per patient. The antifungal agents investigated were fluconazole, itraconazole, voriconazole, caspofungin and amphotericin B and the method used was Sensititre Yeast One® (Trek Diagnostic Systems).
The results for fluconazole and itraconazole were interpreted according to the susceptibility criteria of CLSI (M27-A2). For the other antifungals, in which there are no breakpoints, we determined the MIC90.
Results: We detected 127 invasive samples (belonging to 95 patients) with isolates of *Candida* species other than *C. albicans*: 76 from blood cultures (59.8%), 30 from intra-abdominal samples (23.6%), 14 from respiratory tract samples (11%), 3 from skin / soft tissue (2.4%), 2 from cerebrospinal fluid (1.6), 1 from osteoarticular tissue (0.8%) and 1 from cardiac valves (0.8%). From these samples we isolated 98 strains of yeasts and their susceptibility to antifungal agents as shown in Table 1.
Conclusions:
- It has been verified that on these species fluconazole and itraconazole have a limited activity, except *C. parapsilosis*; voriconazole is the most active tested azole.
- Caspofungin appears to be very effective, including azole-resistant *Candida* species, like *C. krusei.*
- Amphotericin B is still efficient on the treatment of serious infections caused by *Candida* species other than *C. albicans*.

Table 1. Antifungal susceptibility for non-*albicans Candida* species

Candida species (n)	Fluconazole		Itraconazole		Voriconazole	Caspofungin	Amphotericin B
	%S[1]	%SDD[2]	%S	%SDD	MIC_{90}	MIC_{90}	MIC_{90}
C. parapsilosis (32)	100	–	85.2	15	1	0.12	1
C. glabrata (28)	4.17	66.7	0	33.3	1	0.25	1
C. tropicalis (21)	83.3	–	20	40	0.5	0.25	1
C. krusei (15)	0	–	16.7	83.3	0.5	0.5	2
C. kefyr (1)	100	–	100	–	0.03	0.03	1
C. pseudotropicalis (1)	100	–	100	–	0.03	0.03	2
Total (98)	55.3	22.4	40.8	36.3	1	0.5	1

[1] Percentage of susceptible strains; [2] Percentage of dose dependent susceptible strains.

R2310 Fungaemia by *C. krusei:* acquisition of voriconazole resistance *in vivo*

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Fluconazole prophylaxis has been associated to an increased prevalence of *C. krusei* and *C. glabrata* strains. *C. krusei* shows intrinsic resistance to fluconazole, but usually not to the other azoles such as voriconazole. *C. krusei* blood isolates were recovered from a leukemia patient during two months; during this period he was submitted to voriconazole therapy. An increase in minimal inhibitory concentration (MIC) values

of voriconazole was registered among consecutive isolates, which finally developed a resistant phenotype.
Objectives: Aiming to clarify the acquired resistance mechanism, we raised the hypothesis of such resistance being due to overespression of efflux pumps.
Methods: Two clinical *C. krusei* isolates were studied: one susceptible (MIC 1μg/ml) and one resistant (MIC 8μg/ml) to voriconazole. Agar disk diffusion assay was performed in order to study the synergistic effect between FK506 (Tacrolimus, described as an efflux blocker), and voriconazole, in the resistant *C. krusei* isolate, as described by Ricardo E. et al (2009). Ten-fold dilutions of FK506 ranging from 1000 to 1μg/ml were assayed and voriconazole was added to YEPD agar plates at supra-MIC value (1 μg/ml). In order to induce resistance, the susceptible strain was exposed, *in vitro*, to sub-inhibitory concentrations of voriconazole (1μg/ml). Every day 1ml of culture broth was transferred to new fresh medium, with voriconazole (1μg/ml). Susceptibility profiles to voriconazole were assayed until acquisition of resistance. Flow cytometry assays using rhodamine 6G (Rd-6G) 5μM (an efflux pump fluorescent substrate) were performed in order to compare the resistant isolates (*in vivo* and *in vitro* induced) with the susceptible isolate, regarding the role of efflux pumps in *C. krusei* resistance.
Results: Agar disk diffusion assay showed growth inhibition around the disks impregnated with the highest FK506 concentrations (100 and 1000μg/ml). At the 10th day of incubation of the susceptible strain with voriconazole the MIC value was 64μg/ml. Both resistant strains showed less intensity of fluorescence when stained with Rd-6G 5μM comparing to the susceptible isolate.
Conclusions: A synergistic effect was observed between voriconazole and the highest FK506 concentrations. The resistant strains accumulate less intracellular Rd-6G than the susceptible strain, which means that prolonged exposition to voriconazole, *in vivo* and *in vitro*, induced resistance associated to efflux pumps activity.

Fungal infections

R2311 Vulvovaginal candidiasis in a Kuwait hospital during a 2-year period

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Objectives: The purpose of this study was to determine the etiologic agent of all vulvovaginal candidiasis (VVC) isolated in our hospital during a two-year period.
Methods: 178 samples received in the hospital laboratory from July 2007 to July 2009 belonging to 89 women between ages 18 and 68 with a diagnose of VVC were reviewed. All samples were cultured on Sabouraud Dextrose Agar for 24–48 hours at 37 degree C. *Candida* spp. were identified on the basis of the macroscopic appearance of colonies, Gram-stained specimens and the identification to the species level using the API System ID 32C (bioMérieux, France).
Results: From 89 isolated strains, 38 strains (43%) were identified as *Candida albicans*, 27 strains (29.5%) identified as *Candida glabrata*, 22 strains (25%) as *Candida parapsilosis* and 2 strains (2.5%) as *Candida* crusei. When we attemped to sort the 89 cases with *Candida* by age groups, in the 13–20 years age group we included 10 samples (11.2%), in the 21–30 years group 46 samples (51.7%), in the 31–40 years group 21 samples (23.6%), in the 41–50 years group 5 samples (5.6%).
Conclusion: VVC affects female's everyday life. In the last years there has been a rise in the share of VVC attributable to non-*albicans Candida* species. It is important to know the etiological agents in each hospital and in each population in order to obtain the most precise diagnosis and treatment.

R2312 Fluconazole susceptibility testing in candidaemia isolates

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Objectives: Candidemia is a life-threatening disease, requiring early and correct treatment. Fluconazole is the standard antifungal therapy; however, not all *Candida* isolates are susceptible. Different methods for antifungal susceptibility testing are described. Therefore, we compared the E-test and disk diffusion versus broth microdilution. We also evaluated the possibility of direct susceptibility testing on positive haemocultures.
Methods: All records from patients with candidemia from January 2005 until August 2009 were analysed and the susceptibility for fluconazole was calculated. In a substudy the fluconazole E-test (on RPMI 1640 + 2% glucose agar), disk diffusion (on Shadomy agar according Neo-Sensitabs protocol) and a reference method (broth microdilution according CLSI M27-A protocol) were compared.
Results: Overview of data: The total number of patients with *Candida* septicemia decreased from 2005 till present: from 50 to 38 episodes each year. *C. albicans* was most frequently isolated at approximately 25 times a year, almost all susceptible to fluconazole (98.3%). This was followed by *C. glabrata*, *C. parapsilosis*, *C. krusei* and *C. tropicalis*, all with annually decreasing trend. The susceptibility of *C. glabrata* varied most depending on the method used: 22.7% susceptible (S) and 36.6% resistant (R) with disk diffusion on Shadomy and 17.2% S and 41.4% R with broth microdilution.
Technical validation: Strains with known MIC value for fluconazole (with reference method) were used to evaluate E-test and disk diffusion. For *C. glabrata* and *C. parapsilosis* different results were found using different methods. Direct susceptibility testing was conducted on samples spiked with ATCC control strains and patient haemocultures positive for yeasts. If growth was sufficient, correct results were obtained except for *C. glabrata*. However in many cases growth on the RPMI agar was insufficient, so repeated standardized susceptibility testing was needed.
Conclusion: Correct and rapid identification of *Candida* in septicemia is more important than antifungal susceptibility testing. Inconsistent results with different methods and wide MIC distribution for wild-type *C. glabrata* makes the testing of fluconazole susceptibility a challenge in daily practice.

R2313 Effect of non-steroidal anti-inflammatory drugs on *Candida* sp.

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Objectives: The aim of the study was to investigate the action of some non-steroidal anti-inflammatory drugs (NSAIDs) sodium diclofenac, aspirin and piroxicam on cells viability of planktonic cells of *Candida albicans* and *Candida krusei* strains and possible ultra structural changes of *C. albicans* cells treated with diclofenac.
Methods: Four strains of *C. albicans* and one *C. krusei* were used in this study. Strains were isolated from pharyngeal excreta. Isolates were identified by the conventional and molecular typing (amplification of ARN ribosomal 5.8S gene and restriction with enzymes Dde I, Cfo I, and Hind III). Tests of viability of cells in the presence of three NSAIDs were done by the serial dilution method. The standard method of electronic microscopy was used to study the ultra structural changes in *C. albicans* cells, induced by the diclofenac treatment (1mM and 2mM).
Results: The results of study identified strains through conventional and molecular typing showed us that four strains were in *C. albicans* species and one strain in *C. krusei.* The cells viability tests showed us a value of this up to 38–50% for all *C. albicans* strains which were treated with sodium diclofenac to plasmatic concentration (3.6 microg/ml) and 55% for *C. krusei* strain. In the presence of aspirin 1 mM the viability of cells was 76–81% for *C. albicans* strains and up to 51–79% for piroxicam. For *C. krusei* strain the cells viability was 93% for aspirin and 80%, piroxicam respectively. The electronic microscopy study stood out the ultra structural changes of *C. albicans* cells treated with sodium diclofenac 1 mM and 2 mM. We noticed changes to the plasmalema level (separated from the cytoplasm), to outer layer of cell wall (became more electrono-transparent), the invaginations of plasmatic membrane and the disorganization of cytoplasm which appeared completely damaged in the small parts. Cells had necrotic or aged aspects.

Conclusions: Our study stood out the effect of some NSAIDs on *Candida albicans* and *Candida krusei* strains. The greater inhibitor effect was obtained for *Candida* cells treated with diclofenac. The results of the electronic microscopy study showed us the ultra structural changes to *C. albicans* cells treated with diclofenac. We think that use of NSAIDs, especially sodium diclofenac to the specific antifungal treatment can cause a susceptibility of *Candida* cells and facilitate the action of antifungal drugs. The results can be a successful anti-*Candida* therapy.

R2314 Primary cutaneous cryptococcosis due to *C. laurentii* in a renal transplant recipient

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Objectives: We present a renal allograft recipient with primary cutaneous cryptococcosis (PCC) by *Cryptococcus laurentii*, probably caused by repeated skin injury and inoculations while injecting insulin and low molecular weight heparin on the thigh.
Methods: Identification of the yeast was made from the skin biopsy stained with Gram's stain. India ink preparation from the skin biopsy was done. Isolated from culture on Sabourad's dextrose agar. Serum cryptococcus latex agglutination test was also done. Speciation was done using Mini API (bioMérieux).
Results: The Gram's stain revealed spherical and elongated budding yeast-like cells without any pseudohyphae. Sabourad's dextrose agar colonies are cream coloured with a smooth mucoid texture. The fungus was notably absent from blood and lungs. The serum cryptococcus latex agglutination test was negative.
Conclusion: This is first report of primary cutaneous cryptococcosis due to *C. laurentii* in an immune compromised host. Reporting of such patients might further expand the existing clinical manifestations. Awareness and high index of suspicion might assist in early diagnosis and hence institution of treatment.

R2315 Candidiasis associated with bacterial vaginosis in patients from a health district in Madrid

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Introduction: *Candida* spp. is one of the most frequent microorganisms isolated in female genital tract infections. In spite of its theorical incompatibility with bacterial vaginosis (BV), there are certain cases of BV in which *Candida* spp. is also present.
Aim of the study: The aim of this study was to see the prevalence of candidiasis associated with bacterial vaginosis in a group of patients from a district in Madrid.
Methods: 165 samples (7 endocervical, 158 vaginal) from patients with bacterial vaginosis were collected from 1st March to 12th November 2009 in a hospital of Madrid. Samples came from different health care centres associated to our hospital. These samples were taken by vaginal or endocervical swabs. Bacterial vaginosis was diagnosed following Nugent criteria and samples were cultured on blood and chocolate agar at 37ºC for 48 h. *Candida* spp. were identified by selective culture on CHROMagar at 37ºC for 48 h on the appearance of colonies of different colours, being green colonies an indicator of the presence of *Candida albicans*. Other species of *Candida* were identified by Auxacolor system, a commercial yeast identification kit based on colorimetric tests for conventional assimilation substrates.
Results: We found 23/165 cases (13.9%) in which *Candida* spp. was associated with bacterial vaginosis. *Candida albicans* was isolated in most cases (21/23). In the two remaining cases *Candida parapsilosis* and *Candida* sp. were isolated.
Most women who presented this association were young and in childbearing age, being the mean age 27 years old and the standard deviation 9.66. The youngest and oldest patients were 15 and 48 years old respectively.
May and July were the months with the highest proportion of candidiasis associated to BV, 23 and 27.27% respectively (see table) and the average of this association was 3 cases per month.
Conclusion: In spite of the theorical incompatibility of yeast infection and bacterial vaginosis, our data show that *Candida* spp. is present in more cases of bacterial vaginosis than expected, being the prevalence 3 cases per month on average.

Prevalence of candidiasis associated with bacterial vaginosis

Month	Samples	BV	Candidiasis	Isolates	Yeast + BV
March	12 Vaginal	All	1	*C. albicans*	1/12 = 8.33%
April	14 Vaginal, 1 Endocervical	All	0		0%
May	25 Vaginal, 1 Endocervical	All	6	5 *C. albicans* 1 *Candida* sp.	6/26 = 23%
June	34 Vaginal, 2 Endocervical	All	3	2 *C. albicans* 1 *C. parapsilosis*	3/36= 8.33%
July	22 Vaginal	All	6	*C. albicans*	6/22 = 27.27%
August	14 Vaginal, 1 Endocervical	All	1	*C. albicans*	1/15 = 6. 66%
September	12 Vaginal, 2 Endocervical	All	1	*C. albicans*	1/14 = 7.14%
October	15 Vaginal	All	3	*C. albicans*	3/15 = 20%
November	9 Vaginal	All	2	*C. albicans*	2/9 = 22.22%
Total	158 Vaginal, 7 Endocervical	All	23	21 *C. albicans* 1 *C. parapsilosis* 1 *Candida* sp.	23/165 = 13.9%

BV = Bacterial vaginosis.

R2316 Evalution of *Candida* colonization index in patients in intensive care units

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Objectives: Nosocomial candidiasis is a great risk for the intensive care patients. *Candida* infections mainly evolve from endogenous colonization, thus the detection of the colonization is very important. In this study; we evaluated candida colonization in the intensive care unit patients by using candida colonization index (CI) and aimed to predictive value of colonization index for invasive candidiasis.
Methods: From September 2008 to February 2009, 100 patients older than 18 years of age in the intensive care unit were included in this study. Throat, nose, skin (axilla), urine and rectal swab cultures were taken weekly from each patients. Also tracheal aspirates, drain and central vascular catheter cultures were taken if there were. *Candida* colonization index was calculated by the ratio of the number of culture positive sites to the number of sites cultured.
Results: *Candida* colonization was found in 42 of 100 patients. Of these colonized patients, invasive candidiasis developed in nine patients, candidemia in five and urinary tract infection in four. Most of the colonized patients were in the surgical intensive care unit (ICU), staying longer length in the ICU and had more invasive instrument. Additionally candida colonization was diagnosed mostly in the patients with bacterial sepsis and exposed to broad spectrum antibiotics. Colonization index was found to be greater than 0.5 in 8 of 42 patients. Heavy colonization ($CI > 0.5$) was only determined in one of the nine patients with invasive candidiasis. However all of the nine patients who develop candidal infection were colonizated before the infection. The sensitivity and spesifity of colonization index for determining invasive candidiasis were found respectively 100% and 64%. Positive predictive value was 21% and negative predictive value was %100.
Conclusion: *Candida* colonization is frequently met among the intensive care patients. Invasive candidiasis in the intensive care unit setting is thought to be subsequent to colonization. *Candida* colonization index may be a good parameter to predict invasive candida infections.

R2317 Fast identification and susceptibility testing to antimycotics of *Candida* spp. isolates

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Introduction: Precise identification and susceptibility testing of yeasts, especially *Candida*, is essential for obtaining a prompt diagnosis and administering a proper therapy, having in mind the actual problem with resistance to various antimycotics.
Aim: To identify yeast isolates from lower respiratory tract (LRT), urine and genital samples using novel yeast identifying method and to test their susceptibility to antimycotics.

Material and Methods: A total of 140 yeasts, isolated from LRT (41), urine (40) and genital (59) samples in a one year period (October 2008–October 2009), were evaluated. Conventional microbiology methods were used for isolation of yeasts. The identification was using both: growth on Chromogenic *Candida albicans* agar (CALB) (Oxoid, UK) and RapID (Oxoid, UK), a method that utilizes determination of yeast enzymes and provides identification results in only 4 hours. Disc diffusion test was performed to determine the susceptibility to nystatin (N), voriconasole (VOR) and fluconasole (FCA) according to the CLSI standards.
Results: RapID identified *Candida albicans* as the most frequent isolate (98 strains – 70.0%), followed by 25 (17.9%) C glabrata, 10 (7.1%) C tropicalis, 5 C crusei, and one isolate each of Trichosporon beigelii (had turquoise colour like C albicans on CALB) and Rodothorula rubra. A total of 82 (83.7%) C albicans were susceptible to all tested antimycotics, 11 were resistant to N. and 5 strains (urinary isolates) to N and FCA. A total of 18 (72.0%) C glabrata strains were susceptible to all antimycotics. Resistance to N and FCA showed 5, 7 and 6 strains of C albicans, C glabrata and C tropicalis, respectively; 10 of those were urinary isolates. Other 7 isolated yeasts were susceptible to all tested antimycotics.
Conclusion: RapID as fast (4 hours) and simple identifying method meets the needs for accurate and reliable results in mycology diagnosis. Susceptibility testing revealed that voriconasole still has good antimycotic activity on the tested strains, but there is emergence of fluconasole and nystatin resistant strains. Therefore, especially in the cases of urinary infections where fluconasole is the only oral antimycotic that can achieve high urine concentration, a susceptibility testing is highly recommended.

R2318 Indication of empirical antifungal therapy in selected patients with persistent febrile neutropenia according to clinical criteria and risk profile

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Objectives: Universal empirical antifungal therapy (EAT) in every patient with persistent febrile neutropenia (PFN) is the standard of care, but some recent published data suggest that EAT could be applied only in selected patients. The aim of this study is to investigate a diagnostic and therapeutic protocol based on clinical criteria and risk profile, allowing the indication of EAT exclusively in selected patients with PFN without impact on invasive fungal infection (IFI) incidence and IFI-related mortality.
Methods: Prospective observational study including every persistent febrile neutropenia episodes in patients with hematological malignancies or stem cell transplantation (SCT) recipients admitted in the Hematology Service from October 2007 to November 2009. A previously defined diagnostic and therapeutic approach was applied in every PFN episode and EAT was indicated in patients with: (a) severe sepsis or septic shock; (b) focused infection: lung, central nervous system, sinus, abdominal or skin; (c) individualized clinical decision in patients at high risk of IFI. A comparative analysis of incidence of proven or probable IFI and IFI-related mortality was performed according to whether or not EAT was indicated.
Results: Eighty-five episodes PFN in seventy-two patients were included. The 48.2% were male and median age in years was 47 (15–75). The most frequent hematological malignancies were acute leukemia (45.8%) and lymphoma (21.2%). Thirty-two patients were SCT recipients, 53.1% allogeneic, the 24.7% were IFI-high risk patients. The median of duration of neutropenia and fever were 14 days (range: 6–63) and 10 days (range: 5–37) respectively. EAT was indicated in fifty-two episodes (61.2%) during a median of 11 days (range: 2–164); in the rest of episodes (n = 33) EAT was no indicated. The overall IFI incidence was 14.1% (n = 12). In the group that received EAT, twelve patients developed IFI (23.1%), in comparison with no-one patient in the group that did not receive EAT. The 30 days-global mortality was 16.5%, 25% in the group that received EAT and 3% in the group that did not received it. The IFI-related mortality was null in the group that did not receive EAT and 3.8% (2 of 52 patients) in the group that received EAT.
Conclusion: These data suggest that, in the management of patients with PFN, the indication of EAT just in patients selected on the basis of clinical criteria and risk profile, may be safe and avoid over-treatment.

R2319 Molecular identification of fungi of clinical relevance

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Objectives: Culture and conventional identification remain the usual basis for diagnosis of fungal infections, but they have a long response time and a low sensitivity rate. The objectives of this study were to perform molecular identification of clinically relevant fungi not identified at the species level with a conventional approach (culture, API ID32C and morphologic criteria) and to compare the results of molecular detection of fungi in invasive samples (other than blood) in those of culture.
Methods: In a preliminary phase, 10 strains of international collections were tested. After they were all correctly recognized, two prospective studies were done in parallel during one year (Sept. 2008 to Aug. 2009): (i) 36 cultures of difficult-to-identify fungi (12 yeasts and 24 moulds) obtained from different clinical samples were identified by molecular methods, and (ii) the presence and identification of fungi in 39 invasive samples other than blood (10 bronchoalveolar lavages, 5 bronchoalveolar brushes, 7 heart valves and 17 biopsies (joint: 8;lung: 7;brain: 2) were determined using a conventional approach and compared with the results of a molecular method based on sequencing the Internal Transcriber Spacer (ITS) regions 1 and 2, complemented with sequencing the β-tubulin and the elongation factor genes, and the intergenic spacer (IGS) region.
Results: All 36 organisms of objective (i) were identified by the molecular method as concrete species of genera *Aspergillus* (9), *Candida* (7), Trichosporon (5), *Scedosporium* (3), Alternaria (3), fusarium (3), Microsporum (2), Penicilium (2), Sporotrichum (1) or *Acremonium* (1). 35 out the 39 samples of objective (ii) were negative by both culture and molecular methods. Moulds were identified by the molecular approach in two cases in which the same organism grew in culture (a joint biopsy and a heart valve from the same patient yielding *Scedosporium apiospermum*). Additionally, the molecular approach identified an *Aspergillus* sidowii in a lung biopsy and an *A. fumigatus* in a bronchoalveolar brush in two culture-negative cases. The molecular method allowed identification of the organism (from either culture or clinical samples) in 48–72 hours.
Conclusions: Molecular methods reduces the response time for identification of clinically relevant fungi (include those for which conventional identification is difficult). This approach should be considered for the diagnosis of fungal infections in the clinical laboratory.

R2320 Efficacy and safety of anidulafungin for treatment of candidaemia in Asian patients

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Objectives: To evaluate the clinical efficacy and safety of anidulafungin (ANID) in the treatment of Asian patients with proven candidemia.
Methods: Phase 3b, open-label, multicentre, non-comparative study in adults with Acute Physiology and Chronic Health Evaluation (APACHE) II score ⩽20. Patients received once-daily intravenous ANID (single 200 mg loading dose, followed by 100 mg per day thereafter) for 5–42 days. Subsequent oral voriconazole was allowed under predetermined conditions; total overall treatment duration was ⩽42 days. Concomitant medications other than systemic antifungals were permitted. The primary endpoint was global response, defined as clinical cure/improvement together with microbiologic eradication/presumed eradication, at the end of all therapy (EOT) in the modified intent-to-treat (MITT) population.

The relationship between β-D-glucan assay results and response was also examined. Safety and tolerability were assessed throughout the study.

Results: The mean age of the enrolled patients (n = 43, from 13 centres in India, Philippines, Taiwan, and Thailand) was 56.5 (±18.5) years and 54% were male. Global response rate in the MITT population (n = 42) at EOT was 86.1% (95% confidence interval: 70.5%, 95.3%); secondary response rates are listed in the table below. Global response rates at EOT were 72.7% (13/18) for *Candida tropicalis*, 71.4% (10/14) for C albicans, 66.7% (4/6) for C glabrata, and 100% (4/4) for C parapsilosis. In the 21 patients with a central venous catheter up to 1 month before baseline, global response rate at EOT was 81.0%. Global responses in predefined populations were as follows: neutropenic patients 50.5% (n = 2), patients aged ⩾65 years 58.8% (n = 17), and patients with renal insufficiency 54.5% (n = 11). In the overall MITT population, patients with clinical response at EOT had lower mean β-D-glucan levels at baseline than those with clinical failure. Treatment-related adverse events were mild to moderate; the most common were diarrhea and rash, in 2 subjects each.

Conclusions: ANID was effective for the treatment of candidemia in Asian patients with APACHE II scores ⩽20. No new safety concerns were identified. These results can likely be extrapolated to a wide population with candidemia, both in the intensive care unit and medical wards.

Parameter[a]	MITT population (N = 42)		
	Clinical response n/N (%), (95% CI)	Microbiologic response n/N (%), (95% CI)	Global response n/N (%), (95% CI)
End of all therapy (EOT)	32/34 (94.1%), (80.3, 99.3)	34/35 (97.1%), (85.1, 99.9)	**31/36 (86.1%), (70.5, 95.3)**[b]
End of IV therapy	34/35 (97.1%), (85.1, 99.9)	36/37 (97.3%), (85.8, 99.9)	33/37 (89.2%), (74.6, 97.0)
2 weeks after EOT	26/28 (92.9%), (76.5, 99.1)	25/29 (86.2%), (68.3, 96.1)	24/29 (82.8%), (64.2, 94.2)
6 weeks after EOT	17/18 (94.4%), (72.7, 99.9)	17/18 (94.4%), (72.7, 99.9)	17/19 (89.5%), (66.9, 98.7)
12 weeks after baseline	17/20 (85.0%), (62.1, 96.8)	16/19 (84.2%), (60.4, 96.6)	16/22 (72.7%), (49.8, 89.3)

[a] Missing/indeterminate responses excluded. [b] Primary endpoint.

R2321 Comparative pharmacodynamic characteristics of amphotericin B against *Aspergillus fumigatus*, *A. flavus* and *A. terreus* using an *in vitro* pharmacokinetic/pharmacodynamic system simulating amphotericin B human pharmacokinetics

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Background: In conventional antifungal susceptibility testing methodologies of *Aspergillus* species, conidia are exposed to constant drug concentrations. However, in clinical practice, antifungal drugs are administered as intravenous boluses resulting in decreasing plasma concentrations. In order to describe the pharmacodynamics (PD) of amphotericin B (AMB) with a clinically relevant model, we developed an *in vitro* dialysis system simulating human pharmacokinetics (PK) after bolus administration and we studied the effect of standard dosing regimens of amphotericin B against *A. fumigatus*, *A. flavus* and *A. terreus*.

Methods: AMB standard dosing of 1 and 0.5 mg/kg resulting in peak plasma concentrations of 2.4 and 1.2 mg/l, respectively and half-lives of 24 h were simulated in a central compartment (CC) with 700 ml RPMI1640+0.165M MOPS. After injecting the drug, a peristaltic pump pumps into CC fresh medium and pumps out the content of the CC at the same rate as the clearance of the drug. Lung bronchiole was simulated with a dialysis tube (within the CC), which allows free diffusion of molecules smaller than 20kD comprising the peripheral compartment (PC). Different dialysis tubes were inoculated with 105CFU/ml of *A. fumigatus*, *A. flavus* and *A. terreus* conidia with CLSI MICs of AMB of 0.5, 0.5 and 1 mg/l, respectively and incubated at 37ºC for 48 h. For PK studies, 200μl were sampled from the CC and the PC and drug concentrations were determined with a bioassay utilizing an AMB susceptible *Paecilomyces variotii* strain. For PD studies, 200 μl from the inoculated dialysis tubes were sampled regularly to determine the galactomannan (GM) levels with the ELISA Platelia (Biorad). All experiments were performed at least twice.

Results: The drug concentration-time profile in CC and PC simulated reproducibly the plasma-concentration profiles of AMB (intra- and inter-day variation <10%). The simulated AMB dosing of 1 mg/kg inhibited completely *A. fumigatus*, partially *A. flavus* but not *A. terreus*. The simulated AMB dosing of 0.5 mg/kg did not have any effect against *A. terreus* and *A. flavus* whereas *A. fumigatus* was only inhibited for 48 h.

Conclusion: AMB levels from standard dosing of 1 mg/kg were efficient in inhibiting the growth of *A. fumigatus* but not *A. flavus* and *A. terreus*. The new *in vitro* PK/PD system reliably simulates human pharmacokinetics of AMB and it can be used to investigate alternative dosing regimens which may improve AMB activity.

R2322 Fungaemia in an intensive care unit in a tertiary care hospital: a 5-year survey

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Objectives: Fungaemia represents an important cause of morbidity and mortality in critical ill patients. The aim of the study was the evaluation and retrospective analysis of fungaemias in the intensive care unit (ICU) of a tertiary care hospital.

Methods: Patients hospitalized in the ICU longer than 48 h were included in this study, over a 5-year period (2004–2009). Demographic characteristics, predisposing factors, incidence of fungaemia, susceptibility profile, therapy and outcome were analyzed. Laboratory investigation was performed by conventional methods. Chromogenic medium and API 32C were used for the identification, while antifungal susceptibility was assessed by MICs determination using a broth microdilution method according to CLSI recommendations.

Results: In total, 74 strains were isolated from 70 patients. The mean age was 59 years and 55 were male. In 8 patients, the same pathogen was isolated from both blood and i.v. catheter cultures. Predisposing factors included cardiovascular disorders (n = 20), diabetes mellitus (n = 13), malignancy (n = 12), respiratory failure (n = 8), burns (n = 6), chronic alcohol consumption and alcoholic cirrhosis (n = 5) and chronic renal failure (n = 3). Six patients suffered from hematological malignancies, and one was HIV positive. Non-albicans *Candida* strains were the most frequent isolates (n = 39, 53%): *C. parapsilosis* 20 (27%), *C. tropicalis* 9 (12%), *C. glabrata* 9 (12%) and *C. dubliniensis* 1 (1.3%). Thirty strains (40.5%) were identified as *C. albicans*. Thee strains Saccharomyces bullardii (4%), one strain Zygosaccharomyces spp. (1.3%) and one Rhodotorula mucinanginosa (1.3%) were also isolated. All antifungal agents showed excellent activity against most of these potentially lethal pathogens. However, two strains of *C. glabrata* were resistant to itraconazole and fluconazole, three strains of *C. albicans* were resistant to caspofugin and two strains of *C. tropicalis* were resistant to itraconazole. *R. mucinanginosa* was sensitive only to amphotericinB, fluconazole and ketoconazole. All patients were treated with amphotericinB and/or caspofungin. Case fatality was 38%.

Conclusion: Candidemia was the most common fungal nosocomial infection among ICU patients with high mortality rate. *Candida non-albicans* were the most prevalent strains (53%). Early diagnosis and initiation of antifungal therapy, as well as control of the underlying predisposing factors, are the only potentially curative options for this emerging invasive infection.

AIDS and HIV infection

R2323 Utility of rapid HIV-test conducted in pharmacies

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Background: One of the main problems today is the late diagnosis of HIV. Perhaps closer to the people the possibility to realize a rapid HIV

test at a pharmacy to contribute to the further spread of the test and allow earlier diagnosis of HIV.
Methods: The 1–4 2009 is put into practice a pilot in 20 pharmacies in the BAC to perform rapid HIV tests. It analyzes all tests since that date to 15.9.2009.
Results: In that time period 1708 tests have been performed: 608 in Gipuzkoa, 884 in Bizkaia and 206 in Alava. Some 67% of people are men. The average age is 35 years and the age distribution 40% of patients are between 30 and 40. Around 69% of the patients was the first time they were tested. 15 people have positive results: 12 true and 3 false positives. 8 of them have a good immune status (more than 350 CD4/ml) and 4 others not found to have consulted with an HIV specialist consultation. The negative predictive value of the test is 99%.
Conclusions:
– It appears that facilitate the implementation of HIV rapid tests can contribute to a further universalization of the test.
– The negative predictive value of the test is high: 99%.
– It is important to ensure the pharmacy-access medical consultation to avoid loss among patients.

R2324 Bulge of syphilis among HIN-infected patients: epidemiological data from a Greek hospital

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Objectives: To describe an outbreak of early syphilis in HIV seropositive patients treated in the Infectious Diseases Unit in AHEPA University Hospital of Thessaloniki, Greece in 2008.
Methods: A review of infectious syphilis cases in a HIV Unit in 2008. Demographics were recorded with details of presentation, results of diagnostic tests, concurrent infections and treatments, likely route and place of acquisition, and details of contact tracing.
Results: Of 640 patients, 152 were checked and 27 of them (4.2%) were syphilis positive. All were homo- or bisexual men with a median age of 41 years old (range, 27–75 years).16 patients (58%) stated unprotected oral intercourse. 11 patients (42%) reported unprotected anal intercourse. HIV-positive serologic status was known for a median of 8.8 years (range, 0–19 years), only 2 (7.4%) individuals receiving the diagnosis simultaneously. 21 patients (77.7%) were under antiretroviral (ARV) therapy; median time under ARV treatment was 8 years (range, 0–16 years). At time of syphilis seropositivity, median CD4-cells count was below 500/mm^3 (~459/mm^3, range, 77–840/mm^3) and median plasmatic HIV-RNA virus load was 3.0 log/mL (range, −1.8 to −5.8 log/mL). Secondary syphilis was more frequent (9 patients, 33%) than primary (7 patients, 26%), latent syphilis (10 patients, 37%) and symptomatic neurosyphilis (1 patient, 4%). Overall, 20 patients (74%) received parenteral treatment and 7 (26%) oral for 21 days. Lumbar puncture was performed in 17 neurologically asymptomatic (63%) and 1 symptomatic patient (4%). 10 patients (37%) denied undergoing the procedure. 10 (37%) were diagnosed with neurosyphilis. Satisfactory serologic response was observed in 10 patients (37%) at 6 and 9 months follow up and in 17 (63%) at 6–9-12 months follow up, respectively.
Discussion: Multiple sexual partners, unprotected oral sex, and increased age among MSM were the predominant risk factors contributing to this syphilis epidemic. The overall high rate of unprotected sex demonstrates an increasing prevalence of unsafe sexual practices among MSM attributed to faith in antiretroviral therapy (simplified regimen) and to mental fatigue arising from years of protected sex to reduce the risk of HIV. Therefore, it is essential that prompt diagnosis, treatment, and contact tracing occur in order to control this major outbreak.

R2325 Expanded post-exposure prophylaxis for simultaneous multiple source HIV exposure in a healthcare worker

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Background: Guidelines about post-exposure prophylaxis (PEP) in healthcare workers are mostly based on retrospective data and expert opinion. The role of new drug classes in PEP is largely undefined.
Materials and Methods: We report an extreme case of high-grade needlestick exposure of a healthcare worker to serum from multiple HIV-infected patients after trying to prematurely remove the respective tubes from an automated biochemical analyzer.
Results: After review of the medical records of the 8 source patients (Table 1), we offered the healthcare worker an expanded PEP regimen including the entry inhibitor enfuvirtide. She refused to take subcutaneous injections, so we recommended use of the integrase inhibitor raltegravir. The CCR5 inhibitor maraviroc was not commercially available at that time. She completed therapy without problems and periodic evaluation for HIV transmission up to 9 months after the incident was negative.
Conclusions: We believe there are several important issues pertinent to this extremely unusual event. These include 1. the choice of PEP in cases of exposure to potentially resistant HIV virus(es), 2. the use of newer classes of antiretroviral drugs for PEP and the theoretical advantages of some of them in the PEP setting due to their mechanisms of action and 3. the practice of differential labeling of specimens from HIV-infected patients, applied for a long time in several hospitals in our country.

Year of HIV diagnosis (range)	1994–2008	Patients with undetectable HIV viral load (<50 copies/mL) on last measurement	7/8
Age range (years)	36–82	Patients with available HIV viral load measurement within last 3 months	4/7
Hellenic ethnicity	8/8	Range of most recent CD4 count (cells/μL)	119–1294
MSM as risk factor	8/8	Range of baseline CD4 count (cells/μL)	126/1060
Patients receiving ART	7/8	Patients with baseline CD4 count below 350 cells/μL	5/8
Patients receiving stable ART last 3 months	7/8	Patients with AIDS-defining illness last 6 months	1/8
Patients with recent satisfactory compliance with ART (clinician's judgment)	8/8	Other significant illnesses/malignancies last 6 months	2/8
Current NNRTI-based regimen	3/8	Known chronic hepatitis B and/or C	0/8
Current PI-based regimen	5/8	Evaluation for hepatitis C last 3 months	1/8
Patients with history of ART failure	2/8	Failed ART regimens	Pt1: AZT/3TC; Pt2: D4T/3TC/EFV, D4T/DDI/NFV
Year of ART failure	1999, 2000–2001	Available genotype resistance testing during failure	0/2

R2326 Tuberculosis in HIV–HCV co-infected patients

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Objectives: Coexistence of tuberculosis (TB) in human immunodeficiency virus and hepatitis C virus (HIV–HCV) co-infected patients, is a unique clinical and public health challenges. Medications for treatment of TB, HIV and HCV are hepatotoxic. So this condition made us to determine prevalence, risk factors and factors that may predispose for anti-tuberculosis therapy induced hepatoxicity.
Methods: In a retrospective study medical records of all hospitalized HIV–HCV co-infected patients from January 2001 to December 2009 at the National Research Institute of Tuberculosis and Lung Disease (NRITLD) were reviewed. A standardized case record form was applied to collect demographic, clinical, laboratory and microbiologic data. Patients with coexisting TB were identified as a case group and patients who were not infected with TB considered as the control group. Presenting signs and symptoms, and co-morbidities and widespread lab data (including biochemical, haematologic and serologic assay) in both groups were measured.
Results: 126 HIV–HCV co-infected patients (all of them were smoker males, 25.5% jobless, 47.7%single, 43.1%Married, 9.2% divorced, 80% IVDU, 84.6% opium user, 13.8% HBsAg+, 84.6% imprison and 12.3% in hospital mortality) were recruited in this study.60 out of them had coexisting TB. Significant statistical differences were seen in marital status, hospitalization length, CD4 count, clinical sings and symptoms (cough, sputum, fever, weight loss) microcytic anemia, ESR and CRP between TB and non-TB groups.

Conclusion: In multivariable analysis, injection drug use and imprison were significant independent risk factors for HIV–HCV co-infection. Among HIV–HCV-infected patients admitted in NRITLD, TB was a common infection. No significant difference in LFT and other biomarkers found in TB and non-TB infected patients. Tuberculosis infection was not associated with in hospital mortality. These findings imply that as the rate of anti-tuberculosis therapy induced hepatoxicity in HCV-HIV co-infected patients is similar to other patients, standard treatment could be pursued in the usual manner.

R2327 Genetic characterization of *Trypanosoma cruzi* isolated from HIV patients with reactivation and its correlation with kDNA profiles and LTCD4+ counts

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Objectives: The factors involved in chagas disease reactivation are not clear, they may be related to selective host immune depletion and/or to specific parasite populations. Herein, the characterization of *T. cruzi* DNA nuclear and kDNA was performed in samples from HIV patients with blood and / or central nervous (CNS) system reactivation, HIV patients not reactivated and from HIV negative chagasic patients.
Methods: The parasite characterization was performed by amplification of the D7 domain of the gene 24S, 18S rRNA and the mini-exon gene intergenic region, and kDNA using low stringency single specific primer - PCR. *T. cruzi* populations were classified according to the new nomenclature (Consensus 2009).
Results: *T. cruzi* reactivation was detected in 10 patients HIV positive and two HIV negative immunocompromised by chemoterapy or transplants, 91.7% (11/12) *T. cruzi* II and 8.3% (1/12) *T. cruzi* V. Parasite kDNA showed high variability, but identical genetic profiles in blood and CNS fluid from a same patient. These populations were clustered in to three branches, two with 72.7% of isolates and low LTCD4+ counts (42.0 cells/mm^3) and the other with 27.3% of samples and 158.0 cells/mm^3. Among the 29 HIV co-infected patients not reactivated the *T. cruzi* I was detected in 6.9% (2/29); *T. cruzi* II in 86.2%(25/29) and *T. cruzi* V in 6.9% (2/29). In 50 HIV negative patients, *T. cruzi* I was found in 4% (2/50), *T. cruzi* II in 94% (47/50) and *T. cruzi* V in 2.0% (1/50).
Conclusions: There was no difference in the distribution of *T. cruzi* groups among HIV positive and HIV negative patients (fischer test $p = 0.7791$), or between HIV reactivated and not reactivated patients (fischer test $p = 0.8281$) or associated with blood and/or CNS invasión in reactived patients. Both *T. cruzi* II and *T. cruzi* V had potential for reactivation and were associated with specific kDNA profiles and LTCD4+ counts.
Support: Programa Nacional de DST/AIDS – Ministério da Saúde–UNESCO (VS0106/2006) – FUNEPU – CNPq (Universal 2008).

Hepatitis

R2328 Evaluation of three immunoassays for hepatitis C virus antibody detection

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Objective: Hepatitis C Virus is a major cause of acute hepatitis and chronic liver disease, worldwide. Diagnostic tests for hepatitis C include serological assays that detect antibodies to hepatitis C virus (anti-HCV). Various automated immunoassays are widely used in clinical laboratories in order to detect anti-HCV. The aim of this study is to compare the sensitivity and specificity of three different immunoassays for the detection of HCV antibodies.
Methods: Sera were obtained from 320 adult patients (both sexes, aged 25 to 75 years old) of unknown serological status. All samples were screened for anti-HCV using Chemiluminescent Microparticle Immunoassay (CMIA), Microparticle Enzyme Immunoassay (MEIA) and Electrochemiluminescence Immunoassay (ECLIA). Architect i2000SR (Abbott), Axsym Plus (Abbott), and Elecsys 2010 (Roche) were the automated analyzers used respectively. Prevalences were calculated according to S/CO ratio. Any positive or dubious (low S/CO ratio) result was confirmed using the Line Immunoassay INNO-LIA HCV.
Results: Among the 320 samples tested, 18 were found to be positive for anti-HCV by all three methods (S/CO > 200 for ECLIA, S/CO > 10 for MEIA and S/CO > 5 for CMIA). The positive samples were subsequently tested using INNO-LIA and were all confirmed showing 100% sensitivity for all three assays. Moreover, 6 samples were found positive with a low S/CO ratio by ECLIA and CMIA (S/CO < 200 for ECLIA and S/CO < 5 for CMIA) and 4 of them were also found positive with a low S/CO ratio by MEIA (S/CO < 10). INNO-LIA confirmed that two of the above samples were positive (2 out of six for ECLIA and CMIA, 2 out of 4 for MEIA), showing an overall specificity of 98.7% for ECLIA and CMIA and 99.3% for MEIA.
Conclusions: Our study suggests that MEIA, CMIA and ECLIA indicated excellent sensitivity and almost identical high specificity. Thus, any of the above methods can be used for routine detection of HCV antibodies in serum samples. Supplemental anti-HCV tests such as INNO-LIA, could also be used in order to resolve false-positive testing.

R2329 Initial laboratory predictors of severe hepatitis in patients with acute hepatitis A

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Objectives: Hepatitis A virus (HAV) infection commonly causes acute self-limited hepatitis. However, severe hepatic or renal dysfunction may occur infrequently. We investigated the initial predictors for the development of severe acute hepatitis A (S-AHA) and acute renal failure (ARF) in patients with AHA.
Methods: We retrospectively reviewed the medical records of patients with AHA, from January 2007 to March 2009, at Chung-Ang University Medical Center. The definition of AHA was based on the detection of IgM antibody against HAV using enzyme immunoassay. S-AHA was defined as prothrombin time (PT) <40% of control activity during the course of AHA and ARF was defined as the increase of serum creatinine >0.5 mg/dL over the baseline level.
Results: During the study period, 192 patients developed AHA. The majority of patients were young adults (<40 years of age, 91.7%) without underlying illness. S-AHA and ARF developed in 22 (11.4%) and 10 (5.2%) patients, respectively. The patients with S-AHA more frequently had male gender (86.4% vs. 62.1%, $p = 0.025$) and ARF (19.0% vs. 3.6%, $p = 0.015$) than non-severe groups. The following initial laboratory findings were more commonly observed in patients with S-AHA; lactate dehydrogenase (LDH) >660 IU/L (90.5% vs. 40.0%, $p < 0.001$), C-reactive protein (CRP) >20 mg/L (52.9% vs. 19.2%, $p = 0.005$), albumin <3.4 g/dL (68.2% vs. 26.6%, $p < 0.001$), total cholesterol <95 mg/dL (81.8% vs. 26.5%, $p < 0.001$) and platelet <150,000/μL (95.4% vs. 39.0%, $p < 0.001$). Independent predictors for S-AHA were high LDH (OR, 11.35; 95% CI, 2.00–64.38; $p = 0.006$), low albumin (OR, 12.67; 95% CI, 3.54–45.29; $p < 0.001$) and thrombocytopenia (OR, 17.16; 95% CI, 1.99–148.10; $p = 0.01$) in multivariate analysis. Patients with ARF had following initial laboratory findings more commonly than patients without ARF; LDH >660 IU/L (100% vs. 42%, $p = 0.001$), CRP >20 mg/L (57.1% vs. 20.9%, $p = 0.047$), total cholesterol <95 mg/dL (70.0% vs. 30.3%, $p = 0.014$) and platelet <150,000/μL (80.0% vs. 42.8%, $p = 0.045$).
Conclusion: Thrombocytopenia, high LDH and low albumin at admission were independent predictors for S-AHA. Initial laboratory profiles may provide useful information for predictor of subsequent development of S-AHA.

R2330 Retrospective study of increasing incidence of acute hepatitis A in Area 2 of Madrid. A report from the microbiology department at a university hospital, Madrid, Spain

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Objectives: Study of incidence of acute hepatitis A (AHA) during the period of 4 years. (January 2006 to November 2009).
Methods: 12666 serum samples obtained from Outpatients, Hospitalized Patients (HP) and patients from Primary Care (PC) were analyzed. HA-IgM antibody, by enzimoimmunoassay (EIA, Arquitect Abbott Diagnostic) was determinated.
Results: In 2006, of the 2794 samples studied, 3 males cases were reported: 1 of SC, 1 of HP and 1 of PC. In 2007, of the total of 3094, 15 were reported: 9 PC, 3 SC, 3 HP (ages 14–52 years, 4 females/11 males). In 2008, we reported 32 cases of 3235 samples: 18 PC, 12 SC, 2 HP (ages 16–46 years, 10 females/22 males). And in 2009 of the total of 3544 samples, we obtained 17: PC 8, SC 9, HP 1 (ages 20–29 years, 17 males). With our results of 67 positive samples, in patients with sintomatology of acute disease, an important group mentioned high-risk sexual practices with other persons of the same/different sex, with prevalence of same sex.
Conclusions: An increase of AHA was observed since May of 2007 to July of 2009, and is becoming more pronunced between september of 2008 to July of 2009. Furthermore we observed the transmission was in most of cases due to sexual practices. In the future, the epidemiological anamnesis of sexual transmission should be considered.

Year	Total (n = 12666)	Positive	PC	OP	HP	Range	M/F
2006	2794	3	1 (33.33%)	1 (33.33%)	1 (33.33%)		2/1
2007	3094	15	9 (60%)	3 (20%)	3 (20%)	14–52	11/4
2008	3235	32	18 (56.25%)	12 (37.50%)	2 (6.25%)	16–46	10/22
2009	3543	17	7 (41%)	9 (53%)	1 (6%)	20–29	17/0

R2331 Effect of changes in bio-energy on the severity of the disease in patients with chronic hepatitis C

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Currently, biological oxidation is defined as a set of substrate oxidation reactions in living cells whose primary function – providing energy metabolism. Oxygen consumption of tissues depends on the intensity of the reactions of tissue respiration. The highest rate of tissue respiration characterized by kidney, brain, liver, and the lowest – skin, muscle tissue (at rest). Multilevel control of cellular metabolism, providing maintenance of homeostasis under changing environmental conditions, including as one major factor regulation of redox potential and redox state of nicotinamide nucleotides, which can be measured by the ratio of oxidized and reduced kofermentov. Ustanovleno that the increase in the ratio of oxidized coenzymes to Retreaded increases oxidative properties of tissues and body fluids, activates the functioning of glycolysis, tricarboxylic acid cycle, while inhibition of lipogenesis and gluconeogenesis reaction.
Aims: To define the mechanisms by which NADH/ NAD and of lactate/ pyruvate plays an antifibrogenic role.
Methods: Examination of lactate and pyruvate levels was carried out by enzymatic method which is based on the oxidation of lactic acid to pyruvic by enzyme lactate dehydrogenase with the parallel reduction of NAD+ to NADH2.
Results: Metabolic disorders, which are characterized by the correlation NAD+/NADH2 and increase of NAD+ (0.494+0.03 mmole/l) concentration in comparation with NADH2 (0.002+0.001 mmole/l) what makes the reaction slower in connecting with acceleration of lactate/pyruvate correlation and in result the speed of gluconeogenesis is decreased, are observed at the patients with the chronic hepatitis C. The most excessive metabolic disorders are observed at the patients with virus genotype 1b, which can be undesired sign of antivirus therapy and can require of its correction.

Virology non-HIV/non-hepatitis

R2332 Mumps infection in the period 2007–2009

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Objectives: To present the most common clinical manifestations and epidemiological characteristics of mumps infection in our patients, in period of two years (2007–2009).
Methods: 632 patients had been analyzed. 138 (22%) of them, were hospitalized. They were diagnosed according to the clinical manifestations, epidemiological characteristics, biochemical analyses and ELISA (IgM Ab and IgG Ab).
Results: 470 (74.5%) patients were male, and 162 (25.5%) were female. According to age, most of the patients were from 15 to 19 years old – 252 (39.5%). The largest frequency of patients was registered in the months from September till March. In hospitalized 138 patients, the most common clinical manifestations were: parotitis in 88 patients (63.7%) – bilateral 65 (73.8%) and unilateral 23 (26.13%); orchitis in 44 patients (31.8%) – bilateral 17 (29.7%) and unilateral 27 (61.3%); mumps meningitis in 3 patients (2.2%); oophoritis in 3 patients (2.2%). Hepatic lesions appeared in 82 patients (59.4%).
Conclusion: Most common clinical expressions of the mumps infection are parotiits (63.7% of patients) and orchitis (31.8% of patients). There is also high percentage of hepatic lesions (59.4% of patients). According to age, most of the patients were from 15 to 19 years of age (39.5%). The epidemics in our region started in march 2007 in non-immunized child from gipsy population, with tendency to spread, and was terminated in the spring of 2009. Most of the infected patients were non-immunized or not completely immunized, and mostly people from the gipsy population.

R2333 DNA-based drug against DNA virus infections

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Objective: There are certain problems in ethiotropic therapy of viral infections caused by DNA viruses: herpes simplex virus (HSV) and human papillomavirus (HPV). Therefore, the use of immunomodulators seems perspective. Previously, it was found that immunomodulator ferrovir (FV) (sodium salt of DNA from salmon's milt conjugated with Fe3+) induced production of inflammation cytokines and antiviral factor with low m.m. (Acta Virologica, 1999;43:32–7) and was effective in immunocompromised patients with human immunodeficiency virus infection and hepatitis C infection. The aim of the study was to analyze the efficiency of FV against HSV- and HPV-infection.
Methods: FV was used as monotherapy in open clinical study enrolled 305 adult patients (pts) with genital HSV-infection with no less than 6 recidivations per year and as the part of complex therapy in 63 pts with HPV-infection. Pts were treated with 75 mg FV twice daily during 10 days in the enema per rectum or intramuscular injections.
Results: FV administration was well tolerated and no side effects were observed. In 87.8% cases of pts with HSV-infection long remission (only 1–2 recedivations per year) was registered and shortening of time of recidivation was found. In pts with HPV-infection FV also provided long period (12 months) without recidivation which had happened only in 6.4% of cases.
Conclusion: FV demonstrated good antiviral properties; is well tolerated by patients, is useful in case of HSV- and HPV-infection, and the low price makes it accessible to population in limited resources context.

R2334 Efficacy and safety of topical acyclovir/hydrocortisone 1% formulation for the treatment of recurrent labial herpes simplex in adolescents

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Objective: To investigate efficacy and safety of topical acyclovir / hydrocortisone 1% formulation for the treatment of recurrent labial herpes simplex in adolescents.
Methods: Non-immunocompromised subjects 12–17 years old who had more than 2 episodes of recurrent labial herpes during the previous 12 months were eligible. Patients were not allowed to use systemic or topical antivirals and steroid agents two weeks prior and during the study drug treatment period. The study drug was administered for the new episode of labial herpes simplex and was applied 5 times per day for 5 days. Clinical assessment was performed daily for 5 days, 1 week, 3±1 weeks and 6 months after the last dose of the study drug.
Results: A total of 22 patients (11 boys and 11 girls) were treated with the study drug. The labial herpes simplex recurrence rate during the last year in most patients varied from 4 to 8 times, one patient had 12 relapses during the previous year. During the new episode of labial herpes simplex all patients noted disappearance of clinical symptoms (pain, burning) on the second day of the study treatment. Eighteen of 22 subjects had non-ulcerative herpetic lesions – the process stopped on papule stage. In most patients (12 persons) the symptoms of relapse resolved on the forth day, in 5 persons – on the fifths day, and in one person – on the sixths day. The skin was normal during the first week and 3±1 weeks after the last dose of the study medication in all patients. Four patients have been diagnosed a recurrence of herpes leading to development of a lesion with vesicle, ulcer and hard crust. They had hard crust stage of labial herpes simplex on the end of treatment period. On the first week after last dose of study drug there were determined residual abnormalities in those patients and the normal skin was fixed on 3±1 week visit. None subjects had adverse events related with the study drug. Phone contact was performed post 6 months after last dose of the study drug and all of treated patients had no recurrence labial herpes during the period.
Conclusions: Topical acyclovir/1% hydrocortisone showed good efficacy for the treatment of recurrent labial herpes simplex. All patients noted quick resolving of symptoms (pain, burning). Application of the drug stopped the recurrence process on the papule stage. There were no adverse events due to study drug.

R2335 Influenza A H1N1: Shell vial culture vs. PCR

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In the beginning of the H1N1 Influenzavirus pandemia, we started working with PCR, culture and antigen detection (the last was refused due to its low sensibility), but culture was performed in certain circumstances like immunocompromised or admitted in Intensive Care Units patients.
Objectives: To compare shell-vial culture and PCR for Influenza AH1N1 detection considering PCR as "gold standard".
Methods: 214 samples were processed during the period April-November 2009 by PCR [AgPath-ID™One-Step RT-PCR Kit (Ambion Applied Biosystem), artus® Influenza/H1 LC/RG RT-PCR-Kit (Qiagen)] and cultured in MDCK cell line shell-vial (Vircell™) with Trypsine, incubated at 35–37ºC for 24 or 48 hours, and stained with monoclonal antibody against respiratory viruses, including Influenza A (Chemicon™).
Results: See Table 1.
We also observed that 24 hours of incubation are enough and correlates with 50–75% cell monolayer infection (detected by monoclonal antibody stain).
Conclusion: MDCK cell line is an adequate cell line for Influenzavirus H1N1 isolation and Influenza A monoclonal antibody stain identifies it correctly in a pandemic situation (without distinguish between virus subtypes).
We consider that virus isolation is necessary for later studies. Furthermore, we may identify other viruses when we are not looking for them on purpose or in samples not considered at first.

Table 1. Culture and PCR comparison results

	Culture +	Culture –	Total
PCR +	102	32	134
PCR –	2	78	80
Total	104	110	214
Shell-vial culture sensibility		76.12%	
Shell-vial culture specificity		97.5%	
Shell-vial culture positive predictive value		98.08%	

R2336 The usefulness of IgG avidity for determining primary cytomegalovirus infection in pregnant women

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Objectives: The diagnosis of infection with human cytomegalovirus remains difficult on symptoms alone as many of them are mild and asymptomatic. Primary CMV infection in early pregnancy bears a high risk of fetal damage. The prenatal diagnosis of CMV has focused on first-trimester screening since the time of maternal CMV infection is an important variable and the rate of transmission from mother to fetus is much higher. If fetal infection occurs earlier in gestation, it appears to present a greater threat to the fetus.
Methods: 8.768 pregnant women in the first trimester of the pregnancy were tested for CMV during the last 18 months May 2008 – Oct 2009 in "Mitera" general, maternity & children hospital. Women were aged between 23 and 44 (mean age 33.5 years). Samples positive for CMV IgG were tested farther for IgM antibodies. Finally samples positive in both CMV IgG and IgM antibodies were farther tested for CMV IgG avidity. All tests were performed by chemiluminescent microparticle immunoassay (CMIA, Abbott Park, US).
Results: Of the 8.768 women tested, 3.558 (40.6%) had never been infected with CMV, 5.082 (58.0%) found to be positive in CMV IgG and negative in IgM. 117 (1.3%) samples found to be positive in both CMV IgG and IgM and 11 (0.1%) were in the CMV IgM grayzone. Of the 117 positive samples, 23 (19.7%) had a low avidity and 93 (79.5%) had a high avidity. One sample (0.8%) was in the grayzone. All samples with CMV IgM in the grayzone had high avidity.
Conclusions: Measurement of CMV IgG avidity may help to improve the serodiagnosis of CMV infected women by determining the time of infection. The presence of high avidity indicates that primary infection occurred well before conception and the fetus is most likely protected against debilitating CMV infection. This information is important in the clinical management of pregnant women found to be positive for CMV antibodies at their first-trimester of gestation when the risk of fetal damage is greater.

R2337 Patients with 2009 influenza A/H1N1 virus in a tertiary hospital in Spain, April-October 2009

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Background: During the first weeks of April 2009 North America and Mexico detected the initial cases of a novel swine-origin Influenza A/H1N1 virus: a triple-reassortant Influenza A virus. This was quickly followed by detection in other countries and by the end of April, the virus had spread to over 123 countries.
Methods: Using medical charts, we collected data on 212 patients who were attended for influenza-like illness (fever, cough, sore throat) in our Hospital and who tested positive for the 2009 H1N1 virus; we regarded age, hospitalization (at least 24 hs), and underlying diseases.

Two types of real-time reverse-transcriptase–polymerase-chain-reaction assays, were used; Influenza RT-PCR Kit RUO (Artus) as a screening test and a confirmatory RT-PCR (Applied Biosystems ™), according to the CDC protocol.
Results: Of the 190 patients for whom data were available, 134 (70.5%) were admitted to the domiciliary hospitalization unit, 55 (28.9%) were hospitalized (median age 27.07, range 2–85), 4 (2.1%) were admitted to an intensive care unit (median age 30, range 14–51), and 2 (1.05%) died (median age 18.5 range 14–23): 110 (57.8%) were adult patients, between 18 and 65 years of age, 74 (38.9%) were children under the age of 18 years, and 5 (2.6%) were adults 65 years of age or older. 80 (42.1%) of the patients had at least one underlying medical condition; asthma; diabetes; heart, lung disease, obesity and pregnancy, considered risk factors for severe disease 26 (13.6%), had recently traveled to Mexico. (including the first reported case at the Valencian Community) 21 (38.9%) of the 55 hospitalized patients and 1 of the 4 (7.2%) admitted to an intensive care unit patients did not have any risk factors for severe illness.
Conclusions: During the evaluation period, 212 patients tested positive for the 2009 A/H1N1 Influenza. More than 95% of the affected patients were under 65 years; 55 (29.1%) patients required hospitalization because of severe illness. 21 (38.9%) of these hospitalized patients as well as 1 of the ICU admitted patients, did not have any risk factors for severe illness. It's seems to be important to continue advancing in the knowledge of the pathogenicity of this microorganism.

R2338 Different clinical presentation between male and female patients with Puumalavirus infection. Are the clinical differences related to expression of different oestrogen receptor subtypes?

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Objectives: To investigate the expression of estrogen receptor (ER) mRNA in peripheral blood mononuclear cells during Puumala virus infection (nephropathia epidemica).
Methods: Ambulatory and in-patients (n = 20, male/female: 10/10) at Umeå university hospital with confirmed nephropathia epidemica (NE) were followed with routine blood chemistry and sampled for peripheral blood mononuclear cells (PBMC) during day 1 and 3 and a follow-up convalescent sample after 12 weeks. The PBMC were stored in −70ºC until RNA was extracted using RNeasy kit (Quiagen). cDNA synthesis was performed and expression of ER α, ER β and ER β cx (mRNA splice variant of ER β) were quantitatively estimated using real-time PCR (Applied Biosystems). The multiple variables from the blood chemistry, relative expression levels of ERs mRNA, sex, and day of onset of the disease were related to each other in a principal component analysis (PCA)(SIMCA-P, v 11.5.0.0).
Results: ER α is expressed in higher quantities than ER β cx. ER β (wild type) could not be detected. ER α is correlated to a rise in white blood cells (WBC) ($p = 0.0004$) in the total group of patients (n = 20) and divided into males (n = 10) and females (n = 10), a correlation is seen between ER α and urea ($p = 0.03$), C-reactive protein (CRP) ($p = 0.04$) and WBC ($p = 0.01$) in the male patient group, compared to a correlation between ER α and WBC ($p = 0.04$) in the female patient group. The PCA show a distinct dichotomy of male and female samples. Female samples tend to be correlated to a higher level of ER α, and male samples to higher levels of ER β cx.
Conclusion: Our results from the PCA indicate a different clinical presentation in men and women with NE. This could explain that more men than women (2–5:1) receive the clinical diagnosis NE although the serological distribution is 1:1. Females were associated with higher levels of ER α and males with ER β cx. ER β cx is known to dimerize with ER α and thereby prevent the normal function of ER α. A larger study is needed to confirm the role of ER α and ER β cx in this disease.

R2339 Nosocomial transmission of Crimean-Congo haemorrhagic fever

R. Caylan, D. Yapar, S. Keske, I. Hasanoglu, M.A. Tasyaran (Ankara, TR)*

Introduction: Crimean-Congo haemorrhagic fever is a tick-borne viral disease. The virus is acquired by the bite of infected ticks. Transmission also may occur from direct or aerosol contact of blood or secretions of infected patients. Health-care workers are at increased risk of acquired infection while caring for haemorrhagic patients. Nosocomial CCHF mortality rate is higher than the other routes (respectively 80%, 10–50%). Ribavirin is recommended during postexposure prophylaxis and treatment of CCHF, however it is controversial.
Case report: In June 2009, 56-year-old female patient who was diagnosed as CCHF with positive PCR result from Refik Saydam National Public Health Agency, was developed oral mucosal and vaginal bleeding, melena during the sixth day of therapy. Patient was confused and she had seizures due to intracranial haemorrhage in sixth day of hospitalization. The patient's existing oral and nasal haemorrhagies increased during these seizures. Physician intervented only with disposable gloves because the patient needed emergency airway management and placed oral airway immediately without surgical mask or eye protection. Patient died after a few hours with widespread bleeding.
Five days after this intervention 33-year-old female physician presented fever, headache, myalgia and malaise. Her temperature was 38ºC, pulse 88 beats/min; blood pressure was 90/60 mmHg. Laboratory findings; Blood biochemical tests were normal, haemoglobin 11 g/dL leukocytes 900 K/mcL and platelet count of 76000 K/mcL. Oral ribavirin started quickly for the possible nosocomial transmission. The ribavirin dose was 2 g for loading, followed by 4 g/day for 4 days and 2 g/day for 3 days. She had no history of percutaneous exposure. Probably close contact with aerosolisation of blood or excretions are suspected route of transmission. Diagnosis was confirmed by RT-PCR. During the follow-up platelet counts persisted on decreasing and the liver enzymes slightly increased. No fever was detected after the third day. Haemorrhagic manifestations didn't develop. Significant side effects of ribavirin were not observed. Patient's symptoms improved after seventh day of admission. In conclusion, to prevent transmission of CCHF in close contact with haemorrhagic patients, droplet and contact precautions must be performed. Although it is controversial early onset of ribavirin had been found to be effective in our case.

R2340 Evaluation of Crimean-Congo haemorrhagic fever patients: epidemiological, clinical and laboratory features

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Introduction: Crimean Congo haemorrhagic fever (CCHF) is a disease caused by CCHF virus of family Bunyaviridae and transmitted to humans generally by Hyalomma tick bites or by direct contact with blood or other excretes of infected humans. There has been annual increases in case numbers in Turkey since 2002.
Materials and Methods: From April 2007 to September 2009 CCHF virus ELISA and/or RT-PCR confirmed cases in Ankara Ataturk Education and Research Hospital were included in the study. All the informations of the patients have been provided from patient charts.
Results: From 2007 to 2009, 94 comfirmed cases were hospitalized in our hospital. Female to male ratio was 1.14. Median age was 49 (15–76 years) years. 93% of the cases were from rural areas. 74% of patients had tick bite or contact history. Of these 94 patients one (1.06%) was nosocomial infection. The most seen clinical complaints were malaise (90%), fever (85%) and myalgia (81%). Cholecystitis were seen in 6 patients (6.4%). The mean hospitalization duration was 10 (1–62 days) days. Mortality rate was 6.4% (6/94). Ribavirin treatment were administered at 26 patients and also supportive treatment to all patients.
Discussion: CCHF is a big seasonal health problem for Turkey with increasing cases annually. The main risk factor for transmission of

disease is mostly tick bite. The cholecystitis seems to be a new defined clinical finding in CCHF. There is not a specific treatment for CCHF; efficacy of ribavirin treatment is controversial. The mortality rate was similiar with those reported in literature from Turkey.

R2341 Assessment of serological markers for Epstein–Barr virus in patients for transplantation

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Objectives: Viral infections constitute the single greatest cause of infectious-disease related morbidity and mortality in organ transplant recipients. Cytomegalovirus (CMV), which frequently causes latent asymptomatic infection in healthy adults, may evade immune surveillance in immune compromised patients, as well as in renal transplant recipients and to start to replicate. Epstein–Barr virus (EBV) infection sometimes results in clinical symptoms (fever, leucopenia, pharyngitis, hepatitis, lymphadenopathy) and eventually may lead to uncontrolled proliferation of B cells terminating in post-transplant lymphoproliferative disease (PTLD). The risk of PTLD is mostly determined by the prevalence of anti-EBV sero-positivity in transplanted patients.
Methods: This prompted us to investigate the serum samples submitted to our laboratory from 25 post transplanted patients (18–35 years old) and 30 healthy blood donors (20–42 years old). Serum samples were collected from all patients before transplantation and at three months after transplantation. Sera were stored at −70ºC. The samples were tested for EBV- and CMV-specific serology, including VCA IgM, VCA IgG, early antigen (EA) and EBNA antibody. The levels of antibodies were determined using commercially available sensitive enzyme-linked immunosorbent assay (ELISA) method.
Results: Antibody avidity test results were added to provide an expanded serological profile in which patients with low antibody affinity were defined as having primary infections while those with high antibody affinity were regarded as having past infections. 25 samples (48.2%) were found to be seropositive. Recent infection was diagnosed in 32.6% of patients while prior infection in 15.6%. Past infection was diagnosed by detecting VCA IgM antibodies in 20.5% of the examined samples. Our results confirm the thesis that VCA IgM in the absence of antibody to EB nuclear antigen (EBNA) is regarded as suggestive of acute primary EBV infection because EBNA antibodies develop only in late convalescence.
Conclusions: In conclusion, specific serological anti-EBV IgG markers (EBNA and EA) must be used for the serological diagnosis of EBV infectious mononucleosis. In order to obtain the highest sensitivity, VCA IgM, VCA IgG, EBNA and EA antibodies need to be measured.

R2342 Unusual nosocomial transmission of Crimean-Congo haemorrhagic fever; two cases report from Turkey

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Objectives: Crimean Congo Hemorrhagic Fever (CCHF) is a severe hemorrhagic fever caused by a Nairovirus, belonging to the family Bunyaviridae. In the spread of this zoonosis to humans, the main role is that of ticks; however, transmission is also possible via blood, tissue and bodily fluids of infected people or animals. Nosocomial transmission is also possible and, health care workers are one of the major risk groups for CCHF virus acquisition especially when caring for patients with hemorrhages from different body sites. In this paper we are reporting CCHF in two health care workers whose did not have any contact to blood or body fluids of a patient.
Case 1: 30 years old and pregnant nurse (ten weeks) admitted to our clinic with sudden onset high fever, myalgia, arthralgia and fatigue. She was caring the patients with CCHF in our clinic. Four days ago, in her shift, one patient died because of CCHF, the patient had respiratory symptoms and renal insufficiency. Although we don't have negative-pressure room, all the patients had to be isolated in a private room, and all healthcare workers are using barrier-nursing techniques that include disposable gloves, masks and goggles and hand-washing or use of alcohol based desenfectans are the main way of protection. We isolated the nurse and sent serum samples to the national reference laboratory for CCHF tests. Next day she was diagnosed as CCHF with positive PCR and with her informed consent, ribavirin treatment was given. On the following days, fever was continued and alanine aminotransferase, lactat dehydrogenase, creatine phosphokinase levels were increased. After five days, clinical and laboratory findings improved and she discharged with a medical abortus decision.
Case 2: 26 years old, male resident admitted to our clinic with the same complaint after two days of nurse's admission. He had cared the same patient on same day with the nurse. He was diagnosed CCHF with laboratory RT-PCR test. He had been taking the oral ribavirin with a decision of himself and we continued the therapy. His symptoms began to resolve in third day and he discharged from hospital.
The only history of their contacts to inside the patient's room without mask once or twice.
Conclusion: Although the main way of transmission of CCHF to health care workers is close contact to blood and other body fluids, transmission with aerosol or air droplets may be possible.

R2343 Central nervous system infections due to herpesviruses in immunocompetent population

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The objectives of the present study were the detection of the most common herpesviruses from cerebrospinal fluid (CSF) samples of patients with central nervous system (CNS) infections from Northern Greece, in order to:

1. estimate the incidence of CNS infections caused by herpesviruses.
2. correlate the causative viruses with clinical manifestations and CSF laboratory findings.
3. compare the results of this study with results published from other countries.

Methods: From March 2003 to June 2008, 156 immunocompetent patients with possible viral CNS infection, hospitalized at Northern Greece hospitals, were included in this study. From these patients, 60 (39%) had encephalitis, 64 (41%) patients had meningitis, 7 (4%) patients had encephalomyelitis, 4 (2%) patients had Guillen-Barre syndrome and 21 (14%) patients had various unspecified neurological diseases. Polymerase chain reaction (multiplex consensus nested PCR), as well as serology, were performed for the detection of herpesviruses in CSF.
Results: Herpesviruses from CSF were detected in 11 of 156 (7%) patients. Ten of them were adults and one was child. Herpesvirus genome was detected by PCR from 10 patients and there was a patient with encephalitis, the etiologic agent of which, HSV, was identified by intrathecal andibobies production.
From 11 patients with herpetic CNS infection 6 had encephalitis, 4 had meningitis and one patient had Guillain–Barre syndrome. Herpes simplex virus type 1 (HSV-1) genome was detected from 5 patients with encephalitis and a patient with Guillain–Barre, herpes simplex virus type 2 (HSV-2) from a patient with meningitis and varicella-zoster virus (VZV) was detected from 3 patients with meningitis. From a patient with encephalitis, the diagnosis was established by HSV intrathecal antibodies detection. The detection of herpesvirus genome from this patient' CSF was impossible.
The incidence of herpes simplex encephalitis in our study was 1 patient per 1.000.000 people per year. All patients with meningitis had excellent outcome; however, poor outcome, which led to death, was observed in two of six patients with encephalitis, (mortality 33%). The poor outcome was associated with low Glascow coma scale at the admission to the hospital.
In conclusion, HSV-1 was the most common herpesvirus detected in this study, which was the cause of encephalitis with high mortality. HSV-2 and VZV were less frequently detected and caused milder disease, with good outcome.

Mycobacterial infections (including diagnosis)

R2344 Miliary TB-mimicking advanced ovarian cancer with osteolytic lesions of the spine

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Objective: to present a case of miliary TB mimicking advanced ovarian cancer with thoracic spine metastases.
Results: A 24-year old Somalian female complained for backache, constipation and low grade fever for the last 5 months. Basic laboratory tests revealed anemia (Hb = 9.7 mg/dl) and severe thrombocytosis (PLT = 1027×10^3). She also had an elevated CA-125 of 982 U/ml (<35U/ml) and a positive tuberculin test (17 mm). She underwent C/T scan which revealed pleural effusions, ascites, a solid lesion of the right ovary and osteolytic and slerosing lesions of the lower thoracic vertebrae, and transvaginal ultrasound which showed a large cystic mass arising from the right ovary. Paracentesis of the ascites was performed; the fluid was an exudate with positive cytology for atypic cells, possibly adenocarcinoma, and negative for AFB. Gastroscopy and barium enema were normal. As the above findings suggested of an advanced ovarian cancer, laparotomy was performed; the macroscopic picture was of multiple whitish lesions over the peritoneum and the intestines (omental cake). Two liters of ascetic fluid were removed and an ADA test was done which came back positive: 55U/ml (13–23U/ml). The right ovary and salpynx were removed; the biopsy showed granulomatous tissue. C/T guided biopsy of the vertebral lesions was performed, and PCR for Myc. tuberculosis on the material was done which was positive. The patient was put on anti-TB treatment with isoniazid, rifampicin, pyrazinamid, and ethambutol with gradual improvement.
Conclusions: As TB is rare in the developed world, ovarian and peritoneal TB is often misdiagnosed as advanced ovarian cancer, leading young women to unnecessary laparotomy and surgery. Ascites, peritoneal nodules and elevated CA-125, even in the presence of osteolytic lesions of the vertebrae, do not necessarily indicate malignancy. Tuberculosis should always be suspected, especially in young women from endemic countries. As cultures for mycobacterium are time consuming tests, and peritoneal fluid is often negative for AFB, PCR and ADA are useful tools for the differential diagnosis.

R2345 Evaluation of Alpha Tec Nac-Pac™ mycobacteria digestion and decontamination system on pulmonary samples

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Objectives: Clinical samples sent to the Mycobacteria Laboratory for culture confirmation of Mycobacterial infection are contaminated by non-mycobacterial organisms and require digestion and decontamination to allow effective diagnosis of mycobacterial infection. The optimal recovery of mycobacteria requires a tightly regulated pH. A basic pH quickly eliminates non-mycobacterial organisms from the patient sample. However, prolonged exposure to a high pH is toxic to Mycobacterial organisms. A carefully controlled pH through-out samples preparation is essential.
Methods: We assessed the performance of the Alpha Tec NAC-PAC™ Digestion and Decontamination System in a high throughput laboratory in Johannesburg, South Africa, in 100 pulmonary samples. The NAC-PAC™ method was compared to the currently implemented BD MycoPrep™ Kit.
The pH of the decontaminated specimen should be less than 8.1 immediately after buffering and maintained between 6.8–7.1 for culturing and diagnosis. At these pH levels optimum survival of the Mycobacterial organisms can be ensured. The Alpha Tec NAC-PAC™ system is the only commercially available system that can effectively control pH and help reduce Mycobacteria die-off during the specimen preparation process. Samples were split and processed using BD's MycoPrep™ system and Alpha Tec's NAC-PAC™ system. Both methods were performed as per the manufacturers' procedure and assessed in the BACTEC™ System.
Results: Of the 100 samples processed using the Alpha Tec NAC-PAC™ kit contamination levels decreased significantly: 90 samples were positive for TB, 8 were MOTTs and a 2% contamination rate was noted. The same 100 samples processed using the BD MycoPrep™ kit showed a 7% contamination rate, 90 samples were positive for Mycobacteria infection, of which 8% had to be re-cultured.
Conclusion: The advantage of Alpha Tec NAC-PAC™ vs. BD MycoPrep™: contamination rate decreased from 7% to 2%; Recovery time improved by 3–5 days, decreasing TAT for results. NAC-PAC™ NALC-NaOH reagent is stable for up to 72 hours after preparation, whereas MycoPrep™ needs to be used within 24 hours of reagent reconstitution. Pellet Resuspension Buffer allowed for tight, standardized, reproducible pH which increased specimen uniformity for growth detection, molecular procedures and conventional culture techniques.

R2346 Characterization of multidrug-resistant strains of *M. tuberculosis* in Bulgaria

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Background: The rate of MDR TB in Bulgaria continue to be high (WHO, 2009). Here we report about results of a first study on the MDR TB strains from the clinical isolates in 2008 in the country.
Methods: The study panel included 26 MDR TB strains isolated from new and retreated patients from all over the country. MGIT 960® first and second line drugs and Geno Type MTBDR plus® were used to strain characterization.
Results: A collection of 26 strains shoved a phenotypical resistance at least to INH and RMP by MGIT 960®. MDR TB strains were confirmed as: 9 resistant to STR, INH, RMP and EMB; 9 to STR, INH and RMP; 3 to INH, RMP and EMB; 5 to INH and RMP. All the MDRTB strains were retested by Geno Type MTBDR plus® and were INH and RMP resistant. Second line drugs testing shoved only one strain resistant to OFL. All others 25 MDRTB strains were fully susceptible to OFL, AMK, KNM, CAP.
Conclusion: Using the classical methods was confirmed that no XDR TB cases was found in last year in Bulgaria. To complete the characterization of Multi-Drug resistant strains of *M. tuberculosis* MIRU-VNTR analysis are performing.

R2347 Identification and major antituberculous drug sensitivities of 2,301 *Mycobacterium tuberculosis* strains by TK automated system

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Objective: A tuberculosis (TB) laboratory for diagnosis, drug resistance tests and treatment follow are necessary to achieve the goals that WHO defines. In developing countries diagnosis of TB which is an air-borne infection; rapid, easily performed, inexpensive tests are important. We evaluated major antituberculous drug sensitivities of 2301 *Mycobacterium tuberculosis* (MTB) strains by TK automated system.
Methods: *Mycobacterium* strains isolated from sputum between August 2006 and September 2009 were studied identification and antibiotic susceptibilities by TK automated system (Salubris, Inc. MA, USA) in class 2 biosafety cabins. One susceptibility test was performed on recurrent positive culture for each patient except treatment follow culture positive cases.
Results: 62012 sputum samples were examined in a three year period. AFB positivity was 13.3% and culture positivity was 11.3%. Smear negative culture positive cases were 1146, smear positive culture negative cases were 2218. Strains susceptible to all 4 drugs were found 1833 (79.6%). Resistance to one drug were found isoniazid (INH) 5.9%,

rifampicin (RIF) 1.7%, streptomycin (SM) 3.2% and ethambutol (EMB) 2.1%. Multidrug resistance (MDR) rate was 6%.

Conclusion: Around the same time TB laboratory was instituted, direct observation therapy (DOT) was started on all the TB dispenceries in Istanbul. The data to evaluate the positive effects of DOT was not ready yet. Istanbul is a metropolis which has majority of the TB cases. Rapid diagnosis, treatment follow and drug resistance tests has an important role in controlling the disease.

	Sample	AFB(+)	Culture(+)	MTB	INH %	RIF %	SM %	EMB %	MDR %
2006	5171	672	460	131	3	1.5	9.1	0	5.3
2007	17701	2585	1391	898	6.6	2.2	2.5	7	7.1
2008	20633	2715	1108	661	7.8	1.5	1	1.3	6.2
2009	18507	2291	1019	611	6.2	1.6	0.4	0.4	5.5
Total	62012	8263	3978	2301	5.9	1.7	3.2	2.1	6.0

AFB: Acid fast bacilli.

R2348 Synergistic activity of three antituberculous drug combinations against clinical isolates of *Mycobacterium tuberculosis* resistant to different drugs

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Objectives: To determine the synergistic activity of 3 drug combinations: isoniazid (H) plus rifampicin (R) plus ethambutol (E), ofloxacin (O) plus R plus E and O plus R against *M. tuberculosis* clinical isolates resistant to different drugs compared with drug susceptible isolates.

Methods: Clinical isolates were collected in the Hospital Clinic of Barcelona: HRE combination: 11 H-resistant isolates; OER combination: 12 H-resistant and 3 multiresistant isolates (MDR); OR combination: 21 H-resistant isolates; and the 3 combinations: 11 drug susceptible isolates. The individual MICs of the isolates studied were evaluated with the proportional method in 7H11 solid medium. The above combinations were studied crossing 7 concentrations of each antibiotic (corresponding to their MIC, two greater and four lesser dilutions), with a 2-dimensional chequerboard assay and an adaptation to a 3-antibiotic chequerboard assay in 7H11 medium. After analysing the results of all the combinations, the fractional inhibitory concentration (FIC) was calculated. Two-dimensional chequerboard assay: the FIC was calculated as FIC index=MICA in combination/MICA alone+MICB in combination/MICB alone where A and B were the two respective antimicrobial agents tested. The FIC index was interpreted as FIC index$\leqslant$0.5, synergistic activity, FIC $>$ 0.5–4, indifference and FIC $>$ 4 antagonistic activity. Three-dimensional chequerboard assay: was calculated as above adding one more drug, C. In this case, the FIC index was interpreted as FIC$\leqslant$0.75, synergistic activity. As a control a 1/100 inoculum was seeded in antibiotic-free medium. All the plates were incubated at 37ºC, being read at the end of 3 and 4 weeks.

Results: HRE Combination: Most H and R MICs of the H-resistant isolates decreased up to 3 dilutions compared to their individual MIC displaying synergism of all the H-resistant isolates. OR Combination: No strain showed synergism or antagonism in either the drug resistant or the susceptible isolates. OER Combination: Most O, E and R MICs of H-resistant and MDR isolates decreased up to 2 or 3 dilutions compared to their individual MIC. Therefore, most of these isolates showed synergism and susceptible isolates.

Conclusions: The isolates studied did not show antagonism in either the HRE, OR or OER combination. Accordingly, the HRE regimen may be effective against low-grade H-resistant isolates (MIC=0.8mcg/ml). The OER combination is more effective in susceptible isolates than the HRE combination.

R2349 Evaluation of Chromogenic in situ hybridization method as a new tool for *Mycobacterium tuberculosis* detection in samples from tissue embedded in paraffin

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Objective: To establish the utility of Chromogenic In Situ Hybridization (CISH) methodology for *M. tuberculosis* detection in samples from tissue embedded in paraffin (TEP).

Methods: As control positive we used a biopsy of lymphatic nodule embedded in paraffin previously obtained in Pathology laboratory from a patient with tuberculosis (TB) diagnosis and negative control was skin biopsy from a patient with Leprosy. Initially, we confirmed the TB diagnosis trough both histopathology (Hematoxilin-Eosin stain and Ziehl Neelsen stain) and molecular methods (PCR IS6110). In order to obtain an adequate material for the last method we started for evaluate different sizes (5, 10 and 15μm) in the TEP cuts, and methods of DNA extraction: CHELEX, CHELEX-Triton, Inorganic solvents and Quiagen columns were evaluated. Standardizing conditions for CISH included evaluation the follow variables: enzyme digestion (Pepsin or Proteinase K), formamide concentration, the detection system (Peroxidase or Alkaline Phosphatase), chromogen (Diaminobenzidine or Texas red), microwave treatment on samples and for probes four different probes Biotin labeled and based in IS6110 sequence were evaluated. Final evaluation was realized by visual examination at light microscope.

Results: The tissue cuts from 5μm showed the best results and the best method for DNA extraction from TEP and for use in PCR was CHELEX. The CISH conditions standardized in our laboratory included: Pepsin digestion, Formamide 50%, Peroxidase, Diaminobenzidine and microwave treatment as easy and useful option to improve the hybridization. Regarding probes, all probes showed positive results for CISH, however the best results regarding bacilli number detected were obtained with Probe 4(see figure 1).

Conclusions: We selected CHELEX as simple and economic tool for DNA extraction from TEP. The method CISH was standardized for *M. tuberculosis* detection in TEP.

We established for first time the potential utility from CISH method using a fragment of IS6110 for *M. tuberculosis* detection in TEP. Other assays with higher number of samples and bacilli number known are necessaries in order to establish the CISH impact as new tool for tuberculosis diagnosis.

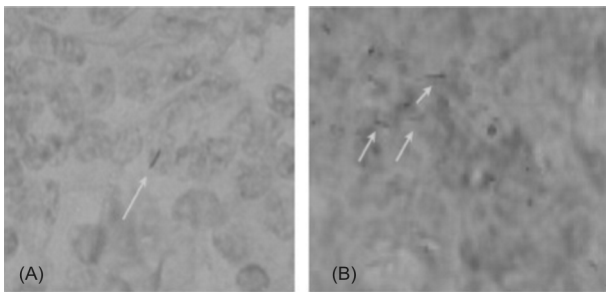

Figure 1. CISH results. (A) Probe 1; (B) Probe 4.

R2350 Utility of molecular biology in the diagnosis of mycobacterial infections

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Objectives: The emergence of tubercolosis/HIV co-infection and the increase in the number of cases of infection with nontuberculous mycobacteria (NTM) require rapid laboratory test results in the isolation and identification of mycobacteria. The objective of this study was to evaluate the identification of mycobacteria by Real-Time PCR and Microarray in comparison with that obtained using classical biochemical methods.

Methods: Between 2007 and 2009, 195 clinical specimens were analyzed using Ziehl–Neelsen staining, culture by Migit (Becton Dickinson, Italy), Real-Time PCR for *M. tuberculosis* (Quiagen SpA, Italy) and microarray method (LCD-Array Myco Direct 1.7 Chipron, Germany).
Results: Of the 195 patients, 30 (15.4%) were positive for *Mycobacterium tuberculosis* complex by Real-Time PCR and 28 (14.4%) were positive for NTM by microarray.
M. tuberculosis complex was found in 20 (66.67%) pulmonary specimens and in 10 (33.33%) nonpulmonary specimens (liquor, urine, stool and others).
The microarray method identified: 9 (32.14%) *M. avium* complex, 5 (17.86%) *M. xenopi* and *M. chelonae,* 3 (10.72%) *M. kansasii,* 2 (7.14%) *M. gordonae* and *M. phlei,* 1 (3.57%) *M. genavense* and *M. marinum.*
When comparing the methods, the sensitivity of the Ziehl–Neelsen staining, culture and Real-Time PCR were 16.66%, 83.33% and 100% respectively.
The sensitivity of Ziehl–Neelsen staining, culture and microarray was 10.71%, 82.14% and 100% respectively.
Conclusions: Despite the cost, the identification of mycobacteria using the molecular technique is faster: maximum 6 h vs. 28–30 days for classical methods.
These methods have high specificity and sensitivity, this justifies its implementation and routine use in referral laboratories, since it facilitates the diagnosis providing opportune treatment. Current recommendations advice that, in general, the use of molecular tests for the diagnosis of tuberculosis should always be interpreted together with patient clinical information.

Infection in the immunocompromised host and transplant recipients

R2351 Galactomannan detection in bronchoalveolar lavage fluid and serum in critically ill adult liver transplant recipients on mechanical ventilation at risk for invasive aspergillosis

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Objectives: To prospectively assess and compare the galactomannan (GM) diagnostic performance on bronchoalveolar lavage fluid (BALF) and serum samples in critically ill liver transplant recipients (LTR) at risk for invasive aspergillosis (IA). GM performance in BALF remains poorly defined as a diagnostic adjunct in LTR at risk for IA.
Methods: Conventional microbiologic methods, tissue biopsies and necropsies, with the assessment of risk factors, signs, symptoms and radiologic imaging were used for the diagnosis of IA as defined by the Pauw et al (Clin Infect Dis 2008; 46:1813). GM detection (cutoff above 0.500) in BALF and serum samples was performed at the discretion of the Intensive Care Unit clinical team. Patients were stratified in 3 groups (high, intermediate and low risk) as proposed by Hellinger et al (Liver Transpl 2005; 11:656).
Results: There were 5 and 7 patients in the low and high risk group respectively. 4 patients were colonized on respiratory samples with *Aspergillus* (1 *A. terreus,* 1 *A. fumigatus*, and 2 *A. niger*). A total of 36 GM serum and 12 BALF samples were performed for 12 LTR patients on mechanical ventilation at risk for IA. There was 1 proven disseminated IA. The sensitivity (S), specificity (SP), positive and negative predictive values (PPV and NPV) for GM on BALF were 100, 90.90, 50 and 100% respectively and in serum samples were 100, 100, 100 and 100% respectively.
Conclusions: In this small LTR cohort there was one false positive GM assay on BALF in a patient colonized with *A. niger*. GM detection appears to be a good diagnostic adjunct for IA on LTR with a suggestive clinical syndrome and high probability of IA. Further investigations including a larger number of patients are needed to establish the usefulness of the GM assay in LTR on mechanical ventilation at risk for IA.

Acknowledgments: This investigation was supported by grants Fondo de Investigacion Sanitaria, Instituto de Salud Carlos III, Proyecto Investigacion PI 070134 (to MSC), PI 070107 (to A d P) and PI 070376 (to JP), grant IT-26407 of Departamento de Educacion, Universidades e Investigacion del Gobierno Vasco (to JP) and Saiotek from Departamento de Industria, Comercio y Turismo del Gobierno Vasco (to JP) and an Educational grant from Pfizer (to A d P) and Gilead Spain (to A d P).

R2352 Gram-positive nosocomial infections in patients in a general intensive care unit

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Objectives: To access the incidence, to recognize risk factors and to determine the rates of antimicrobial resistance in nosocomial Gram-positive strains isolated from patients treated in ICU.
Methods: This is a retrospective study of 277 patients who were hospitalized in a seven bed ICU during one year period (July 2008 to July 2009). Data on demographic characteristics, primary diagnosis, comorbidity, number of indwelling devices and current antibiotics were cross-tabulated according to the presence and type of Gram (+) pathogens isolated. The identification and antimicrobial sensitivity of Gram-positive pathogens were performed with MicroScan (Dade Behring) according to CLSI instructions.
Results: Sixty patients (49, 6% of 121 with documented nosocomial infection) with gram (+) isolates were identified. Methicillin-resistant *Staphylococcus epidermidis* (MRSE, n = 33) and methicillin-resistant *Staphylococcus aureus* (MRSA, n = 18) were most commonly isolated, followed by *E. faecalis* (n = 5) and *E. faecium* (n = 4). There were no significant differences between the groups according to demographic characteristics. The following independent risk factors for Gram (+) nosocomial infection were identified. For MRSE: chronic obstructive pulmonary disease comorbidity, previous isolation of *Acinetobacter* sp. and *Pseudomonas* sp. and previous/current treatment with carbapenem, and for enterococcus sp. previous/current treatment with third generation cephalosporins. The number of indwelling devices was not linked with increased risk of coagulase negative staphylococcal infections. All staphylococci strains were sensitive to vancomycin, teicoplanin and linezolid while three strains of enterococci (one *E. faecalis* and two *E. faecium)* were resistant to vancomycin and teicoplanin.
Conclusion: To reduce the emergence and spread of antimicrobial resistant Gram (+) pathogens in ICU, monitoring and optimisation of antimicrobial use should be considered carefully. Identification of associated risk factors for Gram positive nosocomial infections would aid initial antibiotic choice in such patients at risk.

R2353 *Rhodococcus equi* infections among hospitalized HIV-infected patients in a new infectious disease centre in Malaysia: a 2-year analysis

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Objective: *Rhodococcus equi* infection has been regarded as an opportunistic infection in immunocompromised hosts. Although the organism is easily cultivated from specimens, it may be misdiagnosed as a contaminant or commmensal due to its diphteroid appearance. The AIDS epidemic has resulted in an increase in awareness on the part of the microbiology laboratory in identifying cases of *R. equi* infections. A prevalence study of *R. equi* infections in our 3 year-old institution is presented.
Methodology: Clinically significant isolates of *R. equi* that were cultured in the Microbiology Laboratory of Sungai Buloh Hospital, Malaysia between January 2008 until October 2009 were included. The laboratory used the Analytical Profile Index (API) system for the identification of the organism. The case files of the patients were reviewed and discussions with the physician were done to determine the clinical significance of the isolates.
Results: *R. equi* that was deemed clinically significant was isolated from 10 patients over a two-year period (2007–2009). The organism was cultured from blood, sputum and bronchoscopy specimens. All

the isolates were recovered from HIV-infected patients. Pneumonia was the main manifestation with fever and productive cough being the presenting complaints. Two patients developed the infections while being treated for underlying pulmonary tuberculosis while another two patients had underlying histoplasmosis. Chest radiographs revealed consolidation (without lobar predilection) and in half of the cases, cavitations were seen. Pleural effusion was not present in any of the cases. The average CD4 lymphocyte count at the time of presentation was 10 cells/μl. All the patients were treated with a combination of vancomycin plus either imipenem or erythromycin. No mortality was seen.

Conclusion: Our observation underlines the importance to suspect *R. equi* pneumonia in patients with HIV who have low CD4 lymphocytes count (<20 cells//μl) and presence of lung cavities on chest radiograph. The microbiology laboratory plays an important role in the diagnosis of *R. equi* infection as its diagnosis ultimately relies on the isolation of the organism. A prompt identification and notification helps in the management of the patient.

R2354 Clinical experience of daptomycin use on a haematology unit in the United Kingdom

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Background: In 2007, following an outbreak of glycopeptide-resistant *Enterococcus* raffinosus involving 18 patients on a haematology unit, a decision was made to exclude the use of glycopeptides on the unit in an attempt to limit the spread of this organism. Daptomycin (DAP) and taurolidine line locks replaced intravenous teicoplanin and vancomycin line locks in March 2008. Here we report the clinical outcomes of 25 haematology patients with catheter-related bacteraemia (CRB) treated with daptomycin and enrolled in the UK EU-CORE programme between March and August 2008.

Methods: EU-CORE is a retrospective, non-interventional records review evaluating outcomes of patients (pts) treated with DAP in Europe. Data is collected on patient demographics, antibiotic usage, microbiological and clinical outcomes and adverse events from pts treated with DAP between January 2006 and August 2008. Pts from Newcastle with CRB were entered into the database. All pts had received at least one dose of DAP. Outcomes were assessed as cured, improved, failure and non-evaluable.

Results: Data from 25 haematology pts were collected. All pts were included in the safety population. Most pts had significant underlying disease. Clinical outcomes were success, defined as 'cure plus improved' (88%), failure (4%) and non-evaluable/switched therapy (8%). The most frequently isolated pathogens were coagulase-negative staphylococci (CoNS) (18/26) of which 7 were *Staphylococcus epidermidis*. Oxacillin- or penicillin-resistant organisms were isolated from 14 patients and 3 patients were culture negative. Doses of DAP ranged from 4.5 to 8 mg/kg. DAP was frequently used as first-line therapy (76%). 9 patients had received prior antibiotics. Duration of therapy ranged from 3 to 21 days. 3 patients received DAP as out-patient parenteral therapy (OPAT). One patient who had improved on therapy later died from a herpes simplex viral infection.

Conclusions: DAP was administered in both the hospital and out-patient settings. The overall clinical success rate in this population was 88%. After the introduction of DAP, glycopeptide resistant enterococcal (GRE) colonisation declined significantly. Since August 2008, no new GRE have been isolated on the Unit.

R2355 The challenge of respiratory viral infections in onco-haematological patients

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Objectives: Respiratory virus infections in immunocompromised patients with haemoblastosis have been associated with significant morbidity and mortality. While influenza, parainfluenza viruses and respiratory syncytial virus (RSV) are well known for their potential to cause severe pneumonia, information has only recently emerged regarding the significance of the newly discovered viruses such as human coronaviruses NL63, HKU1 (CoV), Bocavirus (HBoV) and human metapneumovirus (HMPV).

Methods: Two cohorts of patients (pts) with different forms of haemoblastosis were studied during the period from 2008–2009. Group 1 (n = 34) had clinically diagnosed infection complications, the second (group 2) (n = 14) had no clinical symptoms of infection. Diagnosis of viral, mycoplasmal and chlamidial infections was based on positive PCR test. Clinical specimens (nasal and throat swabs and blood) were collected in these groups of pts and studied in PCR for detecting genomes of RSV, influenza virus A and B (IVA, IVB), parainfluenza 1, 2, 3, 4 (PIV-1, -2, -3, -4), rhinoviruses (rhV), adenoviruses (Ads), CoV, HBoV, HMPV, *Mycoplasma pneumoniae* (Mp) and *Chlamydophila pneumoniae* (Chp). Herpes viruses – herpes simplex 1,2; human herpes virus 6; cytomegalovirus (CMV) and Epstein–Barr virus (EBV), were detected only in blood by means of PCR.

Results: In group 1 respiratory viral infections (RVI) were diagnosed in 14 (41.2%) pts: IVA – 4 (28.6%), rhV – 4 (28.6%), CoV – 3 (21.4%), PIV-3 – 1 (7.1%), HMPV – 1 (7.1%) cases. Interestingly that herpes viruses (CMV and EBV) in blood were detected in 37.5% cases of etiologically determined episodes of respiratory viral infections. Bacteria (*Escherichia coli*) was isolated in one patient. In this case neither respiratory viruses, nor herpes viruses were detected. In group 2 RVI were diagnosed in 3 (21.4%) cases – 1 CoV, 1 – rhV and in 1 case – IVA and HMPV were detected simultaneously. It is important that in latter case the clinical material was received during the period of intensive anticytostatic treatment and profound neutropenia. So that the clinical diagnostic of infection in the case was very difficult.

Conclusion: Our data suggest that respiratory infections as well as herpes group viruses must be controlled in immunocompromised leukaemia patients. PCR is adequate method for detecting viral infections.

R2356 Differences in resistance pattern of urinary pathogens of hospital inpatients with diabetes mellitus at a 6-year interval

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Objectives: To estimate differences regarding resistance (R) to antimicrobials of urinary tract infection (UTI) pathogens in patients with Diabetes Mellitus (DM) at a 6 year interval.

Methods: Prospective demographic and microbiology data entry of hospital patients with DM documented UTI. The periods of study were 2001–02 (period A) and 2007–08 (period B). Data entry and analysis in IBM compatible PC using SPSS programme; sensitivity as by Kirby-Bauer, statistics by Yates corrected x2.

Results: We studied 58 urine culture specimens from patients (36 F-22 M, mean age±SD: 74.6±14.8 years) hospitalized for UTIs in period A and 148 (99 F/ 49 M, mean age±SD: 72.9±11.6 years) in period B. The most frequent UTI pathogens for periods A and B respectively were: *E. coli* (55.1% vs. 44.6%, P = 0.17), *Klebsiella* spp. (15.5% vs. 15.2%, P = 0.81), *Pseudomonas* spp. (12.1% vs. 13.5%, P = 0.78), *Proteus mirabilis* (6.9% vs. 9.5%, P = 0.56), *Enterobacter* spp. (6.9% vs. 2.1%, P = 0.09), *Enterococcus* spp. (1.7% vs. 14.2%, P = 0.008) and *Acinetobacter* spp. (3.4% vs. 1.4%, P = 0.33). The rates of antimicrobial resistance between periods A and B were: ampicillin 70.2% vs. 72.8% (P = 0.77), 1st /2nd generation cephalosporins 51.4% vs. 47.1% (P = 0.57), cotrimoxazole 36.8% vs. 34.6% (P = 0.83), ciprofloxacin 22.8% vs. 32.9% (P = 0.14), gentamicin 16.8% vs. 21.1% (P = 0.36) and imipenem 2.7% vs. 11.2% (P = 0.03).

Conclusion: The constant variability of UTI pathogens, others rising, others decreasing, the appearance of unexpected ones, and mainly R profile changes deem continuous surveillance and awareness, to ensure the optimal empirical antimicrobial choice based on the most recent data at the given milieu. Designing and implementing guidelines for hospital, but also the community, setting is obviously warranted. Most resistance rate did not vary considerably, but the overall resistance rate is justifiably alarming.

R2357 Bacterial bloodstream infections in neutropenic children with haematologic/oncologic disorders at a tertiary care centre in Saudi Arabia

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Background: The aim of this study is to determine predominant pathogens and their susceptibility patterns among our pediatric neutropenic patients for proper selection of empiric antibiotic therapy.
Methods: Retrospective chart review of pediatric patients with hematologic/oncologic disorders with bacteremia between January 1998 and December 2008. Demographic data, underlying diseases, bacterial isolates, and antibiotic susceptibility were analyzed.
Results: One thousand eight hundred and eighty nine (1889) bacteremia episodes were identified. Gram negative bacteria (GNB) were more frequently isolated causing 954 episodes (51%). Of these, *E. coli* (23%), *P. aeruginosa* (21%), *K. pneumoniae* (18%), *Enterobacter* spp (7.9%), and *S. maltophilia* (7%). Seventy four percent of GNB were susceptible to pipracillin/tazobactam, 66% to ceftazidime, 66% to gentamicin and 41% to pipracillin. Eighty-seven percent of those tested were sensitive to imipinem/meropenem. Gram positive bacteria (GPC) caused 935 episodes (49%). Of these, coagulase negative staphylococcus was the most frequent (30%), followed by *S. aureus* (24%), *S. pneumoniae* (17%), viridans streptococcus (13%), and *Enterococcus* spp (10%). No VRE was isolated.
Conclusion: Our results concur with observations of other studies that GNB is emerging as major cause for bacteremia in children with cancer. This supports not including vancomycin in the initial empiric therapy for febrile neutropenic patients. Further, there is a potential need for better utilization of conjugate pneumococcal vaccine to decrease the incidence of invasive pneumococcal diseases in our patient population.

R2358 Parvovirus B19 infection in patients with colo-rectal cancer

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Objectives: The study was designed to determine the prevalence of parvovirus B19 (B19) infection in patients with colorectal cancer and to investigate the hematologic and immunologic features related to B19 infection.
Subjects and Methods: 39 primary diagnosed colorectal cancer patients and 32 sex and age matched apparently healthy persons were enrolled in this study. Specific B19 IgM and IgG antibodies were assessed by enzyme-linked immunosorbent assay, presence of B19 DNA in serum samples – by nested polymerase chain reaction and viral load – by real time PCR. Clinical and laboratory data including haemoglobin value, number of leukocytes, lymphocyte, monocyte and neutrophile were collected by examination. The CD3, CD4, CD8, CD19, CD38, CD16, CD95 and CD25 subpopulations were determined in the patients by cytofluorimetry. The IL-6 level in patients' serum samples was assessed using quantitative ELISA.
Results: B19 DNA was detected in 14 (35.9%) patients with viral load 382 – 1.5×10^5 copies/ml and 3 (9.4%) controls with viral load 350–475 copies/ml. B19 specific IgM antibodies have been revealed in 2 (5.1%) patients and 2 (9.3%) controls while IgG antibodies in 21 (56.4%) patients and 21 (65.6%) controls persons. The frequency of active B19 infection was significantly higher in patients with colorectal cancer compared with control persons ($p = 0.01176$). The patients had significantly higher frequency of anaemia and significantly lower haemoglobin value compared with control persons ($p = 0.019$ and $p = 0.025$, respectively). The PCR positive patients had also significantly lower number of CD16 cells and significantly higher IL-6 level compared with control persons ($p = 0.010$ and $p = 0.001$, respectively).
Conclusion: The results of this study suggest that patients with colorectal cancer may have a significantly increased risk of B19 reactivation and B19 infection have impact on haematological and immunological parameters in these patients. Screening of these patients with PCR is recommended when infection is suspected.

R2359 The incidence, epidemiology and risk factors of bloodstream infections in febrile neutropenic patients with haematologic malignancies

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Objectives: Bacteremia is considered as the most significant cause of mortality and morbidity in febrile neutropenic patients. The epidemiology and risk factors might differ among institutions and over the time period. The aim of this study is to evaluate the frequency, epidemiology and factors predictive of bacteremia in neutropenic patients in Gazi University Hematology and Hematopoietic Stem Cell Transplantation (HSCT) units.
Methods: Between November 2007 and November 2008, 177 febrile neutropenic episodes of 115 patients with hematological malignancies were included in this study. Cases were defined as patients with bloodstream infection and controls were the patients without bloodstream infections. We evaluated the cases and controls for the risk factors, complications and mortality rates. Microorganisms isolated from blood samples and their susceptibility patterns were also analysed.
Results: The prevalence of bacteremia was 61% and mortality rate was 12.4%. Duration of severe neutropenia (neutrophile count $<100/mm^3$), underlying hematologic malignancy, stem cell transplantation, relapsing or refractory disease, presence of central venous catheter and presence of mucositis were significant predictive factors for bacteremia. Presence of central venous catheter and relapsing or refractory disease were independent risk factors. The incidence of hypotension and intensive care necessity were higher in cases. Candidemia and Gram-negative bacteremia were significantly associated with higher mortality rates.
Gram-positive microorganisms were the most common isolates (76.8%) with the predominance of coagulase negative staphylococci (63.6%) with methicillin resistance rate of 64%. The most frequent Gram-negative pathogen was *E. coli* with extremely high quinolon resistance rate of 82.1% and the resistance rate of 50% to 3–4th-generation cephalosporin.
Conclusion: Monitorization for the epidemiology of bacteremia and prediction of significant factors associated with bacteremia in febrile neutropenic patients are considered to be important for the choice of initial antibiotic therapy.

R2360 Epstein–Barr virus-associated post-transplant lymphoma

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Objectives: As the number of transplant patients is increasing, the prevalence of malignancies after transplantation is getting more and more common. Among post-transplant malignancies, the importance of lymphomas is significant, because of their increasing prevalence and their poor prognosis; most cases are of B-cell origin, and EBV-associated. Our aim was to show an EBV-associated lymphoma in a kidney transplant patient.
Case description and Methods: A 12-year-old boy underwent kidney transplantation because of congenital urologic malformation. After this, the patient received immunosuppressive treatment and urinary chateterization was necessary due to bladder dysfunction. However the graft function was appropriate, urinary tract infection was diagnosed about 4–5 times a year. In June, 2009 the patient was admitted to the Department of Paediatrics, because of sudden grand mal seizure. Brain MRI examination revealed blood-brain injury, and the possibility of multifocal, granulomatous encephalitis or lymphoma has been arisen, this affects white matter and cortex too. PCR for EBV (Artus® EBV LC PCR Kit, Qiagen) was performed from CSF and brain biopsy specimen, and these gave positive results 1464 copies/ml and 49 200 copies/ml, respectively while CMV PCR (Artus® CMV LC PCR Kit, Qiagen) was negative. After this, the EBV viral load was determined once a week. Cytologic examination justified post-transplant lymphoproliferative disease (PTLD). According to these findings, parenteral acyclovir and rituximab treatments were started,

besides these everolimus and mycophenolic acid as immunosuppression was applied. During the therapy, the viral load was decreased and after 6 weeks negative EBV PCR result was obtained. Control brain MRI exam revealed total regression of previously recognised abnormalities. Recently, the patient was treated with valagancyclovir.
Conclusions: Due to the increasing number of immunosuppressed patients, the occurrence of post-transplant lymphoproliferative diseases becomes more frequent and we have to pay attention to the emergence of EBV-associated cases. To achieve correct diagnosis in these cases, besides serology, molecular methods have crucial role in the detection of pathogen and monitoring the effectiveness of antiviral therapy. This case may draw attention to the fact that screening for EBV in transplant patients should be considered.

R2361 Procalcitonin in neutropenic patients with sepsis

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Objectives: Procalcitonin (PCT) concentrations increase in a setting of systemic bacterial infection and may be used as a marker of the severity of sepsis. Studies showed that its levels are elevated also in neutropenic patients with sepsis and can be used as a prognostic factor of febrile neutropenia. The aim of our retrospective study was to find out whether PCT levels are a prognostic factor for multi-organ failure and mortality in neutropenic patients with sepsis.
Methods: 57 adult patients admitted to the intensive care unit with a diagnosis of sepsis participated in this retrospective study. Based on their absolute neutrophil count the patients were divided into two groups of 27 neutropenic patients and 30 non-neutropenic patients. Neutropenia was defined as the absolute neutrophil count below 1×10^9.
Results: The average PCT level in sepsis patients was 15.6 ng/ml (range 0.08–129 ng/ml) for the neutropenic and 14.6 ng/ml (range 0.08–161.1 ng/ml) for the non-neutropenic group. This difference was not significant ($p=0.45$). In the group of neutropenic patients the average PCT concentration was higher for those with multi-organ failure than for those without (21.37 ng/ml vs. 4.67 ng/ml), but the difference was not significant ($p=0.07$). Among neutropenic patients who died the average PCT concentration was 12.9 ng/ml (range 0.29–50.6 ng/ml) and among survivors it was 18.2 ng/ml (range 0.08–129 ng/ml), however the difference was not significant ($p=0.32$).
The group of 11 neutropenic patients with septic shock was further analysed. Among them there was found to be no significant difference in PCT levels between those who died and survivors ($p=0.07$) and between those with and without multi-organ failure ($p=0.11$). When this group was compared to neutropenic patients without septic shock, however, they were found to have significantly higher values in PCT level (26.4 ng/ml (range 0.42–129 ng/ml) vs. 6.3 ng/ml (range 0.1–36.5 ng/ml), $p<0.05$), mortality rate (69.2% vs. 35%, $p<0.05$) and rate of multi-organ failure (84.6% vs. 35%, $p<0.05$).
Conclusion: Our retrospective study confirmed previous findings that PCT levels are increased also in neutropenic patients with sepsis. PCT concentrations in neutropenic and non-neutropenic patients with sepsis are similar. However, our study shows that increased PCT concentrations in neutropenic patients with sepsis can be used as a prognostic factor for septic shock, but not for multi-organ failure and mortality.

R2362 Development of a unified febrile neutropenia policy for adult and paediatric haematology/oncology patients

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Objective: In our paediatric and adult (P&A) haematology/oncology (H/O) unit in Leeds we treat various groups of patients (young adults, older teenagers both H/O) forming the Teenage & Young Adult unit (TYA). We aim to simplify the management of these patients by unifying 3 policies, defining febrile neutropenia and identifying potential antimicrobial agents for use as empirical monotherapy aiming for one FN policy across all 3 groups (P&A&TYA). We collected data relating to Gram-negative bacteraemias aiming to review the feasibility of a suitable agent for monotherapy for all 3 patient groups. Certain patients may need to be managed differently e.g. post allogeneic stem cell transplant (SCT). We will present this data and our lessons learned in achieving this important goal of unification of policies.
Methods: From our computerised microbiology database we identified all Gram-negative bacteraemias in our patient group between February 2008 and May 2009. For each organism we recorded susceptibilities to 7 antimicrobial agents (gentamicin, tobramycin, piperacillin/tazobactam, aztreonam, ceftazidime, meropenem and ciprofloxacin). We recorded the absolute neutrophil count (ANC) at the time of bacteraemia, diagnosis and treatment received. Where the isolate was resistant to piperacillin/tazobactam, we determined the initial antimicrobial therapy prescribed and subsequent changes made including the reason for change.
Results: We identified 192 bacteraemias in adult haematology patients and 54 bacteraemias in paediatric H/O patients. 93 adult patients had a ANC of <0.1 (48%), with 74 having a ANC of >1 (39%). Of these isolates 12 (6%) were resistant to piperacillin/tazobactam. In the paediatric group, 54 bacteraemias were identified. 24 (44%) had a ANC of <0.1, with 19 (35%) having a ANC >1. In adult patients who were post allogeneic stem cell transplant, the incidence of resistance to piperacillin/tazobactam was higher (15%) as compared to other groups of patients.
Conclusion: We have shown that the majority of Gram-negative bacteraemias in P&A&TYA H/O patients occur in either severely neutropenic patients or patients who are not neutropenic. In the majority of patients, single agent piperacillin/tazobactam is a suitable agent as monotherapy. Patients who have undergone allogeneic SCT may need to be risk stratified to receive different antimicrobial therapy. A unified policy is feasible and we will share the valuable lessons learned in achieving this goal.

R2363 Infectious complications in children with acute myelogenous leukaemia in a Mexican hospital: a 5-year analysis

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Objective: To know the epidemiology and microbiology of the infectious complications that the patients with acute myeloid leukemia (AML) during the treatment in Hospital Infantil De México (HIM), and this way to improve the management in these children.
Method: We conducted a retrospective, observational study, and included all consecutive patients with AML that presented to the hospital with the diagnosis fever and neutropenia (FAN) from January 2004 through December 2008 in HIM. We collected information regarding AML treatment phase, type of infections and its severity (septic shock), number of infections in each patient for each infectious event, number of infectious events in each patient, infectious agents isolated, site of isolation and chemotherapy phase, and number of hospitalization days for infectious events. Descriptive statitistical anális were done.
Results: Fifty children suffered 164 infectious events. The most frequent AML type was the M4 in 30%; then M3 and M2 (18%). Average FAN event per patient were 3.28 (n 1–7). We analyzed 164 infectious events, 32.9% (n = 54) were events of FAN without an identifiable focus. Among the infectious complications; septic shock and colitis were the most frequent complications with 21.3% (n = 35). Nosocomial pneumonia and infections related to catheter were 12.8% for both diseases (n = 21); these pathologies were the second most important; severe sepsis and mucositis grade II where the third most frequent complications (8.5% n = 14). A total of 59% (n = 158) of the infectious complications happened in induction and 40.3% (n = 106) occurred in maintenance. Infections were clinically and microbiologically documented in 46% (n = 76) of cases. Coagulase Negative *Staphylococcus* (CNS) represented the major cause of septicemia (25%), whereas *Escherichia coli* were the second pathogen most frequently isolated from blood 13.6%). Gram-negative bacteria accounted for 41.8% of all isolates and Gram-positive accounted for 37.5%. Fungal infections were observed in 15.6%.
Conclusions: Our results indicate that the main infectious complications in AML at HIM were tiphlytis, nosocomial pneumonia, septic shock, and

community acquired pneumonias. Septic shock was more frequent during maintenance; the most common pathogens were *E. coli*, Coagulase Negative *Staphylococcus*, *P. aeruginosa*, *S. aureus*, *S. pneumoniae*.

R2364 Are respiratory infections related to immunological and genetic data in common variable immunodeficiency, IgG subclass deficiency and IgA deficiency?

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Background: Patients with common variable immunodeficiency (CVID), IgG subclass deficiency and IgA deficiency have clinical heterogeneity not always related to immunoglobulin concentrations. Few studies have evaluated the relationship between immunological data and respiratory tract infections. Genetic or immunological markers combined with IgG concentrations may be useful to establish the prognosis and treatment of these patients. We describe clinical, immunological and genetic variables in patients with CVID, IgG subclass deficiency and IgA deficiency and evaluate whether the number of IgM memory B and dendritic cells and TACI mutations justify the clinical heterogeneity.
Methods: Prospective observational study of patients with CVID, IgG subclass and IgA deficiency from 1989–2008 with descriptive analysis of clinical, immunological and genetic variables. Two groups were made according to clinical manifestations: Group 1 with high illness burden; Group 2 with low illness burden. We compared IgG concentrations, IgM memory B and dendritic cell count and TACI mutations in both groups with Fisher statistics.
Results: 20/29 patients were evaluated: 9 CVID, 9 IgG subclassdeficiency, 2 IgA deficiency. Recurrent lung infections (85%) were the most common manifestation. 9 (45%) patients were included in group 1. The rate of B memory IgM cells <15% and dendritic cell count <5 cells/μL were significantly more frequent in group 1. Mutations encoding for TACI were only detected in Group 1. The sensitivity and specificity of at least 1/3 positive tests to detect patients with high illness burden were 100% and 90.9%, respectively. There was no significant correlation between IgG < 500 mg/dL and illness burden.
Conclusions: Immunological and genetic variables allow better characterization of CVID, IgG subclass and IgA deficiency. IgG concentration alone is not sufficient to predict patient outcome.

R2365 Clinical characteristics and B cell immunology in patients with functional or anatomic asplenia

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Objective: Asplenia is an immunodeficiency that predisposes to life-threatening infectious complications, also known as overwhelming post-splenectomy infection (OPSI) with severe sepsis and septic shock as clinical sequelae. The information from recent studies on clinical characteristics of asplenic patients and infectious complications in this cohort are limited. One of the most likely reason for the highly increased incidence of infections by pneumoocci and other encapsulated bacteria relates to the function of the spleen as it is the only secondary lymphoid organ which allows the generation of plasma cells producing antibodies against bacteria bearing a polysaccharide capsule.
Methods: Since beginning of 2009, all asplenic patients treated at the University Medical Center Freiburg including those recently splenectomized have received care by a specialized outpatient clinic and were followed up prospectively. Patient demographics, comorbidity, reason for splenectomy, vaccination status and infectious complications were documented using a structured questionnaire. Pneumococcal antibodies and B cell phenotype were measured.
Results: During the study period, 23 patients were seen in our outpatient clinic. The mean age of asplenic patients was 59 (range 19–88). The most frequent reason for splenectomy was abdominal malignancies (35%), followed by benign tumors (14%), iatrogenic surgical complications (12%), trauma (9%) and lymphoma (9%). 4 patients with previous OPSI were seen. Three of these patients had OPSI due to pneumonia and one due to meningitis. Complete compliance with current vaccine recommendations and patient awareness was overall low. Compared to individuals with intact spleen, asplenic patients had lower marginal zone B cells.
Conclusions: Follow up of this cohort of asplenic patients offers the unique possibility to study clinical and immunologic risk factors for infectious complications prospectively.

Community-acquired infections including CAP, sepsis, STD, . . .

R2366 Rectal *Chlamydia*, an underdiagnosed infection in men who have sex with men

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Objective: To determine the number of rectal *Chlamydia* (CT) screens carried out in men who have sex with men (MSM) attending an urban sexually transmitted infection (STI) clinic between the 1st January 2005 and the 30th of June 2009. Also to determine the prevalence of rectal CT in the screened population, the indication for screening for rectal CT, concurrent STI's and the HIV status of those who tested positive for rectal CT.
Methods: A retrospective analysis of all STI screens on MSM's attending the clinic between 1st of January 2005 and the 30th of June 2009 was carried out. All positive rectal CT samples were identified and the medical notes were reviewed to determine the indication for rectal CT testing, symptoms, HIV status and concurrent STI's. Rectal swabs had been tested for *Chlamydia* using three different assays in the time frame. From January 2005 to June 2005 Abbott LCR was used. From June 2005 to June 2008 Becton Dickenson ProbeTec CT Assay was used with positives confirmed by Roche Cobas Amplicor. From June 08 June 2009 Abbott Real time CT PCR assay was used.
Results: A total of 1991 MSM STI screens were carried out. 310 (15.6%) were tested for rectal CT. Of those tested, 33(10.6%) were positive for rectal CT. The majority, 22/33 (67%) were asymptomatic. Only 2/33 (6%) had concurrent urethral chlamydia infection. However 32/33 (97%) had a concurrent STI. 12/33 (36.3%) were HIV positive. There were no cases of lymphogranuloma venereum.
Conclusion: Our data shows a high rate of rectal CT in the cohort of MSMs screened, the majority of which were asymptomatic. It identifies that routine rectal CT screening has not been carried out on this population, but would now be recommended. We also identified a high rate of rectal CT occurring with concurrent STI's, including HIV.

R2367 Neonatal sepsis in a tertiary care hospital of eastern Nepal: laboratory perspective of five years duration

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Objectives: Present study was undertaken to determine the prevalence of bacterial etiological agents associated with neonatal sepsis and pattern in the antimicrobial susceptibility over the duration of last five years in BP Koirala Institute of Health Sciences (BPKIHS), a tertiary care hospital in eastern Nepal.
Materials and Methods: All the blood culture samples obtained from suspected cases of neonatal sepsis and submitted to microbiology department of BPKIHS for culture and sensitivity over the duration of five years were included. Isolation, identification and antimicrobial susceptibility testing was done by standard microbiological method.
Results: A total of 39542 blood culture specimens were received in microbiology laboratory from the year 2005 to 2009 which included 9090(22.98%) samples from the suspected cases of neonatal sepsis. Fifteen hundred thirty two (16.8%) samples yielded the growth of bacteria which was slightly higher (22.5%) in the year 2005. Rate of positivity in the following years ranged from 14.4% to 17%. Common Gram-positive organism isolated were *Staphylococcus aureus* 38.5%, followed by CoNS 9.6% and *Enterococcus* spp 8.9%.

Whereas *Acinetobacter* spp (13.5%), *Klebsiella pneumoniae* (9.9%), *Enterobacter* spp (9.3%) remained the most common Gram-negative organisms.
During the period of 5 years prevalence of *S. aureus* increased from 28.2% of total culture positivity in 2005 to 53.5% in 2009, similarly Enterococci raised from 2.5% to 13%. Coagulase negative Staphylococci declined from 21.7% to 1.2%.Prevalence of other bacteria remained more or less same during the study period.
Most of the bacteria exhibited resistance to commonly used antimicrobials. Methicillin resistant *S. aureus*(MRSA), Vancomycin intermediate S aureus (VISA), Vancomycin resistant Enterococci (VRE). High level gentamicin resistant enterococci (HLGR) and ESBL producing Gram negative bacilli were found to be emerging specially in later part of the study.
Conclusion: Neonatal blood stream infection is common in our set up. Various bacteria were associated as etiological agents. Predominance of Gram positive cocci was observed.. Resistance to commonly used antimicrobial is an emerging problem. Continuous monitoring and rational use of antimicrobial and strict adherence to infection control practice may prove useful for prevention and spread of antimicrobial resistance.

R2368 Predictive factors for quinolone resistance in *Escherichia coli* strains isolated from men with febrile urinary tract infection

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Background: Most febrile urinary tract infection (FUTI) in men are acute prostatitis. Although quinolones are the indicated therapy, about 20–30% of our community *Escherichia coli* (*E. coli*) strains are quinolone-resistant (QR). The aim of the study was to assess the risk factors for QR in males with FUTI.
Methods: This was an ambispective study (January 2008 to October 2009) in which we collected clinical data from 90 males (mean age 59.7+16.6 years; mean Charlson score 2.7+2.6) with community acquired FUTI due to *E. coli*. Inclusion criteria were age >18 years and clinical symptoms of FUTI (armpit temperature >38ºC, urinary symptoms) and a positive urine culture to *E. coli*. Susceptibilities to pipemidic acid and to ciprofloxacin were tested by disk diffusion techniques. A strain was considered susceptible if the inhibition zone was below CLSI limit and resistant if otherwise. Statistical analysis was performed by the chi-square or Fisher exact test. Variables associated to QR in the univariate analysis were included in a binary logistic regression analysis with QR as the dependent variable. In the logistic models, age and Charlson score were analyzed with the median as the dichotomizing value. Statistical significance was defined as a two-tailed P value of <0.05.
Results: Among *E. coli* isolates, 26.6% were resistant to pipemidic acid and 15.5% resistant to ciprofloxacin. In the univariate analysis compared to the quinolone susceptible strains the following variables were associated with QR: older age (39.4% vs 79.2%; P = 0.01), diabetes mellitus (DM) (12.1% vs 33.3%; P = 0.029), high Charlson score (51.5% vs 83.3%; P = 0.006), past urinary tract infection (UTI) (13.6% vs 66.7%; P < 0.001), urinary catheterization (1.5% vs 16.7%; P = 0.017), urinary tract abnormalities (43.9% vs 70.8%; P = 0.024), recent antibiotic treatment (9.1% vs 45.8%; P < 0.001) urological manipulation (3% vs 20.8%; P = 0.013) and previous hospitalization (3% vs 16.7%; P = 0.041). In the logistic model factors that remained associated to QR were DM (OR 4.78, 95% CI 1.03–22.16, P = 0.045), past UTI (OR 8.15, 95% CI 2.04–32.5, P = 0.003) and recent antibiotic treatment (OR 5.94, 95% CI 1.23–28.76; P = 0.027).
Conclusions: Our study shows that FUTI that occur in males with DM, past history of urinary infection or recent antibiotic treatment have a higher risk to be caused by QR *E. coli* strains and therefore fluorquinolones are probably not the best therapeutic empirical option in these patients.

R2369 Clinical outcome of anal HPV-associated cancer in HIV-infected patients

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Objectives: Anal HPV infection and HPV related cancers are increasing among HIV infected people despite the introduction of HAART. Screening and treatment strategies are still undefined. In this retrospective study we investigated the outcome characteristics of 20 HIV infected patients with anal cancer diagnosed at L Sacco University hospital in the period 1998–2008.
Methods: Clinical, immuno-virological and treatment parameters for HIV infection and anal cancer were collected from clinical records of 20 HIV infected patients admitted in the II Surgical Unit of our hospital. HPV DNA tests for high and low risk HPV genotypes was performed on fixed histological samples.
Results: HIV infection was acquired by sexual route in 70% of the 16 males and 4 females included in this study. At time of anal cancer diagnosis, 80% of the patients were on-HAART and 4 patients were off-HAART according to national guidelines for antiretroviral treatment of HIV infected patients; 13 patients had a previous diagnosis of AIDS. Median CD4 count was 379.7 cells/microL (range 87–863) and median viral load 7421.4 copies/mL (range 50–26000). HPV infection by a combination of high and low risk genotypes was detected on histological samples in 80% of patients. Combination therapy (radio and chemotherapy) was performed in 13 patients, 2 patients received only surgical treatment, 2 patients only radiotherapy and 3 patients only chemotherapy. Median survival after anal cancer diagnosis was 26.9 months (range 6–69) with 13 patients alive after 12 months of follow up and 8 patient disease free after 12 months. Survival rate was slightly higher in patients treated with combination therapy than those treated with a single intervention.
Conclusions: HIV positive patients remains at high risk of HPV anal related cancers also in the HAART era. Survival rate is closely related to the treatment regimen, to the HIV immunovirological parameters ant to the stage of the disease at time of diagnosis. There is a need of anal cancer screening programs for HIV infected people for the early diagnosis and treatment of HPV related anal cancers.

R2370 First report of *Helicobacter cinaedi* infective endocarditis

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Objective: *Helicobacter cinaedi* is a Gram-negative spiral rod, and was initially isolated from homosexual men with proctocolitis. *H. cinaedi* has been reported as a causative agent of enteritis, cellulitis, bacteraemia, arthritis, and meningitis. We experienced a case of infective endocarditis (IE) due to *H. cinaedi* infection, which has not been reported previously.
Case report: A 71-year-old female with end-stage renal disease on haemodialysis, aortic valve stenosis, abdominal aortic aneurysm was transferred from a haemodialysis clinic because of low-grade fever for 5 days and elevated C-reactive-protein (CRP) levels for 10 days. On admission, the patient complained of fatigue, WBC was 9.6×10^9/L, and CRP was 150 mg/L. Transthoracic echocardiogram showed a 8 mm vegetation on the tricuspid valve. Vancomycin was started empirically. On day 12 a Gram-negative spiral rod was recovered from blood cultures collected on the day of admission and day 2. The antibiotic was switched to ceftriaxone 2g once daily and gentamicin 80 mg after every haemodialysis for empirical coverage of *Campylobacter* fetus, a Gram-negative spiral rod known as a cause of IE. The organism grew on a blood agar at microaerophilic condition at 35 degrees Celsius but could not be identified at that time. Despite the administration of the antibiotics, fatigue and the level of CRP did not improve, ESR was elevated from 35 to 54 mm/hr, and the size of vegetation was increased to 17 mm. The isolate was analyzed by polymerase chain reaction (PCR) using 16S rDNA universal bacterial primers and gyrB gene-based *H. cinaedi* specific primers, and was concluded to be *H. cinaedi.* Based on this result, the antibiotic regimen was switched on day 32 to intravenous

ampicillin 2g once daily and gentamicin 80 mg after every haemodialysis. The levels of ESR and CRP 28 days after the commencement of the new regimen were decreased to 12 mm/hr and 7 mg/L, respectively. The size of vegetation was decreased to 3 mm, and eventually disappeared with no obvious embolic events. The new regimen of antibiotics was administered for 42 days and the patient was discharged.

Conclusion: To our knowledge this is the first report of *H. cinaedi* IE. The patient was initially covered for *Campylobacter* fetus, which is known as a Gram-negative spiral rod causing IE. PCR analysis guided the correct diagnosis of *H. cinaedi* IE and the appropriate treatment. Six weeks course of ampicillin and gentamicin was effective against *H. cinaedi* IE.

R2371 Emergence of vancomycin intermediate resistance in community-acquired methicillin-resistant *Staphylococcus aureus*

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Objectives: Community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) is well recognised as an important pathogen that causes predominantly skin and soft-tissue infections and infrequently life-threatening infections, mostly among children. Vancomycin (VA) is the drug of choice for the treatment of these infections. We describe the isolation of a CA-MRSA with intermediate resistance to VA from a 4-year old girl admitted to the hospital with sepsis and severe multifocal infections including pericarditis, pleural effusions, septic arthritis and osteomyelitis. She was initially treated with combination of VA and clindamycin and had a long complicated course.

Methods: Three CA-MRSA strains were isolated from blood, wound and pericardial pus. Bacterial identification and initial susceptibility testing was performed with the VITEK2 automated system. MICs of vancomycin, teicoplanin, erythromycin, clindamycin, daptomycin, linezolid and tigecycline were determined by E-test. All isolates were subjected to PCR in order to identify the presence of Staphylococcal Cassette Chromosome type IV (SCCmecIV), Panton-Valentine leukocidin (PVL) and Van A and B genes.

Results: Susceptibility testing results demonstrated that two isolates had intermediate susceptibility to VA (MIC, 3 mg/L). All three were susceptible to erythromycin, clindamycin (both MIC, $\leqslant$0.25 mg/L), teicoplanin (MIC, 1–1.5 mg/L), daptomycin (MIC, 0.19–0.25 mg/L), linezolid (MIC, 2 mg/L) and tigecycline (MIC, 0.25–0.38 mg/L). According to the updated CLSI and EUCAST interpretive criteria the isolates recovered from blood and wound fluid are considered as VISA. All isolates carried SCCmecIV and PVL genes. No isolate was positive for Van genes.

Conclusions: The emergence of VISA in CA-MRSA is of great concern. These isolates are not easily detected with routine laboratory antimicrobial testing. As outcome of severe staphylococcal infections depends on the rapid and appropriate therapy, investigation for the presence of VISA in cases of CA-MRSA infections is warranted.

R2372 Clinical characteristics and treatment of prostatic abscess in Korea

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Objectives: Prostatic abscess can cause significant morbidity and mortality in elderly patients usually with diabetes mellitus. But little is known concerning the epidemiology of prostatic abscess because of it's low incidence. We evaluated the pathogen, clinical characteristics and treatment associated with prostatic abscess in Korea.

Methods: This descriptive study was based on a retrospective review of clinical records from January, 1985 to October, 2009 at 3 university hospitals in Korea. Diagnosis of prostatic abscess was based on enlarged gland with ring enhancement lesions on computed tomography, hypoechoic area with thick walls on transrectal ultrasound, or pathologic findings.

Results: Were identified 31 patients. Most of patients were elderly (mean age 63$\pm$11.86). Underlying conditions included diabetes mellitus (15/31, 48%), hypertension (12/31, 39%), benign prostatic hypertrophy (11/31, 35%) and stroke (4/31, 13%). The common symptoms were fever (14/31, 45%) and dysuria (14/31, 45%). Pyuria was found in 27 (87%) patients. Serum level of prostate-specific antigen were determined in 26 (84%, mean 10.76 ng/ml$\pm$16.16) patients and were elevated in 14 (54%) of these patients. Initial digital rectal examination were performed in 9 (29%) patients. The prominent finding was tenderness (3, 33%). Causative pathogens (Table1) isolated from blood (8/24), urine (15/31) or prostate aspiration (5/7) were *K. pneumoniae* (8/19, 42%), *E. coli* (5/19, 26%), *P. aeruginosa* (3/19, 16%), *P. mirabilis* (1/19, 5%) and MSSA (2/19, 11%). Fourteen patients had undergone invasive procedures, including transurethral resection of the prostate(12/31, 39%), transrectal needle aspiration (2/31, 6%). All patients were cured.

Conclusions: *K. penumoniae* is the major pathogen of prostatic abscess in Korea. Prostatic abscess should be considered in old patients with unexplained fever and nonspecific urinary symptoms.

Table 1. Bacterial isolates and treatment of 31 patients with prostatic abscess

No.	Urine	Blood	Abscess	Diagnostic tool	Drainage procedure	Antibiotics (days)
1	*K. pneumoniae*	NG	ND	CT	TURP	Ciprofloxacin (28)
2	NG	*E. coli*	ND	CT, TRUS	ND	Ciprofloxacin (54)
3	NG	*K. pneumoniae*	ND	CT	ND	Ciprofloxacin (24)
4	*K. pneumoniae*	*K. pneumoniae*	ND	CT	ND	Ciprofloxacin (23)
5	*E. coli*	NG	*K. oxytoca*	CT	TURP	Ceftriaxone (14)
6	NG	ND	ND	TRUS	TURP	Cefotiam (27)
7	*K. pneumoniae*	*K. pneumoniae*	ND	CT	ND	Ciprofloxacin (26)
8	*P. mirabilis*	*P. mirabilis*	ND	CT	ND	Meropenem (55)
9	*K. pneumoniae*	*K. pneumoniae*	ND	CT	ND	Ciprofloxacin (61)
10	*K. pneumoniae*	*K. pneumoniae*	ND	CT	ND	Ceftriaxone (43)
11	NG	ND	ND	TRUS	ND	Levofloxacin (9)
12	NG	ND	ND	TRUS	ND	Cefotaxime (9)
13	*P. aeruginosa*	NG	ND	TRUS	ND	Levofloxacin (48)
14	*K. pneumoniae*	NG	ND	TRUS	ND	Ciprofloxacin (22)
15	NG	NG	ND	CT	ND	Ciprofloxacin (21)
16	NG	NG	*K. pneumoniae*	CT	TURP	Levofloxacin (23)
17	NG	ND	ND	TRUS	ND	Ampicillin+AG (13)
18	*P. aeruginosa*	ND	ND	TRUS	ND	Cefomenoxime (6)
19	NG	ND	ND	TRUS	ND	Cefuroxime (28)
20	*P. aeruginosa*	ND	ND	CT, TRUS	TRNA, TURP	Ceftazidime+AG (34)
21	NG	ND	*E. coli*, MRSA	TRUS	TRNA	Vancomycin+AG (11)
22	NG	NG	ND	TRUS	ND	Ceftriaxone+AG (9)
23	*P. aeruginosa*	NG	ND	CT	TURP	Levofloxacin (7)
24	NG	NG	ND	CT	ND	Levofloxacin (15)
25	MSSA	MSSA	MSSA	CT	TURP	Levofloxacin (17)
26	*E. coli*	NG	ND	CT	TURP	Ceftriaxone + metronidazole (16)
27	NG	NG	*K. pneumoniae*	CT	TURP	Moxifloxacin (30)
28	NG	NG	ND	CT	ND	Levofloxacin (29)
29	NG	NG	ND	CT	TURP	Ciprofloxacin (58)
30	MSSA	ND	ND	CT	TURP	Levofloxacin (57)

ND: not done. NG: no growth. TRUS: transrectal ultrasound. CT: computed tomography. TRNA: transrectal needle aspiration. TURP: transurethral resection of the prostate. MSSA: methicillin sensitive *S. aureus*. MRSA: methicillin resistant *S. aureus*. AG: Aminoglycoside.

R2373 Brucellar spondylitis: review of 22 cases

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Objectives: Osteoarticular disease is the most common complication of brucellosis, and spondylitis is the most prevalent and important clinical form of osteoarticular involvement in adults with infections due to *Brucella* species. This study was carried out to describe the demographic, clinical, laboratory findings and treatment modalities of patients with brucellar spondylitis.

Methods: The clinical and laboratory characteristics of 22 patients with brucellar spondylitis followed in our clinic between January 2005 and October 2009 were evaluated retrospectively. The diagnosis was established on the basis of standard tube agglutination titre of 1/160 of antibodies for brucellosis and/or positive blood sample cultures. The diagnosis of spondylitis was based on clinical symptoms confirmed by Magnetic Resonance Imaging.

Results: In the study period, 64 patients were diagnosed as brucellosis. Twenty-two patients had spondylitis (%50 male). The mean age was 58.64$\pm$14.42 (17–81). All patients except one were over the age of 40. Nearly half of patients (45%) were over the age of 60. Acute, subacute and chronic forms of infection's rates were 36.4%, 59.1% and 4.5%, respectively. Severe back pain (100%), fever (63.6%), weight loss

(63.6%) were the most common clinical symptoms. Lumbar vertebrae were the most frequently involved regions (54.5%). Thoracolumbar, thoracal and cervical involment were seen in the 4, 4, and 2 patients, respectively. Spinal epidural abscess was found seven cases (31.8%). One patient had a psoas abscess. Standart tube agglutination test was positive ($\geqslant$1/160) in 68.2% of the patients. Total culture positivity was 45.5% (blood 8, abscess specimen 2). Three patients underwent surgical intervention for diagnostic purposes. Other three patients required surgical treatment for vertebral stabilization. Nineteen patients (86.4%) received a combination of gentamycine (3 mg/kg/day for the initial 14 days) plus doxycycline (100 mg bid), and rifampicine (600 mg/day). Two patients received doxycycline plus rifampicine, one patient received doxycycline plus streptomycin. Duration of therapy varied according to clinical response and the presence of epidural and psoas abscess. The shortest duration of treatment was three months.
Conclusion: Brucellosis should be included in the differential diagnosis of back pain especially in the countries such as Turkey where this infection is endemic.

Lyme borreliosis, toxoplasmosis

R2374 Epidemiological and clinical characteristics of influenza A (H1N1) V infection in Isfahan, Iran, July–October 2009

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Objectives: Following the declaration by the World Health Organization (WHO) of human cases of infection with a new influenza A (H1N1) virus, the Iranian Ministry of Health (MoH) launched a system to monitor and report the presence of this new virus on 10 May 2009. Here we report the confirmed cases of this new virus identified in Isfahan, a province in Iran.
Methods: In a laboratory-based reporting system, the Provincial Health Centers were supplied by the MoH with case definition and patient information forms to be disseminated to all health care institutions in their province. Any person who fulfilled the case definition criteria was directed to one of the three designated health facilities that were prepared to receive suspected cases. The nasopharyngeal samples were sent to the National Influenza Reference Laboratory at Tehran school of Public Health in a viral transport medium (virocult, Medical wire & Equipment, UK) and were tested with the real time RT-PCR protocol and reagents supplied by the WHO.
Results: A total of 376 samples were taken from suspected cases between 1 July and 21 October 2009.50 from these samples were positive for influenza A (H1N1). The date of symptoms onset of the first confirmed case of pandemic H1N1 influenza was 13 July 2009. She had traveled to Mecca (Saudi Arabia) and had already become symptomatic while staying there. 58% of the cases were female. The median age was 27 years (range: 10–75 years). The majority of cases (34%) were from 25 to 29 years old. 9 of them (18%) were hospitalized. 58% of the patients had a travel history to other affected areas. Most travel-associated cases had been returning travelers from Saudi Arabia (55.2%), followed by Malaysia (17.24%).12.24% had close-contact with other patients. The Median duration between developing symptoms and referring to health centers was 3 days (range: 0–9 days). Fever was the most common symptom, presented in 95.9% of the cases, followed by cough (85.7%) and myalgia (77.5%). One death was reported.
Conclusion: Influenza A (H1N1) v entered Isfahan through travelers, mainly coming from Saudi Arabia. The Majority of the confirmed cases consisted of young adults as reported from other countries. They mainly manifested clinical symptoms were similar to those reported in other areas. Because of many patients with influenza like symptoms may not visit the physicians, it is possible that the cases have been under diagnosed.

R2375 Clinical manifestations of Lyme borreliosis in Bulgaria – comparison with tick studies

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Objectives: Lyme borreliosis is endemic in Bulgaria. About 1000 cases are officially reported every year giving an incidence of 12/100,000.
Methods: Using ELISA with recombinant *B. burgdorferi* antigens, a total of 1257 patients were diagnosed.
Results: The main part of them (857/68.2%) presented with erythema migrans. The second most common clinical manifestation was neuroborreliosis, detected in 239 (19%) of the patients – radiculoneuritis, cranial neuritis, encephalopathy, meningoradiculoneuritis and myelitis were found in 13.7%, 2.3%, 1.8%, 0.9% and 0.3% of the patients resp. Lyme arthritis was much less common – found in only 101 (8%) of the patients, followed by heart and ocular manifestations, borrelial lymphocytoma and acrodermatitis chronica atrophicans. *Borrelia garinii* is the species most frequently associated with neuroborreliosis. On the contrary, when 202 Bulgarian *Ixodes ricinus* ticks collected from vegetation were examined by PCR for infection with different *Borrelia* species, *Borrelia afzelii* but not *B. garinii* was found to be predominant, found in 17% of the ticks, followed by *B. burgdorferi* sensu stricto (5.4%), *B. garinii* (1.8%), *B. valaisiana* (1.8%), and *B. lusitaniae* (0.9%).
Conclusion: To what extent this discrepancy could be due to a higher pathogenic potential of *B. garinii* or to variations in host susceptibility remains to be determined.

R2376 Evaluation of H1N1 pandemic influenza cases hospitalized in an infectious diseases clinic

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Objective: The H1N1 pandemic influenza virus is a new influenza virus causing illness in people. This virus was first detected in Mexico in April 2009. It is spreading from person-to-person worldwide. In Turkey, the number of cases is increasing day by day and 73 patients died until 16th October.
Methods: In this prospective study, H1N1 pandemic influenza cases hospitalized in our clinic between October and November 2009 were evaluated. During this period, totally 85 patients having severe disease were hospitalized with suspicion of H1N1 pandemic influenza. Of these patients, 40 had positive nasopharyngeal swab specimen for H1N1 virus by PCR test.

Table 1: Risk factors for development of pneumonia in H1N1 pandemic influenza

Risk factors	Patients without pneumonia (n = 26)	Patients with pneumonia (n = 14)	p value
Female gender	21 (80.8%)	9 (64.3%)	0.220
Pregnancy	8 (38.1%)	4 (44.4%)	0.528
Underlying disease	6 (22.9%)	6 (42.9%)	**0.047**
Initiation of oseltamivir in 48 h	13 (50%)	9 (64.3%)	0.386
Hyperglycemia	1 (5.3%)	7 (50%)	**0.005**

Results: The age range of the H1N1 cases were between 17–77 years and 30 (75%) were female. Twelve (40%) patients were pregnant. Underlying disease was present in 12 (30%) patients. Fifteen patients (38%) had history of contact with H1N1 positive people. At the admission, the duration of complaints was between 1–10 days. The most frequent complaints were fever (90%), myalgia (90%), cough (85%), headache (85%) and sore throat (75%). Physical examination revealed fever (85%), oropharingeal hyperemia (60%), shortness of breath (18%) and crepitan rales (28%) on lung auscultation. In laboratory tests, leucopenia (20%), thrombocytopenia (15%), elevation of liver enzymes (18%), and hyperglycemia (24%) were detected. Most of the patients (80%) were treated with oseltamivir and 55% were given the

treatment in the first 48 hours. The only complication of H1N1 infection was pneumonia. Pneumonia was detected in 35% of the patients and 60% were administered antibiotics. Two patients needed mechanical ventilation and one died. The risk factors for development of pneumonia during H1N1 pandemic influenza infection are shown in table 1.
Conclusion: Pneumonia is the most important complication of H1N1 pandemic influenza and the risk is high in the patients with hyperglycemia or an underlying disease.

R2377 Lyme borreliosis in the United Kingdom: trends and travel

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Lyme borreliosis (LB) is not notifiable under general public health legislation in England and Wales. Case ascertainment is based on voluntary reports to the Health Protection Agency's Centre for Infections (CfI), supplemented by direct reporting from the HPA Lyme Borreliosis Unit. Laboratory-confirmed cases of LB in England and Wales have increased year-on-year with 813 cases identified in 2008 (1.52/100,000 total population, including cases known to have been acquired overseas). The age, sex and seasonal distributions of serologically confirmed cases in 2008 were similar to those seen in previous years. Tick bites were reported by 342 (42%) patients and erythema migrans by 268 (35%) patients. Neuroborreliosis was identified in 83 (10%) patients, of whom 43 (43/83) (52%) had a facial palsy. Arthritis was identified as a clinical presentation in 10 (1.2%) patients. No clinical details were available for 350 (43%) patients.
LB occurs only in people who have been bitten by an infected ixodid tick, the vector host. Peak feeding times for tick blood meals are late spring, early summer and autumn, coinciding with peak periods for many countryside leisure activities at home and, increasingly, abroad. Over half of all patients undergo serological testing in the months of July, August and September; representing a likely peak onset of symptoms in the early summer months.
Popular UK holiday destinations such as Exmoor, the New Forest, the Lake District, the Yorkshire moors and the Highlands and Islands of Scotland, are areas where ticks are abundant and from where cases of LB are often reported. Cases are not confined exclusively to these regions, but any area, large or small, which provides suitable environmental conditions for *Ixodes ricinus* and their bird and small mammal hosts, may be a local LB focus.
In recent years, there has been a significant rise in the number of infections acquired overseas (15–20% annually), especially in North America, France, Germany, Austria, Italy, Hungary, Poland, Slovenia, Bulgaria, Slovakia, Croatia, Romania, Scandinavia and the Baltic republics. These are all countries which have been identified in case-reports from England and Wales residents, a significant proportion of whom were on activity holidays such as walking, trekking or mountain biking. Some travel-associated cases occurred in migrants from other European countries who acquired infections prior to moving to the UK or during holidays in their home countries.

Antimicrobial clinical trials

R2378 Efficacy and safety of linezolid compared with vancomycin for treatment of methicillin-resistant *Staphylococcus aureus* complicated skin and skin structure infections in patients from long-term care facilities

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Objective: The long-term care facility (LTCF) patient presents unique challenges for the treatment of complicated skin and skin structure infections (cSSSI). The objective of the present analysis was to examine the efficacy and safety of linezolid (LZD) versus vancomycin (VAN) in LTCF compared with non-LTCF patients with culture-proven methicillin-resistant *Staphylococcus aureus* (MRSA) cSSSI.
Methods: Data from a multicentre, prospective clinical study were evaluated based upon origin of the patients from LTCF compared with non-LTCF. Patients received either LZD 600 mg PO/IV every 12 h or VAN 15 mg/kg every 12 h with doses adjusted for creatinine clearance. Clinical efficacy was evaluated in the modified intent-to-treat (mITT) population, which required a baseline culture-proven diagnosis of MRSA. Safety was assessed from the intent-to-treat (ITT) population.
Results: Nineteen of the 640 mITT patients presented with MRSA cSSSI from LTCF: 10 and 9 randomised to LZD and VAN, respectively. Average age was 75 y compared with 49 y from non-LTCF. Surgical wound infections were the most frequent cSSSI in LTCF whereas abscesses were most frequent in non-LTCF. Duration of treatment was 1.5 d less for LZD compared with VAN (7.8 d vs 9.4 d) in LTCF patients. Clinical and microbiological outcomes at end-of-study (EOS) are shown in the table below. Twenty-five of the 1052 ITT patients presented with MRSA and were evaluated for safety. Two of 16 LZD patients reported treatment-related adverse events (TRAE) of nausea; both cases were mild in severity. No TRAE were reported in the VAN group (n = 9). In non-LTCF patients, 24% reported TRAE, independent of treatment.
Conclusion: The percentage of TRAE was numerically lower among MRSA cSSSI LTCF patients compared with non-LTCF patients. The clinical success rate was also numerically lower among LTCF patients treated with both LZD and VAN; however, the numbers in the LTCF group are small. Further research needs to be completed to confirm these pilot data in this critically important patient population.

Table: Clinical and microbiological success rates at EOS

	LTCF		Non-LTCF	
Treatment	LZD	VAN	LZD	VAN
Clinical success (%)	50[a]	62.5	81.7[b]	74
Microbiological success (%)	50[a]	62.5	74.3[b]	66.2

[a]NS; [b]$P < 0.05$.

Paediatric infections

R2379 Improving clinical quality and patient safety in cystic fibrosis paediatric patients: first multidisciplinary clinical audit at a large district hospital of northwest England

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Cystic fibrosis (CF) is a multisystem disorder with pulmonary disease the leading cause of morbidity and mortality. Royal Blackburn hospital in northwest England has a large paediatric CFunit. Preliminary to proposing an integrated care pathway for management of CF – a multidisciplinary clinical audit was undertaken. Standards were recommendations of cystic fibrosis trust consensus (CFTC) [www.cftrust.org.uk].
Methods: Between Jan-Dec 2008, clinical data and laboratory database on 24 CF patients was audited. Information on demographics, screening, testing protocols, reporting and management of these patients was obtained and compared against guidance from CFTC. The data specific to *Pseudomonas aeruginosa* [PSA], MRSA and *Burkholderia cepacia* complex [Bcc] is presented here.
Results: 24 paediatric patients aged 1–17yrs [male – 37.5% and female 62.5%] were included in this audit. Data on PSA, MRSA and BCC is presented here. 33 – organism were isolated [PSA – 36% (12/33); MRSA – 9% (3/33) and BCC – 6% (2/33)]. Respiratory samples must be screened every 2-months – a compliance of 71% (17/24) was observed. All new isolated of BCC and PSA must be sent to a reference laboratory for genotyping – 100% compliance with BCC and 100% non-compliance with PSA was noted.
Absolute compliance was noted for use of a combination of nebulised colistin and oral ciprofloxacin for 3-weeks for new isolates of PSA. The standard has no firm consensus on screening from non-respiratory sites or eradication of MRSA. Compliance to screening of non-respiratory sites [nose and groin] as per local policy remains variable; however cough swabs and/or sputum are collected on every visit [100% compliance]. IV teicoplanin or vancomycin is used locally for eradication.

62.5% compliance to a turn-around-time of 72-hours was achieved while the average time to reporting of samples was 77.6-hours. Significant non compliance was observed in local standard operating procedures [SOP] for processing of samples from cystic patients [to be presented].
Conclusions: CF is one of UK's most common life-threatening inherited diseases. A multi-disciplinary team initiated integrated care pathway for enhancing clinical quality and patient safety was envisaged. Results from this audit have been used to draw up an action plan. Significant non-compliance were observed with laboratory SOPs; screening of non-respiratory sites for MRSA; referring new PSA isolates for genotyping. Details of result to be presented.

R2380 Human metapneumovirus and human bocavirus infections among children in Russia

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Objective: to determine the role of HMPV and HBoV in pattern respiratory illnesses among children in Russia.
Methods: 2826 children in the age from 1 month to 15 years hospitalized with upper and lower respiratory tract illnesses in children's hospitals of Moscow city were examined for 2004–2009. Virological diagnosis was made with a polymerase chain reaction on specimens obtained from nasopharyngeal washing (primers got from Gen-Bank).
Results: HMPV was identified in 340 of 2826 children (12.0%) and HBoV in 306 of 2189 children (14.0%). Detection frequency of these viruses fluctuated during different years of investigation from 8.3% till 14.2% for HMPV and from 9.6% till 17.0% for HBoV. The presence of viruses in population was registered during all calendar year. The highest detection of these viruses was occurred between March and June, and between September and December.
A majority of children who was positive for HMPV were aged <12 months and for HBoV were aged from 1 to 3 years.
New viruses were detected in patients with both upper and lower respiratory tract illnesses. HMPV was associated predominately with pneumonia (29%), bronchitis (29%), bronchiolitis (16.7%), croup (14%), and, and HBoV – with croup (19%).
Clinical symptoms of HMPV infection such as rhinorrhea, cough, wheezing and fever are similar to those of respiratory syncitial viral infection. The most severe course was observed among children aged up to 6 months. The duration of HMPV infection was 3–20 days.
HBoV infection is clinically similar to typical acute respiratory viral infections, however, in the most cases it resulted in obstructive syndrome evolution and was frequently characterized by dyspepsia. The duration of HBoV infection was 3–16 days.
Conclusions: HMPV and HBoV are the important cause of acute respiratory infections in hospitalized children in Russia. HMPV and HBoV circulate in Moscow (Russia) during all calendar year and have seasonal peaks. Morbidity peak of these viral infections is observed twice a year. These viruses are more frequently detected among children aged up to 3 years. HMPV and HBoV are associated with both upper and lower respiratory tract illnesses, but HMPV is mainly associated with pneumonia and bronchitis, and HBoV – with croup.

R2381 Prevalence of urinary tract infections in neonates

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Objectives: Describe the prevalence of urinary tract infections and distribution of common pathogens in neonates in our hospital.
Methods: Retrospective study of urinary tract infections in neonates from January 2004 to October 2009.
Results: We analyzed all the urine cultures from neonatal units and neonatal emergencies. The total samples were 699, of which 464 (66.40%) from neonates admitted and 235 (33.60%) of out-patients. In-patients 589 (84.30%) samples were negatives with positives 87 (12.40%) and urine contaminated 21 (3%). Samples represented the 385 (55.10%) were obtained by suprapubic puncture urine by catheter 217 (31%), urinary catheterization in patients 10 (1.40%), pediatric bag 12 (1.70%) and spontaneous voiding urine 75 (10.70%). The distribution of UTI by gender, showed that the positive results were more common in males (55.70% vs 44.30%, $p < 0.01$). Considering the age of the patients, we have seen that are more frequent in infants under 15 days (69.8%). The most common organism was *E. coli* (63.20%) followed by *E. faecalis* (10.30%), *E. faecium* (4.60%), *K. pneumoniae* (4.60%), and followed by a smaller percentage for *P. mirabillis* (2.30%), *S. epidermidis* (2.30%), *E. cloacae*, (2.30%). *E. aerogenes* (1.10%), *C. albicans* (1.10%), *M. morganii* (1.10%) *S. agalactiae* (1.10%), ESBL *E. coli* (1.10%) and BLEA *K. pneumoniae* (1.10%). In 90% of patients, treatment was used intravenous ampicillin and cefotaxime. In the 7 patients in whom bacteremia was detected had a history of prematurity and abnormal urinary passages, still causing Gram negative bacilli, *E. coli* being the most frequent, followed by *K. pneumoniae* ($p < 0.01$).
Conclusion: In our hospital, (1) urinary tract infections in infants are more common in males and under 15 days. (2) The predominant organism in the etiology of urinary tract infections in infants remain Gram-negative bacilli, *E. coli* being the most frequent, followed by *Enterococcus*. (3) It is confirmed that prematurity and abnormalities in nephrourological via are risk factors for urinary infection.

R2382 *Bacillus pumilus* bacteraemia in a term neonate

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Objectives: Despite the widespread distribution of *Bacillus* spp (aerobic spore-forming rods) in nature, they rarely cause infection. They can be pathogenic in immunocompromised hosts. We report for the first time a case of bacillus pumilus bacteremia in a term neonate in Greece.
Methods: A female neonate, 38 wks of gestation, weighting 3390 g was born by caesarean section and Apgar Score at $1' - 7$ and at $5' - 8$, was admitted to the NICU, with abdominal distention and gastric residue. At day 2, milk was commenced, which she tolerated well. On the 8th day, she looked unwell, became lethargic, and hypotonic. Her condition deteriorated and required Dopamine and FFP transfusion.
Results: A full septic screen was carried out. Total leukocyte count 20,180/mm (neutrophils – 89%, lymphocytes – 6%, monocytes – 5%), Hb – 12 g/dl, Hct – 36%, PLT – 28,000/mm, CRP – 120 mg/L. Chest radiograph was normal. Blood culture grew a motile Gram positive rod and the identification with API 50 CHB (bioMérieux, France) show bacillus pumilus which was reported as a contaminate. The organism was susceptible to penicilline, vancomycin, trimethoprim/sulfomethoxazole, erythromycin, chloramphenicol, tetracycline, ciprofloxacin, cefalothin, gentamycin, rifambicine with the disc diffussion and Etest method according to the CLSI guidelines. Urine culture was negative. CSF study was negative. Blood culture repeated two days latter continued to grow the same organism. By now, it was apparent that the isolate could not be considered a contaminant. Vancomycin – cefotaxime were started, based on susceptibility pattern. There was clinical and laboratory response to the antibiotics, which she received for a total of 10 days.
Conclusion: Despite the presence of *Bacillus* spp. in air, soil and dust these organisms have rarely been implicated in human disease. Predisposing risk factors include prematurely, mechanical ventilation and indwelling catheters. We describe a rare case of bacillus pumilus bacteremia in a term neonate with no predisposing factors.

R2383 Meningococcal sepsis in children – a 15-year review

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Meningococcal sepsis (meningococcaemia) is a rare disease that affects primarily the paediatric population. Because of its rapid onset and devastating consequences it represents an important paediatric health concern.
Objectives: To assess the epidemiologic features, clinical presentation, bacteriological and therapeutic findings and outcome in children with meningococcal sepsis admitted to the Hospital of Infectious Diseases of Iasi during the last 15 years.

Methods: Retrospective study including all children aged ⩽18 years, with meningococcaemia, hospitalised in our clinic from 1994 through 2008.
Results: We found 311 cases with invasive meningococcal disease treated in last 15 years. Clinically, 61 of patients with age ⩽18 years presented with meningococcaemia (95% from all sepsis cases). Yearly incidence was about 4 cases (range, 0–7) and 61% of patients were from rural communities. Peak incidence (69%) was recorded in the late winter and early spring months. More than half of the cases (77%) occurred in the first three years of life and median age of patients was 2 years. Clinical condition at admission, included after a sudden onset, manifestations such as: fever (⩾38ºC) (57%), petechial rash (100%), hypotension (54%), meningitis (43%), respiratory failure (16.4%), septic arthritis (3.3%). The blood culture was positive in 35% of the patients (16 from 45). Microbiological confirmation was also based on direct microscopic examination after Gram staining (28% positive), culture (23% positive), and detection of soluble antigens in cerebrospinal fluid (42% positive from 14 cases). Serogrouping was available only to 25% of the patients, the most frequent being group B (67%) of 15 patients. The clinical form was severe in 26 cases (43%). Unfavourable outcomes occurred in 22 of 61 patients, all with purpura fulminans. Death appeared at around 17 hours from admission, by endotoxic shock and disseminated intravascular coagulation (mortality rate of 36%). Among survivors, one patient had gangrene. Only 46% of all cases were treated before admission, 89% of them receiving preadmission βlactamins. The treatment was based upon penicillin G (68%). All isolates were sensitive to penicillin. The average duration of antibiotic therapy was 5.9±4.3 days (m±SD). In all cases, we administrated cortisone therapy.
Conclusion: Prompt recognition of the signs and symptoms of the disease and aggressive treatment remain the mainstay of survival in meningococcaemia.

R2384 Antimicrobial susceptibility of invasive *Streptococcus pneumoniae* isolates from children in Athens, 2003–2008

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Objectives: To investigate the resistance patterns of *S. pneumoniae* isolates from patients with invasive pneumococcal disease (IPD), hospitalized at "P.&A. Kyriakou" Children's Hospital during a six-year period (Jan 2003 to Dec 2008).
Methods: Invasive isolates were reviewed using the laboratory archives and the patient charts. Susceptibility to penicillin (PN), cefotaxime (CTX), erythromycin (ER), clindamycin (CL), trimethoprim-sulfamethoxazole (SXT), tetracycline (TE), rifampin (RF), chloramphenicol (C), vancomycin (VAN), ofloxacin (OFL) was tested using the disk diffusion method, following the CLSI guidelines. MICs to PN, amoxicillin (AMX) and CTX were determined using Etest.
Results: During the study period, 126 invasive pneumococcal isolates (IP) were identified (114 blood, 9 CSF, 3 pleural fluid). Yearly distribution of IP from 2003 to 2008 was as follows: 30, 20, 21, 20, 20, 15. Susceptible to all antimicrobials tested were found to be 60% (76/126). Non-susceptible to PN (PNSP) were 18% (23/126). According to the MICs 21/23 (91.3%) were intermediate and 2/23 (8.7%) were fully resistant to PN. Resistance rates to other antimicrobials were significantly higher in the group of PNSP isolates in correlation with PN susceptible isolates ($p < 0.05$). Analytically, among the PNSP isolates, resistance to ER, CL, SXT, and TE was 56.5%, 34.7%, 43.4%, and 17.3%, while among PN susceptible isolates resistance to ER, CL, SXT, and TE was 20.3%, 4.8%, 6.8%, and 2.9%, respectively. Multidrug resistance reached 47.8% of PNSP strains, but only 3.8% of PN susceptible isolates. Various antibiotic resistant phenotypes were observed. Macrolide-resistance was 27% [M-phenotype 62% (21/34), MLSBc 38% (13/34)]. Yearly incidence of PNSP from 2003 to 2008 was as follows: 7%, 20%, 14%, 30%, 10%, 40%. Yearly incidence of ER – resistant isolates was: 3%, 20%, 14%, 15%, 0%, 13%. The most active antimicrobials were AMX, CTX, VAN, RF, C to which no resistance was found.
Conclusions: Antimicrobial resistance of *S. pneumoniae* remains a significant problem in our area. The increase of penicillin and macrolide resistance from 2003 through to 2008 was worrisome. The decrease of resistance observed during 2007 could be related with the introduction of PCV7 into the national immunization program in 2006. Continuous surveillance of antimicrobial resistance is needed to determine the impact of PCV7 and other newer vaccines in pediatric population.

R2385 Common pathogens isolated from wound infections in children in western Greece

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Objectives: Wound infections are common in children. The aim of this study is to record the most frequent microorganisms isolated from wound infections of children who were examined or hospitalized in a paediatric hospital in Western Greece.
Methods: A total of 991 clinical samples were collected from children 0–15 years old, during a two year period (2007–2009). The samples were cultured in the conventional growth materials. Isolates were identified at species level by conventional tests and the antibiotic susceptibility by the disk diffusion method (Kirby-Bauer), according to the guidelines of CLSI.
Results: From the 991 cultures, 553 (55.8%) were positive for microbial etiologic agents. The most commonly isolated microorganisms, according to their frequency were: *S. aureus* 373 strains (67.4%), CNS (coagulase-negative staphylococci) 55 strains (9.9%), *S. pyogenes* 34 strains (6.1%), *P. aeruginosa* 23 strains (4.1%) and E.Coli 20 strains (3.6%). From the 55 CNS, 20 (36.36%) were considered to be contaminations, based on the patient's symptoms and clinical history. From the 373 *S. aureus* strains, 212 strains (56.8%) were methicillin resistant (MRSA), and 122 of them (57.5%) were collected from children hospitalized in the orthopaedic, surgical or paediatric clinic, whereas 90 strains (42.5%) were community-acquired.
Conclusions: *S. aureus* seems to be the most common cause of wound infections in children. It is worth to mention the high percentage of methicillin-resistant strains (MRSA-56.8%), as well as their prevalence in hospitalized patients in comparison to those of the community. Among the Gram(−) microorganisms, there is a prevalence of *Pseudomonas aeruginosa*, which was isolated in 23 cases (4.1%), mainly from ear secretions.

R2386 Clinical aspects of boutonneuse fever in children

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Background: The disease caused by *Rickettsia conorii* is known by various geographically recognized names, including Mediterranean spotted fever, boutonneuse fever, Kenya tick typhus, Indian tick typhus, Israeli spotted fever, and Astrakhan fever. It is a moderately severe vasculotropic rickettsiosis that is often initially associated with an eschar at the site of the tick bite.
Objective: Retrospective analysis of 63 children and 18 adolescents hospitalized with boutonneuse fever in Children Infectious Diseases Clinic of Clinical Infectious Diseases Hospital of Constanta.
Material and Method: Retrospective analysis of boutonneuse fever hospitalized in Children Infectious Diseases Department during a period of 6 years (2003–2008). We evaluate demographic, clinic, serologic and therapeutic data.
Results: During a period of 6 years (January 2003-December 2008) in Children Infectious Diseases Department we followed 81 cases of boutonneuse fever. From the total of cases 61.27% were from urban area, 53.08% were male. The majority of cases were registered in warm season. The eschar (tache noir) was present in 56 patients. Fever had a 6 days mean duration and disappears often in first 3–4 days of etiologic treatment. Maculopapular rash with nodular boutonneuse lesions was detected in 72 cases, 5 having petechial lesions. Only 34 children had leucocytosis, 9 with thrombocytopenia. Serological diagnosis was accomplished in 61 patients. Etiologic treatment was done for 5–7 days with Chloramphenicol in 61 patients, Clarithromycine in 5 cases;

Ciprofloxacine in 6 cases, and Azithromycine in 9 cases. Mean duration of the illness was of 7 days, especially in moderate disease.
Conclusions: From the total of cases with boutonneuse fever childrens represents 13.25%. Boutonneuse fever is a problem of actuality in the urban areas, of our county, especially in warm season. The epidemiological and clinical diagnosis, confirmed by ELISA for *R. conorii* requires beginning of etiologic treatment.

R2387 Prevalence of toxocariasis in paediatric population

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Objective: Toxocariasis is one of the most common zoonotic helminthiasis that is frequent in Turkey. Clinical signs are often non-specific, thus, its diagnosis is made by specific serology, such as ELISA and Western blot (WB). The serological diagnosis remains the main tool for the diagnosis of toxocariasis. The objectives of our study was to determine the frequency and distribution of toxocara seroprevalence in the paediatric population.
Methods: From January to October 2008, a total of 267 sera were collected from children with asymptomatic for toxocariasis (mean age, 8.5±2.3 years, range: 4–16 years).
Eosinophilia counts were performed by using automatic blood cell counter (Kell-dyn 3500).
Toxocara seroprevalence was measured with ELISA IgG kit (CELISA, Cellabs, Australia). In cases where negative or low-positive values obtained by ELISA, the results were confirmed by WB (LDBIO, France).
Results: The sera of 78 people of 267 asymptomatic individuals were found to be positive for *Toxocara* IgG antibody (29.21%). Seropositivity rapidly increased by age reaching 25.65% at the age of 12–16 years. The borderline anti-*Toxocara* IgG ELISA results were positive with WB. A significantly higher percentage (26%, $P = 0.01$) of *Toxocara* seropositivity was found in children with eosinophilia when compared with 13% seropositivity measured in children without eosinophilia. The borderline anti-*Toxocara* IgG ELISA results were positive with WB.
Conclusions: The highest positivity (18.11%) were found mostly under-developed regions and the lowest positivity (11.10%) were found in and around the center of the city. Probably, this reflects the differences in hygienic conditions. The *Toxocara* seroprevalence rapidly increasing with aging within the age limits of this study. This can be explained by the frequent contact of children with contaminated soil. A significantly higher percentage of *Toxocara* seropositivity was found with eosinophilia compared with the asymptomatic children. The western blot technique was very useful in confirming the borderline and negative anti-*Toxocara* IgG values obtained by ELISA method.

Vaccines

R2388 Evolution of serotype distribution of pneumococcal infections among children in the region of Tarragona, Spain, 2002–2008

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Objective: To know the evolution of the distribution of different serotypes causing pneumococcal infections among infants and analyze possible serotype replacement after the introduction of the 7-valent conjugate pneumococcal vaccine (CPV-7).
Methods: Retrospective cohort including all cases of invasive infections which had a serotype of *Streptococcus pneumoniae* identified during 2002–2008 among patients ⩽14 years of age from the region of Tarragona (Catalonia, Spain). Prevalence of infections caused by serotypes included in CPV-7 (types 4, 6B, 9V, 14, 18C, 19F and 23F) was determined for early cases (2002–2005) and contemporary cases (2006–2008).
Results: During the total study period, 72 cases were included (19 early and 53 contemporary cases). The most dominant serotypes were type 1 in 19 cases (26.4%), type 14 in 15 cases (21%), type 19A in 9 cases (12.5%) and type 6A in 4 cases (5.6%). Globally, 32% (23/72) of cases were due to vaccine serotypes, 21 (15/72) due to vaccine-related serotypes and 47.2% (34/72) due to non-vaccine serotypes. Cases caused by serotypes included in CPV-7 were 63.2% in 2002–2005 and 20.8% in 2006–2008 respectively ($p = 0.002$). Infections due to vaccine-related serotypes represented 10.5% and 24.5% in early and contemporary cases, respectively ($p = 0.336$). Infections caused by serotypes 1 (from 10.5% to 32%) and serotype 19A (from 5.3% to 15%) increased between 2002–2005 and 2006–2008 periods, whereas infections caused by serotype 14 (from 36.8% to 15%) and serotype 19F (from 10.5% to 1.9%) decreased during the study period.
Conclusions: In our population, although the potential impact of the CPV in preventing pneumococcal infections is considerable, it still remains a large proportion of cases not covered by the currrent seven-valent vaccine. Some serotypes not included in the vaccine (especially types 1 and 19A) have increased, which point to a certain degree of serotype replacement after the introduction of conjugate vaccine.

R2389 Serotypes distribution and susceptibility to antibiotics of Streptoccus pneumoniae isolated in blood cultures in Madrid, Spain

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Objective: This study was to determine the serotypes distribution of Streptoccus pneumoniae isolated from adult patients with bacteremia for three years.
Methods: 77 samples were isolated from blood cultures in adult patients. Samples were received at the Department of Microbiology (Hospital Universitario de la Princesa, Madrid, Spain) from January 2007 to November 2009. Seventy seven patients were studied of whom 41 males and 36 females and were a age range between 25–98. The microorganism was identified by being susceptibility to optochin and soluble to bilis. Susceptibility to penicillin, cefotaxime, erithromicin and levofloxacin was determined by E-test. Clinical resistance was based on CLSI (M100S13) 2008 breakpoints. Serotype was determined using rapid latex agglutination (Pneumotest Latex, Statens Serum Institut, Denmark) and specific factors of serotyping were performed through Quellung reaction.
Results: Seventy nine strains (89.6%) were included in the 23 valent vaccine (VP23V) and 15 (19.5%) were included in the 7 valent vaccine. Four serotypes were the most frequently found in our strains: 7F (14.3%), 22F (11.7%), 3(10.4%) and 1 (6.5%). The thirteen valent conjugate vaccine confers efficacy against four of these serotypes (7F, 3, 19A and 1). The percentages of no susceptibility to penicillin and cefotaxime and resistant to erithromicin and levofloxain were 2.6%, 3.9%, 19.5% and 0%, respectively. Strains no susceptibility to penicillin or cefotaxime were included in the serotypes 14 and 6B. Thirty three percentage of erithromicin resistant strains were included in the serotype 19 A.
Conclusions: Serotypes most frequent found (7F, 22F, 3, 19A and 1), are included in the VP23 vaccine but no in VC7 vaccine. The thirteen valent conjugate vaccine confers efficacy against four of these serotypes (7F, 3, 19A and 1). The percentage of no susceptibility to β-lactams is low and it is in relation with the serotypes 14 and 6B. The erithromicin resistant is high and it is relation with the serotype 19A.

R2390 *Streptococcus pneumoniae* isolates in paediatric population: evaluation of emerging serotypes in the era of 7-valent pneumococcal conjugate vaccine and their antibiotic susceptibility

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Objectives: To estimate the serotype distribution of pneumococci and their antibiotic susceptibility patterns among paediatric patients.
Methods: Clinical isolates were obtained from 84 children, aged 4 months to 8 years, between March and October 2009. Vaccination with 7-valent pneumococcal conjugate vaccine (PCV7) was considered appropriate if it was done according to the following scheme: a three-

dose series if starting vaccination at ⩽12 months of age; a two-dose series if starting vaccination at 12–24 months of age; and one-dose series if starting vaccination at ⩾24 months of age. *Streptococcus pneumoniae* strains were collected from the following clinical cases: acute otitis media (69.0%), pneumonia (14.3%), sepsis (7.1%), conjunctivitis (2.4%), acute tracheitis (2.4%), acute sinusitis (1.2%), acute tonsillitis (1.2%), bronchiectasis (1.2%). Antimicrobial susceptibility of all isolates was tested by the disk diffusion method in accordance with the guidelines of CLSI, against erythromycin, clindamycin, vancomycin. Penicillin and ceftriaxone MICs were determined by E-test. Pneumococci were serotyped by primer specific PCR.

Results: Of 84 children, 73 (87.0%) were vaccinated with PCV7, 9 (10.7%) were not vaccinated, 2 (2.3%) dropped-out. Of 84 strains, 61 (72.6%) were serotyped and 4 of them (6.5%) belonged to vaccine type (4, 9V, 14, 23F). A total of 10 (16.4%) isolates were serotype 19A, all susceptible to penicillin, while 4 of them were resistant to erythromycin and clindamycin (phenotype C). A total of 7 (11.5%) were resistant to penicillin, erythromycin and clindamycin and belonged to 7 different non-vaccine types. All isolates were susceptible to vancomycin and ceftriaxone.

Conclusions: Pneumococcus is a leading cause of invasive and non-invasive infection among children and its seriousness is the increasing prevalence of drug-resistant strains. As reported in literature although the widespread PCV7 vaccination, an increase in non-vaccine types has been observed also in our study. Moreover the proportion of resistant pneumococci was higher among non-vaccine types. This study could be the a platform for future surveillance.

Internet and electronic resources

R2391 Application of information and communication technologies in the course "Cultivation of viruses, riketsii, chlamids in the laboratory conditions"

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Introduction: The development and distribution of information and communication technologies and Internet expand the opportunities for communication, exchange of information and change the work environmental and life style to everyone. The Blended learning is characterized by integration of different information and communication technologies in a traditional educational context. Regarding the content and organization this integration may be very diverse in different co-relation of the traditional and online educational technologies. The technologies can be used to support the teaching, learning and pedagogical communication.

Objective: The objective of the research is to perform on mosaic organized online educational resources with the relevant structure corresponding to the goals of the course "Cultivation of viruses, riketsii, chlamids in the laboratory conditions" from the post graduated education of masters' and bachelors' medical specialists. In this publication we propose a technology with description of the steps for developing a web-based course with a dynamic content with interactive possibilities. The course also integrates the legal documentation and includes the following topics: general theory, main terms of virus vaccines production and their application in practice, and therapy of the virus infections.

Materials and Methods: Theoretical analysis and synthesis, questionnaire, information and communication technologies.

Results: Full with content resources were created as a decision of some lections and practical lessons. They could be obtained from online resources, adapted and amended as well as tasks on different education activities. The text resources and cases are oriented to self education for finalizing of learning in regard with specification in the students education.

Conclusion: The new information and communication technologies allow the places, time, speed and level of the education to be defined by the student. It gives a different overview of the educational process, leading to faster results with lower expenses, free access to educational materials and clear concept of every participant in the educational process.