Abstracts accepted for publication only

Pathogenesis

R2405 Microbiological characteristics of follow-up blood cultures at seven university-affiliated hospitals in Korea

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Objectives: The aims of this study were to evaluate the patterns of the request of repeated blood culture and analyze the microorganisms detected in follow-up cultures.
Methods: The frequency and intervals of repeated blood cultures during January and February 2010 were analyzed at seven university-affiliated hospitals in Korea. The microbiological culture results were categorized according to the isolated pathogens or skin contaminants during the first and follow-up cultures.
Results: Among 3072 patients who underwent repeated blood culture, 53.5% were requested to undergo two repeated cultures, while 16.3% were requested to undergo more than four repeated cultures. For the 5241 events of repeated blood cultures, intervals of 1, 2, and 3 days were noted between the first and repeat cultures in 23.1%, 21.4%, and 15.0%, of the patients, respectively. Persistent growth of pathogens isolated in the first culture was noted in 2.6% of the repeat cultures, clearance of pathogens in the follow-up culture in 8.5%, new pathogens in the follow-up culture in 5.2%, growth of contaminants in 7.6%, and no growth of microorganisms at any time in 76.1%.
Conclusion: Two or three follow-up cultures were most commonly requested, with the interval between the initial and repeat culture being <3 days in most cases. Among follow-up cultures, 16.3% showed either persistence or clearance of the original pathogen or a new pathogen. Follow-up culture should be considered after receiving the first result, which takes at least 3–4 days.

R2406 Reduction in interleukin-2 serum levels but lack of evidence of the Th1 to Th2 cytokine shift during the course of HIV infection

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Objectives: Infection with human immunodeficiency virus (HIV) results in dysregulation of the cytokine profile. A switch from a T helper 1 (Th1) to a Th2 cytokine has been proposed as an important factor in progression of HIV infection to AIDS. The aim of the present study was to assess the levels of Th1 and Th2 cytokines in treatment naïve and under treatment HIV infected individuals in order to identify the switch from Th1 to Th2 cytokines.
Methods: This study was carried out in 140 HIV infected patients (21 treatment naïve and 119 under treatment) and 35 matched healthy controls. The serum samples were checked with enzyme-linked immunosorbent assay (ELISA) for interleukin (IL)-2, IL-4, IL-10 and interferon (IFN)-gamma.
Results: A total of 140 HIV positive patients with mean age 36.9 ± 9.2 years and 35 matched controls were enrolled in the study. IL-2 level was relatively higher and IL-10, IL-4 and IFN-gamma levels were relatively lower in the treatment naïve group than the under treatment group. Except for IL-2, all of the other cytokines exhibited a negative correlation with the CD4 cell counts and IFN-gamma levels showed the strongest negative correlation.
Conclusion: Our observations did not demonstrate switching of the type 1–2 T helper cells cytokine profile in HIV infected patients and suggested more complex changes in Th1 to Th2 cytokine patterns in HIV infection.

R2407 Last situation of Crimean-Congo haemorrhagic fever in Iran and its public health importance

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Objectives: Crimean-Congo Haemorrhagic Fever (CCHF) is a viral zoonotic disease. The virus is from Nairovirus genus, Bunyaviridae family. Humans are infected by tick bite, handling of infected blood or tissues and nosocomially. The disease is asymptomatic in livestock (only mild fever) but in human mortality rate could be 50%. CCHF has been observed for decades in Iran but since 2000, as a public health importance, has been extensively studied.
Methods: Since 2000, the laboratory of Arboviruses and Viral Haemorrhagic Fevers of the Pasteur Institute of Iran, as the National Reference Laboratory, has performed advanced laboratory diagnosis consisting of specific Elisa for IgM and IgG detection and also gel-based and Real time RT-PCR for detection of a fragment of CCHFV genome in the sera of CCHF probable patients.
Results: From June 2000 to 20 September 2011, 2382 serum samples from CCHF probable patients have been collected from different provinces of Iran and transferred according to safety procedures to the Laboratory of Arboviruses and Viral Haemorrhagic Fevers (National Reference Laboratory). We confirmed (serologically and/or molecularly) the disease of 853 patients, and between confirmed cases we have 122 death cases. Our data showed the disease has been seen in Iran 23 out of 30 provinces in Iran, but we observed the most infected province in Iran is Sistan-Baluchistan, this province is near to Afghanistan and Pakistan (endemic area) and the genome isolates from different part of Iran have close relationship to Matin (Pakistan) strain, and also we found some isolated from central part of Iran which are near to Iraq strain.
Conclusion: By representing a major public health problem, CCHF is in the most important rank of viral hemorrhagic fevers in Iran and it is important to expand control program in all country especially in infected province by public awareness and also information to high risk group. Our phylogenetic study showed that rather than Matin strain we have another strains such as Iraq strain in Iran. As Iran is one of the CCHF infected countries in the world, so our experiences on different aspects on CCHF can be very useful for prevention and public health in this regard in the region and also in the world.

R2408 Pathogenesis of coxsackievirus B3 isolate from clinical and environmental specimen in Swiss albino mice

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Objective: Coxsackievirus B3 causes asymptomatic infection to more severe disease like myocarditis, aseptic meningitis, encephalitis and neonatal sepsis like illness. Environmental surveillance of sewage is a suitable method for detection of enterovirus serotypes circulating in the community. The present study was undertaken to compare the pathogenesis of predominant coxsackievirus serotype isolated from environmental and clinical specimen.
Methods: Coxsackievirus B3 (CB3) isolates in this study was isolated from cerebrospinal fluid and sewage. Fifteen days old Swiss albino

mice were infected with oral route and intracerebral route with 0.2 mL of 107 TCID50/mL of virus of different origin. Sterile PBS was inoculated in age and sex matched control mice. Mice were sacrificed at 5, 10, 15 and 20 days after post inoculation. Isolation of virus in heart, spleen, thymus, pancreas, brain, small intestine, large intestine and blood was done in RD cell line and virus titres was determined by Karber method. Histopathological changes of infected tissues were studied.

Results: Virus was isolated from several organs (heart, pancreas, spleen, thymus, small and large intestine) on different days depending on the route of infection but not their origin. Organs became negative for virus isolation after 20 days with the exception of spleen tissue in intracerebral route and large intestine by oral route. Histopathology showed mild inflammation and necrosis in heart, intestine and pancreas tissue of all infected mice.

Conclusions: It is concluded that the pathogenesis of coxsackie b3 does not depend on their source but depend on the route of infection. These observations suggest the prominence of environmental surveillance of the enterovirus which might be latent in nature until they get a suitable host to infest their pathogenesis.

R2409 Prevalence of PAPI-1 in clinical isolates of *Pesudomonas aeroginosa*

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Objectives: The aims of this study were to evaluate frequency of PAPI-1 in *Pseudomonas aeroginosa* isolated in References laboratory of Ilam, Milad and Emam Khomaini hospital in Iran and to study frequency of extended spectrum β-lactamases (ESBLs) among isolates which were positive and negative for PAP-1.

Methods: Forty-eight clinical isolates of *P. aeroginosa* were obtained during April 2010 to September 2010. The isolates were evaluated for ESBLs by screening and confirming disk diffusion methods and for PAPI-1 by PCR methods.

Results: The results of the current study showed that 31.5% (n = 15) of 48 isolates of *P. aeruginosa* isolates were positive for ESBLs by screening and confirming disk diffusion methods. In this study, of 48 *P. aeruginosa* isolates in all laboratories, 10 isolates were resistant to azteronam and 3rd generation of cephalosporin and produced ESBLs, While in Imam Khomaini hospital 4 and in References laboratory of Ilam 5 isolates were ESBLs positive. Generally, 15 isolates were ESBLs positive that also confirmed by confirming disk diffusion methods. The results of PAPI-1 detection showed 35.4% (n = 17) of isolates were positive for PAPI-1, which 42.1% (n = 8) were found in isolates in milad hospital, 29% (n = 5) of PAPI-1 positive were detected in References laboratory of Ilam and 23.5% (n = 4) in Imam khomaini hospital. Interestingly, all the PAPI-1 were ESBLs positive and no PAPI-1 detected in non-ESBLs *P. aeroginosa*.

Conclusion: This was first study of prevalence of PAPI-1 in clinical isolates of *P. aeroginosa* which showed most of PAPI-1 positive strains had high levels of resistance and produced ESBLs. It is suggested that PAPI-1 maybe has an important role in antibiotic resistance of *P. aeroginosa.*

R2410 Colistin in management of multidrug-resistant *Acinetobacter baumannii* infections

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Objective: In this study, it is aimed to evaluate the outcome of colistin therapy as monotherapy vs. combination therapy in the multidrug-resistant Acinetobacter baumanii infections.

Methods: This case-control study was conducted at a 800-bed training hospital in Istanbul, Turkey, from August 2008 to May 2011. Patients, who received colistin as monotherapy or combinated with one of other antimicrobials <24 hour or received antimicrobial regimens without colistin were excluded from the analysis. Response to colistin treatment was defined as clinical and microbiologic evaluation.

Results: Totally 44 patients, who were treated with colistin as a monotherapy or combinated with one of those antibiotics including carbapenems, rifampicin and tigecycline due to their 44 attacks with *A. baumannii* and fulfilled the study criteria, were included into the study. Of those, 31 patients (70%) were male, mean age was 51.71 ± 18.82 years (14–87), length of stay at hospital prior to *A. baumannii* infection was 19.25 ± 17.51 days (3–95 days) and also 39 patients were supported with mechanic ventilation during 38.58 ± 29.96 days (2–205 days) in intensive care unit (ICU). Comorbid conditions were reported in 15 patients, and also 26 patients had been treated with other antibiotics due to accompanying another infections. There was no significant difference between colistin monotherapy and colistin combinated therapy in treatment of VAP and blood stream infection in terms of clinical and microbiologic response ($p > 0.05$). Microbiologic response was found significantly higher in 31 of 44 patients than clinical response that achieved in 18 of 44 patients in overall response ($p = 0.005$, Table 1). Ten-day mortality rates was found 27% (12/44), 30-day mortality was found 38% (17/44). Mortality rates were found similar in patiens that received colistin within 72-hour of identified *A. baumannii* infection and in patients that received colistin after 72-hour of identification (57% vs. 58%, $p = 0.651$). Mortality rate was significantly higher in patients, who were supported with mechanic ventilation more than 10 days (n: 20, 31%, OR = 9.09; 95% CI 0.940–87.95; $p = 0.023$).

Table. 1 : Clinical response rates of colistin monotherapy and combination therapies with respect to site of infections

Site of infection	Therapy	Clinical response	Microbiologic response
VAP (n:30)	Colistin monotherapy	5/11	8/11
	Colistin+ Rifampicin	5/8	8/8
	Colistin+ Carbapenem	0/3	0/3
	Colistin+ Tigecycline+ Rifampicin	0/2	0/2
	Colistin+ Tigecycline	1/4	2/4
	Colistin+ Carbapenem+ Rifampicin	1/2	1/2
Blood stream infection (n:6)	Colistin monotherapy	1/2	2/2
	Colistin+ Sulbactam-orphaperazone	1/1	1/1
	Colistin+ Rifampicin	1/3	2/3
Nosocomix pneumonia (n:1)	Colistin+ Carbapenem	0/1	0/1
Urinary tract infection (n:1)	Colistin+ Carbapenem+ Rifampicin	0/1	1/1
Surgical site infection (n:2)	Colistin monotherapy	2/2	2/2
Meningitis (n:2)	Colistin+ Carbapenem+ Rifampicin	0/2	2/2
Surgical site infection +VAP (n:1)	Colistin monotherapy	0/1	0/1
Central-line associated blood stream infection (n:1)	Colistin+ Rifampicin	1/1	1/1
Total (n:44)		**18/44**	**31/44**

Conclusion: Colistin should be thought as a monotherapy for treatment of *A. baumannii* infections decreasing the cost and drug burden compared to combination therapy that did not decrease mortality. Patients with prolonged duration of ventilation are likely more to have increased mortality.

R2411 The effect of oxygen on the growth characteristics and virulence of *Clostridium difficile*

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As an obligate anaerobe *Clostridium difficile* cannot tolerate oxygen within the environment. This intolerance may be due to an inability to either prevent damage from reactive oxygen species (ROS) through neutralisation mechanisms e.g. catalase or efficiently repair the damage

which ROS have caused. Whilst the effect of oxidative stress has been investigated in other anaerobic microorganisms, the area has not been well researched in *C. difficile*. Our research to date has investigated oxygen tolerance in *C. difficile* and also how characteristics such as sporulation are influenced by changes in redox potential.

Five *C. difficile* PCR ribotypes (001, 017, 027, 078, 106) and NCTC 11204 were grown overnight in Wilkins-Chalgren anaerobe broth and adjusted to a density of 107 CFU/mL. These cultures were then to inoculate sterile volumes of Wilkins-Chalgren anaerobe broth and incubated in concentrations of between 1% and 15% oxygen at 37°C for 48 hours. Samples were taken at the beginning of the incubation period and periodically throughout the experiment, diluted accordingly and spread onto both Wilkins-Chalgren anaerobe agar and fastidious anaerobe agar supplemented with 0.1% (w/v) sodium taurocholate and 5% (v/v) horse blood. Agar plates were then incubated for 48 hours at 37°C in anaerobic conditions and following incubation, colonies quantified. At each time point, samples were also taken and malachite green staining used to visualise spore formation.

Each of the PCR ribotypes investigated in this study grew in the presence of up to 2% oxygen with no observable detrimental effects. Above concentrations of 2% oxygen however, growth was less consistent across separate observations. Above concentrations of 3% oxygen, growth began to become inhibited and the numbers of vegetative cells present in the samples began to decline. Although growth was inhibited in higher concentrations of oxygen, vegetative cells were found to survive in 15% oxygen for 24 hours. PCR ribotypes 017 and 078 appeared to posses a greater tolerance to oxygen compared with the other PCR ribotypes tested, growing in concentrations of 3% oxygen and surviving for over 24 hours in atmospheric conditions.

Although regarded as a strict anaerobe, *C. difficile* can grow in the presence of small amounts of oxygen. The results here also suggest that oxygen tolerance may vary between different PCR ribotypes and strains and therefore further studies into the mechanisms behind this are clearly warranted.

R2412 Analysis on the diversity of vaginal microbiota in healthy chinese women

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Objectives: PCR-denaturing gradient gel electrophoresis (PCR-DGGE) has been used to analyze the vaginal microbiota of healthy Chinese women in different physiological states. Our research provided a basis for the development of vaginal probiotics adapted to Chinese female population.

Materials: Women were enrolled during routine gynecologic examinations in the Peking University 1st Hospital from October 2009 to January 2010, and included 30 cases of reproductive age, 30 cases of post menopause age, 90 cases for routine prenatal care (first trimester: 30 cases, second trimester: 30 cases, third trimester 30 cases), as well as 30 cases for 6–8 weeks postpartum. Vaginal discharge was collected and total bacterial DNA was extracted.

Methods: Universal bacterial primers were used to amplify the V3 region of 16S rDNA gene. PCR products were analyzed by denatured gradient gel electrophoresis (DGGE).

Results: (i) Vaginal flora of healthy Chinese women in their reproductive age is relatively simple. The most common bacteria include: *Lactobacillus crispatus*, *Lactobacillus iners*, and *Lactobacillus gasseri*. *L. iners* is the predominant vaginal bacteria that cannot be recognized by traditional method. (ii) The vaginal flora of pregnant women includes: *Lactobacillus iners*, *Lactobacillus crispatus*, *Lactobacillus delbrueckii*, *Lactobacillus gasseri* (first trimester); *Lactobacillus iners*, *Lactobacillus crispatus* (second trimester); *Lactobacillus crispatus*, *Lactobacillus iners*, *Streptococcus anginosus* (third trimester). (iii) The vaginal flora of postpartum women is mostly represented by *Lactobacillus iners*, *Prevotella* spp., *Streptococcus anginosus*, *Lactobacillus crispatus*, *Streptococcus agalactiae*, *Streptococcus gallolyticus*, *Lactobacillus gasseri*, *Veillonella* sp., *Anaerococcus lactolyticus*, *Megasphaera* spp. (iv) The vaginal flora of post-menopause women is more complex than in the reproductive age; the most common bacteria include: *Lactobacillus iners*, *Lactobacillus crispatus*, *Escherichia coli*, *Streptococcus agalactiae*, *Streptococcus gallolyticus*, *Veillonella* spp., *Streptococcus intermedius*, *Streptococcus anginosus*, *Prevotella* spp., *Anaerococcus lactolyticus* and *Bacteroides fragilis*.

Conclusions: (i) The most common vaginal bacteria of Chinese healthy women are *Lactobacillus crispatus*, *Lactobacillus iners*, and *Lactobacillus gasseri*. (ii) Dominant vaginal flora of healthy women during pregnancy and reproductive age is similar, whilst the postmenopausal and postpartum flora is more complex. (iii) PCR-DGGE is a useful tool for analyzing the vaginal microbiota.

R2413 Significance of anti-*Chlamydophila pneumoniae* IgA and IgG determination in patients with acute and post-acute ischaemic stroke

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Objectives: Accumulating evidence indicates that recent acute infections and chronic infectious diseases are important triggers or risk factors for ischemic stroke (IS) mediated by atherogenic process in the arteries. *Chlamydophila pneumoniae* (Cp) has been associated with first IS by enhancing atherosclerosis. Serological data however are limited and results often contradictory. We aimed to investigate the seroprevalence of *C. pneumoniae* antibodies in patients with acute IS and its impact on admission and follow-up.

Methods: We evaluated in a retrospective case-control study performed between January 2007 and October 2011 anti-Cp IgA and IgG (BU/mL) serum samples from 78 consecutive patients (mean age, 66.7, range 35–82 year) with first IS at different times (admission, 0–9 hour), 7 and 90 days after, in relation to age, sex, NIHSS score (mean values on admission vs. 90 days after), CT scan and stroke subtype (TOAST).

Results: Anti-Cp IgA have shown statistically significant values in 53.8% (42/78) IS patients vs. 10.5% (9/85) age and sex matched controls ($p < 0.021$), compared to Cp IgG (71.7%, 61/78) vs. 84% (72/85); $p < 0.38$). IgA titres were evaluated according to the following score: low (10–30), intermediate (30–60), elevated (>60), more elevated (>90). Of IS patients, 12 (15.3%) had intermediate IgA titres on admission which became more elevated 90 days after (Group 1; NIHSS, 10 vs. 4.08, $p < 0.022$); 10 (12.2%) had more elevated IgA titres on admission but decreased at 90 days (Group 2; NIHSS, 10.7 vs. 4.4, $p < 0.063$); 6 (7.6%) had intermediate IgA titres on admission but low 90 days after (Group 3; NIHSS, 11 vs. 5.2, $p < 0.091$); 4 (5%) had low/intermediate titres on admission but increased at 90 days (Group 4; NIHSS, 7.3 vs. 1.7, $p < 0.014$); 6 (7.6%) had negative IgA titres on admission that slightly increased at 7 and 90 days (Group 5; NIHSS, 11 vs. 1.2, $p = 0.000053$); 4 (5%) patients had low titres on admission and intermediate at 7 days but was negative 90 days after (Group 6, NIHSS, 12.3 vs. 5.5, $p < 0.274$). All patients showed intermediate Cp IgG titres from admission to 90 days.

Conclusions: Patients from Group 1, 4 and 5 showed a NIHSS score statistically significant after 90 days. Positive or negative IgA values at IS onset which increase and become elevated at 90 days, appear to be associated with clinical and neuroradiological improvement. No significant statistical difference in achieving of good outcome was seen between IgA in relation to age, sex or TOAST.

R2414 Ability of greater omentum (omentum majus) to eradicate Klebsiella biofilm from surgical biomaterials implanted to Wistar female rats – initial evaluation

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Introduction: Metal or silicone implants used to recover bone structure and function after fractures or osteomyelitis often serve Klebsiella as a scaffold for biofilm establishment. Inability of physical removing these biofilm covering implants lead to chronic infections, resistant to treatment with antibiotics. The only solution are alternative methods, such as covering wounds with different types of pedicled or vascularised flaps. The aim of this study was in vitro assessment of Klebsiella strains ability to form biofilm on surgical implants and in vivo (animal model) evaluation of greater omentum influence on the Klebsiella biofilm on this implants.
Materials and Methods: Quantitative cultures, crystal violet staining and modified Richard's technique were used to in vitro assess the ability of 30 clinical Klebsiella strains to form biofilm on silicone tendon endoprostheses, surgical steel screws, polypropylene surgical meshes and vascular prostheses. In vivo assessment covered 100 Wistar female rats. In experimental group, omentum greater was closed around infected biomaterials, whereas in control group, this biomaterials were implanted into subcatenous tissue, periosteum or left in abdomen. Implants were restored in day 2, 5, 9, 14, 30 after implantation. Biofilm was removed from it's surface using mechanical and chemical methods. Rats' lungs, kidneys, livers, pancreas, spleens and greater omentum were uptaken for histopathological assessment.
Resuts: Among investigated *Klebsiella pneumoniae* strains, 53% of them proved to be effective biofilm–formers in vitro, however level of bacterial adhesion was associated with the type of biomaterial. The Klebsiella strain (K66), which was able to very effectively form biofilm structure on all tested biomaterials, was chosen to be used in research in vivo. In experimental group, after 2 days from implantation, number of cfu was reduced 1000-fold. Between 14 and 30 day after experiment beginning, implant was sterile. In control group, in 1–5 day from implantation, number of cfu was 10 times higher than on the begining of experiment.
Conclusions: Obtained results indicate high ability of greater omentum to erradicate Klebsiella biofilm from surgical implants. Histopathological assesments indicated that number of organs pathologies related to bacterial activity (necrosis, ulcers) was lower in experimental group than in control group. Studies were granted by Ministry of Education and Science (grant No N401038138).

R2415 The skin as a key organ by its innate immunity in Lyme disease transmission

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Objectives: The skin is a key organ in the transmission of arthropod borne diseases like Lyme disease. We investigated whether a specific cutaneous innate immunity might explain a part of the organotropism of some *Borrelia burgdorferi* sensu lato strains, and which factors in the skin might influence the outcome of the disease, responsible of multi-systemic disorder.
Methods: Using different clinical isolates (human pathotypes) of *B. burgdorferi* ss inoculated to C3H/HeN mice, we compared the transmission and the dissemination of these *B. burgdorferi* ss strains and measured the induction of antimicrobial peptides (AMPs), chemokines and cytokines in kinetics of skin inflammation.
Results: Different inflammatory profiles were observed in the skin, with induction of AMPs in the course of infection. A strong induction of MCP-1, IL 6 and TNF-alpha at day 7 was observed that corresponds to a peak of intense bacterial multiplication whatever the strain. We also evidenced that the surface lipoprotein OspC, was essential in the inflammation.
Conlusion: The skin is an essential interface in the development of Lyme disease by its skin innate immunity, represented by an induction of AMPs and MCP-1 that might play a role in the dissemination of *B. burgdorferi* ss strains. OspC and BBK-32 are two bacterial lipoproteins with a critical role in the skin pathogenesis.

Biofilm

R2416 Inhibitory effect of coral-associated bacterial extracts on methicillin-resistant and susceptible *Staphylococcus aureus* biofilms

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Objectives: Biofilms are biotic and abiotic surface-associated, sessile bacterial communities entrench themselves in an exopolysaccharide matrix. Biofilm- associated bacteria renders huge tolerance towards antibiotics and host immune defence mechanism, leaving behind their planktonic counterparts. In this study, ethyl acetate extract of three Coral Associated Bacteria (CAB) were assessed for their ability to modify the adhesion properties, such as cell surface hydrophobicity (CSH), and to inhibit synthesis of biofilm in vitro by clinical strains of methicillin resistant (MRSA) and susceptible *Staphylococcus aureus* (MSSA).
Methods: Five clinical strains each from MRSA and MSSA along with two reference strains MRSA ATCC 33591 and *S. aureus* ATCC 11632 were taken for the study and their ability of slime production was evaluated using Congo red agar (CRA). CAB extracts were tested for antibacterial activity prior to antibiofilm activity using agar well diffusion method. Subsequently, the extracts showing no bactericidal action were assayed qualitatively and quantitatively for antibiofilm activity respectively, through CLSM and spectrophotometry at 570 nm against all test strains. The percentage hydrophobicity and reduction in slime production of test strains with and without extracts were assessed by microbial adhesion to hydrocarbon assay (MATH) and CRA plate method respectively.
Results: Out of nine CAB screened, three have shown excellent antibiofilm activity (79–87%) against MRSA and MSSA biofilms. CLSM images also revealed the potential of CAB extracts in effective disruption and reduction of biofilms. MATH assay revealed that the hydrophobicity of MRSA was comparatively high upto 44–62% with that of MSSA showing a maximum of 33%. Two extracts were able to strongly decrease the hydrophobic property of both MRSA and MSSA cell surfaces. Classic black color appearance of biofilm forming test strains were highly reduced and changed to pink on CRA plate incorporated with three extracts individually. This proves that all three CAB extracts have a significant role in reducing the slime production of biofilm forming *S. aureus* isolates.
Conclusion: This study unveils that coral ecosystem remains an untapped resource with surplus biotechnologically potent bacteria in marine environment. Characterization of lead molecules from these extracts may end up with novel bioactive agents which can be targeted to these dreadful biofilm forming pathogens.

R2417 A fatal case of *Trichosporon asahii* endocarditis two and a half years after aortic valve replacement

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Objectives: *Trichosporon* species is a yeast like ubiquitous fungus extensively distributed in nature. Here we present a case of *Trichosporon asahii* endocarditis in a 57-year old woman, occuring two and a half years after aortic valve replacement.
Methods and Results: A 57-year old woman who had undergone aortic valve replacement in the past, presented herself at a peripheral Hospital with high fever, right hemiparesis and aphasia. A brain CT scan revealed thrombotic infarctions at the left temporal lobe and the left cerebellum hemisphere. A yeast like but unidentifiable fungus was isolated in five consecutive blood cultures and so antifungal chemotherapy was added to the antimicrobial treatment (Table I). An intraesophageal U/S performed 2 days after, revealed an aortic pseudoaneurysm of 2.4 cm close to the metallic prosthetic valve as well as a large vegetation measuring 1.5×0.9 cm. Using the Vitek 2 System method we isolated and identified *Trichosporon asahii* in nine consecutive blood culture sets and with the same method we determined

the susceptibility profile of the microorganism who appeared to be sensitive in vitro to Fluconazole, Amphotericin B and Flucytosine with an MIC of 0.5, 2 and 1 μg/mL respectively. Despite intravenous and pos combined antifungal chemotherapy (Table II) the patient showed no signs of improvement and finally expired on March 19, 2011 because of sepsis. It is important to underline that the patient was not considered at any time during her hospitalization able to undergo any surgical operation for her underlying disease.

Table I
Chemotherapy administered at the Peripheral Hospital

Chemotherapeutic agent:	Period of administration:
Gentamicin iv 80mgx3	12/02/2011 to 28/02/2011
Vancomycin iv 1grx2	12/02/2011 to 02/03/2011
Imipenem iv 500mgx3	25/02/2011 to 02/03/2011
Amphotericin B liposome iv 6flx1	15/02/2011 to 02/03/2011

Table II
Chemotherapy administered at Evangelismos Hospital of Athens

Chemotherapeutic agent:	Period of administration:
Amphotericin B liposome iv 6flx 1	02/03/2011 to 11/03/2011 & 14/03/2011 to 19/03/2011
Micafungin iv 100mgx1	04/03/2011 to 06/03/2011
Voriconazole iv 300mgx2	06/03/2011 to 19/03/2011
Meropenem iv 2grx3	12/03/2011 to 19/03/2011
Vancomycin iv 1grx2	12/03/2011 to 19/03/2011
Vancomycin p.os 125mgx4	09/03/2011 to 19/03/2011
Metronidazole iv 500mgx4	14/03/2011 to 19/03/2011

Conclusion: *T. asahii* has already been reported as a cause of endocarditis in patients who have undergone heart valve replacement, therefore it is important to be suspected as a possible pathogen when clinical manifestations of endocarditis are observed. Due to the small number of cases reported so far, our data concerning mortality and epidemiology is quite poor. However, it emerges that *Trichosporon* spp. endocarditis presents much higher mortality rate than that observed in other infective endocardites. It is also acknowledged that chemotherapy alone is unable to control deep mycosis infections and that patient's rescue can only be achieved when antifungal treatment is followed by effective management of the underlying disease. Nevertheless, early detection of the pathogen may improve the prognosis of the patient in terms of improving patient's clinical condition so that further surgical treatment can be offered.

R2418 Evaluating the ability of in vitro biofilm formation of *Escherichia coli* isolated from urine samples and analysis of inhibition of biofilm growth by *Lactobacillus* species

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Objectives: To investigate the ability of biofilm formation of urinary *E. coli* isolates and how they are affected after treating the surfaces with *Lactobacillus plantarum*, *Lactobacillus fermentii* and *Lactobacillus casei* species.
Methods: Eighty-two *E. coli* strains isolated from urine specimens were identified by standard microbiological methods, and investigated for biofilm production. The study consisted of two phases; in the first phase, adhesion capabilities of isolates to polystyrene microwells were evaluated and accepted as biofilm formation capability of bacteria. Crystal violet microtiter plate based biofilm assay described by Christensen and colleagues was used for quantification of biofilm formation. The optical densities (OD) of microwells were measured at 550 nm. Spectrophotometrically using an enzyme linked immunosorbent assay reader. Bacteria were classified as non-biofilm formers, moderate biofilm formers, and strong biofilm formers according to their abilities of biofilm formation. In the second phase, polystyrene microwells were coated with *L. casei*, *L. plantarum* and *L. fermenti* spp. by incubation at 37°C for 48 hours with suspensions of these bacteria, and biofilm formation of urinary *E. coli* isolates were evaluated thereafter. Quantitation of biofilm formation was performed by ultrasonication and colony counting methods.
Results: In the first phase of the study; 11 (13.42%) *E. coli* were detected as strong, 39 (46.34%) *E. coli* as moderate, and 32 (40.24%) *E. coli* as non- biofilm forming strains. In the second phase; microwells coated with *Lactobacillus* spp. were detected as 93% less biofilm formation allowing compared to the plates in the first phase of the study. The decrease in biofilm formation was statistically significant.
Conclusion: Although the results of our study indicated the fact that, coating inorganic surfaces with *Lactobacillus* spp. suspension may reduce *E. coli* colonization significantly, we would like to underline the need for further studies before accepting this as a strategy for the prevention of biofilm formation of urinary devices.

R2419 Decreasing antifungal susceptibility of different phases of biofilms formed by *Candida albicans* and *Candida parapsilosis*

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Objectives: Mature *Candida* biofilm, with its typical 3D structure consisting of cells, hyphae/pseudohyphae and matrix, can strongly and effectively protect cells against antifungal drugs. Biofilms are often resistant to commonly used antifungal agents and are formed on catheters and surfaces of other medical devices thus acting as clinical complication. The aim of this study was to evaluate susceptibility of different phases of biofilms formed by *C. albicans* and *C. parapsilosis*.
Methods: Six strains of *C. albicans* (two strains) and *C. parapsilosis* (four strains) clinical isolates were included and biofilm formation under impact of fluconazole (FLU) and caspofungin was evaluated. Biofilms were formed in 96-well microtiter plates. Antifungals were added to forming biofilm (i) in the beginning, (ii) after 90 minutes (after adherence phase) and (iii) after 24 hours. Effect of added antifungals was evaluated using XTT-reduction assay.
Results: Adding of antifungal in the very beginning of biofilm formation led to its full inhibition with MIC′s maintained mostly on low level. Higher MIC′s both in FLU and CAS arm were consequence of antifungals added to biofilm after adherence phase. When antifungals were added to preformed 24-hrs old biofilm, any inhibition of its forming did not occurred. Increase of MIC′s is shown in Table 1 below.

Table 1: FLU and CAS MIC in different phases of biofilm

Isolate	FLU MIC80 [mg/L]			CAS MIC80 [mg/L]		
	T = 0	T = 90 m	T = 24 h	T = 0	T = 90 m	T = 24 h
C. parapsilosis 3430/1	1	4	≥64	0,125	≥16	≥16
C. parapsilosis 5055/2	0,5	8	≥64	0,03	2	≥16
C. albicans 16755/1	≥64	≥64	≥64	0,03	0,5	≥16
C. parapsilosis 16755/2	4	16	≥64	≥16	≥16	≥16
C. albicans 21922/1	1	1	≥64	0,25	1	≥16
C. parapsilosis 21922/2	≥64	≥64	≥64	≥16	≥16	≥16

Conclusion: Direct adding of FLU or CAS to cells with ability of biofilm formation can lead to full inhibition of its forming. Adding antifungals in later phase of biofilm formation has no inhibiting effect.

R2420 Expression of ALS1, ALS3 and CPAG_05056 genes in dispersal cells and in biofilm formed by *Candida parapsilosis* and *Candida albicans*

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Objectives: Adherence is the first and critical step in process of biofilm formation and is associated with change in gene expression. There are eight ALS genes involved in adherence of *C. albicans* and, previously, some of them were proved to be upregulated during biofilm formation. In *C. parapsilosis*, five genes were described as ALS adhesins. In this

study we aimed to evaluate the expression of one of those gene in *C. parapsilosis* as well as expression of ALS1 and ALS3 in *C. albicans* during different phases of biofilm formation.
Methods: Real-time PCR was used to determine the level of gene expression in three *C. albicans* strains and three *C. parapsilosis* strains. We focused on expression of ALS1 and ALS3 genes in *C. albicans* and CPAG_05056 gene in *C. parapsilosis*, compared to expression of ACT1 gene as houskeeping gene. Primers for ACT1, ALS1 and ALS3 were prepared according to published data. Primers for CPAG_05056 were designed by our team. Expression was monitored in yeast cells, in mature biofilm and in dispersal cells detached from biofilm. Obtained data were evaluated using delta-delta-Ct method.
Results: ALS1 expression was higher in dispersal cells (6–26-times) than in biofilm or yeasts. Expression of ALS3 was considerably higher in one *C. albicans* isolate dispersal cells, but was also increased in biofilm (3–24-times and 34-times respectively). *C. parapsilosis* CPAG_05056 expression was considerably high in dispersal cells and was increased in biofilm cells too in case of two strains. Unfortunately third *C. parapsilosis* strain showed no increase in expression.
Conclusion: In these experiments, we found out that expression of adherence genes is high dispersal cells derived from biofilm and in biofilm of *C. albicans* and *C. parapsilosis*, too. Our results suggest that dispersal cells detached from biofilm are, in some way, adapted to form biofilm in advance. We suppose, that more experiments should be done to evaluate expression of other ALS genes in *C. parapsilosis*.

R2421 Dynamic in vitro of urogenital *E. coli* biofilm by oestradiol influence

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Bacterial biofilms are associated with a large number of persistent and chronic infections like recurrent urinary tract infections (RUTI) in posmenopausic women. The uropathogenic *E. coli* (UPEC) adherence to perineal areas and vaginal mucosa is one of the first steps to initiate RUTI. The patients with this pathology were subjected to different prophylactic antibiotic regimens but not always the improvement occurs. Despite significant advances in the understanding of UPEC biology, it is not clear which factors are involved in the recurrence and how to control the RUTI. In previous works we demonstrated the different estrogen activity against vaginal and urogenital biofilms.
Aim: To study the in vitro influence of Estradiol Hemisuccinate (EH) on the *E. coli* BF dynamic as a model for non antibiotic strategy of RUTI control.
Methods: one-Qualitative assay: we study 30 *E. coli* isolates from urinary tract infections (UTI). The UPEC BF was investigated using a previously described method. Briefly, each UPEC were placed in six tubes with Trypticase Soy Broth (TSB). After overnight incubation at 36°C, one glass coupon (GC) was placed in each tube as an abiotic surface. EH was added to four tubes (200 μg/mL) from the beginning (2) and after 12 hours (2). The other two were used as controls. All glass coupons were stained with crystal violet and readed at 12 and 24 hours after incubation at 36°C, by optical microscope. Two-Quantitative assay: we used microtiter assay (MA) to study the early stage of BF formation and the EH concentrations (200–25 μg/mL) influence. Following 12 and 24 hours of incubation and the remotion of planktonic bacteria, the crystal violet coloration was employed. For reading a RT-2100C microprocessor (absorbance mode with lambda 450 nm) was employed.
Results: EH improves UPEC BF in the early stage: is more thick and homogeneous than the ones without EH, but at 24 hours fragmentation and dispersion was shown in all isolates tested mainly in presence of exuberant exopolisacharide. The EH activity could not be concentration dependent because the different dilutions have not shown influence on BF quantification.
Conclusions: *E. coli* BF detachment and dispersion are factors to consider as possible strategy to control postmenopausal RUTI. The possibility of local estradiol application on women perineal area is important to restrict the BF development, and the selective pressure due to continuous employ of antibiotics in these patients.

R2422 Antimicrobial activity in catheter model *E. coli* biofilm susceptibility to adamant containing compound AM-166

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Purpose: Study of adamant derivative AM-166 potency to disturb mature biofilms developed by *E. coli* clinical isolate, received from a patient with pyoinflammatory disease.
Materials and Methods: Compound's Minimum Inhibitory Concentration (MIC) was found by serial micro-dilutions in Mueller-Hinton Broth (Guidelines MUK 4.2.1890–04). Catheter Dialock Hemodialysis Access system was used to investigate aminoadamantan derivative effect on *E. coli* film forming disturbance. Test microorganisms were incubated in Trypticase Soy Broth (TSB, bioMérieux SA, France), subsequently diluted to 5–60 CFU/0.1 mL in TSB. Of 0.15 mL of the broth with test microorganisms was introduced in the catheter lumina and the catheter was incubated overnight at 37°C. After completion of the incubation, 1.0 mL of AM-166 was introduced with syringe to the catheter. The catheter distal end was then sealed and incubated in flat position for 72 hour at 37°C. After completion of the incubation, the catheter surfaces were treated with alcohol. Clip opened, liquid from the catheter was transferred to a sterile test tube. The catheter was washed with 0.1 mL of TSB and the fluid was transferred to a sterile test tube. From the central part of the catheter, a section (approximately 5 cm) was aseptically cut out and put into a sterile test tube with 3 mL of TSB. After ultrasonication during 1 minute and shaking during 15 second, colour density was assessed by Adsorbance Microplate Reader Elx808 (BioTeK, USA). AM-166 was studied at 1.0 MIC, 2.0 MIC, and 5.0 MIC.
Results: Our experiments have shown, that MIC of AM-166 with respect to *E. coli* is 0.39 μg/mL. Experimental results evidence, that all studied AM-166 concentrations cause damage to mature *E. coli* biofilms. It was established that the compound's efficiency at 5.0 MIC was 94%, at 2.0 MIC – 86%, and at 1.0 MIC – 42% with respect to control.
Conclusion: So, aminoadamantan derivative causes dose-dependent damage to *E. coli* biofilm. In future, the task will be to investigate inhibitory effect of AM-166 with respect to biofilms developed by various microorganisms, specifically by *S. aureus*, *P. aeruginosa*. In the longer term, it is necessary to investigate in order to establish effect of catheter chemical composition on biofilms formation and AM-166 efficiency with various composition catheters.

R2423 Biofilm and virulence factors in Proteus bacteria from catheter-associated urinary tract infections

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Objectives: The number of the catheter associated urinary tract infections (CAUTIs) increases every year. Over 40% of nosocomial infections are infections of the urinary tract, especially infections of catheterised patients. With the inserted catheter, the bacteria can more easily attack urinary tract. For effective combat with CAUTIs, it is necessary to understand virulence factors and possible linkages among their production. In this work we focused on the proof of biofilm formation in various conditions and selected virulence factors in the bacteria of the genus Proteus.
Methods: From the CAUTIs we isolated 102 strains of the genus Proteus. As a control group we included 50 strains of the genus Proteus isolated from stool of healthy people. With isolated strains we performed tests for virulence factors: biofilm formation in Brain Heart Infusion (BHI) and in BHI with addition of 4% of glucose (BHI-g), tests for swarming, swimming and twitching motility, the ability to attach to various types of catheters, the ability to swarm over various types of catheters, the production of urease, and the susceptibility to selected antibiotics.

Results and conclusion: There were differences between the group of strains isolated from CAUTIs and control group in several virulence factors – the biofilm formation in BHI with and without glucose supplementation ($p << 0.001$; $t = 5.105$ and $p << 0.001$; $t = 6.859$, respectively), the twitching motility ($p = 0.012$; $t = 2.548$), and the swarming over one type of catheter – the Argyle Catheter ($p = 0.023$; $t = 2.297$). The ability of the isolates to swarm over particular catheters was different in both tested groups of isolates ($H [6, N = 714] = 233.7864$; $p << 0.01$; $H [6, N = 350] = 81.07407$; $p << 0.01$, respectively). The differences may be caused by different hydrophobicity of the catheters. The surface hydrophobicity varies even in catheters from same material, but from different suppliers. The results of this study show, that genus Proteus is well adapted to the survival in the host body. Identifying and understanding of particular virulence factors is necessary for prevention and treatment of CAUTIs. The choice of the catheter based on the knowledge of its properties, especially as the bacterial adherence and swarming ability are concerned, could lead to decrease of risk of CAUTI in patients, which is an important aim. The research was funded by grants IGA MZ 9678 and MSMT INGO LA10037.

R2424 Evaluation of relationships among virulence factors of *Pseudomonas aeruginosa* strains isolated from catheter-related infections

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Modern health care is associated with increasing ratio of invasive surgery and immunocompromised patiens, which are highly susceptible to nosocomial infections. *Pseudomonas aeruginosa* is common nosocomial pathogen. Due to the high antibiotic resistance these infections are difficult to eradicate. *P. aeruginosa* is equipped with a large arsenal of cell associated and secreted virulence factors. The aim of this study was to evaluate production of selected virulence factors of 80 catheter-related isolates and to find potential relationships among them.

Antibiotic resistance was evaluated by disc diffusion method, assay for detection of beta-lactameses was performed as double-disc synergy test (metallo-beta-lactamase secretion was confirmed by spectrophotometric assay). Formation of submersial biofilm was tested in microtiter plate, whereas air-liquid interface (A–L) biofilm was observed in test tube. Effectiveness of the antibiotic lock technique (with amikacin, ciprofloxacine or ethanol) against selected biofilm-positive strains was tested as well. Different types of motility were detected by plate test with medium solidified with different amount of agar. Elastolytic activity of the strains was tested by elastin-congo red assay. Haemolysins were evaluated by spectrophotometric assay as the amount of released haemoglobin. Siderophores occurence was observed by plate test.

Production of hemolysins, LasB elastase, pyoverdine and biofilm formation (both submesial and A-L) were commonly observed among strains. All strains further showed one or more types of motility. In contrast, secretion of pyocyanine and pyochelin was poor. Colistine and amikacin were the most effective antibiotics. One day biofilm was eradicated by the solution of 30% ethanol with 4% trisodium citrate, amikacin 1 and 1 mg/mL ciprofloxacine. Positive correlation was observed among production of several extracelular virulence factors (LasB elastase and haemolysins, pyochelin and pyocyanine; pyocyanine and LasB elastase) and between submersial and A-L biofilm formation. On the other hand we detected negative correlation between submesial as well as A-L biofilm and LasB elastase production. Interestingly, siderophores sectetion was negatively correlated with submersial biofilm and positively with A-L biofilm. All correlations were significant at $p = 0.05$ and correlation coefficient gamma > 0.50. This work was supported by the Grant Agency IGA MZ CR 9678 and INGLO LA 10037.

R2425 Antibiotic susceptibility patterns of biofilms of vancomycin resistant and susceptible enterococci under conditions of oxygen limitation in vitro

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In-vitro studies of biofilms (BF) of pathogenic microorganisms are typically carried out under standard aerobic conditions, however this does not always reflect the true nature of in-vivo infections & the possible effects of reduced oxygen environments encountered throughout the body. Furthermore, the interior of mature biofilms have been shown to be anaerobic in nature. Previous studies have established that planktonic forms of enterococci can exhibit increased resistance the aminoglycosides under anaerobic conditions, but whether a similar trend exists for BF of the organism has yet to be examined. A study was therefore carried out to establish whether BF of enterococci exhibit altered antibiotic resistance patterns when cultured and exposed under reduced oxygen environments in-vitro. Twelve clinically derived isolates obtained from active infections comprising three each of VRE-faecium, VRE-faecalis, VSE-faecium and VSE-faecalis were cultured as BF using an established 96-well microtitre plate platform under strict aerobic, microaerophilic and anaerobic conditions. The isolates were chosen from a larger pool of 90 organisms due to the fact that they produced BF of similar mass, to minimise any effect this may have on antibiotic response. Forty-eight hour old BF were exposed to eight antibiotic agents commonly used against enterococci for clinical infections (amikacin, gentamicin, rifampicin, tetracycline, imipenem, meropenem, ampicillin and linezolid) for a period of 24 hours. The resultant reduction in metabolic activity was measured against untreated BF using the XTT assay. Testing was also carried out against the same organisms in planktonic form using NCCLS methodology. Variance in antibiotic response to the aminoglycosides was noted for planktonic forms of the organism under anaerobic conditions, as was expected, however no variance in activity to any of the antibiotics was observed against BFs regardless of atmosphere. This suggests that the mechanisms contributing to altered antibiotic resistance for planktonic forms of the organism do not exist in BF state, or the variances were too small to be measured against the higher antibiotic concentrations using in BF testing. These results indicate that a range of antibiotics, including the aminoglycosides, may be suitable for use against BFs in environments where reduced oxygen concentrations are encountered, or in applications such as in antibiotic lock therapy.

R2426 Microbial biofilm on laryngeal voice prosthetic devices

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Objectives: The durability of voice prostheses after their implantation is variable, and the most frequent reason for replacing a voice prosthesis is leakage of fluids into the trachea due to valve insufficiency, which mainly is caused by biofilm formation. The physiological flora of the neopharynx and the tracheostoma created in laryngectomized patients are predisposing factors for adherence of microorganisms to the surface of voice prostheses. The aim of this presentation was to characterize with great precision the presence of individual microorganisms and the most frequent microbial combinations in the biofilm of the indwelling voice prosthesis in situ.

Methods: The study was performed by retrospective analysis of data archived at medical documentation of the Clinical Medical Centre and the Teaching Institute of Public Health in Rijeka. During the analyzed period, voice rehabilitation in laryngectomized patients was performed by implanting a Provox2 voice prostheses (Provox2; Atos Medical AB, Sweden). In total 100 implanted prostheses were microbiologically processed. The biofilm was removed from the valve by scraping and rigorous homogenization in saline. The composition of the microflora found in the biofilm was determined by plating 0.1 mL of the homogenate on nutritional substrates for cultivation of fungi Sabouraud agar, chromagar *Candida* plate (CHROMagar™ Candida, BD Diagnostics, USA), and the blood agar for the culture of bacteria.

Identification of isolated strains was performed using standard laboratory protocols and commercial kits (API bioMerieux, France).
Results: Out of the total of 292 isolates, 67% were bacteria and the remaining 33% were yeasts. Simultaneous presence of bacteria and fungi was established in 83% of the processed voice prostheses; in 16% of samples the biofilm contained only one or more bacterial species. The most frequently found yeast species on voice prostheses biofilms was *C. albicans*, followed by *C. krusei* and *C. tropicalis*. The most frequently isolated bacteria included *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus mirabilis* and Streptococcus agalactiae. The median lifetime of the voice prosthetic devices was 180 days.
Conclusion: Dividing the prostheses in four groups according to the composition of biofilm revealed that the device lifetimes varied significantly between groups. The longest lifetime of voice prostheses was associated with the presence of single fungal isolate in combination with bacteria.

R2427 Detection of autofluorescence in biofilms formed by rapidly growing mycobacteria

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Objectives: To study the distribution and the behaviour of the autofluorescence in a biofilm formed by rapidly growing mycobacteria.
Methods: *Mycobacterium fortuitum* ATCC 6841 and *Mycobacterium abscessus* DSM 44196 were used in the experiments. The biofilm development test was made using hydrophobic, uncoated sterile 2 × 4-well plates, which were incubated at 37°C on an orbital shaker (80 rpm) during 24, 48, 72 and 96 hour. The medium was changed daily until the end of the experiment. The wells were stained using Live/dead© BacLight© and Nile Red© stains and analysed with a confocal laser scanning microscope. One set of wells was used to study both autofluorescence and Nile Red© stain, and the other one to analyse the proportion of live and dead mycobacteria. Thickness was measured eight times/well in predefined points of each well. All experiments were performed in triplicates for each strain.
Results: Both strains increased their thickness according to the incubation time: *M. fortuitum* biofilm reached a maximum thickness at 72 hours (23.11 ± 4.35 μm), while *M. abscessus* biofilm reached it at 96 hours (13.60 ± 6.21 μm). The sample standard deviation of *M. abscessus* is higher because its biofilm surface is more irregular than that of *M. fortuitum*. Percentage of covered surface at 96 hour was also higher for *M. fortuitum* (43.15 ± 15.34%) than for *M. abscessus* (39.10 ± 32.24%). The highest value of covered surface proportion was achieved at 48 hour in *M. fortuitum* (69.35 ± 1.63%), while achieved at 96 hour in *M. abscessus*. Mature biofilm of both strains tends to be detached partly at long incubation times, a fact that could explain these findings.
The detection of autofluorescence in both species is similar, being higher when the percentage of covered surface is lower. There is a concordance between the Nile Red© stained bacteria and those who emitted autofluorescence. At the same time, the proportion of live and dead mycobacteria in the biofilm showed that all bacteria were alive. We also detected the presence of autofluorescence outside bacterial cells in both species, with irregular distribution inside the biofilm.
Conclusions: Mycobacterial biofilms showed the presence of autofluorescence not only inside the cells but also in the extracellular matrix. Detection of autofluorescence could be useful for future studies of the mycobacterial biofilm structure.

R2428 The effects of different physico-chemical stressors on biofilm-associated *Legionella pneumophila* survival

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Objective: The aim of this study was to evaluate the survival against different physical and chemical stressors of biofilm-associated *Legionella pneumophila*, which is the etiologic agent of Legionnaires' disease. Legionnaires' disease is usually seen as a serious form of pneumonia that carries with it a fatality rate in the order of 10–15%.
Methods: Heterotrophic mixed biofilms were allowed to develop for six months on glass coupons in a bench-scale biofilm reactor, which was experimentally infected by *L. pneumophila* ATCC 33152 strain. Coupons have been analyzed for *L. pneumophila* bacteria using BCYE plating, after 24 hours exposition to different conditions of temperature (4 and 60°C), pH (pH 3, 5 and 11), osmotic stress (ultra pure water and 3 M salt solution) and disinfectant (2, 500, 1000 ppm monochloramine).
Results: Legal dose of 2 ppm of monochloramine was found ineffective against biofilm-associated *L. pneumophila*, whereas shock treatment (500 and 1000 ppm) was found significantly successful to fight against this bacterium where applicable. Exposure to 60°C was inefficient to kill all *L. pneumophila*, which is generally used as heat treatment method in man-made water systems for decontamination. Significant amount of *L. pneumophila* bacteria also resists pH 11 and 3 M salt solution. But the acid-tolerant bacteria showed significant decrease on exposure to pH 3, whereas pH 2.2 acid solution is widely used to isolate this bacterium from mixed microflora. No significant change was observed after exposure to 4°C, ultra pure water, pH 3 and 5.
Conclusion: Biofilm is essential for the survival and multiplication of *L. pneumophila*. Interestingly, the growth of other environmental organisms stimulates the growth of *L. pneumophila* in aquatic environments. An infectious dose level for *L. pneumophila* has not been established, however low numbers of this bacterium possess risk for public health. Since man made water systems are ideal incubators, *L. pneumophila* could multiply in a very short time. The prolonged persistence of pathogen bacteria in aquatic biofilms serves as a reservoir and detachment through shear forces or another mechanisms permits continuous pathogen intrusion into bulk water. Due to the problem of resistance and potential environmental impacts, alternative strategies for Legionella control need to be investigated and put to practice.

R2429 The prevalence of implant-associated biofilm infections

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Objectives: Currently, the presence of an implant associated biofilm infection is primarily suspected when the patient shows clinical symptoms of infection or repeated positive cultures with low virulent microorganisms. However, since biofilms may consist of extremely slowly growing persisters and/or microorganisms only inducing minor inflammation, we believe that the actual amount of such infections may be underestimated. Therefore, the present study was designed to investigate the prevalence of biofilm on implants and in the surrounding tissue of deceased humans.
Methodology: Implants and implant-associated material from deceased humans without a history of implant failure or inflammation associated with the implant were included in the study. From each case the following was collected; tissue adjacent to the implant and if possible the implant itself as well as control tissue from an unrelated part of the body. The collected samples were subjected to Peptide Nucleic Acid – fluorescent in situ Hybridization (PNA-FISH) and visualized by Confocal Laser Scanning Microscopy (CLSM) to determine the presence of any microbial infections. To determine the presence of an immune response, HE (Hematoxylin and Eosin) stained samples was also included in the study. To identify the present microorganisms, part of the 16 second and the D2 LSU rRNA genes

were sequenced and the results compared to validated databases. When deemed necessary and when possible, deep sequencing was performed to get a more accurate description of the infecting microorganisms.
Results: Including controls, our preliminary study consisted of 42 tissue samples and eight implants from a total of 21 cases. We found an infection rate of approximately 52% (11 out of 21), when correcting for suspected post mortem contamination. Interestingly, the microbial findings were almost solely pathogenic and non – pathogenic fungi, which is in contrast with what is seen after revision surgery where findings primarily are bacterial.
Conclusion: The data from the present study on post-mortem material shows that there is a much higher incidence of implant colonization than initially anticipated, especially with fungi.

R2430 Antibiofilm and quorum quenching activity of bio-emulsifier from coral-associated *Streptomyces* sp.

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Objectives: Emergence of multidrug resistant pathogens made the world to switch over to post antibiotic era. Biofilm formation is an important mechanism for microbial survival and resistance to many antibiotics. Pathogens living in biofilms are more resistant to antibiotics than their planktonic counterparts. Biofilm formation on medical devices causes severe nosocomial infections and poses critical threats to human wellbeing. Biofilm infection can be minimized by inhibiting the initial stage of biofilm formation and subsequent growth. Novel antibiofilm compounds are required to combat biofilm related infections. Coral ecosystem, an untapped reserve for novel bacteria was explored for bio-emulsifier producers anticipating that biomolecules from such bacteria will be of new type.
Methods: Antibacterial activity of Coral Associated *Streptomycetes* sp. (CAS) was tested against representative Gram negative bacteria (*Chromobacterium violaceum*, *Pseudomonas aeruginosa* ATCC 10145 and *Serratia marcescens*), since an ideal quorum quenching agent should not possess any bactericidal activity. Quorum quenching activity of CAS was tested against *C. violaceum* (QS reporter strain). Quorum quenching and antibiofilm activity of CAS was evaluated by quantitative and qualitative methods. The bio-emulsifier extracted was characterized by TLC and FTIR spectroscopy. Minimum biofilm inhibitory and disruption concentrations were determined.
Results: Out of 23 coral associated bacteria screened, seven isolates were found to be bio-emulsifier producers. Bio-emulsifier from strain U23 was tested for antibiofilm activity against representative Gram-negative bacteria (*C. violaceum*, *P. aeruginosa* ATCC 10145 and *S. marcescens*). 16S rRNA gene sequence analysis revealed that strain U23 is *Streptomyces* sp. (JN315778). CAS completely inhibited violacein production of *C. violaceum*. Bio-emulsifier from CAS has shown inhibition of biofilm formation and disruption of mature biofilms at significantly lower concentrations (200 μg/mL). It inhibits the QS controlled virulence factors like swarming, biofilm formation, production of pigment and extracellular enzymes in *S. marcescens* and disrupts EPS in *P. aeruginosa*.
Conclusion: Bio-emulsifier from coral associated *Streptomycetes* sp. thus effectively acts as potent antibiofilm agent. Further purification and characterization will reveal the nature of the antibiofilm agent.

R2431 Combined activity of colistin with human polymorphonuclear neutrophils against *Pseudomonas aeruginosa* biofilms

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Background: Chronic *Pseudomonas aeruginosa* (PA) airway infection constitutes the primary cause of bronchial inflammation and respiratory deterioration in cystic fibrosis (CF) patients. It is predominantly caused by biofilm (BF)-producing strains frequently resistant to multiple antibiotics. Little is known about the effects of polymorphonuclear neutrophils (PMNs) against PA BFs in combination with clinically useful antibiotics. Our aim was to examine the activity of PMNs alone and in combination with colistin (CST), an antibiotic active against multi-resistant PA, against PA BF in comparison to their activities against planktonic cells (PL).
Methods: Two clinical PA isolates from CF patients were used, one resistant (CLSI MIC 4 mg/L) and one susceptible (1 mg/L) to CST. Both strains were grown in Muller-Hinton broth. BFs were grown in 96-well plates at 37°C for 48 hour. CST (1, 4, 16 mg/L) and human PMNs (E:T ratios 1:20 or 1:10), alone or in combination, were incubated with BF or PL at 37°C 5% CO_2 for 24 hour. BF formation and antibacterial activities were assessed by metabolic XTT assay. ANOVA was employed (n = 6). Synergy was defined when the observed bacterial damage produced by the combinations of PMNs and CST was significantly higher than the expected sum of damages produced by PMNs or CST alone; additivity was defined when the observed damage produced by the combination was significantly higher than the damages produced by PMNs or CST alone.
Results: PMNs damaged PL and BF in an E:T ratio-dependent pattern; however, PMN-induced damage of BF was significantly lower than that of PL even when PMNs were combined with CST for both strains. When PMNs at 1:20 were combined with 16 mg/L CST for BF of R or 4 mg/L for BF of S strain, synergistic effects were noted. By comparison, additive effects were noted when PMNs were combined with 1 or 4 mg/L CST for BF of R strain. At E:T 1:10, PMNs combined with CST (1 and 4 mg/L) had synergistic effects on BF of S strain. No significant interaction was noted between PMNs and CST on PL damage for both strains at all conditions tested. At all conditions tested (CST alone or combined with PMNs), no significant differences in BF damage were noted between the two clinical isolates (S and R).
Conclusions: BFs of PA strains that are either S or R to CST are significantly less susceptible than PL to the activities of PMNs and CST alone or in combination. However, PMNs and CST combinations tested exhibit additive or synergistic effects on BFs of PA.

R2432 Interactions of the triple combination of voriconazole, amikacin and polymorphonuclear neutrophils against mixed biofilms of *Candida albicans* and *Pseudomonas aeruginosa*

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Objectives: *Candida albicans* (CA) forms multispecies microbial communities, known as mixed biofilms (MBF), with various bacterial species including *Pseudomonas aeruginosa* (PA). CA and PA also interact with each other to cause mixed infections in humans. The establishment of MBF of CA and PA has important clinical implications requiring vigorous treatment as microorganisms exhibit a high level of resistance to antimicrobials and are protected from host defenses. Our aim was to study the combined activities of PMN with two antimicrobial agents, AMK and VRC, against MBF of CA and PA in comparison to their corresponding planktonic cells (PL).
Methods: CA-M61, a BF-producing CA strain, and a clinical PA isolate from a cystic fibrosis patient were used. MBF were grown in 96-well plates in RPMI at 10^6 cells/mL at 37°C/48 hour. PL of CA and PA were grown in RPMI or in Muller-Hinton broth, respectively, at 37°C/24 hour. PL and BF were then incubated for 24 hour with twofold dilutions of amikacin (AMK) (1–64 mg/L for PL and 4–256 mg/L for BF) and VRC (0.125–64 mg/L for PL and 0.5–256 mg/L for BF) and PMNs. PMNs from healthy donors at E:T ratios 1:1 or 1:2 were incubated further for 24 hour alone or in combination with AMK and VRC, simultaneously in a checkerboard format. Damage induced by both antimicrobial agents to PL and MBF was assessed by XTT assay. The interactions between AMK combined with VRC and PMNs were analyzed using the Bliss surface model. Synergy was concluded when the observed damage was significantly higher than the expected sum of damages; whereas, additivity was defined when the observed damage

was significantly higher than each component but where synergy was not achieved. ANOVA (n = 6) with Dunnett's test was performed.
Results: Against PL, a synergistic interaction was observed when 8–64 mg/L of AMK were combined with 0.125–4 mg/L of VRC in the absence of PMN or in the presence of PMN at E:T 1:1. At E:T 1:2, a synergistic interaction was observed when 16–64 mg/L of AMK were combined with 0.25–1 mg/L of VRC. Against BF, the above drug-drug and drug-PMN combinations demonstrated indifferent interactions.
Conclusions: The triple combination of voriconazole (VRC) with AMK and polymorphonuclear neutrophils (PMN) against PL mixtures exhibits synergistic interactions. By comparison, the interactions against MBF are indifferent. These in vitro interactions between an antipseudomonal and an antifungal agent with PMN suggest differential interactive effects of these antimicrobial agents in the treatment of mixed infections.

R2433 Phenotypic and genotypic characterisation of coagulase negative staphylococci isolated in blood cultures from patients with haematological malignancies

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Introduction: We studied the production of biofilm in coagulase negative staphylococci (CNS) isolated from bacteremia in patients hospitalized in the bone marrow transplant centre of Tunisia during 10-year period (1998–2007). In addition, we analyzed the relationship between biofilm production and detection of adherence and antimicrobial resistance genes.
Material and Methods: Forty nine CNS were isolated in 45 patients with bacteraemia: 13 catheter related bacteraemia (CRB) and 36 non CRB (NCRB). Quantitative determination of biofilm production was performed by using a microtiter assay. PCR was used to detect the presence of icaA, icaB, icaC, atlE, aap gene and 13 antimicrobial resistance genes. The chromosomal DNA of methicillin resistant isolates was digested with SmaI and was separated by pulsed-field gel electrophoresis (PFGE).
Results: CRB did not differ significantly from NCRB isolates in their ability to form biofilm but they were significantly more resistant to tetracycline ($p < 0.01$) than NCRB isolates. In addition, the ica A, B and C genes were significantly ($p < 0.05$) more prevalent in CRB than were in NCRB isolates. Resistance to clindamycin (50% vs. 20%, $p < 0.001$) and rifampin (64.3% vs. 48.6%, $p < 0.05$) was comparatively higher among biofilm producers than non-biofilm producers. In addition, there was a significant association ($p < 0.0001$) between the strong biofilm-producer phenotype and harboring the ica operon and atlE and aap concomitantly.
Among the erythromycin-resistant strains the most prevalent macrolide determinant was msr(A) (53.6%). The most important gene coding for aminoglycoside resistance, aac(6′)-Ie-aph(2″), was detected in 77.6% of the isolates. Seven out of eight tetracycline-resistant strains harboured the tet(K) gene and one strain carried tet(K) and tet(M) genes.
Conclusion: Significant association between biofilm production and resistance to antimicrobials (clindamycin and rifampin) and between strong biofilm-producer phenotype and harboring concomitantly ica operon and atlE and aap. Moreover, significantly higher resistance to tetracycline and higher prevalence of the ica A, B and C genes in CRB than in NCRB isolates.

R2434 The effect of low molecular weight chitosan upon biofilm formation by *C. albicans* and *E. faecalis*

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Objectives: The use of the antimicrobial properties of chitosan in dentistry is at present the subject of several studies. We aim the development of new formulations of this compound to prevent the growth of microorganisms responsible for pulpal and periapical inflammation and disease.
Methods: In this work, we have determined the minimal inhibitory concentration (MIC) accordingly to the reference protocols CLSI (M27-A3 for yeasts and M07-A8 for bacteria) and the minimal lethal concentration (MLC) on planktonic cells of *C. albicans* ATCC 90028 and *E. faecalis* ATCC 2912 of 5 diferent chitosans. The chitosans used were: low molecular weight (LMW), medium molecular weight (MMW), high molecular weight (HMW), a mixture in equal parts of high and medium molecular weight (H + M) and a mixture in equal parts of high and low molecular weight (H + L). The effect of LMW chitosan was also accessed upon adhesion, biofilm formation and disaggregation using a model that simulated the teeth – hidroxyapatite discs, and in RPMI medium.
Results: The results showed that MIC for all simple chitosans samples is similar. Since low molecular weight chitosan has been described to be more biocompatible and with less secondary effects, we decided to use this compound in subsequent studies. The results showed a reduction in adhesion of 56%, 37% and 26% for 7.5, 3.75 and 0.029 mg/L of LMW chitosan respectively. The effect upon biofilm formation was at MIC and over MIC concentrations, higher ((90%). These concentrations managed to inhibit almost completely biofilm formation. Preliminary results on disaggregation also showed the efficiency of this compound.
Conclusions: Our results show the potential of LMW chitosan on blocking the formation and on removing teeth biofilm, preventing the development of periapical and pulpal diseases.

R2435 Phenotyping and genotyping of 57 *S. epidermidis* isolates from colonized CVC. Is strain ability to produce slime related to microscopic biofilm characteristics?

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Objectives: To test if a relation is present between phenotypic or genotypic characteristics of *Staphylococcus epidermidis* strains isolated from colonized central venous catheters (CVC) and micromorphological features of biofilm formed on catheter tips in patients.
Methods: Of 57 *S. epidermidis* strains were isolated, in a significant load, from 57 CVC tips (Cleri method). Immediately after cultural test, CVC tips were washed twice in HEPES buffer and stored in 2.5% glutaraldehyde in HEPES. A 5 mm long segment was then aseptically isolated with a scalpel from the distal portion of the tip, dehydrated in ascending alcohol, dried, gold sputtered and observed by scanning electron microscopy (SEM). Each lumen and the external surface of the segment were checked for the presence of the following micromorphological features: fibrin network (FN), white blood cells (WBC), and microbial biofilm (MB) (Fig. 1). Phenotypic characteristics of the isolated strains were assessed by congo red agar test (tryptone soy agar, 36 g/L dextrose, 0.8 g/L congo red) according to the colorimetric scale proposed by Arciola et al. (2002). Almost black, black, and very black colony former strain were considered as slime producer. Specific primers for icaA, icaB, icaC, icaR, icaD were used for the identification of the genes encoding for slime synthesis. Strains were considered positive to ica locus when all genes were detected. Contingency tables comparing each SEM feature to phenotypic and genotypic characteristics were realized and Fisher's exact test was performed for statistical significance.
Results: Positive percents for phenotypic and genotypic tests were 28.0% (16/57) and 52.6% (30/57) respectively. Strains exhibited the following colonies colour: very red (22.8%), red (29.8), bordeaux (19.3%), almost black (15.8%), black (5.3%), very black (7.0%). All bordeaux colonies were ica positive. None of the tested ica genes was present in ica negative strains. FN, WBC and MB were found in 40, 21 and 31 catheters respectively. However, no statistically significant relation was found between any of the three micromorphological features and the strain phenotype or genotype.

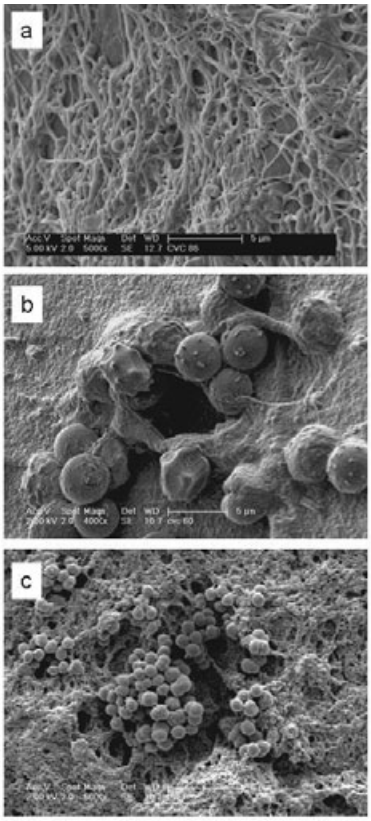

Figure 1: Scanning electron microscopy on biofilm formed on CVC during patient catheterizazion. Representative images of the three micromorphological features considered in the study: a) fibrin network, b) white blood cells, and c) microbial biofilm. Original magnification 5000 (a), 4000 (b), 5000 (c) times.

Conclusion: Microscopic features, as FN, WBC and MB, are frequently observed by SEM on CVC removed from patients but no significant relation to the strain ability of producing slime was found. A larger number of samples is desirable for considering biologic variability of biofilm and patients.

R2436 Biofilm producing ability of Salmonella Typhimurium BK4, isolated From Turkey

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Objectives: The biofilm forming characteristics of 140 Salmonella strains (99 serotyped and 41 genus level identified), originated from Turkey was investigated. Salmonella strains were evaluated for their biofilm morphotype properties on Congo Red agar plates. Additionally, all strains evaluated according to their pellicle structure forming, physical differences of pellicle, and the alterations in the media along with the pellicle forming and for cellulose existence in the biofilm matrix.
Methods: One hundred and fourty strains were tested for biofilm forming characteristics on Congo Red (CR) agar plates. Also, all Salmonella strains were screened on LB agar wo/NaCl containing 200 μg/mL calcoflour for screening absence or presence of cellulose under UV light. For pellicle production, overnight cells were diluted (1:100) in fresh LB agar wo/NaCl and incubated at 20°C for eight days. The strains were visually examined according to their pellicle forming abilities and classified. Furthermore, effects of temperature (5, 20 and 37°C), pH (4.5, 5.5, 6.5, and 7.4), NaCl concentration (0.5%, 1.55, 5.5%, and 10.5%) on biofilm forming was assessed in microtiter plates. Same parameters were also tested on stainless steel surfaces. All experiments were performed in triplicate.
Results: Turkey originated Salmonella Typhimurium BK4 strain was determined as the strongest biofilm producer (OD: 3.418). The optimum temperature for biofilm producing was determined as 20°C for Salmonella Typhimurium BK4. When NaCl concentration increased in the growht media, biofilm production of BK4 was dramatically decreased. On the other hand, pH values exhibited variable effects on biofilm forming feature of BK4 on both polystyrene and stainless steel surfaces.
Conclusion: Salmonella Typhimurium BK4 strain can adhere and form biofilms on both polystyrene and stainless steel surfaces. Biofilm formation is significantly influenced by pH, NaCl concentration and temperature.

R2437 Multi-drug resistance in canine pyoderma isolates of *Staphylococcus pseudintermedius* associated to biofilm formation and to IS256 insertion element

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Objectives: *Staphylococcus pseudintermedius* is the most frequent staphylococcal species isolated from canine pyoderma. The control of *S. pseudintermedius* infection is often difficult due to the expanded antimicrobial resistance phenotypes. Antibiotic resistance in staphylococcal pathogens is often associated to biofilm formation and to mobile genetic elements such as the insertion sequence IS256 that was first described as a part of the transposon Tn4001, which confers aminoglycoside resistance in *S. aureus* and in *S. epidermidis*. The present study was carried out to determine the prevalence of antibiotic resistance, the biofilm formation and the presence of IS256 aspects not yet investigated in *S. pseudintermedius*.
Methods: Thirty-six *S. pseudintermedius* isolates from canine pyoderma were identified using RFLP assay. Susceptibility to a panel of fourteen antimicrobial agents was determined by the disk diffusion method in Mueller-Hinton agar and MRSP isolates were detected by subculture on screening agar base medium. Biofilm formation was investigated by the tissue culture plate method. The presence of IS256 insertion element was characterized by PCR.
Results: All *S. pseudintermedius* isolates showed multidrug-resistance ((6 antibiotics); in particular the highest antibiotic resistances were observed toward to penicillin and trimethoprim-sulfamethoxazole (94%) and clindamycin (92%). Noteworthy MRSP rate (56%) was higher than elsewhere reported (Fig. 1). Interestingly all isolates showed biofilm production and PCR analyses detected the presence of IS256 in 61% *S. pseudintermedius* isolates. Statistical analysis revealed a strict association between the presence of IS256 and gentamicin- and oxacillin- resistance (χ^2 p-Value <0.05).

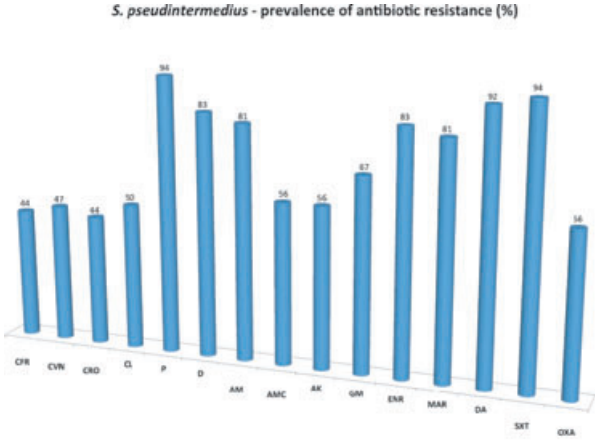

Figure 1. Prevalence of antibiotic resistance among S. pseudintermedius isolates.
Cefadroxil (CFR), cephalexin (CL), ceftriaxone (CRO), cefovecin (CVN), penicillin (P), ampicillin (AM), doxycycline (D), amoxicillin-clavulanic acid (AMC), gentamicin (GM), amikacin (AK), enrofloxacin (ENR), marbofloxacin (MAR), trimethoprim-sulfamethoxazole (SXT), clindamycin (DA), oxacillin (OXA).

Conclusion: To our knowledge this is the first report showing the biofilm formation and the detection of IS256 in *S. pseudintermedius* isolates. Our preliminary results suggest that multi-drug resistance may be related to biofilm formation also in *S. pseudintermedius* as reported in *S. epidermidis* and *S. aureus*. Moreover the association between the presence of IS256 and the resistance to gentamicin and oxacillin indicate that *S. pseudintermedius* may acquire antibiotic resistance genes through mobile genetic elements which may play an important role in the dissemination of multi-drug resistance.

R2438 Kinetics of the biofilm formation of ATCC 27853 *Pseudomonas aeruginosa* strain on silicone catheter under the effect of aminoglycosides: evaluation with the scanning electron microscopy

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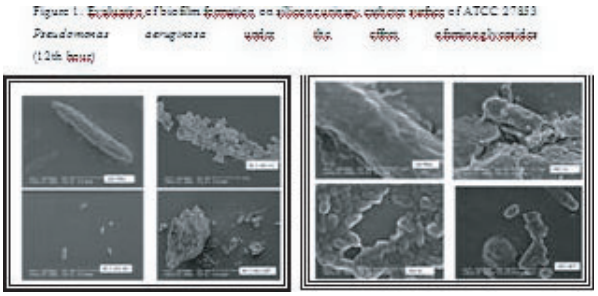
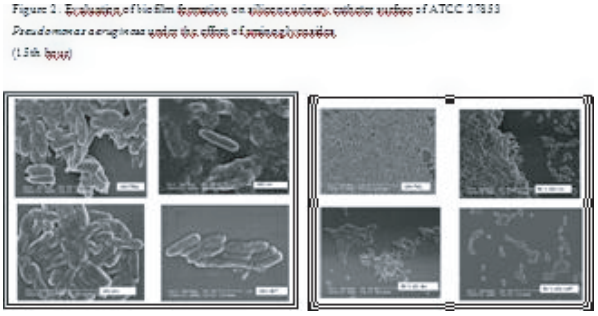
Objective: *Pseudomonas aeruginosa* is adapted to the changing conditions of microenviroment and survived by biofilm forming, defined as a phenotypic change. At the beginning of biofilm formation, bacteriae interact with the signal molecules Quorum Sensing (QS) systems by reorganizing the presentation of virulence factors. In planning the therapy it is getting necessary to identify the relationship between the host (in vitro system) and the Pseudomonas following the exposure of the decreasing concentrations of antibiotics (subminimal inhibitory concentrations) mimicking tissue concentrations.
Methods: In our study, following the determinations of minimum inhibitor concentrations (MICs) of gentamicin, netilmicin and amikacin; las and rhl systems responses of standard ATCC 27853 *Pseudomonas aeruginosa* strain, which was exposed to MIC and 50% MIC of these antibiotics, were determined at the 12th and 15th hours by micro AHL method. The MIC and 50% MIC concentrations of gentamicin, netilmicine, amikacin together with bacterial suspensions prepared in the appropriate sizes (1 × 1 cm) of silicone urinary catheter (Rusch, Germany) in the Eppendorf tubes were incubated for 12 and 15 hours at 37°C. Biofilm formation on the silicone urinary catheter was examined with ''Scanning Electron Microscope'' (SEM, Izmir Institute Technology) at 12th and 15th hours of bacteriae exposures.
Results: Signal molecules of las and rhl systems were present under MIC and 50% concentrations at 12th and 15th hours. Biofilm formation started at 12th hours of infection instead of MIC and 50% MIC concentrations of aminoglycosides. Biofilm formation started at 12th hours of infection instead of MIC and 50% MIC concentrations of aminoglycosides. Silicone surface had no ''preventive'' effect on *Pseudomonas aeruginosa* attachment.
Conclusion: MIC and 50% MIC concentrations of different aminoglycosides have different ''architectual'' effects on biofilm formation of ATCC 27853 *Pseudomonas aeruginosa*. According to control experiments, at the 12th hour bacteriae formed biofilm while bacteriae seemed to be in dispersal phase at the 15th hour of exposure but in aminoglycoside exposured bacteriae showed different cell wall types, probably because of the differences in lipopolysaccharide formation. Natural (gentamicin) and semi-synthetic (amikacin, netilmicine) aminoglycosides have same effect on QS but different effects on the biofilm formation and morphology of *Pseudomonas aeruginosa*.





R2439 Candida biofilms, sodium metasilicate and antifungal drug susceptibility

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Objectives: Slime or biofilm represents a structured community of fungal cells embedded in a self-produced polymorphic matrix adherent to the artificial surface. It is also a fact that antifungal agents frequently fails to eradicate these biofilms despite the use of drugs with proven in vitro activity. This experiment was done to find out whether biofilm produced by susceptible strains are less organized and can be removed easily by sodium meta silicate.
Methods: Using a modified tube adherence test the biofilms produced by 24 different *Candida* species including two international strains were treated with 4/8% sodium metasilicate solutions for 30 minutes and then the cell suspension in the tubes were poured or aspirated out and the walls of the tubes were stained with 1% safranin and scoring of the biofilms were done before and after treatment by use of pair tubes. Although in some previous experiments sodium metasilicate has been used in removing biofilms but it is used only as a minor component of chemical formulations in very low concentrations which is not properly effective. Antifungal sensitivity patterns were studied according to M27-A2, NCCLS protocols and then the biofilm removal scores were analysed in relation to the antifungal sensitivity patterns.
Results: Strong slime production was seen in *C. tropicalis* and weak in *C. glabrata*. Micafungin and amphotericin-B were more effective against biofilm producing *Candida* spp. than fluconazole. Original mean biofilm score of 1.481 became only 0.3 after application of sodium metasilicate solution even in amphotericin B antifungal drug resistant *C. tropicalis* biofilm.
Conclusion: In this study we observed a definite Candida biofilm lowering activity of sodium metasilicate even up to 70% in antifungal drug resistant Candida strains. Sodium metasilicate can be removed easily by washing and it is nontoxic, thus simple occasional washing with this chemical is sufficient to prevent Candida biofilm in many medical devices.

R2440 Mapping and bacterial diagnosis of biofilms on central venous catheters

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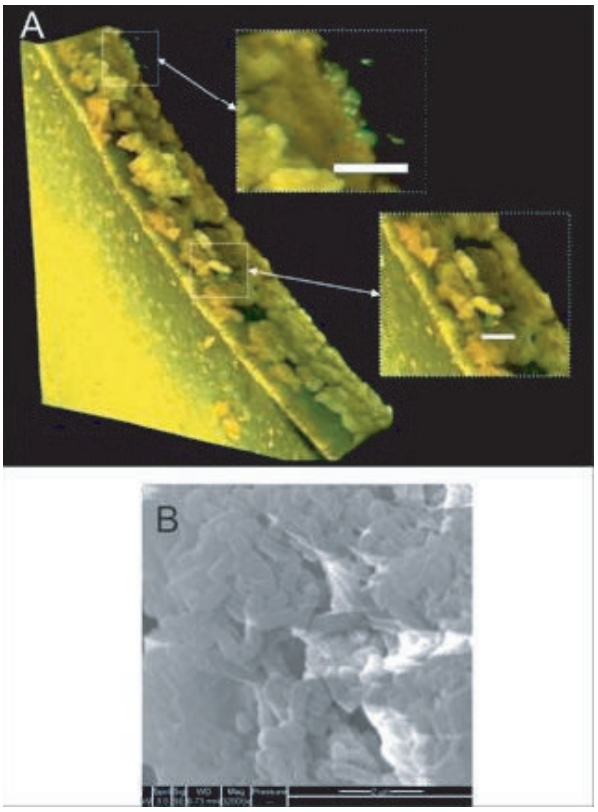
Objectives: Central venous catheters (CVCs) are widely used in adult and paediatric medicine for medium to long-term vascular access. Complications include thrombus and infection. Diagnosis of CVC infection is conventionally done by blood and roll-tip culture, but is suspected of producing a high rate of false negatives, in part because

bacteria can be present as biofilms. We aimed to develop methods to map the distribution of biofilm and thrombus on the surface of peripherally inserted CVCs (PICC) from adults, and subcutaneously-tunnelled or totally-implanted CVCs in paediatric patients using high resolution imaging and sonication-culture.

Methods: CVCs from 11 Peripherally Inserted Central Catheters (PICC) (adult) and 24 tunnelled or implanted (paediatric) patients were collected, of which twenty six were removed for end of treatment (EOT), three for blockage, two for exit site infection and four for other reasons. Four 1 cm sections were cut from each of the tip, middle and entry site regions. One section from each region was (i) cultured following sonication, (ii) examined with scanning electron microscopy (SEM) and c) confocal microscopy to visualise biofilm and proteins in situ (Fig. 1).

Results: SEM showed that all catheters contained some degree of deposit in the lumen. Based on the images we developed a scoring system for semi-quantification. Paediatric CVCs had a median scoring of 3 (small occlusions or 50–80% surface cover) compared to a score of 2 (10–50% surface cover) for adult PICCs. SEM revealed bacterial and yeast biofilm in the lumen of one adult catheter which, interestingly, was culture-negative (Fig. 1 B). Confocal microscopy suggested that bacteria might be obscured by overlying protein (Fig. 1 A). Paediatric CVCs had a higher culture-positive rate than adult CVCs (10 of 23 vs. 1 of 10, one excluded from each patient population due to possible contamination). Bacteria were recovered most commonly (eight of 11) from non-tip CVC sections.



Conclusion: The CVC tip is not necessarily the most reliable section for the detection of infection. Sonication-culture was more sensitive for detecting bacteria than SEM. For all CVCs combined culture found 11 of 20 positive for bacteria compared to 1 of 20 for SEM ($p < 0.05$ by McNemar's test). Even though most paediatric CVCs were removed for EOT there was evidence of bacteria suggesting that CVCs from paediatric oncology patients might be more commonly contaminated than currently realised.

R2441 Production of biofilm on different substrates by *Acinetobacter baumannii* isolates

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Objectives: *Acinetobacter* spp. mainly *Acinetobacter baumannii*-Acinetobacter calcoaceticus cluster, are emerging as problematic opportunistic pathogens due to the rapid increase in multidrug resistance. Biofilm formation is an important feature of most clinical isolates of *A. baumannii* allowing for persistence on colonized surfaces and reducing antibiotics' activity.

This study aimed to evaluate biofilm production by clinical isolates of *A. baumannii* on different substrates.

Methods: Eight isolates of *A. baumannii* from hospitalized patients affected by different infections were used. They were identified by biochemical methods and pyrosequencing. Four of them were isolated from urines, and one each from blood, low respiratory tract, wound and cerebrospinal fluid. Biofilm production was evaluated on polystyrene, polypropylene, polyethylene and titanium. Aliquots (about 5–7×10^8 CFU/mL) from a 24 hours in Brain Heart Infusion broth were used to inoculate wells of a polystyrene microtitre plate, polypropylene tubes, and polyethylene and titanium coupons (diameter: 20 mm; thickness: 6 mm) placed in a six wells plate. After overnight incubation, the medium was refreshed in order to remove non-adherent bacteria and samples incubated for 72 hour until a visible biofilm was formed. Detection of biofilm on each substrate was carried out by a spectrophotometric method, involving crystal violet staining and reading at 595 nm. Each test was performed in triplicate, means of OD595 were calculated and used to classify the isolates as strong producers, moderate producers or not producers of biofilm as compared to negative controls consisting of not inoculated substrates.

Results: Production of biofilm was observed in all the tested isolates but with differences among isolates and materials. The ability to form biofilm was greater on polyethylene followed by polystyrene, polypropylene and titanium.

The greater amount of biofilm on all the substrates was showed by the wound isolate, followed by isolates from CSF, blood and respiratory tract; all of them resulted strong producers. Production of biofilm varied in urinary isolates being two of them strong producers and the rest moderate producer.

Conclusion: Production of biofilm characterized *A. baumannii* isolates, but it seemed to depend on the abiotic substrate and on isolate. More studies are needed to better elucidate the links between infection site and production of biofilm.

R2442 Mechanical properties and disruption of dental biofilms

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Objectives: The aim of our research was to characterize and quantify the influence of a high velocity water microdrop impact on the structure and detachment of dental biofilms from the interproximal space of a typodont model using high speed imaging, and image analysis with focus on the viscoelastic parameters and cohesive and adhesive failure.

Methods: General Bacterial Growth Conditions: *S. mutans* biofilms were grown on coupons, typodont teeth and human deciduous teeth and imaged with confocal and epifluorescent microscopy. High speed videography: a high-speed camera was used to record the impact of a high velocity water droplet of 114 μL, burst from a Philips ''AirFloss'' device, on biofilm detachment from both model and human teeth. Compressive testing: uni-axial compression testing using a small scale mechanical tester was conducted on biofilms while submerged. Computational Fluid Dynamics CFD: CFD simulations based on the finite element method (FEM) were performed utilizing ANSYS Fluent software to calculate the shear distribution caused by the drop around a tooth geometry.

Results: The water droplet had an exit velocity of 60 m/second leaving the ''AirFloss'' nozzle. We estimated that 90% of the water was ejected in the first 10 ms. At this time scale the water behaved initially as a continuous jet before breaking up into nanodrops. On impact there was initial cohesive failure as the water burst through the biofilm and subsequent adhesive failure as the shear caused the biofilm to flow over the tooth surfaces. Of 90% of the biofilm was removed from the IP space and confocal microscopy showed that there were no biofilm at the device tip but small patches of biofilm remained on the prominences at the back of the teeth. Mechanical testing showed that the *S. mutans* biofilms were viscoelastic with an elastic modulus of 179 kPa and a relaxation time of 10 second. The CFD simulations predicted lowest shear at posterior aspect of the teeth and were consistent with the experimental observations.
Conclusion: Biofilms made from *S. mutans* were grown on different surfaces, including human teeth. The impact of the water drop was high enough to cause adhesive and cohesive viscoelastic failure contributing to the detachment process. High velocity water microdrops can effectively remove viscoelastic biofilms with minimal volume and time.

R2443 Comparison of various antifungal agents as catheter lock solutions in an in vitro model of *Candida* spp. biofilm

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Objectives: *Candida* spp. is one of the main causative agents bacteraemia. Most of them are originated as catheter-related fungemia (CRF). The antibiotic lock technique (ALT) has been used to treat bacterial catheter colonization. Current guidelines recommends removal of the catheter when the infectious organism is *Candida* spp., however, removal is not always possible. Our objective was to evaluate the efficacy of different antifungal lock solutions using an in vitro model of CVC infection.
Methods: The following lock solutions were evaluated: fluconazol (FLU) 10 mg/mL, anidulafungin (AND) 1 mg/mL, caspofungin (CAS) 1 mg/mL and liposomal amphotericin B, Ambisome® (AMB) 1.5 mg/mL, all alone and combined with doxicycline (DOX) 5 mg/mL, and Ethanol (ETA) 35%. PBS was used as control. Experiments were performed on full introcan Safety® 14G catheters (Braun Medical, Spain) inoculated with two clinical strains of *C. albicans* and two clinical isolates of *C. parapsilosis*. AL solutions were exchanged every 24 for 72 hour. After 72 hour, catheters were reincubated another 24 hour with fresh media. Catheters were drained, flushed and sonicated at 0, 4, 8, 24, 48, 72 and 96 hour to assess CFU/mL. Scanning electron microscopy was performed to evaluate persistence of biofilm at 0, 72 and 96 hour.
Results: All antifungals resulted in significant reductions ($p < 0.05$) of log10 CFU/mL at 72 hour for both *C. albicans* compared with controls, although FLU did not achieve reductions bigger than 2 log10 CFU. AND and AMB were the most active agents and resulted in significant reductions of log10 for both organism vs. CAS and FLU ($p = 0.001$). Both AND and AMB were able to reduce logCFU below the limit of detection at 72 hour, however neither AND nor AMB prevent regrowth after 24 hour of ALT removal. ETA and DOX combinations showed similar results, but prevented regrowth with AMB and AND. Only AND and AMB resulted in significant reductions of log10 CFU/mL at 72 hour for both strains of *C. parapsilosis*. Neither FLU nor CAS were able to achieve reductions >2 log10 CFU/mL compare to initial inoculum. ETA and DOX combinations did not resulted in a faster sterilization of the lumen but did prevent regrowth after 24 hour of ALT removal.
Conclusion: Our CVC model demonstrated that ALT with 1 mg/mL of AND and 1.5 mg/mL of AMB with either ETA or DOX eradicated biofilm and prevented regrowth suggesting it possible role in ALT and should be explored in clinical trials

R2445 Relationship between multiple arthroplasty revisions for aseptic failure and biofilm-associated infection

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Background: Prosthetic joint infection (PJI) is a biofilm-related disease. Vortex-sonication is used to dislodge biofilm bacteria. The aging population makes that patients will be undergoing multiple arthroplasty revisions (MAR) and it may increase the risk of implant infection. We evaluate the risk of infection when repeated revisions are performed at the affected joint and whether sonication improves the microbiological diagnosis in PJI.
Methods: From February 2009 to September 2011 all consecutive patients undergoing partial or total removal of hip or knee implants (index surgery) were included. We study patients with MAR at the affected joint, whom all of the revisions were performed due to aseptic failure (AF). Retrieved implants were vortexed and sonicated in 400 mL Ringers solution (sonicate fluid). PJI was diagnosed if (1 of the following was present: intraoperative purulence, histopathologic acute inflammation, sinus tract, positive synovial fluid culture or at least two peri-implant tissue cultures positive (same organism) and sonicate fluid culture (100 cfu on either plate.
Results: Of 199 patients with no prior revisions were studied; 46 had PJI (Hip 32, Knee 14) and 153 AF (Hip 94, Knee 59). Sensitivities of peri-implant tissue and sonicate fluid cultures were 48% and 74% ($p = 0.01$), respectively. Sixty-three patients with MAR (1–8) on the affected joint were studied; 25 had PJI (Hip 19, Knee 6) and 38 AF (Hip 24, Knee 14). Sensitivities of peri-implant tissue and sonicate fluid cultures were 68% and 80% ($p = 0.33$), respectively. The infection rate increased from 23.2% to 33.3% to 54.6% to 57.1% as the number of revisions increased from zero to three or more ($p = 0.002$). The risk of PJI in the index surgery was 2.4-fold times higher in patients with two o more revisions than among patients with no prior revisions (RR = 2.4; 95% CI 1.5–3.9) ($p = 0.003$).
Conclusion: Biofilm-sampling technique is useful for the microbiologic diagnosis of PJI. There was a tendency of higher risk of PJI in patients with prior revisions. Two revisions performed at the affected joint is the cutoff point associated with a significant increased risk of PJI in the index surgery.

R2446 Alpha-toxin neutralisation decreases *Staphylococcus aureus* biofilm density and increases vancomycin efficacy in a rat endocarditis infection model

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Objectives: Our lab has previously demonstrated that alpha-toxin (Atox), a cytotoxin, influences *Staphylococcus aureus* biofilm formation on plastics. Further experiments were performed to investigate the effects of Atox neutralization on in vitro and in vivo *S. aureus* biofilm formation. The following describes the results of Atox neutralization on *S. aureus* biofilm formation and the effects of Atox neutralization on vancomycin (VAN) treatment of *S. aureus* biofilms in vitro and in a rat endocarditis (REI) model.
Methods: In vitro biofilm assays were performed with a MBEC assay device that was inoculated with *S. aureus*, treated with the anti-Atox antibody or serum control, and crystal violet stained. MBEC assay biofilms were also treated with titers of VAN, and well-associated cells were harvested and plated to determine VAN susceptibility. The mouse biofilm model involved pre-treating *S. aureus* overnight cultures with the anti-Atox antibody or serum control, infecting Teflon catheter segments, and subcutaneously implanting infected catheters into female mice. The REI model utilized aortic catheterized male rats that were intravenously challenged with *S. aureus* cells pre-treated with the anti-Atox antibody or the serum control. Rats were administered VAN at 6,

24, and 48 hours after infection, and hearts were removed and processed for *S. aureus* counts.
Results: Crystal violet staining of 24-hour biofilms indicated that the density of anti-Atox antibody treated *S. aureus* biofilms was significantly less (p Value <0.0001) than the density of serum control treated biofilms. In the mouse biofilm model, 48-hour CFU counts of catheters infected with anti-Atox antibody treated *S. aureus* were 2.6 log10 CFU lower than the counts associated with serum control treated *S. aureus* infected catheters. VAN susceptibility of 24-hour in vitro *S. aureus* biofilms was increased by eightfold after pre-treatment with the anti-Atox antibody, and when compared to animals infected with serum control treated *S. aureus*, VAN treatment in the REI model reduced 72-hour heart counts by 4.7 log10 in animals infected with anti-Atox antibody treated *S. aureus*.
Conclusions: Neutralizing Atox decreased the densities of in vitro and in vivo *S. aureus* biofilms. Furthermore, anti-Atox antibody treatment increased the susceptibility of *S. aureus* biofilms to VAN, which suggests that the anti-Atox antibody could be utilized in combination with antibiotics to treat *S. aureus* biofilm infections.

R2447 In vitro activity of tigecycline, fosfomycin and colistin against ESBL-producing *Escherichia coli* biofilm investigated by microcalorimetry

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Background: Prosthetic joint infections caused by quinolone-resistant Gram-negative rods are considered difficult to treat. We investigated the activity of tigecycline (TIG), fosfomycin (FOS) and colistin (COL) against planktonic and biofilm ESBL-producing *E. coli* by using conventional microbiological tests and measuring the growth-related heat production (microcalorimetry).
Methods: An ESBL-producing, ciprofloxacin-resistant *E. coli* clinical strain (kindly provided by Pr. Nicolas-Chanoine). In vitro susceptibility testing were performed by macrobroh dilution. *E. coli* biofilm was formed on porous sintered glass beads (diameter 4 mm, pore size 60 μm) in Luria broth (LB) at 37°C. After 24 hour, beads were washed with 10 mL normal saline 3 × and incubated in 2 mL of LB containing serial dilution of TIG, FOS and COL (0.12–256 μg/Ml) for 24 hour. After three washes, beads were placed in ampoules containing 3 mL LB to detect residual bacteria by measuring growth-related heat production at 37°C with a 48-channel batch calorimeter (thermal activity monitor, model 3102 TAM III; TA Instruments, New Castle, DE, USA). The calorimeter measures the heat flow at 37°C controlled to within 0.00001°C and had a sensitivity of 0.25 μW. All experiments were performed in triplicate. The minimal heat inhibition concentration (MHIC) was defined as the lowest antibiotic concentration delaying heat production by >24 hour.
Results: Table summarizes MIC, MBClog, MBCstat and MHIC (in μg/mL) for tested antibiotics.

	MIC	MBC_{log}	MBC_{stat}	MHIC
TIG	0.25	32	32	128
FOS	0.12	0.12	8	<0.12
COL	0.25	0.5	2	32

Conclusions: TIG exhibited a bacteriostatic activity, whereas FOS and COL were bactericidal in the logarithmic and stationary growth-phase. A complete inhibition of biofilm heat production was observed with FOS, while COL and TIG inhibit heat production only at high concentrations. As an alternative to Calgary device method, a challenge/recovery antibiotic activity detection against biofilm with calorimetry seems a repeatable and easy-to-use method. Antibiotic activity against biofilm (alone or in combination) may be evaluated by calorimetry to determine the optimal strategy for eradication of bacteria biofilms

R2448 *Staphylococcus aureus* biofilm formation on fresh, fresh-frozen and processed human cancellous bone grafts

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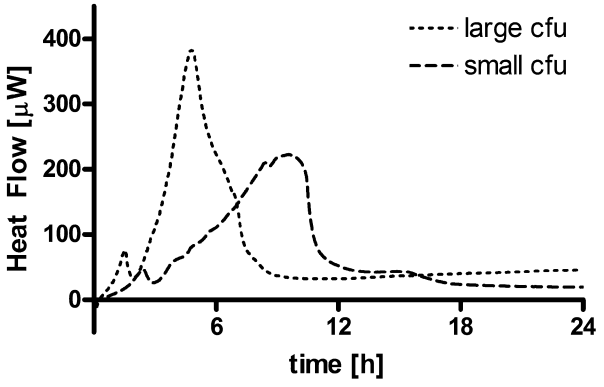
Objective: Bone grafts are often used for filling bone defects. We investigated the characteristics of *Staphylococcus aureus* biofilm formation on fresh, fresh-frozen and processed human bone grafts.
Methods: Standardised cylinders (6.5 × 10 mm) of human cancellous bone grafts were used. Fresh bone grafts were harvested from femoral heads during total hip replacement and transferred in PBS within 2 hour. Fresh-frozen bone graft were frozen at -80°C for 10 days before investigation. Processed BG was sterilized by chemical treatment (osmolysis, NaOH, H_2O_2 and acetone). *S. aureus* (ATCC 29213) biofilm was formed by inoculation of approximately 10^6 CFU in 3 mL TSB containing bone graft sample at 37°C under static conditions for 24 hours. Biofilm characteristics were investigated by sonication at 40 kHz, 0.2 W/cm^2 for 5 minute (morphology and enumeration of removed biofilm bacteria in sonication fluid) and by isothermal microcalorimetry (heat analysis of bone grafts with biofilms). All experiments were performed in five replicates.
Results: Biofilm density was lower on fresh bone grafts (6.7 ± 0.2 CFU/mL) than on fresh-frozen (7.8 ± 0.2 CFU/mL) or processed bone graft (7.6 ± 0.2 CFU/mL) ($p < 0.01$). While fresh-frozen and processed bone grafts grew only one colony morphology, fresh bone grafts grew additional small-colony variants. After re-plating the different colonies, the morphology reverted to normal colony-size. In microcalorimetry, fresh bone grafts showed a double peak corresponding to the two morphologies (Figure), but only one peak was observed with fresh-frozen and processed bone grafts.



Conclusion: Despite structural similarities in the architecture of the three tested bone grafts, marked differences in *S. aureus* biofilm formation was observed. Fresh bone graft was more resistant to biofilm formation than fresh-frozen or processed bone graft, possibly reflecting the presence of host factors such as serum, bone marrow, fibrous tissue and viable bone cells. Observation of small-colony variants in fresh bone graft reflects the interaction between *S. aureus*, viable bone and other host factors. Microcalorimetry might be used for further investigation and understanding of biofilm formation on bone grafts.

R2449 Antibiotic resistance and biofilm in *Stenotrophomonas maltophilia* isolates from cystic fibrosis and non-cystic fibrosis patients

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Objective: To assess antibiotic resistance, biofilm formation ability/efficiency, and their possible relationship among *S. maltophilia* isolates collected from cystic fibrosis (CF) and non-CF patients.
Methods: 86 *S. maltophilia* isolates were investigated: 40 from the sputa of CF patients, and 46 from different sites of non-CF patients. Antimicrobial sensitivity to nine antibiotics was carried out with the agar disk-diffusion technique according to CLSI. Biofilm formation on polystyrene was quantitatively determined by using cristal violet colorimetric assay.
Results: Overall, resistance to erythromycin, imipenem, meropenem, ciprofloxacin, rifampicin, piperacillin/tazobactam, chloramphenicol, levofloxacin and cotrimoxazole was observed in 100%, 98.8%, 87.2%, 80.2%, 72.1%, 50.0%, 47.7%, 26.7% and 18.6% of isolates, respectively. Compared to non-CF strains, CF ones exhibited significantly ($p < 0.01$) higher resistance rates to meropenem (78.6% vs. 63%), rifampicin (78.6% vs. 21.7%), piperacillin/tazobactam (85.7% vs. 71.7%), and chloramphenicol (85.7% vs. 28.3%). Most isolates showed a high level of multidrug resistance (i.e. >50% of strains showed multi-resistance to (6 antibiotics), although no differences in multi-resistance level were found between CF and non-CF groups. CF and non-CF strains showed comparable capabilities of biofilm formation (90% vs. 97.8%, respectively), although the mean biofilm formed by CF strains was significantly lower (OD492, mean ± SD: 0.504 ± 0.350 vs. 0.903 ± 0.813, respectively; $p < 0.05$). Cotrimoxazole, rifampicin, chloramphenicol, levofloxacin, ciprofloxacin, piperacillin/tazobactam, and meropenem-susceptible strains produced significantly ($p < 0.01$) more biofilm than resistant ones. Multidrug-resistance level was negatively associated with mean biofilm formed (Spearman $r = -0.253$; $p < 0.05$).
Conclusions: Our results suggest that: (i) high-level of resistance to meropenem, rifampicin, piperacillin/tazobactam, and chloramphenicol appears to be a trait among CF isolates; (ii) biofilm formation is not a trait among CF strains, although their reduced efficiency in forming biofilm suggests an adaptation to a stressed environment such as CF lung; (iii) high efficiency in forming biofilm may be considered as an alternative strategy adopted by antibiotic-susceptible strains to escape antimicrobial treatments and survive within the host.

R2450 Biofilm production ability of carbapenem-resistant *Acinetobacter baumannii* lineages and blaOXA-24/40-plasmids

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Objectives: Biofilm formation ability has been associated with persistence of *Acinetobacter baumannii* (AB) in the hospital environment and propensity to cause infection, despite the lack of evidences of this feature in epidemic AB carbapenem-resistant (CRAB) lineages. In this study we investigated the ability to form biofilm of disseminated CRAB lineages and the possibility for this feature to be encoded by blaOXA-24/40-carrying plasmids.
Methods: Of 17 isolates producing carbapenem-hydrolyzing Class D β-lactamases (CHDL) were selected from a collection of 213 Portuguese CRAB clinical isolates. They were obtained from bronchial secretions (8); urine (2); catheter (4); intraperitoneal fluid (2) and others (1). Isolates belonged to two different lineages: ST2/ST92-carrying blaOXA-23 (5; 2006–2008) and ST2/ST98-carrying blaOXA-40 (12; 2001–2007). They were resistant to all b-lactams; and presented variable susceptibility to aminoglycosides, tigecycline and colistine. The study also included two transformants harbouring blaOXA-24/40-carrying plasmids and the receptor strain *A. baylyi* ADP1. blaOXA-24/40-carrying plasmids were characterized by a PCR mapping strategy and further sequencing. Adherence was tested with LB Broth cultures incubated in 96-well plates. Biofilm production was confirmed with ultramicroscopic analysis (CLSM and FESEM).
Results: Only one OXA-40 producing AB was classified as non-adherent. The remaining isolates comprising OXA-40 producers and the OXA-23 producers were classified as strongly adherent Confirmation of a complex structure compatible with biofilm production was observed by ultramicroscopic analysis in one blaOXA-24/40-carrying isolate. Nevertheless, transformants of *A. baylyi* ADP1 with plasmids encoding for blaOXA-24/40 and ton-B-dependent receptor (virulence determinant involved in iron uptake) demonstrated identical adherence values (moderately adherent) to *A. baylyi* ADP1 receptor strain.
Conclusion: Isolates of globally disseminated AB (CRAB) lineages demonstrated strong adherence properties and ability to form biofilms in abiotic surfaces which might contribute to their emergence and persistence. In this study we also demonstrate that blaOXA-24/40-carrying plasmids do not contribute to improve adherence and biofilm formation in *A. baumannii*.

R2451 In vitro effect of antimicrobial agents incorporated into resilient denture relines on the *C. albicans* biofilm

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Denture stomatitis associated with Candida is prevalent in 65% of denture wearers. To extend the clinical longevity of resilient denture relines and reduce biofilm accumulation, incorporation of antimicrobial agents into these materials has been suggested. Advantages of this method of drug delivery include no need for patient compliance, simultaneous treatment of injured denture-bearing tissues and candidal infection and reduced application frequency.
Objectives: This study evaluated the antimicrobial action on *Candida albicans* biofilm and determined the minimum inhibitory concentration (MIC) of drugs for treatment of Candida-associated denture stomatitis incorporated into temporary denture relines.
Methods: For MIC determination, *C. albicans* (SC5314) biofilm was formed on disc specimens (10×1 mm) of the relines Trusoft® and Softone® ($n = 6$) modified (experimental) or not (control) by the addition of one of the five tested drugs (nystatin, miconazole, ketoconazole, itraconazole, and chlorhexidine diacetate). For this procedure, standardized *C. albicans* cell suspension (107 cells/mL) was applied to the surfaces of the samples placed in 24-well tissue culture plates. The cells were allowed to adhere for 90 minute at 37°C and non-adherent cells were then removed by washing with PBS. The samples were submerged in RPMI-1640 and incubated at 37°C for 24, 48 hour, 7 or 14 days for biofilm formation. Different dosages of the antimicrobials were tested and cellular viability was determined by spectrophotometric using the XTT [2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide sodium salt] assay at the time periods. The spectrophotometric measurements were converted to percentage reduction in candidal growth and the MICs were determined as the concentrations necessary to inhibit 90% or more of fungal viability.
Results: MICs of the antimicrobials incorporated into both materials were: 0.032, 0.256, 0.128, 0.256 e 0.064 g/mL for nystatin, miconazole, ketoconazole, itraconazole and chlorhexidine, respectively. No fungal inhibition was observed in control specimens.
Conclusion: The incorporation of all tested drugs inhibited the *C. albicans* biofilm on the materials up to 14 days. The lowest MIC values were exhibited by nystatin followed by chlorhexidine. Within the limitations of this in vitro study, it can be concluded that the addition of antimicrobials into denture relines could be a reliable treatment for Candida-associated denture stomatitis.

Antimicrobial pharmacokinetics, pharmacodynamics, pharmacogenomics, pharmacoeconomics and general pharmacology

R2452 Optimal dosing of Daptomycin in the morbidly obese: which body weight is it?

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Objectives: Dosing Daptomycin (DAP) based on total body weight is expected to result in higher drug exposure in the obese patient compared to patients with normal body weight. Prior analysis of patients from a randomized trial suggested the use of lean body weight for dosing of DAP in the obese, but provided no analysis for the population of morbidly obese. The objective of our study was to assess for the bases of optimal dosing of DAP in the morbidly obese.
Methods: Published DAP pharmacokinetic data of matched morbidly obese patients with subjects of normal body weight was used in this analysis. Dosing of DAP based on total (TBW), ideal (IBW), adjusted (ABW), lean (LBW), and fat free (FFW) weight of 4 and 6 mg/kg doses were evaluated with Monte Carlo Simulation (MCS, n = 10000) to assess for the magnitude of drug exposure using the simulated AUC0-24 values. The dosing method that results in the AUC0-24 most similar to that of the matched normal body weight patients was then compared with the exposure achieved by the patients in the randomized trial of bacteremia and endocarditis.
Results: Dosing based on ABW best approximates the exposure provided by DAP at the dose of 4 mg/kg in the morbidly obese when compared to the matched subjects. Simulated mean AUC0-24 values of dosing DAP based on IBW, LBW, and FFW were up to 20% smaller in the morbidly obese. Simulated exposure provided by DAP 6 mg/kg based on ABW in the morbidly obese provides mean ± sd AUC0-24 similar or slightly higher when compared to the population of the bacteremia and endocarditis clinical trial.
Conclusion: We conclude that dosing of DAP based on IBW, LBW, and FFW would result in sub–optimal exposure based on the simulated AUC0-24 values. Since the pharmacodynamic index most predictive of outcomes is the ratio of AUC/MIC, dosing of DAP based on IBW, LBW or FFW should be avoided in this population. Instead, dosing based on ABW should be considered in the morbidly obese.

R2453 PK/PD research of linezolid in different weight Chinese male volunteers

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Study design: This is an open-label phase 1 study involving 20 healthy male participants. The study participants were assigned to two groups according to their body weight: low-weight group (LW): 50 kg < body weight ≤ 55 kg, and high-weight group (HW): body weight (80 kg. The study participants were administered an intravenous single-dose of linezolid (600 mg/30 minute) at constant speed. Seventy-two hour later, all participants were administered an intravenous single-dose of linezolid (10 mg/kg/30 minute) at constant speed.
Objective: Primary, to study features of pharmacokinetics after single dose administration of linezolid injections in different body weight healthy Chinese subjects. Secondary, to obtain serum bactericidal activity at different times after single administration of linezolid injections.
Methods: The study participants were hospitalized for a 9-day/8-night period starting on study Day 1. Testing field located at Unit of Phase I Clinical Trial of National Institute of Clinical Trial For Drugs in PLA General Hospital. Twenty healthy subjects, 10 in LW group and 10 in HW group. HPLC-MS-MS to detect serum concentration of linezolid. Monte Carlo simulation to evaluated probability of target attainment (PTA) of these two linezolid regimens. Main criteria for inclusion: subjects must sign informed consent, male, healthy, aged 18–45 years of age, body mass index (BMI) in the range of 18.0–30.0 kg/m^2, 50 kg < low weight group ≤55 kg, high weight group(80 kg, haven't taken any medicine for at least 4 weeks.
Results: In 600 mg regimen, plasma concentrations (from 0.5 to 16 hour) in LW group were much higher than that in HW group (Cmax: 12002.8 ± 2580.24 vs. 19462.71 ± 3667.33 ng/mL). A persistent inhibitory effect of linezolid trough 7 hour timepoint was observed in LW group. However, inhibitory effect reduced obviously in HW group. PTAs of HW group were much lower than LW group (67.43% vs. 99.76%). While in 10 mg/kg regimen, both HW and LW groups got similar plasma concentrations at each time point (Cmax: 15299.537 ± 1929.733 vs. 16941.891 ± 4704.375 ng/mL). HW and LW groups got similar inhibitory effect. PTAs of HW and LW also showed no difference (98.33% and 97.8%).
Conclusion: Our findings suggest that 10 mg/kg regimen of linezolid may appropriate dosage for different weight patients. However, safety issues should be further evaluated for dosing regimen according to the weight. Register number: ChiCTR-TRC-10001174.

R2454 Voriconazole is stable at high temperatures and released from cement spacers

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Objectives: Voriconazole can be useful for the treatment of prosthetic joint infections due to Candida especially if it can be placed in a spacer. However, its stability at high temperature is not known, and it is an important data, as during cement polymerisation, the temperature can rise up to 80°C.
Methods: We had the opportunity to make in vivo dosage of voriconazole in a patient who presented prosthetic joint infections due to *Candida glabrata* in his both hips. For several reasons (anaesthesia and surgery technique) both removals and spacer insertions were spaced of 1 week. After removal, the surgeon inserted a custom made spacer where he mixed voriconazole powder and cement. The loading voriconazole doses were 600 mg in the right spacer and 400 mg in the left one. The difference was due to the size of the spacer. One week after the second insertion, a needle puncture was performed in both hips, namely 1 week after the insertion of the right spacer and 2 weeks after the left one. The patient did not received intravenous voriconazole during this period. Voriconazole concentrations were measured using high-performance liquid chromatography coupled with a diode array detector method.
Results: Voriconazole was found in both samples. Concentrations were respectively 0.1 mg/L at week 1 and 0.04 mg/L at week 2. The second aspiration needed the instillation of 1 mL of physiological serum, meaning that the concentration measured is far lower than the reality.
Conclusion: (i) Voriconazole was found in the liquid surrounding the spacer, meaning that the heat, produced by the exothermic reaction during polymerisation, did not destroyed it. (ii) Voriconazole is released by the cement spacer. (iii) The concentration observed after 1 week is equivalent to the MIC of some *Candida* species such as *C. albicans*, *C. parapsilosis* or *C. tropicalis*. One could then hypothesise that during the first week the concentrations were over the MIC and thus that the local concentrations obtain could help to cure the patient, in combination with systemic administration of the molecule.

R2455 Simplified oral flucloxacillin absorption test for patients requiring long-term treatment

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Introduction: The absorption of oral flucloxacillin is highly variable between patients. Therefore an oral absorption test is common practice

in our hospital to ensure efficacious continuation of continuous intravenous therapy with an intermittent oral dose regimen. We explored if the test could be simplified

Patients and Methods: In the usual test an oral dose of flucloxacillin is administered while continuous iv flucloxacillin is interrupted (test A) while in the alternative test the iv administration was continued (test B). In both tests serum concentrations of flucloxacillin were measured before and at 1 and 2 hour after the oral test dose. Adequate absorption was defined as an increase in flucloxacillin of at least 10 mg/L after 1 gram at either 1 or 2 hour after oral administration.

Results: The tests were performed in 43 patients with a wide age range (Test A: n = 20; test B n = 23) and identified 10% of the patients as poor absorbers. There was no influence of age, diagnosis, renal function or pre-dose flucloxacillin concentration.

Conclusion: The oral flucloxacillin absorption test without interrupting continuous iv therapy performs well and is simple to implement. The test can be considered in applicable cases to guide transition from iv to oral therapy.

R2456 Pharmacokinetics of high-dose daptomycin in haemodialysis

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Objectives: Pharmacokinetics of daptomycin in patient on haemodialysis has been validated at 4 and 6 mg/kg three times a week. We report two cases where daptomycin at 8 and 10 mg/kg three times a week in patients on haemodialysis was used with concentration monitoring.

Methods: Both patients had conventional haemodialysis during 4 hours three times a week on central venous catheter for chronic kidney failure. Patient A was admitted in the infectious disease ward for a methicillin resistant *Staphylococcus aureus* (MRSA) bacteraemia associated to infected orthopaedic device. Because of multiple arteriovenous fistula thrombosis, CVC had to be saved, and daptomycin was administered at 500 mg (10 mg/kg for a weight of 50 kg) after dialysis three times a week for 14 days with oral rifampicin at 20 mg/kg/day. Patient B was admitted in intensive care unit for a MRSA bacteraemia associated to left endocarditis and spondylodiscitis. Daptomycin was administered at 1 g (8 mg/kg for a weight of 120 kg) after dialysis three times a week for 14 days. Peak concentration, concentration before dialysis (trough before dialysis), concentration after dialysis, area under the concentration curve (AUC) between two doses and minimum inhibitory concentration (MIC) are reported.

Results: The mean (SD) peak/trough were 115.6 (21.2)/16.3 (6.1) and 109.5 (4.5)/19.2 (2.3) μg/mL for patient A and B, respectively. MIC for daptomycin were 0.094 and 0.38 μg/mL for patient A and B. The AUC0-48 hour was 2254 μg/hour/mL for patient A and 2013 μg/hour/mL for patient B. AUC0-72 hour was 2376 μg/hour/mL for patient A and 2971 μg/hour/mL for patient B. The mean reduction ratios due to haemodialysis were 33.5% and 47.5% for patient A and B, respectively. There was no adverse effect, and no increase of CPK. Patients fully recovered from their infection.

Conclusion: In this two patients we reached the recommended target for the peak (approximately 100 μg/mL) and for the 24-hour AUC (approximately 600 μg/hour/mL). The trough was under the recommended limit of 24.5 μg/mL and far above the MIC. Daptomycin could probably be used at high dose in haemodialysis.

R2457 Which is easier to manage? – anxiety of gentamicin-induced nephrotoxicity or gentamicin-induced nephrotoxicity: A surveillance study from a tertiary care cardiac centre in northwest England

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Background: Aminoglycosides have long been recognised for drug-induced nephrotoxicity. Clear recognition of patient and treatment related risk factors including age, pre-existing renal impairment, dose/duration/frequency and therapeutic monitoring [TDM] have improved the situation since early 1980s. However, variation still exists in practice and data on the real impact of this phenomenon. Lancashire cardiac centre [LCC] has 1800 cardio-thoracic [CT] surgeries annually and serves 1.5 million inhabitants in northwest England. We present finding from a prospective study over 3-weeks undertaken to assess gentamicin use, gentamicin TDM and renal functions in emergency and elective cardiac and thoracic surgeries.

Methods: Prospective study of 89 CT surgeries over 3-weeks [September 2011]; gentamicin use, TDM and renal functions [pre and postop]. This study complemented the new prophlaxis policy in CT surgery that replaced previous practice of variable combinations/durations of cefuroxime ± teicoplanin use (subject to surgeon variation), with use of flucloxacillin or teicoplanin, plus gentamicin (3 mg/Kg) at induction/cover over 1st 24 hour postop.

Results: Review of 89 patients included 70.7% [63/89] cardiac and 29.2% [26/89] thoracic surgeries of whom 82.5% [52/63] cardiac/23% [6/26] thoracic patients received gentamicin. Documentation of reason for ommission was missing. No gentamicin detected in blood for 40.3% [22/52] cardiac and 66.6% [4/6]thoracic patients. Remaining thoracic patients and 42.3% [22/52] cardiac patients had a trough <1 mg/L, with 4.5% [1/22] had marginal raise in creatinine. Of 9.6% [5/52] cardiac patients had trough level between 1 and 2 mg and 7.6% [4/52] between 2 and 4 mg/L. Of 13.5% [7/52] had no elevation in creatinine. One patient with gent not detected had elevated creatinine. Four patients had deranged creatinine pre and postoperatively.

Conclusions: The revised guidance on antibiotic prophylaxis for CT surgery followed a drive to reduce use of *C. difficile* driving agents including cefuroxime. None of the patients had any ototoxicity which is the irreversible side effect with aminoglycosides. Elevation in creatinine was reversible when it did increase in patients. There was limited non compliance with using gentamicin as a single dose at induction, documenting reason for omission and missing gentamicin when elevated preoperative creatinine. The results indicate that use of gentamicin as an adjunct with flucloxacillin or teicoplanin in absence of pre-existing renal impairment is safe.

R2458 Factors influencing minimum colistin plasma concentrations in patients with multidrug-resistant Gram-negative bacterial infections

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Objectives: Colistin use has re-emerged for treating multidrug resistant Gram-negative (MDR-GNB) infections but there is a lack of information about which factors can influence its pharmacokinetics. The aim of this study was to correlate the value of the colistin minimum plasma concentration at steady state (Cminss) with patients' characteristics, colistimethate sodium (CMS) dosage and maximum colistin plasma concentration at steady state (Cmaxss).

Methods: Prospective observational pharmacokinetic study performed at a tertiary care hospital in hospitalized patients with MDR-GNB infections treated with different CMS dosages. Cminss (before next CMS dose) and Cmaxss (30 minute after the end of the CMS infusion) were obtained and quantified using a validated HPLC method. Correlations between Cminss and the following variables were analysed: age, body weight, body mass index (BMI), Acute Physiology and Chronic Health Evaluation II score (APACHE II), CMS daily dose (mg/day), serum creatinine and glomerular filtration rate at the beginning of CMS treatment (SCr0, GFR0), at day 3 of CMS treatment (SCr3, GFR3) and at day 4–5 of treatment (just before colistin sample extraction after the steady state had been achieved) (SCr5, GFR5) and Cmaxss. Bivariate correlations were studied by performing the Spearman's rho test (r).

Results: Of 85 patients were included. The bivariate correlations are showed in the table:

	rho de Spearman (r)	P
Positive correlations		
Age (years)	0.385	<0.001
Serum creatinine (mg/dL)		
- SCr_0	0.198	0.069
- SCr_3	0.364	0.001
- SCr_5	0.466	<0.001
CMS dosage (mg/day)	0.266	0.014
$Cmax_{ss}$ (mg/L)	0.970	<0.001
Negative correlations		
Glomerular filtration rate (ml/min)		
- GFR_0	- 0.248	0.023
- GFR_3	- 0.310	0.006
- GFR_5	- 0.457	<0.001

$Cmin_{ss}$ was not correlated with body weight, BMI nor APACHE-II.

Conclusions: Factors influencing minimum colistin plasma concentration at steady state were CMS dosage and age and renal function, mainly after the third day of treatment. These factors should be considered when individual CMS dosage schedule has to be decided. On the contrary, other patients′ characteristics such as body weight, body mass index or severity status did not influence colistin minimum plasma concentration. Minimum and maximum colistin plasma concentration showed a strongly correlation. These findings also suggest the need to closely monitor colistin plasma concentrations in patients treated with CMS.

Mechanisms of action and resistance

R2459 Characterisation of IncFIA, IncFIB, IncF11 and IncN plasmids carrying the CTX-M-15, TEM-1, OXA-1 beta-lactamase genes and the acc (6′)-IB-cr gene in *Escherichia coli*

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Objective: Plasmids have been associated with dissemination of antimicrobial resistance genes in *Escherichia coli* and several studies have shown the association between specific resistance mechanisms and certain plasmid types. We present the distribution and characterisation of Inc F plasmids using the replicon sequence typing scheme (FAB formula) and Inc N.

Methods: Seven cefotaxime resistant *E. coli* strains isolated from blood cultures in the Royal infirmary Edinburgh were examined. Detection of the resistance gene was performed by PCR amplification and subsequent sequencing of the gene. The genetic environments of the CTX M-15 gene were determined by PCR. Plasmids were classified according to their compatibility groups using the PCR replicon typing scheme. Plasmid numbers and sizes were assessed by the alkaline lysis method and S1 nuclease digested linearised plasmid DNA was examined by Pulsed-field electrophoresis (PFGE) profiling. Genotyping of the strains was determined by PFGE with Xba1 restriction.

Results: All isolates were found to have bla(CTX-M-15), bla(TEM-1), bla(OXA-1), and aac (6′)-IB-cr genes. Insertion sequence Isecp1 was found upstream of the bla(CTX-M-15) gene and, in two of the isolates, IS26 was inserted into ISecp1 between bla(CTX-M-15) gene and its normal promoter. Plasmid replicon typing showed four different replicon types; IncF1A (n = 7), IncF1B (n = 5), Inc F11 (n = 7) and IncN (n = 7). On the basis of replicon sequence type (FAB formula) the plasmid groups in two isolates were F2:A1: B-and in the rest F33:A1:B26. PFGE shows that the isolates are genetically divergent. S1 nuclease and alkaline lysis extraction plasmid analysis revealed that all isolates contained detectable plasmid, four of these had seven plasmids but one had eight and two had four plasmids. The plasmid sizes ranged from 3to 194 kb.

Conclusion: Plasmids play a significant role in the dissemination of these genes in *E. coli* since the PFGE showed that these isolates were genetically divergent. These genes were probably mobilised by these plasmids, which can readily spread causing horizontal dissemination of these resistance gene. The co-existence of these resistance determinants in a single isolate increases the selection by one or more of the antimicrobials used in clinical practice.

R2460 Disruption of blaOXA-51-like in clinical isolates of *Acinetobacter baumannii*

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Objectives: Speciation of *Acinetobacter baumannii* using phenotypical methods can be difficult, therefore molecular methods are increasingly used for species identification. One proposed method is the confirmation of blaOXA-51-like, which is intrinsic to this species. We encountered three putative *A. baumannii* clinical isolates that did not amplify blaOXA-51-like when tested by an established OXA-multiplex PCR (Woodford et al., 2006, IJAA). We investigated this phenomenon.

Methods: The isolates were speciated using gyrB multiplex PCR and rpoB sequencing. Susceptibility to imipenem was investigated by Etest. The epidemiological relatedness of the isolates was investigated by rep-PCR (DiversiLab). Analysis of published *A. baumannii* genome sequences allowed the design of primers to amplify and sequence the region surrounding blaOXA-51-like.

Results: Molecular methods confirmed the isolates as *A. baumannii*. The isolates had originated from different countries (Table). Strains 508 and 653 had similar rep-PCR patterns (94.8% similarity) but were considered unrelated. Strain 511 showed <70% similarity to strains 508 or 653. Sequencing blaOXA-51-like revealed that insertion elements had integrated into the gene. In-silico removal of the IS elements allowed the identification of the OXA-51 variants. Two isolates had the novel ISAba19 inserted in blaOXA-78 at nucleotide number 380. ISAba19 is 1309 bp in size, shows 91% DNA homology to ISAba2 and encodes three putative ORFs with 94–97% amino acid identity to those in ISAba2. In strain 511, ISAba15 was inserted in blaOXA-66 at nucleotide number 436. In all strains, the ORF of blaOXA-66 and blaOXA-78 had premature stop codons. The ISAba19 isolates were susceptible to imipenem. Strain 511 (ISAba15 insert) was imipenem-resistant but this strain also possessed an acquired blaOXA-23.

Strain	Country of origin	IPM MIC (mg/L)	OXA-51 variant*	Insertion element	Other OXA present
508	South Africa	0.25	OXA-78	IS*Aba19*	n.d.
653	Turkey	0.25	OXA-78	IS*Aba19*	n.d.
511	South Korea	>32	OXA-66	IS*Aba15*	OXA-23

* OXA-51 variants were identified in-silico; n.d., not detected

Conclusions: These data show that speciation of *A. baumannii* based on the presence of blaOXA-51-like may not always be reliable, therefore additional methods should be employed. These data also show that in clinical isolates, insertional inactivation of the intrinsic blaOXA-51-like is not lethal. Furthermore, a functioning blaOXA-51-like is not necessary for imipenem-resistance.

R2461 Biological fitness cost of macrolide-lincosamide-streptogramin B resistance in *Clostridium difficile*

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Objectives: Antimicrobial therapy plays a central role in the development of *C. difficile* infection (CDI), as this pathogen can colonise the human gut if the normal intestinal microbiota is significantly altered. Clindamycin (CLI), which is part of the macrolide-lincosamide-streptogramin B (MLSB) group, has been recognised as one of the major risk factors for CDI. In *C. difficile*, ermB genes located on mobile elements generally mediate macrolide-lincosamide-streptogramin B (MLSB) resistance. The acquisition of resistance genes also carries a fitness cost influencing the rate of development of resistance, its stability and the rate of its decrease if the antibiotic use is reduced. Here, we evaluated the fitness cost of different mobile elements conferring resistance to MLSB in *C. difficile*.
Methods: Three different macrolide-lincosamide-streptogramin B (MLSB) elements were transferred from resistant clinical isolates to the susceptible *C. difficile* CD13 strain by filter-mating experiments. Minimum inhibitory concentrations (MICs) to CLI and erythromycin (EM) were determined by Etest. Fitness cost of the different elements was assessed by comparison of growth rates of transconjugants and wild-type strain, as well as by competitive growth in vitro. To determine growth curve data, 18 hour cultures of each strain were diluted to an OD600 of 0.05 in BHl, and incubated at 37°C. OD600 was monitored over 12 hours. For competitive assays, 24 hour cultures of one transconjugant and recipient strain were mixed together in a ratio of 1:1. The mixed culture was transferred to fresh BHI broth every 24 hour over 3 cycles. To calculate the competition index, cfu ratio of each strain was determined by plating aliquots on selective and drug-free BHI agar after each cycle.
Results: All mobile elements were transferred to *C. difficile* CD13. Transfer efficiency ranged from 6.1×10^{-7}–2.1×10^{-9} per donor. All transconjugants were highly resistant to EM and CLI (MIC (256 g/L). The acquisition of these elements significantly affected the fitness of the recipient strain, reducing the growth capacities, independently from the genetic organisation of the element acquired.
Conclusion: Results indicate that resistance to macrolide-lincosamide-streptogramin B (MLSB) mediated by ErmB determinant leads to a metabolic cost with a fitness reduction. This could explain the increasing number of ermB-negative isolates resistant to macrolide-lincosamide-streptogramin B (MLSB) circulating in hospitals. At our knowledge, this is the first time that the fitness cost of antibiotic resistance in *C. difficile* is assessed.
This study was funded by the Marie Curie European Project number 237942 CLOSTNET.

R2463 Is daptomycin susceptibility breakpoint appropriate for enterococci?

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Background: The clinical utility of daptomycin (DAP) for the treatment of serious enterococcal infections may be compromised by the emergence of DAP resistance during therapy. The current breakpoint for DAP is ≤4 μg/mL; however, recent data suggest that mutations in genes encoding the LiaFSR system (a three-component regulatory system that orchestrates the cell envelope response to antibiotics) produces slight decrease in DAP susceptibility (MIC between 3 and 4 μg/mL) that may be associated with treatment failures. Our aim was to evaluate the presence of liaFSR mutations in clinical isolates of enterococci exhibiting MICs between 3 and 4 μg/mL.
Methods: Enterococcal isolates recovered from bacteremic episodes were collected from two large hospitals in the Texas Medical Center, Houston, TX. *Enterococcal* species were confirmed by multiplex PCR and DAP susceptibility testing was performed by Etest following the manufacturer's specifications. The liaFSR genes were sequenced in their entirety in all clinical strains with DAP MICs between 3 and 4 μg/mL and in six isolates with MICs ≤ 2 μg/dL. Additionally, DAP MICs were determined in 12 isolates whose genomes have been sequenced and are publically available.
Results: A total of 80 isolates were included in the study. *E. faecium* and *E. faecalis* corresponded to 47% and 53% of the isolates, respectively. The MIC90 for all isolates was 3 μg/mL. A total of eight *E. faecium* and one *E. faecalis* isolate exhibited MICs between three and 4 μg/mL. Mutations in liaFSR system were found in 75% of isolates with MIC 3 and 4 μg/mL, whereas none were found in six clinical isolates with MICs < 2 μg/mL or in any of the sequenced strains of enterococci with MICs < 2 ($p < 0.001$). The most common substitutions were Thr120 for Ala in LiaS and Trp73 for Cys in LiaR.
Conclusions: Clinical isolates of enterococci with DAP MICs between 3 and 4 μg/mL are likely to have mutations in genes encoding the LiaFSR system, which is an important mediator of DAP resistance in vivo. Our data suggest that DAP susceptibility breakpoints should be re-assessed.

R2464 Mechanisms of carbapenem resistance in multidrug-resistant *Klebsiella pneumoniae* isolates

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Objectives: The aim of the present study was to determine the mechanisms of carbapenem resistance among multidrug resistant *Klebsiella pneumoniae* clinical isolates in a tertiary hospital.
Methods: During a 3 year period (December 2008–November 2011), 394 multidrug resistant *Klebsiella pneumoniae* isolates were tested for the detection of KPC-carbapenemase and metallo beta -lactamase (MBL) production. *K. pneumoniae* clinical isolates were collected from blood (76), urine (42), pus (103), catheters (58) and bronchial secretions (192) cultures of patients hospitalized in different wards of our hospital, mainly in ICUs. Identification and susceptibility testing were performed by the Vitek 2 automated system (bioMérieux, France) and MICs were determined by E-test (AB Biodisk, Sweden) when necessary. Phenotypic screening for the presence of carbapenemases was performed with the cloverleaf test. The imipenem-EDTA MBL E-test was used to test for production of MBLs and the boronic acid disc test in combination with meropenem was performed to screen for KPC producers.
Results: All *K. pneumoniae* isolates were multidrug resistant, being resistant to beta-lactams, aminoglycosides, fluoroquinolones and trimethoprim/sulfamethoxazole. The majority of the isolates were susceptible to gentamicin, colistin and tigecycline. Forty-eight (12.2%) isolates were resistant to gentamicin, 148 (37.5%) were resistant to colistin and 64 (16.2%) were resistant to tigecycline. The isolates exhibited various susceptibilities to imipenem and meropenem ranging from 0.5 to 32 mg/L. All isolates were resistant to ertapenem. It should be noted that 37 (9.4%) isolates were initially identified as susceptible to imipenem and meropenem by the Vitek 2 system, but were classified as resistant by the Vitek EXPERT software and were considered 'possible carbapenemase producers'. Of 108 (27.4%) of 394 isolates were MBL producers and the rest 286 (72.6%) were KPC producers. Correlation of MIC values with the two mechanisms of resistance showed that isolates with imipenem and meropenem MICs of ≤1 mg/L produced MBL and KPC at 54% and 46% respectively, while for MICs of >1 mg/L 80% of the isolates were KPC producers.
Conclusion: Multidrug resistant *Klebsiella pneumoniae* isolates, that produce different carbapenemases, pose a most serious therapeutic problem. Isolates with low carbapenem MICs should be routinelly tested so as to accurately detect the presence of carbapenemases and discriminate between the two enzyme types.

R2465 Characterisation of mefA and msrA/msrB multi-drug efflux pumps associated with blaTEM-1 β-lactamase in *Enterococcus faecalis*

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Objectives: *Enterococcus faecalis* is thought to possess a great deal of intrinsic resistance to several antimicrobial agents, in this study, we found ampicillin- and erythromycin-resistant clinical isolates of *E. faecalis*. We attempt to identify the resistance mechanisms among these isolates.
Methods: Twelve isolates of *E. faecalis* collected from 12 different patients were found. Identification and susceptibility pattern were carried out using Phoenix automated phenotypic identification criteria. PCR amplification and sequencing were used to detect β-lactamases production. Colony blotting was performed in order to screen multidrug efflux pumps production. Extraction and N-terminal sequencing of the multidrug efflux pumps were established.
Results: *E. faecalis* isolates shows high resistance to erythromycin and ampicillin with MICs > 16 μg/mL. PCR amplification and sequencing showed that isolates produced TEM-1 beta-lactamase. Colony blotting showed that these isolates harbored multi-drug efflux pumps genes. Multi-drug efflux pumps extraction, purification and sequencing showed the distribution of mefA and msrA/msrB efflux pumps.
Conclusion: Two resistance mechanisms among *E. faecalis* are described, the production of TEM beta-lactamase and mefA and msrA/msrB efflux pumps, these results are very interesting because this is the first report of the co-existence of these resistance mechanisms among *E. faecalis* strains.

R2466 Detection of hlyA and clyA haemolysin genes in clinical isolates of *Escherichia coli* resistant or susceptible to quinolones from blood cultures

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Objectives: Haemolysins are relevant virulence factors of *E. coli*, but most quinolone-resistant clinical isolates of *E. coli* do not produce these proteins. In this study, the phylogenetic group and the presence of genes hlyA and clyA, coding for alpha -haemolysin and cytolisin A respectively, was determined in *E. coli* isolates (resistant or susceptible to quinolones) from blood cultures.
Methods: Four hundred and sixteen isolates of *E. coli* obtained consecutively from blood cultures in our laboratory from January 2006 to December 2008 were studied. Identification was done with MicroScan WalkAway. Susceptibility to nalidixic acid (NAL) was determined by disk-diffusion (CLSI guidelines). Haemolysis production was assessed in 5% sheep blood agar. Phylogroups were determined by multiplex PCR (Clermont et al.). The presence of hlyA and clyA was investigated by PCR using primers hlya-1 GAACTAAAGC TGCAGCAGGTGTTGA, hlyA-2 GCAGCAAGTAAACTGTCACCA TCGT, clyA-1 TAAAATCAGGAAGGAAGCGTATGCC and clyA-2 CGTGTTAGACAGGGTGGTAAAGAAA. Data analysis was performed by SPSS software.
Results: Resistance to NAL was observed in 186 (44.7%) isolates. hlyA and clyA were detected in 88 (21.1%) and 216 (52%) out of the 416 isolates respectively and in another 5 (1.2%) isolates containing both genes (hlyA/clyA). hlyA was detected in 8 (4.3%) NAL-Resistant (R) and in 80 (34.9%) NAL-susceptible (S) isolates ($p < 0.01$). On the other hand, clyA was detected in 127 (68.4%) NAL-R and in 89 (38.7%) Nal-S strains ($p < 0.01$). These values indicate an association between the presence of clyA and NAL-R ($p < 0.01$). The five isolates with both genes corresponded to 1 (0.5%) NAL-R and 4 (1.7%) NAL-S isolates. The 107 isolates lacking hlyA and clyA corresponded to 50 (26.8%) NAL-R and 57 (24.7%) NAL-S isolates ($p = 0.62$). The results of the relationship between phylogroups, NAL testing and haemolysin genes are presented in the Figure 1.

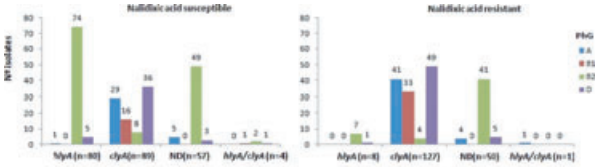

Fig. 1 Haemolysin-encoding genes in *E. coli* isolates from blood cultures. ND= Non detected, PhG= Phylogenetic Group.

Conclusion: *E. coli* from blood cultures produce more frequently clyA than hlyA. In addition, resistance to nalidixic acid is associated with the presence of clyA and not associated with the presence of hlyA. clyA was most frequently detected in isolates of phylogroups A/B1/D than in those of phylogroup B2 which usually contain hlyA and are susceptible to nalidixic acid.

R2468 A study on variable behaviour of *Clostridium difficile* strains to in vitro induction of antibiotic resistance

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Objectives: In order to better understand the influence of metronidazole (MZ), an antibiotic used for treatment, and erythromycin (ERY) or clindamycin (CM), inducing agents of *Clostridium difficile* infection (CDI), on the development of higher MICs on different isolates and different colonies of *C. difficile* strains, in vitro induction assays were performed.
Methods: Twelve *C. difficile* clinical isolates belonging to six different PCR-ribotypes 027, 018, 078, 012, 001, 010, fully susceptible to MZ, ERY and CM were selected for this study. Induction of resistance in presence of MZ was performed in all twelve strains, and in presence of ERY and/or CM in six isolates belonging to the most frequent PCR-ribotypes 001, 027, 078 and 018. The MIC values were firstly evaluated by the Etest method. A further investigation was performed on four parental strains and the respective induced strains, analysing the single colonies' behaviour towards MZ by agar dilution method (ADM), according to CLSI, and by agar incorporation method (AIM) as described by Freeman et al. (2005).
Results: Seven strains belonging to different PCR-ribotypes showed an increase of MIC to MZ after induction but only one achieved a MIC of 8 mg/L by ADM that according to EUCAST is consistent with resistance to MZ. Higher MICs to ERY and CM were easily reached after induction with each of the two antibiotics used separately. All the strains analysed showed a significant MIC increase towards CM, with strains belonging to PCR-ribotype 078 and 027 achieving fully resistance values to this antibiotic. Heterogeneity in the MIC values of the selected colonies studied was found by using the three methods; however a higher diversity of MICs was observed by the ADM. In particular, several colonies showed MICs higher than those detected in the original strains.
Conclusion: These experiments showed that in vitro exposition of susceptible *C. difficile* strains to MZ or ERY/CM can lead to increase of MICs or, in some cases, to resistance. The heterogeneous MICs to MZ observed in isolated colonies of *C. difficile* strains suggest how a recurrent and/or prolonged in vivo administration of this antibiotic could be related with an increase of colonies with higher MICs and with the risk of failure in the treatment of CDI.
This study was funded by the Marie Curie European Project number 237942 CLOSTNET.

R2469 Development of azithromycin resistant-mutant of *Escherichia coli* and *Shigella* spp. in presence of phe-arg-beta-naphthylamide

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Objective: To develop and analyse azithromycin(Azm)-resistant mutants of *Escherichia coli* and *Shigella* spp. in presence of Phe-Arg-beta-Naphtylamyde (PAN), an efflux pump inhibitor.
Methods: Eight clinical isolates of *E. coli* (4) and *Shigella* spp. (4) were spread onto Mueller-Hinton plates containing Azm from 2 to 64 and 20 mg/L of PAN. Previously was evaluating that PAN doesn't affect the viability of the microorganisms. Twenty eight mutants were selected, analyzing the stability of the resistance (20 passages without antibiotic pressure) and the presence of cross-resistance with ampicillin, chloramphenicol, nalidixic acid and tetracycline. Moreover, the mechanisms involved in the development of Azm-resistance like efflux pumps, mutations in the rplD, rplV and rrlH (23S rRNA) genes were searched.
Results: The frequency of mutation ranged between 5.22×10^{-7} and $<6.99 \times 10^{-10}$ for *E. coli* and 1.23×10^{-7} and $<9.99 \times 10^{-10}$ for *Shigella* spp. *E. coli* mutants showed an increase in the Azm MIC of fourfold with one strain achieving a MIC of 256 mg/L. Moreover, *Shigella* spp. presented increases of up to fivefold in MIC levels. All the strains obtained but one (Ec 2.7) showed a stabled Azm resistance. The insertion of six amino acids (IMPRAS) was observed in the rplV gene of Ec 2.7 mutant, which has a MIC of 256 mg/L. This insertion disappears after 20 passages without antibiotic pressure (MIC level decreases to 8 mg/L). No cross resistance with other antibiotics was found. The substitutions H165-Q (rplD) and L46-Q (rplV) were found concomitantly in five *Shigella* spp. mutant strains.
Conclusions: Highly resistant mutants were selected in a single step more easily in *E. coli* than in *Shigella* spp. A low frequency of acquisition of Azm-resistance was found in presence of PAN. Furthermore, the insertion IMPRAS could explain the high MIC level of the *E. coli* mutant Ec 2.7. This insertion reverted in absence of antibiotic pressure.
The association between detected mutations in rplV and rplD genes and resistance to Azm is need to be confirmed by further experiments.

R2470 Characterisation of mobile genetic elements harbouring extended-spectrum β-lactamases and AmpC β-lactamases in clinical *Salmonella enterica* isolates

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Objective: To carry out the plasmid characterization of extended-spectrum-β-lactamases (ESBL) and AmpC beta-lactamase-producing *S. enterica* isolates recovered in three Spanish hospitals during 2003–2009.
Methods: Nine ESBL-positive and two AmpC-positive *S. enterica* isolates [serovars Virchow, 4; Enteritidis, 2; Bredeney, 2; Livingstone, 1; Gnesta, 1 and *Salmonella* spp., 1] were recovered from clinical faecal samples. Seven of these ESBLs were previously characterised. Susceptibility testing to 21 antibiotics was performed by disc-diffusion and microdilution methods (CLSI). The presence of blaCTX-M, blaTEM, blaOXA, blaCMY and blaSHV genes, the blaCTX-M genetic environment and integrons were studied by PCR and sequencing. Clonal relationship among the isolates was studied by PFGE (XbaI and SpeI digestion) and by MLST. Plasmids were analysed by alkaline lysis and S1-PFGE followed by hybridization experiments (blaCTX-M, blaTEM, blaCMY, blaSHV and dfrA12 probes) and typed by PBRT method.
Results: The 11 *S. enterica* isolates of this study carried the genes encoding β-lactamases CTX-M-9, CTX-M-10, CTX-M-14, CTX-M-15, SHV-2, SHV-12 and CMY-2. MIC ranges of beta-lactams detected in ESBL-positive isolates were: cefotaxime (16 to >128 mg/L), ceftazidime (4 to >128 mg/L), aztreonam (4 to >128 mg/L) and cefoxitin (≤8 mg/L). The gene encoding CMY-2 was found in the two AmpC-positive S. Bredeney isolates and they showed the following MIC values: cefotaxime (32 mg/L), ceftazidime (>64 mg/L), aztreonam (8–16 mg/L) and cefoxitin (64 mg/L). PFGE and MLST experiments revealed high clonality and the same ST among *S. enterica* isolates of the same serotype. Table shows the β-lactamases, mechanisms of resistance and integrons detected among the 11 strains. Replicon type and size of plasmids, as well as hybridization results, are also indicated.

Serovar (ST)	β-lactamases	Other resistance genes / Integron	Incompatibility groups detected (pMLST)	S1-PFGE results Size of plasmids (hybridized probes)
Virchow (ST 16)	CTX-M-10	*sul2, strA-strB* / -	HT	297.2 kb (blaTEM-1)
Virchow (ST 16)	CTX-M-10+TEM-1	*aadA7* / -	HT	297.2 kb (blaTEM-1)
Virchow (ST 16)	CTX-M-9+TEM-1	*qnrS*(-), tet(A), *sul2* / Int1	HT	232.2 kb (blaTEM-1)
Virchow (ST 16)	CTX-M-9+TEM-1	*qnrS*(-), tet(A), *sul2* / Int1	HT	-
Salmonella spp. (ST 769)	CTX-M-15+TEM-1	tet(A), *sul2* / Int1	FIB21, A/C	298.4 kb (blaTEM-1, blaTEM-1) (*DfrA12*)
Gnesta (ST 1387)	CTX-M-15+TEM-1	- / -	I1 (ST68, CC31)	174.1 kb (blaTEM-1, blaTEM) 80.7 kb (blaCTX-M, blaTEM)
Enteritidis (ST 11)	CTX-M-14	- / -	I1 (ST105, FIA1, FIB1, FIB22, R	104.5 kb (blaTEM-1)
Enteritidis (ST 11)	SHV-12	- / -	FIA1, FIB1, FIB22	28.7 kb (-)
Livingstone (ST 657)	SHV-2	tet(A) / Int1	I1 (ST25, CC26), F	113.4 kb (-)
Bredeney (ST 396) (2 strains)	CMY-2	- / -	I1	174.1 kb (blaCMY) + 84.2 kb (blaCMY)

NT, non-typable with the PBRT scheme used
-, non-detected

Conclusion: ESBL-positive *S. enterica* isolates contain different plasmids, some of them non typable, although the most frequent incompatibility groups are IncF and IncI1. ESBL genes have been detected in high sized plasmids in *S. enterica* isolates of different serotypes.

R2471 High polymorphism in CMY variants of clonally-diverse clinical and commensal *Citrobacter freundii* and *Citrobacter braakii* isolates

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Objectives: To analyze the polymorphisms of blaCMY gene, probably chromosomally located, in *C. freundii* and *C. braakii* clinical and faecal isolates and to determine their antimicrobial resistance phenotype and genotype as well as their clonal relationship.
Methods: Of 28 *C. freundii* and two *C. braakii* isolates from faecal samples of healthy humans (n = 12) and clinical samples (n = 18) were studied. Antibiotic susceptibility patterns to 25 antibiotics were determined by disc diffusion and agar dilution methods. B-lactam resistance genes (blaTEM, blaSHV, blaCTX-M and blaCMY), blaCMY surrounding regions and characterization of integrons were studied by PCR and sequencing. The presence of 13 resistance genes to quinolones, aminoglycosides and sulfonamides were analysed by PCR. Clonal relationship among isolates was studied by PFGE-XbaI and REP-PCR.
Results: The antibiotic MIC ranges in isolates were as follows (faecal/clinical, in mcg/mL): cefotaxime (0.06 to 0.12/0.125 to 64; ceftazidime 0.025 to 1/0.5 to >256; and cefoxitin 4 to 128/32 > 256. The percentages of resistance to nalidixic acid, ciprofloxacin, trimethoprim, sulphonamides and streptomycin were: 20%, 3.3%, 13.3%, 13.3% and 10%, respectively. All but two Citrobacter isolates presented unrelated PFGE patterns. The blaCMY gene was amplified in all strains, and 26 of them were completely sequenced, detecting 22 new blaCMY variants that were registered at Lahey database. The identity range among detected blaCMY genes was 89–99% (94–99% in case of translated CMY protein). Only the blaCMY-79 variant presented a conservative mutation in H10 helix, whereas none of the remaining variants showed mutations either in Ser84 or Lys87, being these important residues very preserved. The blaCMY-2 variant was found in only one *C. freundii* isolate, and the blaCMY-48, blaCMY-67 and blaCMY-70 variants were detected each one, at least in two isolates. The surrounding regions detected upstream and downstream of all blaCMY genes were ampR and blc genes, respectively. Four strains presented an additional B-lactamase gene (blaTEM in three strains and blaSHV in other one), 11 strains amplified a qnrB gene and two strains

carried a class 1 integron harbouring dfrA12 + orfF+aadA2 and dfrA1 + aadA1 arrangements, respectively.
Conclusion: A high polymorphism was detected in the blaCMY gene of clinical and commensal Citrobacter isolates with high clonal divergence among them. The variant blaCMY-2 was infrequently detected in the studied collection.

R2472 Utilising population analysis to investigate piperacillin/tazobactam differences between broth microdilution and agar dilution for a set of *Escherichia coli*

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Objective: An abbreviated population analysis (PA) was done on 42 *Escherichia coli* (EC) isolates, in order to determine if heterogeneity of resistance expression was a factor in piperacillin/tazobactam (TZP) MIC differences between agar dilution (AD) and broth microdilution (BMD), observed for some of the isolates.
Methods: BMD and AD testing for TZP was performed in triplicate for 42 EC isolates, and a composite MIC (i.e. voted based on the three results) was determined for each method. PA was performed utilizing a range of agar plates prepared with piperacillin at doubling-dilutions from 1 to 256 mcg/mL and tazobactam at a fixed concentration of 4 mcg/mL. PCR for tem, shv, oxa, plasmid-mediated ampC, and ctx-m β-lactamases was done on all isolates.
Results: Twenty EC isolates differed by interpretive category between BMD and AD, utilizing the composite MICs for comparison. When BMD and AD were not in agreement, higher MICs were usually observed for BMD. Seventeen isolates were TZP resistant (R) by both BMD and AD, and five were TZP susceptible (S) by both BMD and AD. Of the 20 isolates that differed between BMD and AD, 85% (17/20) were heterogeneous, and three were homogeneous, by PA. Of the 17 R by both BMD and AD, 65% (11/17) were homogeneous, and six were heterogeneous, by PA. Of the five S by both BMD and AD, three were heterogeneous, and two (including QC EC ATCC 35218) were homogeneous. For the 22 total in category agreement between BMD and AD, 13 were homogeneous (59%), and nine were heterogeneous (41%). The rates of heterogeneity and homogeneity for the respective sets (BMD vs. AD not in agreement, and BMD vs. AD in agreement) were significantly different based on Chi-square analysis p-values of 0.003 and 0.010, respectively. Most isolates (28 of 42) harbored only tem-1.
Conclusion: The majority of isolates with differences between BMD and AD demonstrated heterogeneous growth by PA. When BMD and AD were in agreement, the majority of isolates demonstrated homogeneous growth. PA data support the conclusion that TZP MIC differences between BMD and AD are primarily due to heterogeneity of resistance expression. Further study of the mechanism of TZP resistance for EC harboring tem-1 is in progress.

R2473 Analysis of the mechanisms of resistance to azithromycin and its transferability in clinical isolates of *Escherichia coli* and *Shigella* spp. clinical isolates from Lima, Peru

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Objective: To analyse the mechanisms of resistance to azithromycin (AZM) and its transferability in *Escherichia coli* and *Shigella* spp. clinical isolates from Lima, Peru.
Methods: The Minimal Inhibitory Concentration (MIC) to AZM was determined in 71 clinical isolates (62 *E. coli* and nine *Shigella* spp.) exhibiting a halo to AZM <15 mm. The role of efflux pumps was tested establishing the MIC levels in presence of Phe-Arg-beta-Naphtylamyde (PAN), an inhibitor of efflux pumps. Point mutations in rplD and rplV genes were observed by PCR and sequencing. The presence of 10 established mechanisms of resistance to macrolides (ereA, ereB, msrA, ermA, ermB, ermC, mefA, mefB, mphA and mphB) was searched. Conjugations assays were performed in order to evaluate if plasmid mechanisms are transferable.
Results: The MICs of AZM ranged between 32 and >256 mg/L among *E. coli* isolates and 4 e 8 mg/L among *Shigella* spp. When PAN was added to the media the MICs levels decreased from 1 to eightfold. In seven strains were found substitutions in the rplV gene (K82-N, D-94H, K98-N; L46-Q in two isolates; S101-T, I103-L; I4-L, K6-Q and P80-S in two isolates of *Shigella* spp.). The most frequent plasmid mediated gene was mphA (present in 59% of *E. coli* isolates and 11% of *Shigella* spp.). Also the genes mphB, ermA, ermB, ereA and mefA were found among the isolates. The conjugation assays showed that 25.8% of *E. coli* isolates were able to transfer the AZM-resistance. Thus three genes (mphA, ermA and ermB) were present within conjugative elements. One *E. coli* isolate without any of the searched transferable AZM-resistance mechanisms was able to transfer the AZM-resistance to recipient strain.
Although no analysed plasmid mediated mechanism of resistance was found among *Shigella* spp.
Conclusions: PAN-inhibitible efflux pumps play a role in development of AZM resistance. The possible association between detected mutations in rplV gene and resistance to azithromycin may not be ruled out and need to be confirmed by further experiments. In the area several transferable AZM-resistance mechanisms are present, being the mphA the most disseminated. The AZM should be carefully used in Lima.

R2474 Phenotypic and molecular characterisation of CMY-46 and CMY-50, two novel plasmid-mediated AmpC beta-lactamase carried by *Escherichia coli*

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Objectives: The identification of isolates containing AmpC β-lactamases is epidemiologically and clinically relevant. With this study we performed the phenotypic and molecular characterization of two new CMY-2-types, designated CMY-46 and CMY-50, encountered among a total of 1664 clinical non-duplicate isolates of various *Enterobacteriaceae* species.
Methods: *E. coli* INSRA1169 and INSRA3413 were isolated from the urine of patients with 77 years and 7 months old, hospitalized in the ward and in pediatrics, respectively. The blaCMY genes were cloned in the plasmid pBK-CMV and transformed into electrocompetent *E. coli* DH5 alpha delta ampC by electroporation. Antimicrobial susceptibility (MIC) was determined by a microdilution method. *E. coli* INSRA6015, a CMY-2-producer, was used for phenotype comparison. PCR-mapping of the genetic environment of new blaCMY genes was performed using primers for known antibiotic and mercury resistance genes.
Results: Antimicrobial susceptibly tests showed that all isolates and respective transformants were nonsusceptible to amoxicillin, amoxicillin plus clavulanic acid, cephalothin, cefoxitin, ceftazidime and cefotaxime. INSRA1169 and INSRA6015 were also nonsusceptible to ciprofloxacin and to trimethoprim. Regarding gentamycin, only INSRA1169 was resistant. Its noteworthy that the transformants EcDH5a(pBK-CMY-2) and EcDH5a(pBK-CMY-46) exhibited higher values for extended-spectrum cephalosporins than the respective isolates. All strains were susceptible to cefepime and imipenem, showing synergy between cloxacilin and cefoxitin and/or ceftazidime. No phenotypic alterations were found comparing the new CMY-type with the parental CMY-2. The genetic characterization of CMY-46 and CMY-50-encoding genes revealed a *Citrobacter freundii* chromosome-type structure, encompassing a blc-sugE-blaCMY-2-type-ampR platform in both isolates. In addition, a sul1-type class 1 integron and a truncated mercury resistance operon were encountered.
Conclusion: Although the CMY-type enzymes studied conferred resistance to extended-spectrum cephalosporins, the susceptibility to cefepime lead us to assume that those enzymes are not extended-spectrum cephalosporinases. Otherwise, the presence of three genetic resistance-encoding regions is of great concern, namely the truncated

mercury resistance operon, which may help to promote antibiotic resistance through indirect selection.

Resistance surveillance

R2475 First report of KPC beta-lactamase in *Klebsiella pneumoniae* isolate from Croatia

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Objectives: The aim of the study was to characterize carbapenem resistance in *K. pneumoniae* from Zagreb, Croatia.
Material and Methods: In February 2011. A 78 old male patient was admitted to Clinical Hospital Center Zagreb with subdural haematoma. He was previously diagnosed with acute myeloblastic leukemia. After surgical removal of haematoma he developed purulent meningtis. *K. pneumoniae* with reduced susceptibility to carbapenems was isolated. The patient died from intracerebral bleeding in April 2011.
The antimicrobial susceptibility to a wide range of antibiotics was determined by broth microdilution method in Mueller-Hinton broth and 96 well microtiter plates according to CLSI guidelines. A double-disk-synergy test was performed to detect ESBLs.
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 transferability of meropenem resistance was determined by conjugation (broth mating method) employing *E. coli* A15R-strain resistant to rifampicin. Transconjugant was selected on the combined plates containing meropenem (1 mg/L) and rifampicin (128 mg/mL). The presence of genes encoding ESBLs (blaSHV, blaTEM, blaCTX-M), plasmid mediated ampC β-lactamases and carbapenemases blaKPC, blaOXA-48, blaOXA-NDM, blaVIM and blaIMP was determined by PCR.
Results: The isolate showed resistance or intermediate susceptibility to expanded-spectrum cephalosporins, beta-lactam combinations with inhibitors, carbapenems and gentamicin but remained susceptible only to ciprofloxacin and colistin. Modified Hodge test was consistent with the activity of carbapenemases. The MBL test for metallo-beta-lactamase was negative indicating the absence of metallo beta-lactamase. Imipenem resistance was not transferred to *E. coli* recipient strain by conjugation. PCR revealed the presence of blaKPC, blaTEM genes and blaSHV genes. Sequencing of blaKPC gene revealed the presence of KPC-2 beta-lactamase. Neither plasmid-mediated AmpC beta-lactamase nor OXA-48 beta-lactamase were found. The strain was found belong to ST37 clone by MLST.
Conclusions: Infection control efforts limited the spread of KPC-producing clone of *K. pneumoniae* in our hospital so far. KPC-2 beta-lactamase with similar properties was previously reported from USA, United Kingdom, Israel and Greece. To our best knowledge, this is the first report of KPC beta-lactamase from Croatia.

R2476 Evaluation of the antimicrobial resistance of *Pseudomonas aeruginosa* at a clinical hospital Osijek, Croatia

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Objectives: *Pseudomonas aeruginosa* (PA) is an important nosocomial pathogen causing a wide varriety of infections. The aim of this study is to evaluate the resistance rate of PA isolates between years April 2003 and October 2009, from patients hospitalised at the Clinical Hospital Osijek (1250 beds), Croatia.
Methods: Of 1.531 isolates of PA were tested during 2003/2004, and 1.369 during 2009/2010. The antimicrobial susceptibility was determined by the Kirby-Bauer disc diffusion method and E-test when necessary. Results were interpreted according to CLSI. The isolates were tested for amikacin (AN), gentamicin (GM), ciprofloxacin (CIP), piperacillin/tazobactam (TZP), ceftazidime (CAZ), imipenem (IPM) and meropenem (MEM). The statistical analysis was performed using the chi-square test.
Results: After comparing the resistance rate of PA in the period of April 2003–October 2009, the increasing rate of resistance with statistical significance for tested antimicrobial agents were found as follows: AM 10.84%/18.19% ($p < 0.01$), CIP 25.08%/30.39% ($p < 0.05$), CAZ 1.82%/3.21% ($p < 0.05$), IMP 6.92%/19.28% ($p < 0.01$), MEM 6.92%/17.82% ($p < 0.01$), TZP 1.96%/6.72% ($p < 0.01$). The resistance decreased only for GM 45.33%/39.01% ($p < 0.05$).
Conclusion: According to the mentioned ongoing increasing resistance rate, we can conclude that the number of therapeutic options has continuously been decreasing. Ceftazidime is still the best therapeutic agent for PA infections, due to its low resistance rate. On the other hand, fluoroquinolones and karbapenems show substantional resistance increase which we can contribute to the excessive and irrational usage of broadspectrum antibiotics. The decrease in gentamicine resistance was because gentamicine was not used in the therapy of PA and it was generally used less in last few years because of its high resistance, so it recovered susceptibility. Although there is the increasing rate of resistance, we can still be satisfied that the resistance rate for PA is lower if compared to some other European countries. It is important to continue the monitoring of the antimicrobial resistance in treatment of pseudomonas infections with effective infection control measures, in order to limit the development and spreading of resistance, followed by good clinical practise.

R2477 Fluoroquinolone-resistant urinary isolates of *Escherichia coli* from nosocomial vs. community-acquired infections

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Objectives: Community-acquired (CA) and healthcare-associated (HA) urinary tract infections (UTI) have been associated with high rates of morbidity, and pose a significant economic burden to healthcare systems all over the world. *Escherichia coli* is the primary causative agent of UTI but its susceptibility profile has changed over the last decade. Fluoroquinolones, such as levofloxacin, norfloxacin or ciprofloxacin, are now recommended for the empirical treatment for UTI. However, the increased use of fluoroquinolones has resulted in the rapid emergence of fluoroquinolone-resistant *E. coli*, making the medical community skeptic as to whether fluoroquinolones should remain the drugs of choice for UTI. The purpose of this study was to evaluate the prevalence of fluoroquinolone-resistant *E. coli* in CA-UTI and compare it to cases of HA-UTI.
Methods: We studied all episodes of CA-UTI and HA-UTI, diagnosed in our hospital, due to *E. coli* during the period January 2009 to September 2011. HA-UTI was defined as those UTI affecting patients hospitalized for two or more days. Urine samples were obtained from clean-catch mid-stream urine or from urinary catheters and cultured on Blood agar and MacConkey agar followed by incubation for 24 hour at 37°C. Positive urine cultures were defined by bacterial grow (10^5 colony forming units/mL. Patients with polymicrobial urine cultures were excluded from the study. Identification of *E. coli* was performed by means of standard methods and susceptibilities to ciprofloxacin, norfloxacin and levofloxacin were tested by agar disk diffusion method according to the CLSI criteria. Intermediate and resistant *E. coli* strains to either of the antimicrobials studied were grouped together for data analysis.
Results: We obtained 119 *E. coli* isolates from an equal number of hospitalized patients and 321 from patients attending the Outpatient Clinic of our hospital. Out of the 119 *E. coli* strains isolated from HA-UTI, the resistance to ciprofloxacin, norfloxacin and levofloxacin was 16.8%, 15.1% and 17.6%, respectively. The respective percentages for the 321 *E. coli* strains isolated from CA-UTI were 14.0%, 14.0% and 13.1%, respectively.
Conclusion: Although fluoroquinolones are considered an optimal therapeutic choice in UTI, care should be given before initiating empirical treatment with these agents, at least until antimicrobial susceptibility tests become available for the clinicians.

R2478 Resistance to ciprofloxacin and gentamicin in *Pseudomonas aeruginosa* isolated from ear

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Objective: *Pseudomonas aeruginosa* is a major cause of otitis externa. The aim of this work is to know the susceptibility to ciprofloxacin end gentamicin in *Pseudomonas aeruginosa* isolated from ear.
Methods: All *P. aeruginosa* isolated in our Hospital from January 2006 to October 2011 in ear swab samples were tested to ciprofloxacin and gentamicin. Data of susceptibility of the strains and age and sex of the patients were recorded on LIS. Susceptibility testing were made by disk diffusion method following annual criteria published by CLSI.
Results: Of 635 *P. aeruginosa* were isolated from ear during the six year period. Of 273 (43%) from females and 362 (57%) from males. Ninety-five were isolated from elderly people 331 from adults and 209 from infants. The global rate of resistance was 6.1% to ciprofloxacin and 1.7% to gentamicin. For infants, only four strains (1.9%) were resistant to ciprofloxacin and none to gentamicin. For adults these percentages were 6% and 1.5% and 15.8% and 6.3% in elderly people. During 2006 to 2011 the global data of resistance were 13.3%, 9.6%, 3%, 5.8%, 2.3% and 5.1% to ciprofloxacin and 2.6%, 4.1%, 1%, 0.8%, 1.6% and 1% for gentamicin. For children 5.9%, 6.3%, 0%, 0%, 1.9% and 0% for ciprofloxacin and 0%, 0%, 0%, 0%, 0%, 0% for gentamicin. Adults: 5.4%, 8.7%, 5.6%, 9.1%, 3.4% and 4% for ciprofloxacin and 1.8%, 4.3%, 1.9%, 0%, 1.7% and 0% for gentamicin. Elderly: 37.5%, 18.2%, 0%, 7.1%, 0% and 15% for ciprofloxacin and 8.3%, 9.1%, 0%, 7.1%, 6.3% and 5% for gentamicin.
Conclusion: The rate of resistance to gentamicin both global and stratified by age group remains largely unchanged during the study period but for ciprofloxacin data show a tendency to decrease first and then to stabilize. These data do not match those contained in our records of *P. aeruginosa* isolated from other locations. Mostly of *P. aeruginosa* isolated from ear are from the community unlike in other locations isolated from hospital environment, nursing homes or from patients with underlying diseases. The data presented indicate that both ciprofloxacin and gentamicin are useful in the treatment of otitis externa, and we have to investigate possible causes of differences in antibiotic susceptibility between *P. aeruginosa* isolated from ear and the rest of them.

R2479 Prevalence and antimicrobial resistance pattern of urinary tract pathogens in northern Cyprus

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Objective: Antimicrobial resistance is the major problem for the treatment of urinary tract pathogens (UTPs) in the worldwide. The aim of this study was to determine prevalence and antimicrobial resistance pattern of urinary tract pathogens in North Cyprus.
Methods: This cohort study was performed on positive urine culture samples which were evaluated in the microbiology laboratory of Near East University Hospital between September 2010 and September 2011. Urine samples were cultured on blood agar and EMB mediums. Colonies were counted after 24 hours at 37°C and samples having colony count more than 100 000/mL were considered to be positive. BD Phoenix 100 (Becton Dickinson, USA) system were used for bacterial identification and antimicrobial susceptibility. Commercially available statistical software package was used for analysis.
Results: A total of 174 urine samples were found to be culture positive. One hundred and thirty one patients (75.3%) were female while 43 (24.7%) were male. The mean age of the patients was 40.8. The five most common organisms isolated were: *Escherichia coli* (60.9%), *Proteus mirabilis* (9.8%), *Klebsiella pneumoniae* (9.2%), *Pseudomonas aeroginosa* (5.2%) and *Klebsiella oxytoca* (3.4%). *E. coli* was found to have higher resistance to Trimethoprim-Sulfamethoxazole (45.8%), Ampicillin-Sulbactam (37.7%) and Ciprofloxacin (34.9%) and no resistance to Imipenem (0%). *K. pneumoniae* showed equal resistance to Trimethoprim-Sulfamethoxazole (31.3%) and Piperacillin-Tazobactam (31.3%).
Conclusion: Antimicrobial resistance for common UTPs was found to be lower than expected in this study. We suggest that the low resistance ratio is due to limited usage of wide spectrum antibiotics here in North Cyprus. To our knowledge this study is the first report in English literature for the prevalence of UTPs in North Cyprus. More studies are needed to explore whether this resistance pattern will change in the following years.

R2480 Optimal empirical therapy for male urinary tract infections in Dutch general practices

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Objectives: To optimize empirical treatment of male urinary tract infections (UTIs) in Dutch general practices, we determined the antimicrobial susceptibility of Gram-negative uropathogens, including extended-spectrum beta-lactamase (ESBL) prevalence, and the antimicrobial prescription rates for male urinary tract infections (UTIs).
Methods: From January 2009 to June 2011, general practitioners (GPs) participating in the Dutch Sentinel General Practice Network (n = 42) collected urinary samples from male patients ((18 years) with symptoms indicative of UTI and recorded prescribed antimicrobial treatment. Uropathogens were identified and antibiotic susceptibility of Gram-negative uropathogens was determined.
Results: Of 603 urinary samples were collected, of which 390 (65%) were positive ((10^3 CFU/mL). The majority (83%) of the isolated uropathogens was Gram-negative with *Escherichia coli* being the most commonly isolated (51% of all uropathogens) followed by non-fermenters (10%), *Klebsiella pneumoniae* (6%) and *Proteus mirabilis* (5%). High susceptibility rates were observed to ciprofloxacin, norfloxacin and nitrofurantoin (94%, 92% and 89% respectively), whereas amoxicillin (52%), co-amoxiclav (77%) and co-trimoxazole (82%) showed lower rates. One ESBL (0.3%) was found. An antimicrobial agent was prescribed to 379 (63%) men. Fluoroquinolones (30%) and co-amoxiclav (27%) showed highest prescription rates followed by nitrofurantoin (22%) and co-trimoxazole (15%).
Conclusions: Based on current susceptibility rates, fluoroquinolones are the antimicrobial agents of first choice for male UTIs in Dutch general practices. ESBL prevalence was low. Antimicrobial susceptibility to co-trimoxazole and co-amoxiclav should be carefully monitored, given their low susceptibility rate and relatively high prescription rates. Nitrofurantoin showed high susceptibility rates, although the appropriateness of this antimicrobial agent for male UTIs still needs to be established in clinical trials.

R2481 Trends in the antimicrobial resistance of *Streptococcus pyogenes* during 2007–2011 in a Kuwait hospital

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Objective: To examine select antimicrobial susceptibilities for *Streptococcus pyogenes* (*S. pyogenes*) responsible for acute tonsillitis among the patients in a Kuwait hospital for the years 2007–2011. This study was conducted to evaluate the trends in the antimicrobial resistance of *S. pyogenes* isolates and its implications for empiric therapy.
Methods: Antimicrobial susceptibility of 162 strains of *S. pyogenes* isolated from throat cultures during 2007–2011 was performed by disc diffusion method and results interpreted using CLSI (M2, M7, M100, 2010) breakpoint criteria. The results were compare for 2007–2008, 2009–2010 and 2011 (up to month of October).
Results: The prevalence of resistance for the respective periods were as follow: penicillin 6.7% and 5.6%; ampicillin 33.4% and 50%; erythrom ycin 19.1%, 26.7% and 38.9%; clindamycin 7.2% and 23.1%;

ciprofloxacin 13.4% and 25%. The data of intermediatidy were as follow: penicillin 14.3%, 6.7% and 38.9%; amoxicillin/clavulanic acid 9.5%, 13.3% and 11.1%; erythromycin 47.6%, 26.7% and 11.9%; clindamycin 7.7% and 16.7%; ciprofloxacin 53.3%, 50% and 62.5%. All isolates were susceptible to cefuroxime, ceftriaxone and cefotaxime.
Conclusions: Monitoring of drug resistance of local isolates of *S. pyogenes* is important, since most streptococcal infections are treated on an empirical basis, with increasing reliance upon second, third-generation cephalosporines and beta-lactam/beta-lactamase inhibitor combinations. Continued longitudinal comparisons of worldwide monitored and important pathogen – *S. pyogenes* and changing susceptibility profiles are critical elements in guiding empiric therapies and epidemiologic interventions.

R2482 Frequency and antimicrobial susceptibility of *Pseudomonas aeruginosa* isolated from Makkah hospitals, Saudi Arabia

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Objectives: *Pseudomonas aeruginosa* is the most prevalent pathogen that cause nosocomial infection associated with higher mortality rate in hospitalized patients. This pathogenic bacteria produces different enzymes including metallo-β-lactamases (MBLs) and extended spectrum-β-lactamases that reduce the effectiveness of antibiotics. The aims of this study were to determine the frequency of *P. aeruginosa* isolated from various clinical specimens in different wards of Makkah hospitals. In addition, antimicrobial susceptibility patterns and types of MBLs were also determined.
Methods: A total of 478 *P. aeruginosa* clinical isolates were collected during a period of six months starting September 2009 from clinical wards of Al-Noor Specialist Hospital, Hera General Hospital and King Abdul-Aziz Hospital. All clinical isolates were investigated by routine microbiological methods and antibiotic susceptibility was performed by using automated instruments; Phoenix 100 BD, USA and MicroScan Walkaway 96, Siemens, Germany. MBLs production was determined according to Clinical and Laboratory Standards Institute and confirmed using double disk synergy test. Types of MBLs were detected by using polymerase chain reaction.
Results: A 31% of *P. aeruginosa* were isolated from intensive care unit followed by male medical ward (15.9%). The majority of infections caused by *P. aeruginosa* were respiratory tract infection (52%) followed by wound infection (26%) and urinary tract infection (12%). Higher susceptibility of *P. aeruginosa* was shown against piperacillin (65%), amikacin (64%), merobenem (62%) and imipenem (61%). MBLs producing strains were identified in 31 out of 148 (21%) of *P. aeruginosa* and the most frequent MBLs types found were IMP (22.6%) and VIM (19.4%).
Conclusion: *P. aeruginosa* cause a several nosocomial infection in patients who are hospitalized in different hospital wards in particular intensive care units of Makkah hospital. Antibiotic resistance is increasing overtime rendering the difficulties for patient's treatment. Continuous monitoring of antimicrobial susceptibility is recommended to help to reduce its resistance to antibiotics problem in the future.

R2483 Clarithromycine resistance of *Helicobacter pylori*: prospective study among patients in the northern center of Morocco

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Objective: The aim of our study is to determine the clarithromycine resistance rate of *H. pylori* strains isolated in Moroccan patients with gastric pain.
Method: A prospective and randomized study including adults consenting patients (n = 486) who underwent endoscopy in gastro-enterology unites has been conducted. *H. pylori* detection has been done using Polymerase chain reaction (PCR), histological examination, rapid urease test and culture, directly from collected gastric biopsies. *H. pylori* clarithromycine resistance assessment has been performed on bacterial cultures by disc diffusion technique. A reel times PCR has been done on 41 biopsies randomly selected to confirm our results (detection and clarithromycine sensibility separately).
Results: *H. pylori* infection has been detected by PCR in 65% of cases. From the 144 obtained cultures, 25% were clarithromycine resistant using agar diffusion technique. Only 41 specimens were tested by reel time PCR. The obtained results confirmed those obtained both in detection (by simple PCR) and in clarithromycine resistance. Samples free from *H. pylori* by PCR and positives by histological examination and/or rapid urease test were also negatives by reel time PCR.
Conclusion: The rate of clarithromycine resistance (25%) is concordant with that determined in France (26%) but steel higher than Tunisian one (14.6%). This rate will be confirmed by reel time PCR in larger sampling.

R2484 Aetiological agents of infective endocarditis in Saint Petersburg, Russia

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Objectives: The aim of the present study was to evaluate the spectrum of microorganisms – causative agents of infective endocarditis, and their resistance to antibiotics.
Materials and Methods: Cardiac valves of patients with infective endocarditis were removed during cardiosurgical operations. Valves were homogenized in aseptic conditions and examined microscopically. The cultures were obtained with BactAlert (BioMerieux, France). Identification of bacteria was performed with traditional bacteriological methods and sequencing of 16sRNA gene (ABI Prism 3130, MicroSeq ID v2.0 Software, MicroSeq ID 16s rDNA500 Library v2.0). Susceptibility to antibiotics was tested by agar-dilution method on Muller-Hinton agar (Oxoid, GB).
Results: Totally 51 bacterial strains were isolated from valves (four mitral and 46 aortic), obtained during cardiosurgical operations in patients with endocarditis. In the majority of cases a single bacterial species was isolated, in one case – two species. *Staphylococcus epidermidis* was the most frequent isolate – and was revealed in 24 (47.05%) cases. Other gram-positive cocci were presented with *Staphylococcus aureus* in 9 (17.64%), *Micrococcus luteus* in 3 (5.88%), *Streptococcus saprophyticus* in 2 (3.92%), *Enterococcus faecalis* in 2 (3.92%), *Streptococcus salivarius* in 2 (3.92%), *Streptococcus mitis* in 3 (5.88%) cases. Gram-negative rods were isolated in 2 (3.92%) cases – *Enterobacter cloacae* in 1 and *Acinetobacter baumannii* in 1. Anaerobes were presented with *Propionibacterium acne* in 2 (3.92%) cases. Rare bacteria were Rothia amarae in 1 (1.96%) and Neisseria subflava in 1 (1.96%) case. Resistance to antibiotics was detected in 25 (49.02%) strains. Polyresistant were 7 (13.72%) isolates. The majority of strains were resistant to 1 (23.52%) or 2 (11.76%) antibiotics. Sensitive to all antibiotics were 26 (50.98%) isolates. All Enterococcus strains were susceptible to vancomycin. No resistance to meticillin was observed in *Staphylococcus aureus*. Meticillin-resistant were 6 (25.0%) of *Staphylococcus epidermidis* isolates.
Conclusions: (i) The majority of bacteria, isolated from the excised valves of patients with infective endocarditis, were *Staphylococcus* spp. (ii) Resistant to antibiotics was half (49.02%) of bacterial strains. (iii) Polyresistance to antibiotics was observed rarely. Resistance to meticillin was present in ¼ of *S. epidermidis* isolates.

R2485 *Helicobacter pylori* resistance to metronidazole and clarithromycin in dyspeptic patients in Iran

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The resistance of *H. pylori* to the recently available antibiotic treatment regimens has been a growing problem. The prevalence of high antibiotic resistance of *H. pylori* is the most common reason of its eradication failure. The purpose of the present study is to determine the prevalence of antibiotic resistance among *H. pylori* strains isolated from Iranian patients. We investigated the prevalence of *H. pylori* resistance to metronidazole, clarithromycin, amoxicillin, and tetracycline among 128 *H. pylori* isolates from Iranian patients. After the culture of biopsy specimens and identification, susceptibility tests was performed with Modified Disk Diffusion Method (MDDM) and E-test. Resistance rates to metronidazole, clarithromycin, amoxicillin and tetracycline were 64%, 23%, 2.5% and 0%, respectively. Seventy two percent of the metronidazole resistance strains had MIC>256 mg/mL (High-Level-Resistance). Due to the increasing rate of antibiotic resistance in *H. pylori* strains and in order to decrease the treatment cost, testing of susceptibility to metronidazole and clarithromycin is recommended.

R2486 Spectrum and antibiotic resistance dynamics of uropathogens isolated from pregnant women with community-acquired urinary tract infections in Russia: 2002–2011

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Objectives: To evaluate the dynamics of the resistance of *Escherichia coli* isolates from pregnant women with community-acquired urinary tract infections (CAUTIs) in different regions of Russia during the 9-year period.
Methods: A total of 152 and 186 uropathogens were isolated from urine samples from pregnant women with significant bacteriuria (>10^5 CFU/mL) as a part of two multicenter studies in 2010–2011 and 2002, respectively. Susceptibility of strains was determined by agar dilution and interpreted according to the EUCAST criteria (2011).
Results: Among the identified microorganisms the most frequent were Enterobacteriaceae members [135 isolates in 2010–2011 (88.8%) and 158 isolates in 2002 [83.2%]) out of which there were 100 (65.8%) and 117 (61.6%) *E. coli* strains, respectively. Resistance rates of *E. coli* in 2010–2011 and 2002 are summarized in the Table.

Table. Susceptibility of *E. coli* isolated from pregnant women with CAUTI: 2002-2011

Antibiotic	Year	I+R,%	p value (χ^2 test)	MIC50, mg/L	MIC90, mg/L
Ampicillin	2002	31.7	0.0138*	4	256
	2010-2011	48.0		8	256
Amoxicillin/clavulanate (2:1)	2002	-	-	4	16
	2010-2011	-		8	32
Amoxicillin/clavulanate (2mg/L)	2002	NA	-	NA	NA
	2010-2011	43.0		8	256
Cefotaxime	2002	2.6	0.0002*	0.06	0.125
	2010-2011	17.0		0.06	256
Ceftazidime	2002	1.8	0.0001*	0.125	0.25
	2010-2011	16.0		0.25	2
Cefepime	2002	NA	-	NA	NA
	2010-2011	18.0		0.06	16
Ciprofloxacin	2002	6.0	0.0283*	0.03	0.03
	2010-2011	15.0		0.03	8
Co-trimoxazole	2002	14.5	0.3776	0.125	64
	2010-2011	19.0		0.125	128
Ertapenem	2002	NA	-	NA	NA
	2010-2011	0.0		0.03	8
Fosfomycin	2002	1.8	0.5276	0.5	4
	2010-2011	3.0		1	4
Gentamicin	2002	6.0	0.1817	1	2
	2010-2011	11.0		1	16
Imipenem	2002	NA	-	NA	NA
	2010-2011	0.0		0.06	0.06
Nitrofurantoin	2002	4.3	0.1427	16	32
	2010-2011	1.0		16	32
% ESBL-positive	2002	1.9	0.0005*		
	2010-2011	16.0			

* - statistically significant difference

Conclusions: A statistically significant increase in resistance rates to ampicillin (from 31.7% to 48.0%; $p < 0.05$), cefotaxime (from 2.6% to 17.0%; $p < 0.05$), ceftazidime (from 1.8% to 16.0%; $p < 0.05$) and ciprofloxacin (from 6.0% to 15.0%; $p < 0.05$) was observed between the two study periods. A dramatic growth of ESBL production rate (from 1.9% to 16.0%; $p < 0.05$) was also noted. The changes in susceptibility to other drugs were statistically non significant.

R2487 Prevalence of hGISA heterogeneous glycopeptide intermediate-resistant *Staphylococcus aureus* isolates in Italy

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Objectives: *S. aureus* is an important clinical pathogen causing, bacteriaemia, endocarditis and wound infections in hospitals and community. Glycopeptide Intermediate resistance in *Staphylococcus aureus* (GISA) is associated with treatment failure but is rare. Heterogeneous (h) GISA is more common; prevalence of 0.7%, 0.2%, 7.5%, 0.7%, 6% and 1.1% in UK, France, USA, Belgium, Israel & Italy. It is important to determine the prevalence of hGISA within local and national *S. aureus* strains to inform treatment options. This study investigates the prevalence of hGISA in a local hospital in Bologna, Italy.
Methods: Of 74 strains of MRSA were collected from S. Orsola University Hospital in Bologna, Italy. Strains were screened using 10 μL of McFarland 0.5 inoculum onto a Mueller Hinton agar plate containing 5 mg/L teicoplanin (MHA5T). All isolates were tested with GRD strips using manufacturer's instructions. Any screening plate or GRD positives were confirmed with population analysis profile-area under curve (PAP-AUC) method.
Results: Out of the 74 isolates tested two isolates showed positive results for GRD, with one of these also resulting in a positive MHA5T test (Table). Geometric means for vancomycin and teicoplanin are 1.02 and 0.92 respectively. Both screen positive isolates were confirmed hGISA by PAP-AUC ratio of 1.05 and 1.22. Further screening for second line antibiotic therapies through E-tests, demonstrated a fully sensitive profile of the strains for Daptomycin, Linezolid and Quinupristin/Dalfopristin.

sample #	Etest VA MIC	Etest TP MIC	Etest DAP MIC	Etest LIN MIC	Etest SYN MIC	GRD		MHA5T	AUC ratio
						VA	TP		
7	2	4	0.75 (S)	0.75 (S)	0.38 (S)	2	12	-	1.22
26	2	2	0.75 (S)	0.25 (S)	0.75 (S)	2	8	+	1.05

Conclusions: Of 2.7% of MRSA strains tested were confirmed as hGISA. The prevalence in this hospital is similar to those observed in Northern Italy and has implications for the treatment of hGISA infections.

R2488 Coexistence of extended-spectrum beta-lactamase (ESBL) and ampC plasmidic beta-lactamase in *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis* in a two-year period

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Objective: To study the coexistence of the ESBL and ampC plasmidic (ampCp) beta-lactamase in the *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis* strains isolated from tract urinary infections. To document antibiotics susceptibility in the treatment of urinary tract infections caused by extended-spectrum beta-lactamase (ESBL) producing *E. coli*, *Kl. pneumoniae* and *P. mirabilis*.

Method: Retrospective study of ESBL producing *E. coli*, *K. pneumoniae* y *P. mirabilis* strains isolated from urinary clinical samples of patients from Hospital Miguel Servet from 2010 to 2011. Isolates were identified and tested using MicroScan-WalkAway. ESBL production was confirmed by the double-disk diffusion method according to CLSI. AmpCp beta-betalacmases were confirmed by the use of boronic acid in disk diffusion test according to CLSI in the ESBL strains with cefoxitin concentrarion equal or superior to 16.
Result: In 2010, 4977 *E. coli*, 813 *K. pneumoniae* and 439 *P. mirabilis* were recovered. Of 248 (4.98%) 33 (4.05%) and 2 (0.45%) isolates were positive for ESBL. We tested 16 strains of ESBL *E. coli* with CMI of cefoxitin equal or superior to 16 to find one strain with AmpC plasmidic beta-lactamase. Ciprofloxacin and norfloxacin were resistant in 73.14% and 68.19%. Of 9.18% of the strains were resistant to nalidix acid with quinolones sensible. Fosfomycin was sensible in 86.92%. Gentamicin and tobramycin were sensible in 71.73% and 55.83%. Trimethoprim/sulfamethoxazole was resistant in 69.25%. From January 1, 2011 to October 31, 2011, 4181 *E. coli*, 618 *K. pneumoniae* and 262 *P. mirabilis* were recovered. Of 250 (5.97%), 29 (4.69%) isolates were positive for ESBL. We tested 28 strains of ESBL *E. coli* with cefoxitin equal or superior to 16 to find five strains with AmpCp beta-lactamase. Ciprofloxacin was resistant in 78.13% and norfloxacin in 73.47%. Nalidixic acid resistant with quinolones sensible was found in 6.81%. Fosfomycin was sensible in 92.11%. Gentamicin was sensible in 64.15% and tobramycin 51.97%. Trimethoprim/sulfamethoxazole was resistant in 56.99%.
Conclusion: ESBL strains remains similar in our study. *E. coli* has been the only specie with positive result to AmpCp beta-lacmase in a strain which presented a cefoxitin concentration superior to 16. More further studies are obliged to conclude if the presence of AmpC betalactamases is increasing. Most of the ESBL strains were resistant to quinolones. Fosfomycin remains as an good therapeutic option.

R2489 Activity of moxifloxacin tested against 14 930 clinical isolates of Enterobacteriaceae from Europe

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Objective: To determine the activity of moxifloxacin and selected antimicrobial agents tested against Enterobacteriaceae (ENT) isolated from Europe (EU) over a five year period. Moxifloxacin is a broad-spectrum bactericidal fluoroquinolone (FQ) with potent activity against Gram-positive and -negative pathogens.
Methods: Of 14 930 ENT were analyzed from the SENTRY Antimicrobial Surveillance Program platform collected from 13 countries (2005–2009). The distribution of isolates by infection type (n) was: blood stream infection (12 126), intra-abdominal infection (854), respiratory tract infection (1690), hospital-acquired pneumonia (878), ventilator-associated pneumonia (262), urinary tract infection (1671), and complicated skin and skin structure infection (1114). Isolates were tested for susceptibility by CLSI broth microdilution methods (M07-A8 and M100-S21). Susceptibility interpretations were determined using EUCAST (2010) and CLSI breakpoints.
Results: The table shows the cumulative percentage MIC frequency against the species/groups tested. Against all ENT, 20.8% of isolates were resistant (R) to moxifloxacin compared to 17.8 and 19.3% for levofloxacin (LEV) and ciprofloxacin (CIP), respectively. R against all ENT was lowest for imipenem (0.4%) and meropenem (0.3%). Against *E. coli*, 23.2% of isolates were R to moxifloxacin compared to LEV (22.6%) and CIP (22.9%). R to ceftazidime (CAZ) and ceftriaxone (CRO; surrogate markers for ESBL production) were 5.2 and 8.9%, respectively. Against *K. pneumoniae*, 19.5% of isolates were R to moxifloxacin compared to 16.1% for LEV and 18.4% for CIP (18.4%) with 20.2% R to CAZ and 23.9% R to CRO.

	No. (cum. %) of isolates inhibited at moxifloxacin MIC (mg/L)						
Species (no. tested)	≤0.5	1	2	4	>4	MIC_{50}	MIC_{90}
All ENT (14030)	11439 (76.6)	388 (79.2)	247 (80.9)	262 (82.6)	2594 (100.0)	≤0.5	>4
E. coli (8159)	6167 (75.6)	99 (76.8)	33 (77.2)	60 (77.9)	1800 (100.0)	≤0.5	>4
K. pneumoniae (2180)	1661 (76.2)	94 (80.5)	55 (83)	65 (86)	305 (100.0)	≤0.5	>4
E. cloacae (1147)	901 (78.6)	57 (83.5)	39 (86.9)	22 (88.8)	128 (100.0)	≤0.5	>4
P. mirabilis (607)	439 (72.3)	18 (75.3)	4 (75.9)	16 (78.6)	130 (100.0)	≤0.5	>4
S. marcescens (554)	393 (70.9)	43 (78.7)	48 (87.4)	37 (94)	33 (100.0)	≤0.5	4

Conclusions: Similar to LEV and CIP, moxifloxacin demonstrated a stable susceptibility and MIC distribution pattern in ENT clinical isolates from EU patients over a 5 year period (2005–2009).

R2490 Extended-spectrum β-lactamases are differentially expressed in specimens from normally sterile and non-sterile sites, in a hospital setting

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Objectives: To compare the prevalence of extended spectrum beta-lactamase (ESBL)-producing strains in isolates from normally sterile samples and non-sterile sources, in a Hospital setting.
Methods: We reviewed all cultures of blood or urine (''B/U''), spontaneously expectorated sputum or bronchial aspirates (''sputum'') performed in our Lab, between January 2008 and October 2011, selecting all 12668 positive samples for *Escherichia coli* (EC) or *Klebsiella pneumoniae* (KP). Excluding duplicates, we obtained 10205 isolates, and compared the ratio of ESBL-producing *E. coli* (EPE) and *K. pneumoniae* (EPK) in each type of sample.
Results: We obtained 6351 EC-positive isolates from urine, 1044 from blood and 307 from sputum, and 1632 KP-positive isolates from urine, 429 from blood and 442 from sputum. Considering the EC isolates, we found that 668 urine samples (10.5%), 114 blood samples (10.9%) and 76 sputum samples (24.8%) were positive for EPE ($p < 0.001$), while in the KP isolates, 746 urine samples (45.7%), 200 blood samples (46.6%) and 168 sputum samples (38.0%) were positive for EPK ($p = 0.01$). In both, the differences between urine and blood were not significant. The observed high incidence of EPK reflected a constant upward trend in the period considered, with isolates in B/U climbing from 22.1% in 2008 to 45.4% in 2009 and 53.5% in 2010 ($p < 0.001$), with a similar increase in sputum, from 27.7% in 2008 to 37.1% in 2009 and 45.4% in 2010 ($p = 0.03$). By contrast, EPE plateaued in B/U during the same period (9.1% in 2008, 10.6% in 2009 and 10.8% in 2010, p = NS).
Discussion: We found that the prevalence of EPE was identical in blood and urine (11%), but increased 2.3-fold in bronchial samples (25%). Conversely, the prevalence of EPK was 17% lower in bronchial samples than in blood and urine (38% vs. 46%). Considering that ESBL-producing strains are more frequent in the nosocomial setting than in the community, and that EC is part of the endogenous flora, our results suggest that, in our series, isolates resulting from colonization with Hospital flora colibacilli are more frequent in bronchial samples, while bacteriuria and bacteraemia are due to autologous strains in a higher percentage of cases. On the other hand, the presence of KP in blood and urine is typically of nosocomial origin; the lower percentage of EPK in bronchial samples suggests that a higher proportion of these specimens reflect colonization of the patient's respiratory tract by strains acquired in the community.

R2491 Emerging carbapenemase-producing *Klebsiella pneumoniae* infections in a Greek hospital: clinical and laboratory features

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Objectives: To analyze clinical and laboratory data in 31 infections due to *Klebsiella pneumoniae* isolates with reduced susceptibility to carbapenems.
Methods: All nosocomial infections due to *K. pneumoniae* strains with reduced susceptibility to carbapenems (MIC in meropenem: (1) were recorded in our hospital from November 2010 through November 2011, according to a National Surveillance Study for the management of multi-drug resistant Gram-negative pathogens. Of 31 clinical isolates were recovered from 25 patients in our laboratory. Susceptibility tests

were performed by Kirby-Bauer method, MIC determination by automated system (Phoenix, BD) and E-test method (Biomerieux SA, France) according CLSI standards. Preliminary phenotypic detection was based on the boronic acid disk test, a simple phenotypic algorithm employing three combined-disc tests consisting of meropenem alone and with phenylboronic acid (PBA), EDTA, and both PBA and EDTA. Genotypic confirmation for the presence of the bla-KPC gene by PCR and molecular typing by PFGE.

Results: Of 12 cases of bacteremia, seven catheter related infections, three surgical site infections, two lower respiratory tract infections, five ventilator-associated pneumonia and two urinary tract infections were identified. Twenty-three infections were recorded in ICU, four in Surgical and four in Internal Medicine unit respectively. Ninteen out of 25 patients were mechanically ventilated, 21 had central venous catheter and 25 urinary-bladder catheter. The crude mortality, 28 days after the first positive culture, was 32%.

Multidrug resistance characterized the studied isolates with an MIC range to meromenem ≤1 to (32, with colistin, gentamicin and tigecycline being the most active agents. Boronic acid disk test was able to differentiate 26 KPC, 3 MBL producers and two co-producers of both carbapenemases. PCR confirmed that all 26 isolates produce KPC-2 β-lactamases and belong to the same dominant genotype in Greece.

Conclusion: In Greek hospitals, carbapenem resistance among *Klebsiella pneumoniae* clinical isolates, is alarming and poses a unique challenge for clinical microbiologists and clinicians. Continuation of antibiotic policy and proper infection control practices and barriers to prevent further spread, is mandatory. Boronic acid disk test provides a simple algorithm for phenotypic detection of carbapenemase production and for the differentiation of KPC and MBL enzymes

R2492 Carbapenemase-producing Enterobacteriaceae isolates collected in Portuguese hospitals

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Objectives: In Portugal, little is known on carbapenemase (CARB)-producing Enterobacteriaceae. The aim of this study was to identify the resistance mechanisms of Enterobacteriaceae isolates, identified at hospital laboratories as carbapenem (CA) non-susceptible.

Methods: This study included 61 Enterobacteriaceae isolates (26 *Klebsiella* spp., 15 *Escherichia coli*, nine *Enterobacter* spp., six Morganella morgannii, four *Proteus mirabilis*, one *Serratia marcescens*), collected between April 2006 and September 2011 and sent to the NIH-Lisbon for CA susceptibility confirmation. Antimicrobial susceptibility of clinical isolates was performed by disk diffusion method (CA-SFM). Clinical isolates showing synergism between CA and boronic acid (BOR) (and/or clavulanic acid, CLAV) or with EDTA were considered presumptively CARB-producers from class A or Class B, respectively. PCR and sequencing were applied to detect and identify CARB-encoding genes; the respective genetic environment was revealed by sequencing using PCR mapping. Direct transfer of the CA resistance phenotype was attempted by mating-out assays. Antibiotics susceptibility (MIC) of transconjugants and respective isolates were tested by microdilution.

Results: The majority of isolates were collected from the urine (57.4%) of elderly ((65 years old) male patients (54.1%), admitted at the emergency room/ambulatory (24.6%) and at internal medicine (18.0%) wards. Among all isolates, 50.8% were nonsusceptible to at least one CA, being 67.2% multidrug-resistant; 16 isolates showed synergy between CA and BOR (and/or CLAV). Among those, five were KPC-3-producers (four *Klebsiella pneumoniae* and one Enterobacter clocae), collected in 2010 (2) and 2011 (3). The blaKPC-3 genes were confirmed to be carried by plasmids. Genetic environment of blaKPC-3 gene revealed the presence of a Tn4401 transposon in all but one isolate (*E. cloacae*), suggesting that this last gene was included in other Tn4401-like isoform. We also detected a VIM-2-producing *Klebsiella oxytoca*, collected in 2009, among the seven isolates that showed synergy between imipinem and EDTA. No blaGES, blaNDM or blaIMP were detected.

Conclusion: This study provides new data regarding the molecular epidemiology of CARB-producing Enterobacteriaceae in Portugal. Overall, our results emphasize the need of a concerted action to manage CA use. This is supported by EARS-Net, which reported an increase in CA nonsusceptibility of *K. pneumoniae* isolates from 0.72% in 2008 to 1.58% in 2010.

R2493 Susceptibility comparison of uropathogens isolated from adults with complicated and uncomplicated community-acquired urinary tract infections in Russia, 2010–2011

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Objectives: To compare the resistance of *Escherichia coli* isolates from adults with complicated and uncomplicated community-acquired urinary tract infections (CAUTIs) in Russia.

Methods: A total of 282 and 196 uropathogens from adults with signs of complicated and uncomplicated CAUTI in 17 cities of Russia (Moscow, St. Petersburg, Chelyabinsk, Irkutsk, Kazan, Krasnodar, Omsk, Rostov-on-Don, Samara, Seversk, Smolensk, Surgut, Tomsk, Tyumen, Ufa, Yakutsk, Yekaterinburg) were collected during 2010–2011. The MICs for antibiotics (amikacin – AMK, amoxicillin/clavulanate – AMX/CLV, ampicillin – AMP, cefotaxime – CTX, ceftazidime – CTZ, cefepime – CFPM, ciprofloxacin – CPX, co-trimoxazole – TMP/SMX, ertapenem – ERT, fosfomycin – FSF, gentamicin – GNT, imipenem – IPM, nitrofurantoin – NTF) were determined by agar dilution and interpreted according to the EUCAST criteria (2011).

Results: Among the identified microorganisms the most frequent were Enterobacteriaceae members (225 isolates [79.8%] in the group of complicated CAUTI and 160 isolates in the group of uncomplicated CAUTI [81.6%]) out of which there were 173 (61.3%) and 129 (65.8%) *E. coli* strains, respectively. Resistance rates of *E. coli* in both study groups are summarized in the Table. A statistically significant difference in resistance rates to ampicillin (37.9% and 53.1%; $p < 0.05$), cefotaxime (2.3% and 13.8%; $p < 0.05$), ceftazidime (3.0% and 13.8%; $p < 0.05$), cefepime (3.0% and 11.0%; $p < 0.05$), co-trimoxazole (21.8% and 32.4%; $p < 0.05$) and ciprofloxacin (10.8% and 27.7%; $p < 0.05$) was observed between the groups of adults with uncomplicated CAUTIs and complicated CAUTIs, respectively. A significant distinction in ESBL production rate (2.3% and 13.8%; $p < 0.05$) was also noted. The changes in susceptibility to other drugs were statistically non significant.

Table. Susceptibility of *E. coli* isolated from adults with CAUTI in Russia in 2010-2011

Antibiotic	Complicated CAUTI, I+R,%	Uncomplicated CAUTI, I+R,%	p value (χ^2 test)
AMK	2.3	0.0	0.0821
AMX/CLV (2mg/L)	46.2	35.6	0.0650
AMP	53.1	37.9	0.0088*
CTX	13.8	2.3	0.0005*
CTZ	13.8	3.0	0.0014*
CFPM	11.0	3.0	0.0106*
CPX	27.7	10.8	0.0003*
TMP/SMX	32.4	21.8	0.0408*
ERT	1.8	0.0	0.1328
FSF	1.8	1.6	0.9015
GNT	11.6	10.8	0.8473
IPM	0.0	0.0	-
NTF	2.9	0.8	0.1926
% ESBL-positive	13.8	2.3	0.0005*

* - statistically significant difference

Conclusions: The resistance rates of *E. coli* to ampicillin, co-trimoxazole, III-IV generation cephalosporins and ciprofloxacin were significantly higher in the group of adults with complicated CAUTIs

than in uncomplicated ones. There were no significant differences in resistance to other antimicrobials tested.

R2494 Empiric therapy for sepsis needs adaptation due to increased resistance rates

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Introduction: In 2011 the Dutch Society of Antibiotic Policy (SWAB) issued a new guideline on sepsis therapy. For hospital acquired (HA) sepsis of unknown origin it recommends combination therapies of piperacillin-tazobactam (PTZ), cefuroxim (CEF), cefotaxim or ceftriaxon (CFT) with aminoglycoside (AG) or ciprofloxacin (CIP), for community-acquired (CA) sepsis monotherapy with CEF or CFT or amoxi-clav (AMC) with AG, and for ESBL high risk patients carbapenems. In the present study, we investigated the distribution of species causing sepsis and to what extent the recommended empiric therapy covers sepsis caused by Enterobacteriaceae (ENT).

Materials and Methods: Antimicrobial Susceptibility Test (AST) results, first isolate per patient, from 2008 to 2010 were collected from the Infectious Diseases Surveillance and Information System for Antibiotic Resistance (ISIS-AR) at the RIVM, representing 27 laboratories covering 50% of the hospital beds. The distribution of species and the % I or R Enterobacteriaceae for the recommended regimens was calculated. *E. coli* (ECO) or *K. pneumoniae* (KPN) I or R to 3rd generation cephalosporins were defined ESBLs. Hospital acquired was defined as a blood culture (BC) obtained from an admitted patient and community acquired was defined as a BC obtained at the outpatient department or emergency room.

Results: Of 35.113 (34% ENT) HA and 9.456 (36% ENT) CA isolates were included. The distribution of species was stable over the years, except for a significant decrease among HA and CA pneumococci and increase among HA *E. faecium*. (Intermediate) resistance rates for ENT-BC isolates from 2008 to 2010 are shown in the Figure. Co-resistance rates among HA and CA ESBLs were, respectively: tobramycin (TOBR) 47% and 46%, gentamicin (GENT) 36% and 33%, for amikacin (AMIK) 9% and 5%, CIP 58% and 63%, carbapenems 0.2% and 0%.

Table: (intermediate) resistance rates for Enterobacteriaceae blood culture isolates in The Netherlands from 2008-2010

	Hospital acquired				Community acquired			
Recommenced therapy	2008	2009	2010	trend	2008	2009	2010	trend
AMC	27.0	26.7	30.1	**	26.7	24.9	28.1	
AMC_amik	0.6	0.7	1.2	*	0.5	0.2	0.3	
AMC_gent	2.8	3.1	4.5	***	1.7	3.5	2.3	
AMC_tobr	3.5	3.7	5.0	**	2.1	2.8	3.4	
CEF	17.4	18.1	20.1	**	**13.0**	**12.8**	**14.5**	
CEF_amik	0.4	0.4	1.4	***	0.5	0.2	0	
CEF_CIP	4.1	4.6	***5.5***	**	3.2	4.1	4.7	
CEF_gent	2.2	2.3	3.7	***	0.8	3.1	2.0	
CEF_tobr	2.9	2.9	4.3	**	1.4	2.6	2.7	
CFT	7.0	7.7	8.8	**	**5.7**	**4.6**	**6.0**	
CFT_amik	0.4	0.5	1.0	**	0.3	0.1	0	
CFT_CIP	2.5	2.8	3.5	*	1.9	2.0	2.8	
CFT_gent	1.5	2.0	2.5	**	0.5	1.5	1.0	
CFT_tobr	2.0	2.4	3.3	**	1.0	1.5	1.9	
Carbapenem	0.8	0.7	1.2		1.2	1.3	0.8	
PTZ_amik	0.3	0.5	0.6	*	0.4	0.1	0	
PTZ_CIP	1.5	2.3	3.6	***	1.0	1.1	3.5	*
PTZ_gent	0.9	1.6	2.3	***	0.1	1.0	1.0	*
PTZ_tobr	1.1	2.0	3.0	***	0.6	1.4	1.9	*

* 0.01 =<p<0.05
** 0.001=<p< 0.01
*** p<0.0001

Conclusion: From 2008 to 2010 the distribution of species causing sepsis was stable, except for a decrease among pneumococci and increase of HA *E. faecium*. Although the resistance rates among CA-ENT were stable, the increasing resistance rates among HA-ENT causing sepsis are worrisome. The recommendations of the SWAB guideline are greatly supported by our data, except for CEF-CIP therapy for HA sepsis and CEF or CFT monotherapy for CA sepsis since resistance rates in 2010 were above 5%, the consensus resistance rate above which a drug is no longer eligible for empiric sepsis therapy.

R2495 Bloodstream infections caused by extended-spectrum beta-lactamase producing *Escherichia coli* and *Klebsiella* spp. and associated factors

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Objectives: Antibiotic resistance is an important problem in treatment of Gram negative bacterial infections. Extended spectrum β-lactamases (ESBL) are heterogenous bacterial enzymes that result in clevage and inactivation of extended spectrum cephalosporins, monobactams, and penicillins. These enzymes are found most commonly in the Enterobacteriaceae family, especially *Esherichia coli* and *Klebsiella* spp. (EK). We analysed the clinical characteristics of the bloodstream infections (BSI) caused by ESBL positive EK strains, and compared them with those caused by ESBL negative EK strains.

Methods: This retrospective study was conducted at the tertiary-care teaching hospital in Isparta, Turkey. Hospital records were searched in order to identify all adult inpatients (18 years old) diagnosed with BSI caused by ESBL positive/negative EK strains between January 2007–October 2011. BSI caused by ESBL positive/negative EK in at least one blood culture specimen from a patient with systemic inflammatory response syndrome (e.g. fever, tachycardia, tachypnea, and leukocytosis). Detection of ESBL was performed by using disk diffusion method. Nosocomial acquisition of infection was considered when the infection ocurred >48 hour after admission to the hospital, while community-acquired bacteremia when it occurred <48 hour after admission to the hospital. Data obtained included age, sex, underlying diseases, surgical interventions performed within 30–90 days prior to

Table 1: Demographic, clinical, laboratory characteristics of patients with bloodstream infection due to ESBL positive (and negative) *Escherichia coli* and *Klebsiella* spp.

Data	ESBL (+) EK group (n=54)	ESBL (-) EK group (n=91)	p-value
Sex (Men/	29(53.7)	51(56.0)	0.784
Age,years,mean	66.0 (63.7-68.3)	64.3 (62.9-65.7)	0.529
Acquisition of infection			0.017
Community	18 (33.3)	49 (53.8)	
Hospital	36 (66.6)	42 (46.1)	
Solid tumor	18 (33.3)	28 (30.7)	0.748
Malignant hematologic disease	3 (5.5)	8 (8.7)	0.747*
Diabetes mellitus	14 (25.9)	22 (24.1)	0.814
Chronic obstructive pulmonary disease	5 (9.2)	4 (4.3)	0.293*
Renal failure	6 (10.1)	13 (14.2)	0.584
Liver dysfunction	4 (7.4)	6 (6.5)	0.852
Hepatitis C virus (HCV) infection	3 (5.5)	2 (2.1)	0.207
Hepatitis B virus (HBV) infection	2 (3.7)	2 (2.1)	0.262
HIV infection	0	0	
Renal transplantation	0	1 (1.0)	1.000*
Hemodialysis	2 (3.7)	3 (3.2)	1.000*
Corticosteroids	2 (3.7)	7 (7.6)	0.485*
Immunosuppressants	3 (5.5)	14 (15.3)	0.075
Carrier of urinary catheter	36 (66.6)	30 (32.9)	0.000
Urinary pathology**	26 (48.1)	14 (15.3)	0.000
Corticosteroid use in previous 30 d	2 (3.7)	7 (7.6)	0.485
Corticosteroid use in previous 90 d	2 (3.7)	7 (7.6)	0.485
Hospitalization within 30 d	18 (33.3)	24 (26.3)	0.372
Hospitalization within 90 d	22 (40.7)	29 (31.8)	0.279
Surgical procedure in 30 d	23 (42.5)	14 (15.3)	0.000
Surgical procedure in 90 d	25 (46.2)	16 (17.5)	0.000
Intraabdominal surgery in 30 d	9 (16.6)	3 (3.2)	0.009
Intraabdominal surgery in 90 d	9 (16.6)	3 (3.2)	0.009
Urinary surgery in 15 d	8 (14.8)	5 (5.4)	0.073*
Urinary surgery in 30 d	9 (16.6)	6 (6.5)	0.057
Urinary surgery in 90 d	9 (16.6)	6 (6.5)	0.570
Leucocytosis	21 (38.8)	41 (45.0)	0.434
Leucopenia	5 (9.2)	13 (14.2)	0.362
In ICU at time of infection	17 (31.4)	17 (18.6)	0.790
ICU stay	3 (5.5)	5 (5.4)	1.000
Central venous catheterisation	27 (50)	18 (19.7)	0.000
Antibiotic use in previous 30 d	19 (35.1)	17 (18.6)	0.002
Antibiotic use in previous 90 d	20 (37.0)	18 (19.7)	0.002
Cephalosporin use in previous 30 d	11 (20.3)	7 (7.6)	0.004
Cephalosporin use in previous 90 d	12 (22.2)	7 (7.6)	0.001
Quinolones use in previous 30 d	6 (11.1)	1 (1.0)	0.004*
Quinolones use in previous 90 d	6 (11.1)	1 (1.0)	0.004
Mortality	19 (35.1)	24 (26.3)	0.261

ESBL: Extended spectrum beta-lactamase, EK: *Escherichia coli*, *Klebsiella* spp., ICU: Intensive care unit

*Fisher's Exact Test

**Urinary pathology: Urolitiasis, benign prostat hyperplasia, urinary malignancy, nephrectomy, neurogenic bladder

the episode of bacteremia, antibiotic use within 30–90 days prior to the episode of bacteremia, site of infection, intensive care unit stay, invasive procedures and survival. Statistically analyses were performed using SPSS 15.0. The chi-square test was used to compare dischotomous data unless the cell size was <5, in which case Fisher's exact test was used.

Results: We analysed the records of 145 adult inpatients with BSI due to EK (n = 113 *Escherichia coli*, n = 32 *Klebsiella* spp.) diagnosed between 2007 and 2011. Demographic, clinical, laboratory characteristics of patients with bloodstream infection due to ESBL positive and negative EK cases were seen table 1. Central venous catheterisation, surgical interventions within 30 and 90 days prior to the episode of bacteremia, antibiotic use in 30 and 90 days and urinary pathology were statistically significant.

Conclusion: In conclusion, surgery interventions and previous use of antibiotics is associated factor for bacteremia by ESBL producing organisms.

R2496 Antibiotic sensitivity patterns of *Pseudomonas aeruginosa* strains isolated from various clinical specimens: a comparative study between two periods, 1999 vs. 2011

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Objective: *Pseudomonas aeruginosa* is an opportunistic human pathogenic bacterium that is highly prevalent in hospital environment. The use of new generation of broad-spectrum antibiotics has been responsible for the selection of many multi-resistant strains. We undertook this study to describe the resistance profile of this germ in the Military hospital of Tunisia, to determine its phenotypes and study its distribution among hospital departments. We also compared the recent resistance profile with resistance described from 1990 to 1999 in the same hospital.

Materiel and method: The present study is a retrospective analysis of all positive cultures of *Pseudomonas aeruginosa* found between October 2010 and November 2011 in the Military hospital of Tunisia. Information about resistance profiles, site of sampling and department origin were provided. We have focused on susceptibilities to betalactam family and aminoglycosides family, ciprofloxacin, colistin and fosfomycin. Drug susceptibilities had been evaluated by disk-diffusion method on Mueller-Hinton agar in accordance with the criteria of CA-SFM 2010.

Results: Sampling was taken from patients belonging to many departments and distributed as the following: 35.5% are isolated from ICU, 13.5% from cardiovascular and cardiothoracic surgery, 8.8% from other surgery departments, 8.8% from neonates, 8.5% from urology, and 10.5% from other departments. We included 14.4% emanated from outpatients as well. The site of sampling was also variable, including 37.8% from deep and superficial wounds, 17.5% from tracheal aspirate, 16.3% from urine, 9.3% from auricular infection, 6.4% from blood, 4.7% from catheters, 4.7% from other material and 3.3% from other samplings. *Pseudomonas aeruginosa* was highly resistant to large spectrum penicillin and large spectrum cephalosporin (Table 1). Both of enzymatic and non-enzymatic mechanisms were involved. Resistance to carbapenem was above all related to porin-D2 mutation, whereas the existence of carbapenemase was found rare.

Table 1: Resistance profile of Pseudomonas aeruginosa comparison between 1990-1999 and 2011:

Antibiotics	Ticacillin		Piperacillin		Tic + clavulinate		Ceftazidime	
Period	1990-1999	2011	1990-1999	2011	1990-1999	2011	1990-1999	2011
Antimicrobial resistance rates	52,00%	46,20%	39,50%	31,98%	52,00%	36,53%	26,60%	16,28%
Antibiotics	Cefepime		Imipenem		Ciprofloxacin		Fosfomycin	
Period	1990-1999	2011	1990-1999	2011	1990-1999	2011	1990-1999	2011
Antimicrobial resistance rates	27,50%	11,11%	23,10%	22,94%	39,10%	27,85%	49,60%	38,97%
Antibiotics	Gentamicin		Tobramycin		Netilmicin		Amikacin	
Period	1990-1999	2011	1990-1999	2011	1990-1999	2011	1990-1999	2011
Antimicrobial resistance rates	64,00%	28,24%	35,10%	33,13%	58,60%	36,88%	31,50%	23,49%

Conclusions: Our results showed relatively high rates in the presence of *Pseudomonas aeruginosa* resistant to beta-lactam, amino-glycosides, fluoroquinolones and fosfomycin. Different mechanisms are involved to induce these resistances, including non enzymatic reactions. Treatment options are currently limited. Colistin is known to be still efficient against this germ, even though it is recommended to be used as an ultimate treatment.

R2497 Antimicrobial resistance phenotypes in *Staphylococcus* spp. isolates from mastitis milk of sheep dairy farms in Sicily

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Objectives: Antimicrobial agents are commonly applied to dairy farm to control bacterial infection in lactating sheep. The testing and the monitoring of drug resistance in the main pathogens involved in mastitis represent an important tool to guide veterinarian in selecting the most effective agents for the therapeutic interventions and to avoid the potential resistance spreading through the dairy products and food chain.

Methods: Of 40 strains of Staphylococcus (36 *S. aureus*, two *S. intermedius* and two CNS) used to prepare autogenous vaccine at the IZS of Sicily and isolated during 2009 from mastitis milk of 30 dairy farms were tested for antimicrobial susceptibility by disk diffusion test against the following agents: ampicillin (AMP 30 mcg), amoxicillin + clavulanate (AMC 30 mcg), clindamycin (DA 2 mcg), enrofloxacin (ENR 5 mcg), erythromycin (ER 15 mcg), penicillin (P 10 UI), tetracycline (TE 30 mcg), vancomycin (VA 30 mcg), oxacillin (OX 1 mcg), oxytetracycline (OT 30 mcg) according to CLSI guidelines. MIC values for the strains exibiting ER and DA and methicillin resistance were also detected by E-test. Inducible DA resistance was investigated by DD test. The presence of mecA gene was investigated by PCR.

Results: Of the total 40 strains analyzed 11 were coming from previous tested flocks. The isolates confirmed the same resistance/sensitivity profile except for two farms in which new resistance regarding ER and TE showed up. Of 50% showed resistance to TE and OT and some had multidrug resistance, as showed in the Table 1. The resistance to ER and DA (20% and 15% respectively), was frequently associated with other drugs. High resistance rate for P, AMC, AMP and TE was found. Inducible DA resistance was not observed. The majority of resistant clones to both ER and DA showed low MIC (4–8 mcg/mL), the CNS exhibited high MIC (>256 mcg/mL). The presence of mecA gene resulted in a 2.5% of positive clones holding multiple resistance. None of the tested strains showed resistance to VA and ENR.

Phenotype	Number
TE-OT	9
ER-DA	1
AMP-AMC-P	3
AMPAMC-P-TE-OT	5
AMP-AMC-P-TE-OT-ER	3
AMP-AMC-P-TE-OT-ER-DA	2
AMP-AMC-P-TE-OT-ER-DA-OX	1

Table 1 Resistance profiles

Conclusion: The results confirm the widespread of antibiotic resistance clones in the animals for food productions and highlight the major risk for the increasing drug resistance due to the selective pressure acting in the ecosystem. The finding of phenotipically resistant clones to ER and DA can be related to the frequent therapeutic use of tylosin for the relevant and endemic mycoplasmosis (contagious agalaxia) affecting Sicilian dairy farms. Multiresistant clones mecA+ have been reported in sheep.

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

R2498 Detection of aminoglycoside-resistance genes among 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 hospital environment. Many clinical isolates are resistant to almost all antibiotics including aminoglycosides. Inactivation of these antimicrobial agents by enzimatic modifications is the main mechanism. Different types of aminoglycoside acetyltransferases (ACC), nucleotidylotransferases (ANT) and phosphotransferases (APH) are synthetized by clinical isolates of *A. baumannii*. The aim of the study was detection of selected ACC and APH encoding genes.
Methods: The study included the total of thirty nine strains of multidrug-resistant *A. baumannii*. These isolates were obtained from patients hospitalized in Specialized Hospital in Krakow between 2005 and 2010. *A. baumannii* identification and susceptibility testing (Vitek-2 Compact, bioMérieux, Poland) were performed by standard criteria (CLSI). PCR detection of genes encoding: AAC (6′)-Ih, AAC (3)-Ia, AAC (3)-IIa, AAC (6′)-Ib, APH (3′)-Ia and APH (3′)-VI used in this study was described by Noppe-Leclercq et al. (1999) and Nemec et al. (2004).
Results: PCR analysis showed the occurence of at least one of the following aminoglycoside-resistance genes in 97.4% (38) strains: aph(3′)-VI (66.7%; 26), aac(3)-Ia (64.1%; 25), aph(3′)-Ia (43.6%; 17), aac(3)-IIa (15.4%; 6), aac(6′)-Ih (10.3%; 4) and aac(6′)-Ib (5.1%; 2). A combination of two to four different resistance genes was observed in 66.7% (26) of strains, with a total of eight different combinations. Among analysed isolates the most frequent combination was: aac(3)-Ia + aph(3′)-VI (18%; 7), and the least frequent combinations were: aac(3)-Ia + aac(3)-IIa and aac(6′)-Ib + aph(3′)-Ia + aph(3′)-VI (2.6%; 1).
Conclusions: Our results support that: (i) aminoglycoside-resistance in *A. baumannii* strains might be associated with the production of aminoglycoside-modyfing enzymes, (ii) aminoglycoside-resistant strains frequently contain more than one aminoglycoside resistance gene.

R2500 Otitis-related invasive pneumococcal disease in children: susceptibility and clonal profile of *Streptococcus pneumoniae* isolates

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Objectives: Otic foci have been described as significant source of secondary bacteremia in children. Conjugate vaccines, such as the 7-valent conjugate vaccine (PCV7), alter serotype nasopharyngeal carriage in children, potentially increasing cases of otitis media by non-vaccine serotypes. This study analyses isolates from otitis-related Invasive Pneumococcal Disease (IPD) in children.
Methods: A prospective laboratory-confirmed (by culture and/or PCR) surveillance of IPDs was performed (May 2007–April 2010) in all hospitals with Pediatric department in Madrid (28 centres), a region (6 million inhabitants) where PCV7 was included in the vaccination calendar in 2006. Serotypes, sequence types (STs) of 19A isolates, and antibiotic susceptibility following CLSI recommendations for all isolates from otitis-related IPDs were analysed.
Results: Among 499 IPDs (including 161 bacteremic pneumonia, 60 primary and eight secondary bacteremias), 26 (5.2%) were otitis-related: 18 mastoiditis and eight bacteremias (five secondary to otitis, and three to mastoiditis). Median hospital stay was 8.0 days. All cases (but one mastoiditis) were culture-confirmed (25 isolates): 18 (72%) isolates were serotype 19A (12 from ST320, four from ST276, one from ST63 and one from ST1201), two were serotype 11A, and one serotype 5, 8, 10A, 19F and 24B each. Serotype 19A caused 75% (six out of eight) otitis-related bacteremia. Intermediate/resistance (%) in 19A isolates was: 0.0/94.4 to erythromycin, 0.0/72.2 to clindamycin, 22.2/72.2 to oral penicillin, 55.6/0.0 to parenteral penicillin, 44.4/0.0 to cefotaxime and 0.0/0.0 to levofloxacin. Intermediate/resistance (%) in ST320 was: 8.3/91.7 to oral penicillin, 83.3/0.0 to parenteral penicillin and 66.7/0.0 to cefotaxime. All ST276 isolates were susceptible to parenteral penicillin and cefotaxime. The phenotype for macrolide resistance was inducible in all ST276 (all ermB genotype) and constitutive in all but one ST320 (ermB/mefE in all but two).
Conclusions: After PCV7 inclusion, 72% otitis-related IPDs were caused by 19A. The proportions of otitis-related IPDs among total IPDs (5.2%), of otitis-related bacteremias among non-pneumonic bacteremias (eight out of 68; 11.8%), and of multiresistant ST320 among 19A (66.7%) indicate the benefit of the 13-valent PCV (including serotype 19A among others) for preventing otitis-related IPDs within prevention strategies.

R2501 Molecular epidemiology of imipenem-non-susceptible *Acinetobacter baumannii*: a 5-year study in Daejeon, Korea

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Objectives: *Acinetobacter baumannii* has emerged globally as an increasingly important nosocomial pathogen. The recently discovered clonal complex 92 (CC92) has been reported as the most prevalent clonal complex in many parts of the world. In this study, we investigated molecular epidemiology and various genes involved in resistance to carbapenems, aminoglycosides, and fluoroquinolones in 52 imipenem-non-susceptible *A. baumannii* isolates.
Methods: This study included 52 imipenem-non-susceptible *A. baumannii* isolated in a university hospital in Daejeon, Korea from January 2007 to October 2011. The minimum inhibitory concentrations (MICs) of seven antibiotics were determined by the agar dilution method and E-test. PCR and DNA sequencing were used to identify the genes that potentially contribute to each resistance phenotype. Multilocus sequence typing (MLST) was performed to determine the epidemiological relationships. And pan-European clonal lineages were identified by sequence type multiplex PCR.
Results: The 52 imipenem-non-susceptible *A. baumannii* isolates were classified into seven STs (ST92, ST75, ST137, ST138, ST358, ST69 and ST109) and four allelic profiles. The six STs (except ST109 and four allelic profiles) were clustered into CC92 and the pan European clonal lineage II. ST138 (22 isolates) was the most commonly observed ST and followed by ST137, which was identified in 15 isolates. Interestingly, ST 138 was positive for the ISAba1/blaOXA-51-like and ISAba1/blaOXA-23, which may contribute to carbapenem resistance, whereas ST137 was positive for only ISAba1/blaOXA-51-like. In addition, the armA and aac(6′)-Ib was the most prevalent genes involved in aminoglycoside resistance in 52 isolates, but aph(3′)-Ia was detected only in ST138.
Also, in resistance to fluoroquinolone, ST138 had Ser83–>Leu and Ser80–>Leu mutations in gyrA and parC, whereas ST137 had Ser83–>Leu and Ser80–>Typ mutations.
Conclusions: Imipenem-non-susceptible *A. baumannii* emerged in Daejeon, Korea, over 5 year period was associated with the spread of global epidemic CC92 and European clonal lineage II. Epidemiological surveillance may require to promptly identify the spread of epidemic strains and to adopt adequate containment measures.

R2502 **High genetic diversity and over-expression of the SmeABC efflux pump in ciprofloxacin-resistant *Stenotrophomonas maltophilia***

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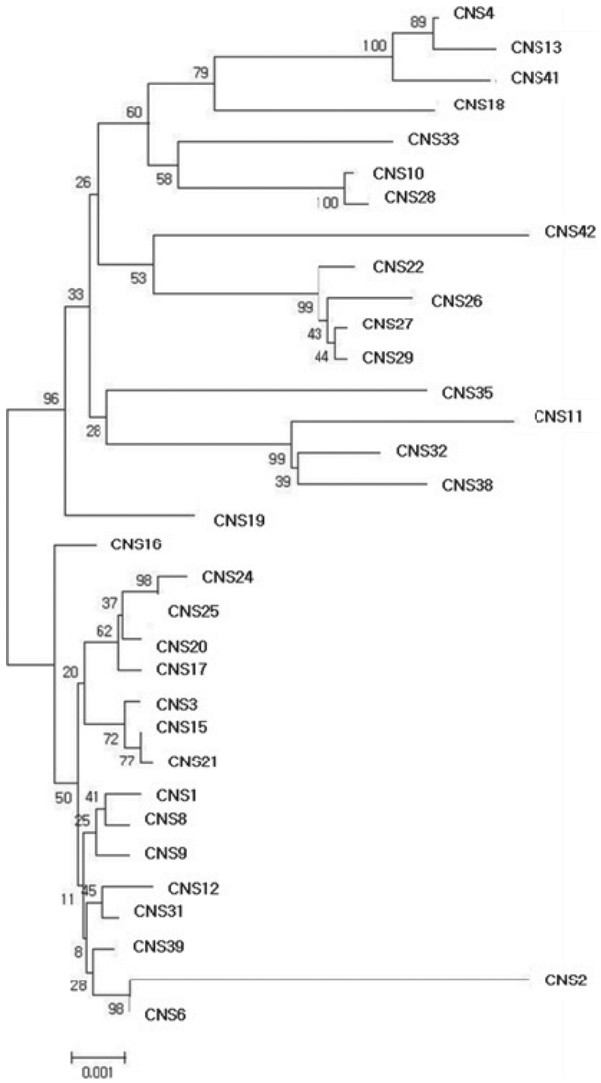
Objectives: *Stenotrophomonas maltophilia*, a non-fermentative Gram-negative bacillus, is one of the multiresistant opportunistic nosocomial pathogens and is being isolated worldwide with increasing frequency. The SmeABC and SmeDEF are tripartite efFLux pumps of which contribution to antimicrobial resistance has been suggested. We aimed to analyze the levels of expression of the SmeABC and SmeDEF and their correlation with antimicrobial susceptibility.
Methods: A total of 33 *S. maltophilia* isolates were collected from a tertiary hospital located in central Korea. The minimum inhibitory concentrations(MICs) of 11 antimicrobials were determined by the agar dilution method or E-test. Real-time PCR was performed to assess the expression of Sme efflux systems. The multilocus sequence typing (MLST) was performed.
Results: The majority of the isolates were resistant to beta-lactams and aminoglycosides; cefepime (90.9%), meropenem (100.0%), aztreonam (97.0%), amikacin and gentamicin (each 93.91%), while for ciprofloxacin (54.5%). Most of the isolates were susceptible to ticarcillin/clavulanic acid (97.0%), levofloxacin (87.9%), trimethoprim/sulfamethoxazole (93.9%), and minocycline (97.0%). Overexpression of smeB and smeF were found in 21 (63.6%) and 19 (57.5%) of the 33 clinical isolates. Fifteen (45.4%) isolates overexpressed both smeB and smeF. The MICs of ciprofloxacin and levofloxacin (MIC50 = 4; 6.83-fold and 1; 3.60-fold increase, respectively) were significantly higher for the isolates with detectable smeB and/or smeF than those without. Overexpression of the SmeABC efflux pump was statistically related to the MICs of ciprofloxacin ($p = 0.033$) and levofloxacin ($p = 0.034$). The MLST showed a high degree of genetic diversity among the *S. maltophilia* isolates: three STs and 23 allelic profiles. The MLST clustering supportively demonstrated the association of SmeABC efflux pump with multidrug resistance, in particular with ciprofloxacin resistance, as the group A and A' isolates harboring smeB showed high-level resistance to ciprofloxacin and the group B isolates showed high-level ceftazidime and cefepime resistance.
Conclusion: The SmeABC efflux pump was considered to play an important role for resistance of *S. maltophilia* to ciprofloxacin. The MLST scheme was thought to be useful for studying the population structure of *S. maltophilia*, which demonstrated a discriminatory typing of groups with resistances to ciprofloxacin and beta-lactams.



R2503 **Aminoglycoside-modifying enzymes in metallo-beta-lactamase producing *Pseudomonas aeruginosa* isolated in Daejeon, Korea**

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Background: Carbapenem resistant *Pseudomonas aeruginosa* has emerged as a serious opportunistic pathogen presenting significant therapeutic challenges. Concurrent resistance to aminoglycoside can lead to a multi-drug resistance threat for nosocomial infections. In the present study, we investigated the prevalence of aminoglycoside resistance and its association with various aminoglycoside modifying enzymes (AMEs) and 16S rRNA methylases in imipenem-resistant *P. aeruginosa* isolates.
Methods: A total of 59 imipenem-resistant *P. aeruginosa* clinical isolates from a tertiary hospital in central Korea were subjected. PCR and sequence analyses for eight AMEs genes including aac(3)-I, aac(3)-II, aac(3)-III, aac(3)-IV, aac(6′)-I, aac(6′)-II, ant(2″)-I, ant(4′)-II and aph(3′)-VI, 16S rRNA methylases including rmtA, rmtB, rmtC and armA, class 1 integrons and three MBL genes, namely, blaIMP-1, blaVIM-2 and blaSIM-1, were performed.
Results: Three types of AME genes [aac(6′)-I, ant(2″)-I and aph(3′)-VI] were detected in 30 isolates (50.8%). Most strains harboring ant(2″)-I gene (17/18) showed the high-level resistance (MIC (1024 μg/mL) to gentamicin. Eighteen strains had two or more types of AME genes and showed resistance to amikacin and gentamicin. Eight strains with AME genes simultaneously had MBL genes; blaIMP-1 (7/8) or blaVIM-2 (1/8), and showed significantly high resistance to imipenem, meropenem, amikacin and gentamicin (MIC (1024 μg/mL). Seven strains contained class 1 integron, which had AME genes. aac(3)-I, aac(3)-II, aac(3)-III, aac(3)-IV, aac(6′)-II, ant(4′)-II, rmtA, rmtB, rmtC or armA were not detected.
Conclusion: AME genes were frequently harboured in imipenem-resistant *P. aeruginosa* and associated with high resistance to aminoglycoside. In particular, simultaneous presence of AME and MBL genes showed significantly high resistance to aminoglycoside and carbapenem.

R2504 **Continuing control of *Clostridium difficile* ribotype 027 in England using long term surveillance: the *Clostridium difficile* ribotyping network for England and N. Ireland (CDRN)**

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Objectives: To describe the key epidemiological findings concerning CDI in England in 2010–2011 vs. the previous 3 years as determined by The *Clostridium difficile* Ribotyping Network for England and N. Ireland (CDRN).
Methods: CDRN provides ribotyping, and enhanced DNA fingerprinting, to identify cross-infection, reduce transmission, optimise management of outbreaks and determine the epidemiology of *C. difficile*.
Results: In November 2010 CDRN processed 7026 faecal samples from 152 health care facilities, which was a 23% increase over 2009/10 when 5720 samples were received from 172 health care facilities. The main reasons provided for submission to CDRN in 2010/2011 were suspected CDI case clusters (68%), increased CDI rate (19%), and severity of symptoms (13%). The number of reports of *C. difficile* recorded by the mandatory *C. difficile* surveillance scheme in England has decreased from 55 498 to 36 095 to 25 604 to 21 698 (in the financial years 2007/2008, 2008/2009, 2009/2010 and 2010/2011). Thus, on average samples were received by CDRN from 1 in 3.1 and 1 in 4.5 reported CDI cases in England in November 2010 and October 2009, respectively. *C. difficile* could not be cultured from 10% to 12% of samples in each of the 4 years. Marked changes in ribotype prevalence have occurred in the 4 years since CDRN was launched in 2007 (Table). There was a striking progressive decrease in the prevalence of *C. difficile* ribotype 027 (from 55% to 13%), with ''compensatory'' increases in the other main types, including ribotypes 002, 015, 014, 005 and 078. Ribotype 106 has also declined markedly but remains the 4th most common type.

Ribotype	2007/08 (n,%)	2008/09 (n,%)	2009/10 (n,%)	2010/11 (n,%)
027	1152 (55.3)	1468 (36.1)	1102 (22.1)	785 (12.7)
015	50 (2.4)	215 (5.3)	330 (6.6)	485 (7.8)
002	57 (2.7)	231 (5.7)	302 (6.0)	475 (7.7)
106	270 (13.0)	517 (12.7)	364 (7.3)	459 (7.4)
001	181 (8.7)	297 (7.3)	371 (7.4)	431 (7.0)
078	37 (1.8)	144 (3.5)	285 (5.7)	389 (6.3%)
014/ 020	57 (2.8)*	218 (5.4)*	128 (2.6)*	358 (5.8) 168 (2.7)
005	29 (1.4)	118 (2.9)	213 (4.3)	344 (5.6)
023	21 (1.0)	109 (2.7)	149 (3.0)	161 (2.6)

*Both ribotypes were grouped together in these years.

Conclusions: Since CDRN was introduced in 2007 there has been a marked decrease in incidence of CDI and associated deaths in England. The changes coincided with a 77% decrease in the relative prevalence of ribotype 027 cases. The improvements may reflect the success of control measures to reduce cross-infection in hospitals caused by epidemic strains. Continued access to timely typing data appears to help to reduce the prevalence of epidemic *C. difficile* strains.

R2505 Molecular characterisation of aminoglycoside resistance in plasmid-mediated AmpC- (pAmpC) and carbapenemase-producing Enterobacteriaceae

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Objectives: Bacterial resistance to antimicrobial agents has become an increasing problem in clinical medicine, limiting the agents available for treatment of many types of infections. Aminoglycosides are widely used in clinical practice and resistance to these drugs may be due to several mechanisms amongst which aminoglycoside-modifying enzymes (AME) are particularly important. We report here the prevalence of the AME present in plasmid-mediated AmpC (pAMPC) and carbapenemase-producing Enterobacteriaceae and which of them were implicated in this resistance.
Methods: A total of 315 pAmpC- or carbapenemase-producing Enterobacteriaceae strains were selected from a collection of 635 clinical pAmpC- and 43 carbapenemase-producing isolates obtained between February and July of 2009. The susceptibility to aminoglycosides (amikacin, gentamicin, kanamycin, netilmicin, neomycin and tobramycin) was determined by the disk diffusion method. The identification of the modifying-enzymes was done by PCR. Some representative amplicons were sequenced.
Results: One hundred twenty three out of the 315 pAmpC- or carbapenemase-producing strains were aminoglycoside-resistant, being the most affected drug kanamycin (K) (111/123; 90.2%), followed by tobramycin (T) (80/123; 65%), gentamicin (G) (64/123; 52%), netilmicin (N) (24/123; 19.5%), and amikacin (A) (18/123; 14.6%). The most frequent enzyme found was APH(3′)-Ia (KNm) (48/123; 39%), AAC(3)-IIa (KGTN) (36/123; 29.3%), AAC-6′-Ib (KTAN) (31/123; 25.2%), ANT-2″-Ia (KGT) (21/123; 17.1, APH(3′)-IIa (KNm) (2/123; 1.6%) and AAC(3)-IVa (TGN) (1/123; 0.8%). Thirty percent of the strains presented more than one enzyme. Finally, 13 strains remain to be concluded. The frequency of the different enzymes found was similar between pAmpC- and carbapenemase-producing strains, with one exception, AAC(3) type enzymes were not been found in carbapenemase-producing strains.
Conclusions: The prevalence of AME in pAMPCs- and carbapenemase-producing strains was 31.4% and 7%, respectively. APH(3′)-Ia was the most frequent enzyme found followed by AAC(3)-IIa.

R2507 Diversity of plasmids in CTX-M-producing *Klebsiella pneumoniae* and *Escherichia coli* in a tertiary medical care centre in the city of Ribeirao Preto, Brazil

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Objectives: The aim of this study was to search the diversity of plasmids in extended spectrum beta-lactamase CTX-M-producing *Klebsiella pneumoniae* and *Escherichia coli* in the University Hospital of the Faculty of Medicine of Ribeirao Preto-University of Sao Paulo (HCFMRP-USP), a tertiary medical care centre, in the city of Ribeirao Preto, Brazil, from April to August of 2007. These bacteria were collected during the outbreak of KPC-2-producing *Klebsiella pneumoniae* in the same hospital.
Methods: Seventy four *K. pneumoniae* and 37 *E. coli* resistant to broad spectrum cephalosporins and/or aztreonam and susceptible to carbapenems were investigated. The blaCTX-M, KPC and SPM genes were investigated using PCR. The PCR-Based Replicon Typing (PBRT) scheme was performed to search replicons of the major plasmid incompatibility groups (Inc) among Enterobacteriaceae.
Results: All bacteria were negative to KPC and SPM-enconding genes. In 59 (79.7%, 59/74) *K. pneumoniae* and 25 (67.5%, 25/37) *E. coli* was amplified CTX-M-enconding gene. IncHI1 (n = 1), I1 (n = 1), N (n = 3), FIC (n = 1), A/C (n = 14) and F (n = 1) were detected in *K. pneumoniae*. IncHI1 (n = 1), I1 (n = 9), N (n = 2), FIA (n = 3), FIB (n = 17), P (n = 3), A/C (n = 9) and F (n = 21) were detected in *E. coli*. No plasmid of the IncHI2, X, L/M, W, Y, T, FIIAs, K and B/O was found. In 38 *K. pneumoniae* and one *E. coli* were not amplified replicons of plasmids, using PBRT scheme. However, more than one plasmid was amplified in the most of *E. coli*. IncFIIAs plasmids were not found in this study, seems to be more related to clones of KPC-2 producing-*K. pneumoniae*, mainly the ST258, as previously reported in the same hospital.
Conclusion: There is a diversity of Inc plasmids in *K. pneumoniae* and *E. coli* in the HCFMRP-USP. The data found suggest that there is a common dissemination of IncA/C plasmids in both bacteria and a prevalence of FIB and F plasmids in *E. coli*. Further studies will be performed to determine the plasmids and clones that carrying CTX-M-encoding genes. These data will contribute to knowledge of dissemination of CTX-producing Enterobacteriaceae.

R2508 First outbreak of carbapenem-resistant OXA-48-producing *Klebsiella pneumoniae* in Russia

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Background and Objectives: An increase in the incidence of carbapenem-resistant *Klebsiella pneumoniae* (Kp) was detected in a 300-bed neurosurgery hospital in Moscow, Russia since August 2010. The aim of this study was to determine the mechanisms of resistance and genetic relatedness among the carbapenem-resistant Kp isolates collected during a suspected outbreak.
Methods: Seventeen carbapenem-non-susceptible Kp isolates were collected from 15 ICU patients between August 2010 and April 2011. All isolates were nosocomial and were obtained from respiratory (n = 11), urine (n = 3) and central nervous system (n = 3) specimens. Susceptibility testing was performed by agar dilution and interpreted by EUCAST criteria. Carbapenemase production was screened by modified Hodge test. Real-time PCR with specific primers and probes was used to detect carbapenemase (VIM, IMP, NDM-1, KPC, OXA-48-like) and ESBL (TEM, SHV, CTX-M) genes. The association of blaOXA-48 gene with IS1999 was determined using PCR mapping and direct sequencing of amplification products. Isolates were typed by ERIC-PCR and RAPD. MLST and plasmid analysis are currently ongoing.
Results: All the isolates studied were resistant to ertapemen (MICs 4–32 mg/L); sixteen were resistant (MICs 4–32 mg/L) and one was borderline susceptible (MIC 1 and 2 mg/L) to imipenem and meropenem. All were also resistant to cefotaxime, ceftazidime, cefepime, ciprofloxacin and fosfomycin, ten to co-trimoxazole and gentamicin, four to amikacin, and one to colistin. The blaOXA-48 gene flanked by the IS1999 was found in all isolates. The blaCTX-M gene was detected as resistance determinant to oxyimino-cephalosporins. The ERIC-PCR and RAPD typing similarly divided the isolates into two groups of ten and six members each, and one single strain. The infection control intervention was implemented which has led to the containment of the outbreak since April 2011.
Conclusions: This study reports the first outbreak of carbapenem-resistant OXA-48-producing Kp in Russia. Results indicate that dissemination of OXA-48 was due to both clonal spread and horizontal transfer. Consistent with other reports, a co-production of OXA-48 carbapenemase with CTX-M ESBL leading to pan-beta-lactam resistance was noted.

R2509 Genetic analysis of the first outbreak of carbapenem-resistant *Klebsiella pneumoniae* in Saudi Arabia

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Objectives: *Klebsiella pneumoniae* has recently surfaced as one of the most antibiotic-resistant organisms in localized outbreaks. Loss of an Outer Membrane Protein (OMP) expression has been implicated for resistance to cephalosporins and carbapenems in certain strains of bacteria. A first documented Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) outbreak involving 23 cases in a tertiary care health facility in Riyadh, Saudi Arabia (King Abdulaziz Medical City, National Guards Health Affairs) was investigated.
Methods: CRKP isolates were identified using standard laboratory methods. Pulse-field gel electrophoresis (PFGE) was done to determine the clonality of the isolates. OXA-A, B, C and D group of OXA β-lactamases were investigated by PCR amplification of their respective gene targets and subsequent sequencing using specific primers. OMP-35 and -36 were also analyzed by PCR amplification and sequencing. Nested primers were designed to sequence and map the whole OMP-36 gene along with the insert.
Results: The isolates were genetically related (77%) by PFGE analysis. All the 23 isolates presented a normal OMP-35 gene sequence. However, there was an interruption in the OMP-36 gene in all isolates. Five out of 23 isolates (22%) showed an insertion of IS-903 in their OMP-36, whereas the remaining 18 showed mutation/deletion of a few nucleotides. This might have resulted in the non-functional OMP-36 protein. Isolates with the insert IS-903 presented A1/A3 PFGE pattern. All 23 isolates were positive for OXA-48, and negative for OXA-A and OXA-D group of enzymes.
Conclusion: This is the first report on the molecular epidemiology of CRKP from Saudi Arabia. The presence of insert in OMP-36 might be responsible in carbapenem resistantnce as is observed in other countries, and same clone may be disseminating in this region

R2510 Diversity of antimicrobial and biocide susceptibility patterns among equine methicillin-resistant staphylococci

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Objectives: Antimicrobial and biocide susceptibility characterization was performed in 14 methicillin-resistant staphylococci isolates (eight *Staphylococcus sciuri*, two *S. aureus*, one *S. lentus*, one *S. fleurettii*, one *S. haemolyticus* and one *S. cohnii cohnii*) previously isolated from 71 sick and healthy horses.
Methods: MICs of the study strains towards several antimicrobial agents, biocides and dyes were determined by broth microdilution according to the CLSI standard. Beta-lactamase production was tested after cephalosporin induction by using the nitrocefin test. PCR amplification was used to detect antimicrobial resistance and plasmid-encoded efflux-pump genes. *S. sciuri* strains were subjected to PFGE. All isolates were characterized by dru-typing.
Results: Beta-lactamase production was detected in three strains and confirmed by the presence of the blaZ gene. Only the *S. lentus* isolate was resistant to chloramphenicol due to the presence of the gene cat pC221. Likewise only one *S. aureus* showed a high MIC (>256 mg/L) to trimethoprim and the dfrK gene was detected. Eight strains were resistant to tetracycline due to the presence of the genes tet(K) (n = 7) and tet(M) (n = 1). The erm(C) gene was seen in two isolates, which showed resistance to both, erythromycin and clindamycin. High MICs to gentamicin and kanamycin were observed in two strains which were encoded by the bifunctional enzyme aacA-aphD (n = 2), and also by aph(3′)-IIIa (n = 1). Fusidic acid resistance and the gene fusC were detected in a single *S. aureus* strain. Two strains had high MICs to ethidium bromide ((64 mg/L), benzalkonium chloride (2 mg/L) and triclosan (2 mg/L) and carried qac genes (*S. haemolyticus* harbouring qacA and *S. cohnii cohnii* carrying both qacB and a qacH-like gene). The same *S. sciuri* clone, with an undistinguishable PFGE profile, was isolated from different horses. The dru type dt11a was the most frequently identified type (n = 7), two single strains (*S. lentus* and *S. haemolyticus*) harboured novel dru types one *S. aureus* had dt10q and three strains were non-typeable.
Conclusions: This study showed that methicillin-resistant staphylococci are important reservoirs of antimicrobial and biocide resistance genes, which circulate in different staphylococcal species, including MRSA. These bacteria/genes seem to be disseminated in the equine population and can be transmitted to humans in close contact, which raises the question of potential interspecies and zoonotic spread.

R2511 Prevalence of plasmid-mediated quinolone resistance determinants (qnr) and aac(6‘)Ib-cr in Enterobacteriaceae from blood cultures in a Greek university hospital

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Objectives: To determine the prevalence of plasmid-mediated quinolone resistance determinants in blood culture isolates of Enterobacteriaceae recovered in Attikon University Hospital, Athens, Greece.

Methods: Over an 18-month period (Dec 2008 to May 2010) a total of 147 Enterobacterial isolates were recovered from blood cultures of single hospitalized patients. Thirty-seven *Escherichia coli*, 32 *Klebsiella pneumoniae*, 13 *Enterobacter* spp., eight *Serratia marcescens*, two *Morganella morganii*, two *Stenotrophomonas maltophilia*, one *Proteus vulgaris* and one *Providencia stuartii* isolates were retrieved from the laboratory collection and tested. Isolates were screened for the presence of qnrA, qnrB and qnrS by multiplex PCR, using universal primers for each gene amplifying all related alleles. Gene aac(6′)-Ib was screened by PCR and the aac(6′)-Ib-positive PCR products were digested with BtsCI to identify the aac(6′)-Ib-cr variant. The qnr and aac(6′)-Ib-cr positive isolates were additionally tested for extended-spectrum β-lactamases (ESBL), metallo-β-lactamases (MBL) and *K. pneumoniae* carbapenemase (KPC).

Results: Among the 96 Enterobacterial isolates tested, five isolates (5.2%) were positive for qnr genes, all harbouring the qnrS determinant. The aac(6′)Ib gene was detected in 21 isolates (21.9%), with four isolates containing the mutated variant aac(6′)Ib-cr (4.2%). Two *Enterobacter* spp. and one *S. maltophilia* isolates harboured only the qnrS. One *E. coli* isolate harboured only the aac(6′)-Ib-cr, while one *K. pneumoniae* and one *M. morganii* isolates harboured simultaneously the qnrS and the aac(6′)-Ib-cr. Aac(6′)Ib was significantly more prevalent among *K. pneumoniae* isolates (50%), but only one harboured the aac(6′)-Ib-cr (3.1%). Isolates carrying only the qnrS had MICs to nalidixic acid ranging from 4 to 8 mg/L and to ciprofloxacin from 0.032 to 3 mg/L, while qnrS and aac(6′)-Ib-cr –positive isolates were resistant to both antimicrobials (MIC >32 mg/L). Trimethoprim/sulfamethoxazole was active against the qnrS-positive isolates but not against those carrying aac(6′)-Ib-cr probably due to co-existing resistance mechanisms. Only the *K. pneumoniae* isolate was an ESBL producer.

Conclusions: This epidemiological survey of the three known qnr determinants and of aac(6′)-Ib-cr shows their presence among Enterobacteriaceae responsible for bloodstream infections in patients hospitalized in a Greek tertiary-care hospital, with the qnrS gene being identified at 5.1% and aac(6′)-Ib-cr at 4.2%.

R2512 First report of a quinolone resistance mutation in the Chlamydia trachomatis gyrA gene from clinical sample

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Objectives: Genital Chlamydia trachomatis (CT) infection is the most prevalent STI worldwide. So far, mutational resistance to fluoroquinolones (FQs), which are used, along with macrolides and tetracyclines, for treatment of CT infection, has not been described in clinical CT isolates, although selection of FQ resistance mutations in primary target (GyrA) has been shown in vitro. In Russia, FQs are used widely and available over-the-counter thus potentially exerting significant selective pressure for resistance development in STI pathogens. This study aimed to assess the presence of FQ resistance mutations in quinolone resistance determining regions (QRDRs) of the CT gyrA and parC genes in clinical samples.

Methods: Two sets of urogenital samples (cervical swabs from females and urethral swabs from males) were screened for mutations in the CT gyrA and parC genes using a newly designed real-time PCR assays. One set comprised 33 samples collected in St. Petersburg in 2006–2008 from 16 patients with posttreatment recurrence of CT infection. The other consisted of 557 consecutive CT positive samples from gynaecological and urological patients collected in Smolensk in 2009–2011 and St. Petersburg in 2010–2011 during routine testing by PCR. The presence of mutations was inferred by postamplification melting temperature (Tm) analysis of fluorescent probes complementary to the sequences encoding amino-acids 80–87 of GyrA and 80–85 of ParC (*Escherichia coli* numbering). Samples showing altered probe Tm as compared to a wild type control (CT L2) were further characterized by sequencing of QRDR containing fragments.

Results: Of the 590 CT positive samples, 551 and 543 were positive in the PCR assays targeting the gyrA and parC genes, respectively. No samples were found to contain mutations in parC, but five samples revealed the presence of mutations in gyrA. Three of them had only silent substitution at gyrA codon for His80. Two samples, both obtained from the same patient in the group of patients with posttreatment CT recurrence, had Ser83-Gly substitution, which is known to be associated with FQ resistance in various Gram-negative bacteria, and two additional mutations, Val61-Ala and His129-Gln, outside the gyrA QRDR.

Conclusion: The results of our study indicate that, although extremely rare, gyrA mutations associated with FQ resistance may occur in clinical CT isolates. Clinical relevance of these findings is to be assessed.

R2513 Diversity of carbapenem-resistant bacteria in untreated drinking water in Portugal

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Objectives: The emergence of resistance to carbapenems in clinical settings may seriously compromise the therapeutic usefulness of these antibiotics. However, the current status of dissemination of carbapenem resistance in natural environments remains unknown. The aim of this study was to assess the prevalence, phylogenetic diversity and antibiotic resistance trends of heterotrophic carbapenem-resistant bacteria (CRB) in untreated drinking waters in Portugal.

Methods: Potable water was collected from ten public fountains located in the North and Central regions of Portugal. Prevalence of CRB was estimated in PCA and R2A media with or without imipenem (4 μg/mL). Clonal relatedness of CRB isolates was established by REP-PCR and phylogenetic analysis was based on the 16S rRNA gene. Antimicrobial susceptibility to 16 antibiotics was assessed by disc diffusion methods. Detection of genes encoding β-lactamases was done by PCR.

Results: CRB were detected in all fountains, ranging from 0.1% to 15.9%. A total of 36 non-clonal carbapenem-resistant isolates were identified as Stenotrophomonas (n = 19), Pseudomonas (n = 3), Janthinobacterium (n = 3), Chryseobacterium (n = 2), Sphingobacterium (n = 2), Acidovorax (n = 1), Caulobacter (n = 1), Cupriavidus (n = 1) and Sphingomonas (n = 1). Seventy two per cent of the isolates were resistant to at least six beta-lactams. From those, 22% displayed additional resistances to at least two non-beta-lactam antibiotics. Apart from beta-lactams, resistances to nalidixic acid (22% of the isolates), streptomycin (22%) and kanamycin (19%) were most frequently detected. Common acquired beta-lactamase genes (blaTEM, blaSHV, blaIMP, blaVIM and blaOXA) were not detected.

Conclusions: Carbapenem resistance in untreated drinking waters was mainly represented by genera previously described as intrinsically resistant to these antibiotics and acquired mechanisms of resistance to beta-lactams were not detected. These results suggest that the dissemination of anthropogenic-derived carbapenem resistance in the environment may be at an earlier stage. Therefore this presents an opportunity to start implementing monitoring strategies to follow this dissemination. Also, the high variability in carbapenem-resistance prevalence justifies efforts to identify factors accounting for these differences.

R2514 Characterisation of blaOXA-48 in *Enterobacter cloacae* strains in southern Spain

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Objectives: The oxacillinase OXA-48 was first identified in a *Klebsiella pneumoniae* but recently it has been described in other

enterobacteria. We report the characterization and genetic environment of OXA-48 carbapenemase in *Enterobacter cloacae* isolated from two patients hospitalized in Cadiz, Southern Spain.

Methods: Identification and antimicrobial susceptibility tests were determined by a commercial microdilution system. MICs of carbapenems were confirmed by Etest. Phenotypic detection of carbapenemases was determined by the modified Hodge's test (MHT). Molecular analysis of plasmid-encoded β-lactamases genes was performed using Check Carba ESBL. The confirmation of OXA-48 was determined by PCR and sequencing. Outer membrane protein (OMP) analysis was performed. The genetic relationship between the strains was studied by Rep-PCR. Plasmids were characterized by PCR-based replicon typing. The genetic environment of the blaOXA-48 gene was determined by PCR and sequencing using blaOXA-48 and Tn1999 transposon primers.

Results: Both strains were resistant to all beta-lactams tested. MICs of imipenem and meropenem were 8 and 16 mg/L, respectively, for the first strain, and 2 and 8 mg/L, respectively, for the second one. MHT was positive. blaSHV-like, blaCTX-M9 group and blaOXA-48 in both strains were detected. Rep-PCR suggests that the strains were clonally related. Both strains expressed three outer membrane proteins in the range of the porins size (OmpF, OmpC and OmpA). A approximately 70 kb plasmid in both strains was detected. Hybridization analysis showed the relationship between OXA-48 and a plasmid of incompatibility group P. Replicons of incompatibility groups Y, T, and R were also detected in both isolates, while IncHI2 was detected only in one. The blaOXA-48 gene was flanked by two copies of IS1999, and the presence of an IS1R element truncating the IS1999 insertion sequence upstream from blaOXA-48 allowed identification of a Tn1999.2 type transposon.

Conclusion: OXA-48 has begun to spread since the first outbreak in Turkey, in particular in the Eastern and Southern Mediterranean region being the Tn1999.2 transposon implicated. We describe two clonally related isolates of *E. cloacae* carrying Tn1999.2. Environmental isolates harboring blaOXA-48 in Morocco suggests that OXA-48 producers may lead to community-acquired infections in the Mediterranean area, with a threatening risk of dissemination to other European countries.

R2515 Biocides and multi-drug resistance associated with the first extended-spectrum beta-lactamase-producing nontyphoidal Salmonella strain isolated in Portugal

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Objectives: Extended-spectrum β-lactamases (ESBLs) in Salmonella is a newly emerging threat worldwide though their distribution is uneven in different geographical regions. This study characterizes the first ESBL-producing Salmonella identified in Portugal and the nature of the co-mobilized genes that can confer selective advantages in the animal setting.

Methods: Susceptibility to antibiotics and b-lactamase production was tested by disk diffusion method and to b-lactams confirmed by E-test. Clonality was established by PFGE (XbaI) and MLST. Search of arsB (arsenic), terF (tellurium), copD (copper), merA (mercury), and silA (copper/silver) genes encoding resistance to compounds found in the animal setting (feed, disinfectants and environmental pollution), antibiotic resistance genes, plasmid and integron backbones (PCR, RFLP and/or sequencing), transferability and genomic location (I-CeuI/S1 nuclease hybridization) was performed.

Results: In February 2011, a Salmonella (ST142) isolate with a positive double disk synergy test, carrying an ESBL identified as blaCTX-M-nine gene, was identified from a hospitalized child with gastrointestinal infection. It was resistant to ampicillin (MIC > 256 mg/L), cephalotin (MIC > 256 mg/L) and cefotaxime (MIC = 4 mg/L), but not to ceftazidime (MIC = 0.5 mg/L), cefepime (MIC = 1 mg/L), aztreonam (MIC = 1 mg/L) and carbapenems (MIC = 0.008–0.38 mg/L). Resistance to gentamicin, kanamycin, tobramycin, streptomycin and sulfamethoxazole was also detected and a class 1 integron carrying aadB-aadA2-qacEd1-sul1 was found. The blaCTX-M-9 gene and the class 1 integron were located on a conjugative IncHI2 (99% homology with pR478) plasmid of 240 kb, also carrying the arsB (arsenic resistance) and terF (tellurium resistance) genes. The ISEcp1 was not detected upstream of the blaCTX-M-9 gene and merA, silA and copD genes described in the early antibiotic resistance IncHI2 plasmid pR478 (reference IncHI2 plasmid) were not detected in our strain.

Conclusion: This is the first description of Salmonella harbouring genes encoding ESBL, conferring resistance to therapeutically important broad-spectrum b-lactams, in Portugal. The finding of blaCTX-M-9 gene in a conjugative plasmid IncHI2 carrying genes that confer resistance to antibiotics and biocides (arsenic and tellurium) that could be extensively used in animal production setting is worrisome, as persistence of this b-lactamase and its emergence in other Salmonella strains could be anticipated.

R2516 Characterisation of multidrug-resistant Enterobacteriaceae producing NDM-1 and OXA-48 carbapenemases from Oman

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Objectives: The successful worldwide dissemination of carbapenemase-producing Enterobacteriaceae is of special concern because of limited therapeutic options and higher mortality. While the dissemination of carbapenemase KPC is predominantly bound to distinct *Klebsiella pneumoniae* strains, spread of OXA-48 and NDM-1 enzymes is mainly due to conjugative transfer of different plasmids carrying these carbapenemase genes and facilitating the transmission among many enterobacterial species. Here we report on molecular analysis of multidrug-resistant Enterobacteriaceae isolates from hospitals in Oman.

Methods: Carbapenem resistant isolates of species *K. pneumoniae* spp. (n = 13), *Escherichia coli* (n = 4) and *Enterobacter cloacae* (n = 1) were collected from three hospitals in Oman between September 2010 and February 2011. All patients were Omani whereby three patients recently travelled to India and Pakistan before their admission to the hospital. Relevant resistance genes were identified by PCR and sequencing. Conjugation experiments and strain-typing by PFGE analysis were performed.

Results: The carbapenemase OXA-48 was identified in five isolates. In ten *K. pneumoniae* isolates we identified metallo-beta-lactamase NDM-1 and the OXA-48-related enzyme OXA-181 was identified in two isolates. XbaI-macrorestriction analysis revealed seven closely related NDM-1-producing *K. pneumoniae* isolates and three identical *E. coli* isolates with OXA-48 indicating several events of clonal transmission of carbapenemase producing strains in two hospitals. Furthermore we detected additional extended spectrum β-lactamases (ESBL) CTX-M-15 (n = 14) and CTX-M-24 (n = 3) as well as other β-lactamases (TEM-1, SHV-11, OXA-1, OXA-9) in the majority of isolates. The blaOXA-48 genes were successfully transferred into recipients and plasmids of ca. Of 60 kb size were isolated from transconjugants. The blaNDM-1 genes were found to be located on conjugative plasmids of different size. The reduced carbapenem MIC values of transconjugants indicate the occurrence of further carbapenem resistance mechanisms like increased production of efflux pumps or loss of porins (outer membrane proteins, OMPs) in the clinical isolates.

Conclusion: We identified various carbapenemase-producing Enterobacteriaceae as colonizers or cause of urinary tract infections or severe blood infections in patients from Oman. There is an urgent need of surveillance to prevent further spread of these multidrug-resistant pathogens.

R2517 Prevalence and mechanism of carbapenem resistance in *Acinetobacter baumannii* isolates from Thailand

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Objectives: To investigate the prevalence of carbapenem resistance and mechanism of carbapenem resistance in *A. baumannii*.
Methods: A total of 200 *A. baumannii* isolated from King Chulalongkorn Memorial Hospital during May 2008 to April 2009 were included in this study. Antimicrobial susceptibility testing of imipenem and meropenem was determined by agar dilution method. Oxa-type carbapenemase and metallo-beta-lactamase genes were determined by PCR. The presence of ISAba1, ISAba2 and ISAba3 upstream the blaOXA-like genes was detected by PCR and DNA sequencing. Carbapenem efflux pump was determined by using CCCP, the effux pump inhibitor. The loss or decrease of outer membrane protein (OMP) was analysed by SDS-PAGE.
Results: The prevalence of imipenem and meropenem resistance was both 92.5%. All carbapenem-resistant *A. baumannii* isolates had carbapenemase activity by modified Hodge test. However, metallo-beta-lactamase enzymes by EDTA-disk synergy were not detected in any isolates. The presence of carbapenemase genes including blaOXA-23, blaOXA-24, blaOXA-51, blaOXA-58, blaIMP, blaVIM, blaGIM, blaSPM and blaSIM was screened by multiplex-PCR. Of 185 carbapenem-resistant *A. baumannii* isolates, 182 (98.4%) carried blaOXA-51-like with blaOXA-23-like and 3 (1.6%) had blaOXA-51-like with blaOXA-23-like and blaOXA-58-like. The blaIMP, blaVIM, blaGIM, blaSPM and blaSIM metallo-beta-lactamase genes were not found in any isolates. ISAba1 was found in the upstream region of blaOXA-23-like in all 25 representative isolates and ISAba3 was present upstream blaOXA-58-like in all three isolates. The effux pump inhibitor, CCCP, could not reduce the MIC of imipenem and meropenem equal or more than fourfold. The result showed no efflux pump mechanism in all of the carbapenem-reststant isolates. The reduction of 43 kDa OMP was detected in two of the 10 representative carbapenem-resistant *A. baumannii* isolates.
Conclusion: High resistance rate of carbapenems was found in *Acinetobacter baumannii* isolates from Thailand and carbapenem resistance mechanism was attributed to the production of OXA-23 carbapenemases and the decrease of 43 kDa OMP. ISAba1 and ISAba3 play an important role in the expression of the blaOXA genes in our isolates.

In vitro antibacterial susceptibility and drug interaction studies

R2518 More than a Gram-positive agent: in vitro activity of dalbavancin against *Moraxella catarrhalis*

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Objectives: Dalbavancin is a semisynthetic lipoglycopeptide antibiotic with in vitro activity to a variety of Gram positive organisms, including one of the primary respiratory tract infection (RTI) pathogens, *S. pneumoniae*. Unlike other lipoglycopeptide antibiotics (vancomycin and teicoplanin), dalbavancin has previously demonstrated some in vitro activity against another key respiratory pathogen, *M. catarrhalis*. Since this initial testing with *M. catarrhalis* was done, the broth microdilution (BMD) method was modified to include preparation of the stock solution in DMSO and subsequent dilution in cation adjusted Mueller Hinton broth (CAMHB) containing 0.002% polysorbate 80 (P80). This study was performed to assess the activity of dalbavancin against *M. catarrhalis* using the current BMD procedure.
Methods: Of 10 *Moraxella catarrhalis* (including nine beta lactamase producing strains) and a quality control strain, *S. aureus* (ATCC 29213), were tested by broth microdilution using CAMHB with and without 0.002% P80.
Results: With addition of 0.002% P80 dalbavancin MIC results decreased one dilution for all study isolates. Using the current BMD method, the dalbavancin MIC was 2 mg/L for nine strains and 1 mg/L for one strain.
Conclusion: Using the current susceptibility method, dalbavancin has demonstrated good in vitro activity to *M. catarrhalis*. In addition to its superior PK/PD profile, dalbavancin stands apart from other lipoglycopeptides with its activity against *M. catarrhalis*, providing a potential option in a regimen to treat community acquired RTI.

R2519 Prevalence of anaerobic bacteria and evaluation of its resistance to antibiotics by E test method

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Introduction: Infections caused by anaerobic bacteria are common, and may be serious and life-threatening. Anaerobes are the predominant components of the bacterial flora of normal human skin and mucous membranes, and are therefore a common cause of bacterial infections of endogenous origin.
Objective: the aim of this study was to evaluate the anaerobic bacteria isolated from clinical samples, and to study the pattern of susceptibility to antibiotics from clinical samples since March 2009 until November 2011 in the ''Hospital Universitario de la Princesa'', in Madrid.
Methods: Of 382 strains were isolated from different clinical samples: abscesses (34.82%), peritoneal fluid (18%), biopsies (17%), drainage (9.4%), wound/pus/tissue (7.32%), ascitic fluid (3.67%), bile (5.24%), and other sites (4.55%). Samples were cultured on blood, phenyl ethyl alcohol (PEA), Schaedler (SCS) and Schaedler Kanamycin Vancomycin agar with 5% sheep blood and all plates were incubated in an anaerobic jar (Gas Pak, Becton Dickinson) for 48 hour. The identification of the microorganisms was carried out through conventional tests and confirmed by Rapid ID32A (bioMérieux) gallery. In 84 strains susceptibility test was performed. The minimum inhibitory concentrations (MICs) for amoxicillin/clavulanate, piperacillin/tazobactam, meropenem, clindamycin and metronidazole were determined by E-test (AB Biodisk) according to Clinical Laboratory Standard Institute (CLSI) recommendations.
Results: The microorganisms more frequently isolated were: *Bacteroides* sp. (40.58%), *Propionibacerium* sp. (18.06%), *Prevotella* sp. (13.09%), Clostridium perfringens (7.59%) and Clostridium other than perfringens (5.50%). We isolated a total of 44 different species. From all the species, *Bacteroides fragilis* group was the most prevalent. The global percentage of susceptibility were as follows: clindamycin (58%), amoxicilline/clavulanic acid (74.02%), piperacillin tazobactam (84.2%), meropenem (90.50%) and metronidazole (98.10%)
Conclusions: Nearly half of the strains belonged to *Bacteroides* sp. We obtained a high percentage of resistance to amoxicillin/clavulanate and clindamycin among the isolates studied. These findings emphasize the need to know the susceptibility patterns of anaerobic bacteria in any context.

R2520 Determination of MIC values of tigecycline against multidrug-resistant Gram-negative bacteria isolated from bacteraemic episodes by using agar dilution and Etest methods

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The aim of this study is to determine in-vitro susceptibility of tigecycline against multi-drug resistant gram negative bacteria which are isolated from bacteremic patients using agar dilution and E-test methods while evaluating the correlation between the two test methods. Two hundred and six ESBL producing *E. coli*, *K. pneumoniae* and multi drug resistant (MDR) *A. baumannii* strains which had been obtained from blood culture of patients hospitalized in service and intensive care units in our hospital between January 2008 and October 2010 were included. MIC50/MIC90 values determined by using agar

dilution and E-test methods were 0.5/0.5 and 0.25/0.5 μg/mL for *E. coli*, 1/2 μg/mL and 0.75/2 μg/mL for *K. pneumoniae*, and 4/4 and 2/4 μg/mL for *A. baumannii*, respectively. It's observed that the correlation between MIC values measured by agar dilution and E-test methods is high for *K. pneumoniae* ($R = 0.776$; $p < 0.001$) and *A. baumannii* ($R = 0.759$; $p < 0.001$) while it is low for *E. coli* ($R = 0.299$; $p < 0.001$). It is observed that the correlation between two tests decreases as MIC values, 1 and 2 μg/mL for *E. coli* ve *Acinetobacter* spp., respectively. Consequently, tigecycline is considered an appropriate agent for treating bacteremia caused by *E. coli*, as the MIC values are less than serum concentration of tigecycline. On the other hand, MIC values for A. baumanni strains which are much higher than the serum concentration, eliminates the use of tigecyline in the treatment of bacteremia due to A. baumanni. Lastly, because *K. pneumoniae* strains with high MIC values are emerging; the decision of using tigecycline should be made after determining the MIC value of the causative strain. As the results of E-test and agar dilution used to determine tigecycline susceptibility are correlated, E-test which is easier to use could be utilized for daily use. However, due to the decrease in the correlation between test results for *E. coli* and A. B baumannii with high MIC values, the susceptibility test should be repeated by using either AD or broth microdilution which are the golden standards if the MIC value is found to be high.

R2521 Susceptibility of urinary coliforms to fosfomycin trometerol in Wales

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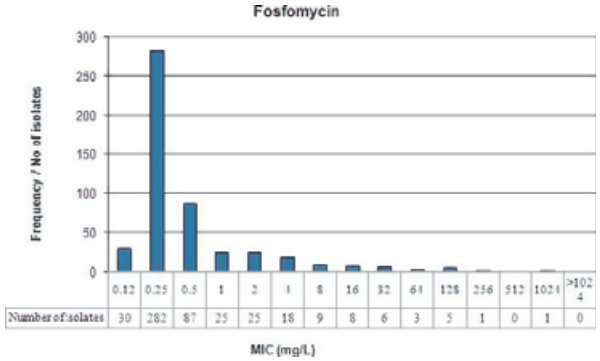
Objectives: Urinary tract infections (UTIs) are among the commonest types of bacterial infections, with antibiotic treatment for UTIs associated with important medical and economic implications. Antibiotic agents such as beta-lactams, trimethoprim, and cotrimoxazole have been used for the treatment of UTIs. However the emergence of uropathogens, mainly *Escherichia coli*, exhibiting high rates of resistance associated with the production of extended-spectrum β-lactamases (ESBLs) is worrisome. Fosfomycin trometerol is a broad-spectrum bactericidal antibiotic agent that inhibits the synthesis of the bacterial cell wall and has a pharmacokinetic profile which encourages use for UTIs; mean peak urinary concentration of an oral single dose of 3 g fosfomycin trometerol (FOS) occurs within 4 hour, while concentrations sufficient to inhibit the majority of the urinary pathogens are maintained for 1–2 days. This study aims to evaluate the susceptibility of 500 recent urinary coliform isolates to fosfomycin trometerol for use as an alternative therapy in uncomplicated UTIs.
Methods: Susceptibilities of 500 urinary Coliforms, including *E. coli* (n = 413), *Enterobacter*, *Citrobacter* and *Klebsiella* species (KES) (n = 63) and *Proteus* species (n = 24) to fosfomycin were determined. Minimum inhibitory concentrations (MICs) were determined using agar dilution incorporating 25 mg/L glucose-6-phosphate to potentiate fosfomycin trometerol activity and categorised using BSAC breakpoints. Any isolate with raised MICs were tested against other agents.



Results: Of 490 (98%) of isolates showed susceptible MICs to fosfomycin trometerol with the mean GeoMIC 0.43 mg/L. Of 2% (10 isolates) exhibited ''resistant'' FOS MICs of >32 mg/L. The frequency of isolates with FOS MICs can be seen in Fig. 1. Of the resistant isolates three were *E. coli* and seven KES; all exhibited susceptible MICs to gentamicin, 3rd generation cephalosporins and mecillinam.
Conclusions: Fosfomycin trometerol is active against Gram negative uropathogens. Of 98% of isolates were susceptible with resistant isolates all proving susceptible to alternative therapies.

R2522 Synergistic effect of linezolid in combination against multidrug-resistant and drug-susceptible isolates of *M. tuberculosis*

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Objective: To evaluate the in vitro effectiveness of two combinations of three drugs: (i) linezolid, levofloxacin and amikacin; (ii) linezolid, levofloxacin and ethambutol against multidrug-resistant (MDR) and drug-susceptible isolates of *M. tuberculosis*.
Methods: Clinical isolates were collected in the Hospital Clinic of Barcelona: nine MDR, 11 drug-susceptible isolates and H37Rv reference strain. The individual MICs of the isolates studied were evaluated with the proportional method in 7H11 solid medium. The abovementioned combinations were studied crossing three concentrations of each antibiotic (corresponding to their MIC and two lesser dilutions), with an adaptation to a three-antibiotic chequerboard assay in 7H11 medium. The fractional inhibitory concentration (FIC) was calculated as: FIC index = MICA in combination/MICA alone + MICB in combination/MICB alone + MICC in combination/MICC alone where A, B and C were the three respective antimicrobial agents tested. The FIC index was interpreted as FIC index ≤ 0.75, synergistic activity, FIC>0.75–4, indifference and FIC>4 antagonistic activity. As a control a 1/100 inoculum was seeded in antibiotic-free medium. All the plates were incubated at 37°C, being read after 21 days.
Results: The individual MIC of the isolates studied was 0.5–1, 0.5, 2.5 and 2.5–5 μg/mL for linezolid, levofloxacin, amikacin and ethambutol, respectively. In the MDR and drug-susceptible isolates the MICs of linezolid, amikacin and ethambutol in combination decreased two dilutions compared to their individual MIC. In the MDR and drug-susceptible isolates the MIC of levofloxacin in combination was either maintained or decreased from one to two dilutions in combination A, with most of the isolates in combination B being decreased two dilutions compared to their individual MIC. Eight and 19 out of the 21 MDR and drug-susceptible isolates showed synergism (FIC = 0.75) in combinations A and B, respectively. Comparing each isolate with the two combinations, 12 MDR and drug-susceptible isolates displayed synergism with (FIC = 0.75) combination A but not with combination B.
Conclusion: The two combinations studied showed indifference or synergism against MDR as well as susceptible isolates of *M. tuberculosis*. The combination including linezolid was more synergistic than that with amikacin, suggesting that it may be useful in MDR treatment.

R2523 In vitro susceptibility trends for MSSA bloodstream isolates over a 3.5 year period in Detroit

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Objectives: In this study, we evaluated in-vitro susceptibility of clinical methicillin-susceptible *Staphylococcus aureus* (MSSA) bloodstream isolates over a 3.5 year period, in Detroit, MI. The study was done to evaluate in vitro susceptibility changes over time to vancomycin (V), and daptomycin (D) and Linezolid (L).

Methods: We evaluated 418 MSSA consecutive bloodstream isolates from patients in urban Detroit from July 2007 to December 2010 to determine susceptibility trends using susceptibility methods for MIC by manual microbroth dilution and Etest (bioMerieux, Inc.) to V, D and susceptibility to L by Etest. The geometric mean was determined for each year and analysed over time.

Results: Statistical analysis was performed using t-test, and significant statistical increases or decreases are described in the following table:

	No.	V (estrip)	p value	V (broth)	p value	D (estrip)	p value	D (broth)	p value	L (e strip)	p value
7/1-12/31/07	43	1.488		0.541		0.487		0.456		1.326	
2008	123	1.557	0.072	0.705	0	0.411	**0.0034**	0.464	0.9523	1.128	**0.001**
2009	116	1.444	**0.0001**	0.823	**0.0014**	0.47	**0.0003**	0.658	0	0.894	0
2010	136	1.452	0.7752	0.763	0.1056	0.456	0.4185	0.633	0.5104	1.02	0.1279

Conclusion: Over a 3.5 year period in urban Detroit, we found a significant decrease in MIC's by Etest from 2008 to 2009, yet a significant increase by manual microbroth of these MSSA bloodstream isolates to V the same time. There was a significant decrease in D MIC by Etest from 2007 to 2008, yet an increase from 2008 to 2009 by Etest to D. A significant decrease was seen in the MIC's by Etest to L from 2007 to 2008. With increasing reports of vancomycin failure in methiciillin -susceptible *Staphylococcus aureus*, it is important to monitor susceptibilities of other relevant treatment options such as linezolid and daptomycin, to determine MIC creep of these agents.

R2524 In vitro activity of tigecycline against Gram-positive and Gram-negative isolates at a tertiary care hospital

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Objectives: Tigecycline (TIG) is a member of the glycylcycline class of antibiotics with a broad spectrum of activity which includes several Gram-positive and Gram-negative bacteria. It is one of the last resort antibiotics for multidrug resistant pathogens like MRSA and ESBL. In this study we evaluate the in-vitro activity of TIG against multiple gram positive and gram negative isolates including ESBL and MRSA. We also evaluate his activity at our Pseudomonas isolates.

Methods: A total of 506 carbapenem resistant isolates of *Acinetobacter* spp.; 50 g positive including 14 MRSA and 5 VRE isolates as well as 80 g negative isolates (12 ESBL, 12 *Pseudomonas aeroginosa*) isolated in our centre were studied. Isolates were recovered from blood cultures, body fluids, pus, bronchial secretions and urine samples in a two year period, from January 2010 till November 2011. The identification and susceptibility testing was performed via the MicroScan Walkaway (Siemens). The TIG susceptibility testing performed using Etest strips (AB Biodisc, Sweden) according to CLSI guidelines. The EUCAST Enterobacteriaceae breakpoints were used to interpret Tigecycline MIC results for *A. baumannii*.

Results: Of the 506 isolates, 420 isolates exhibit an MIC less the 4 mg/L (Susceptible range) which represent 83.00%. There are 86 resistant isolates (16.99%). Of the resistant strains; there were eight samples exhibit very high level of resistance MIC 256 mg/L, and 12 isolates has MIC equal to 4 mg/L and the rest fall between 6 and 24 mg/L. In our study, all these isolates have susceptibility to amikacin at 21%, to cefepime at 0.6%, to imipenem 2.00% and to colistin 98.00%.

Among the 12 pseudomonas isolates; there are 10 isolates resistant to tigacycline with MIC (8 and two were susceptible MIC $\leq$ 1. Of the 12 ESBL isolates there were 11 susceptible to TIG and one are resistant with MIC of 24 mg/L. ALL gram positive isolates including (MRSA, VRE, S. pnemoniae, *S. pyogenes* and enterococci) were susceptible to TIG with MIC 0023–0.125 mg/L

Conclusion: Tigecycline maintains potent in vitro activity against gram positive pathogens and some of highly resistant *Acinetobacter* spp. As for *A. baumannii*, there is a decreased susceptibility but it may be an alternative treatment option when other agents are excluded due to multidrug resistance. TIG shows no activity against Pseudomonas isolates.

New antimicrobials

R2525 Antagonistic activity of *Lactobacillus* spp. against methicillin-resistant staphylococci

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Summary: *Lactobacillus* spp. strains isolated from faeces were divided into three groups depending on the degree of antagonism against methicillin resistant *Staphylococcus aureus* (MRSA) and *S. epidermidis* (MRSE). Lactobacilli with high antagonistic activity may appear to alternative to antibiotics in the control on methicillin resistant staphylococci.

Aim: Assessment of degree of antagonistic effect of lactobacilli on the growth of methicillin resistant staphylococci.

Methods: Nine *Lactobacillus* spp. strains isolated from faeces, one strain *L. plantarum* 38 isolated from officinal probiotic ''Lactobacterin dry'' (''Microgen'', Russian Federation) and 13 strains of methicillin resistant staphylococci (12 – MRSA, 1 – MRSE), isolated from blood under bacteriaemia were studied. For semi-quantitative assessment of the antagonistic action of lactobacilli in vitro the original technique, based on the phased cultivation of cultures of lactobacilli and staphylococci on a combined solid medium was developed. The result of the antagonistic effect of lactobacillar culture on the staphylococcal culture was assessed by the size of the zone of growth inhibition: high antagonistic activity corresponded to 25–40 mm, medium – 15–25 mm, low – 5–15 mm, absence of antagonism – <5 mm.

Results: All 12 MRSA strains and one strain of MRSE only included in the study were sensitive by antagonistic action of strain *L. plantarum* 38. Three strains of lactobacilli (33.3%) had no antagonistic activity on all 13 methicillin resistant staphylococci strains studied. Two strains of lactobacilli (22.2%) showed no antagonism against single MRSE strain, preserving at the same time antagonistic activity varying degree on different (1–10) MRSA strains. The remaining four strains of lactobacilli (44.5%) demonstrated antagonistic activity against single MRSE strain and the majority (83.3%) of MRSA strains; their activity against MRSE strain was medium and low only, for MRSA strains – predominantly high degree of expressiveness.

Conclusion: Most of the investigated strains of lactobacilli possess antagonistic activity against MRSA and/or MRSE. The presence of antagonistic activity of lactobacilli against methicillin resistant staphylococci opens some possibilities for development of a new generation of drugs aimed to these microorganisms, exhibit a high degree of resistance to the vast majority of modern antibiotics.

R2526 Activity of strawberry (*Fragaria vesca*) leaf phenolic extracts on metallo-beta-lactamase VIM-2 producers *Pseudomonas aeruginosa*

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Objectives: *Pseudomonas aeruginosa* (PA) is at present the fourth most common nosocomial pathogen, causing a wide variety of serious infections on compromised hosts and is characterized by an intrinsic resistance to various antimicrobial agents and for the ability to easily develop multi-drug resistance (MDR) under antibiotic pressure. Metallo-β-lactamases (MBLs) confer a MDR profile to the bacteria that present them, since they hydrolyse all beta-lactams, except aztreonam. At present, there is no available inhibitor of MBLs, which augments the risk of infections caused by MBLs producing bacteria. Some polyphenols are generally recognised as quite potent antibiotics. The aim of this study was to challenge clinical isolates of MBL VIM-2 producers PA with an extract and polyphenol-rich fractions from strawberry (*Fragaria vesca*) leaves, in order to screen for antimicrobial activity, with the intent to find possible future therapeutic alternatives to treat MBL producing PA infections.

Methods: *Fragaria vesca* leaves (Fv) were extracted with ethanol (EtOH) and 50% aqueous EtOH. Three fractions from that crude extract were obtained on a Sephadex® LH-20 column by elution with 50% and

75% aqueous methanol (Fa and Fb, respectively) and 70% aqueous acetone (Fc). Fifteen MBL VIM-2 producers PA clinical isolates were used. Standard microplate assays were performed to determine MICs of extract (0.78–50 mg/mL) and fractions (0.39–25 mg/mL).
Results: Crude extract presented MIC50 = 10.35 mg/mL and MIC90 = 20.7 mg/mL. Fraction Fa presented MIC50 and MIC90 of 25 mg/mL; fraction Fb presented MIC50 = 6.25 mg/mL and MIC90 = 12.5 mg/mL; fraction Fc presented MIC50 and MIC90 of 12.5 mg/mL.
The more active fraction was fraction Fb, essentially constituted by tannins: proanthocyanidins and ellagitannins. Curiously, an exacerbation of the pigment in all isolates was detected with this fraction.
Conclusion: A fraction of a crude extract obtained from *Fragaria vesca* leaves presented an interesting activity against MBL VIM-2 producers PA clinical isolates. One or more polyphenols of this fraction can constitute a significant potential for the development of novel antibacterial therapies against these bacteria. Further studies are required to address this possibility.
For an approach to antimicrobial efficacy, MDR clinical isolates should be used and not only ATCC strains, since they represent more accurately the pathovars of the species, which confers higher interest to the obtained results.

R2528 Screening for bio-active and anti-biofilm substances of *Bacillus* and *Paenibacillus* species

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Objectives: Soil living bacteria are known to produce compounds that promote plant growth and confer resistance to plant diseases caused by different pathogens (1, 2). The rhizosphere can be colonized by biofilm formation by various species, thus antimicrobials ensure also survival advantage against competing commensals. For example, *B. amyloliquefaciens* strain FZB42 secrets at least 12 known antibiotics, which inhibit growth and destroy biofilms of other microorganisms and that belong to different chemical classes: lipopetides, polyketides and small peptides (3). The aim of our efforts is to scrren culture supernatants of soli living Gram+ for novel substances with activity against biofilms of multidrug resistant major bacterial human pathogens involved into catheter- and device associated infections.
Methods: We used supernatants of *B. amyloliquefaciens* (n = 5), *B. pumilus* (n = 1), *B. licheniformis* (n = 1) and *P. polymyxa* (n = 3) that were filtered, lyophilized and resuspended in 1/10 volume in sterilized water. Supernatants were used in disc diffusion tests against multidrug resistant isolates of *E. coli* (n = 3), *K. pneumoniae* (n = 2), *P. auruginosa* (n = 4) *S. aureus* (n = 3), *E. faecalis* (n = 2) and *P. mirabilis* (n = 1) as indicator strains. A more detailed analysis of active compounds was performed using bioautography based on thin layer chromatography.
Results: Supernatants of *P. polymyxa* strains exhibited strongest antimicrobial activity against Gram+ and Gram- pathogens. *B. amyloliquefaciens* FZB 42 showed also high activities against all indicator strains. *B. pumilus* and *B. licheniformis* inhibited mainly growth of Gram+.
Conclusion: Gram-positives soil living bacteria secrete a wide spectrum of bio-active secondary metabolites, which can inhibit the growth of human pathogens. Further experiments will concentrate on identification of particular substances with antimicrobial activity and analyze their anti-biofilm activities.

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R2529 In vitro activities of telavancin and three comparator agents against *Staphylococcus aureus*

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Objectives: *Staphylococcus aureus* is becoming increasingly resistant to antibiotics. The worldwide prevalence of methicillin- resistant *Staphylococcus aureus* (MRSA) poses a particular threat due to difficulties in treatment not only with existing ß-lactams, but also with quinolones. Telavancin (TLV) is a novel semisynthetic lipoglycopeptide with a broad spectrum of activity against aerobic and anaerobic gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA). The objective of the current study was to evaluate the in vitro activity of TLV in comparison with vancomycin (VAN), daptomycin (DPC), and linezolid (LZD) against MRSA and MSSA isolates from various clinical specimens of our hospital.
Methods: Thirty six non-duplicate *Staphylococcus aureus* isolates (28 MRSA and eight MSSA) were recovered from blood, bronchoalveolar secretions, urine, trauma, pus and central venus catheter samples. The samples were isolated from patients hospitalized in surgical and internal departments of AHEPA University Hospital in Greece. Bacterial identification and initial susceptibility testing was performed using Vitek2 system (bioMérieux, France). Minimum inhibitory concentrations (MICs) of TLV, VAN, DPC, and LZD were determined with E-test strips (AB Biodisk, bioMérieux, France).
Results: MICs for 50% (MIC50) and 90% (MIC90) for TLV, VAN, DPC, and LZD against MRSA, and MSSA are shown in the table.
Based upon MIC90 comparisons, TLV was two to threefold more potent than VAN, and up to twofold more potent than LZD against MRSA isolates. Comparing the MIC values of TLV, and DPC against MRSA revealed almost similar results. Significantly elevated MIC90 was observed for TLV (threefold), VAN (16-fold), and LZD (sixfold) against MSSA in contrast to DPC.

Organism (N)	MIC (µg/mL)							
	TLV		VAN		DPC		LZD	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
MRSA (28)	0,250	0,380	1,500	1,500	0,064	0,500	0,750	1,000
MSSA (8)	0,250	0,380	1,5	2	0,047	0,125	0,5	0,75

Conclusion: Our results confirm the potent in vitro inhibitory activity of telavancin against important and emerging antimicrobial-resistant *Staphylococcus aureus* pathogens. Based upon MIC90 comparisons, telavancin was consistently more active than vancomycin, and linezolid against all organisms tested and showed potency equal to or greater than daptomycin against MRSA, but lower against MSSA strains. These data highlight the potential therapeutic use of TLV in the treatment of *Staphylococcus aureus* infections.

R2530 Hybrid nanostructurated materials for bio-interface application in developing new antimicrobial strategies

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Objectives: The present work aims to describe the synthesis and characterization of functionalized magnetite nanoparticles for stabilizing essential oils aiming to obtain an efficient antimicrobial strategy for biofilm related fungal infections.
Materials and Methods: The Eugenia caryophyllata buds essential oil microwave assisted extraction was performed in a Neo-Clevenger type apparatus and its chemical composition was settled by GC-MS analysis. The fungal strains were isolated from different clinical specimens and were identified by Vitek II automatic system. Fe_3O_4/Cn (n = 12, 14, 16, 18) – nanoparticles (core/shell) were synthesized and characterized by HR-TEM, XRD, FT-IR and BET. The nanoparticles were used to achieve a core/shell/adsorption-shell based on essential oil nanosystem for covering treatment of the prosthetic devices represented by catheters.

The obtained modified surfaces were subsequently used for the in vitro study of the fungal biofilm development. The biofilm architecture was assessed by Scanning Electron Microscopy (SEM).
Results: The results proved that the nanostructurated materials (core/shell/adsorption-shell) show a significant antibiofilm activity, highlighting the opportunity of using them for the developing of efficient antibiofilm strategies as coating modified biomaterials (prosthetic devices), as well as for the controlled release of different antimicrobial substances for further clinical applications.

Epidemiology of MRSA, VRE and other Gram-positives

R2531 Molecular characterisation of methicillin-resistant *Staphylococcus aureus* with emergence of epidemic clone of sequence type (ST) 772 and novel ST 2129 in southern India

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Background: The emergence of community-acquired MRSA (CA-MRSA) has changed the current epidemiology of *Staphylococcus aureus*, in particular methicillin-resistant *S. aureus* (MRSA).
Objectives: The aim of the work was to study the molecular epidemiology of MRSA strains isolated from throat swab of pharyngitis patients attending Thoracic Science Department of Government Rajaji Hospital, Madurai, Tamil Nadu, India.
Methods: In total, 63 non-repeated MRSA were isolated in the prospective study carried out between December 2009 and March 2010. These isolates were subjected to two conventional multiplex PCR assays including pvl, mecA, nuc genes detection and SCCmec typing. The antimicrobial susceptibility was determined by disk diffusion method. The clonal relationship between the selected strains was determined by Multilocus Sequence Typing (MLST). Based on the PVL prevalence, SCCmec types and difference in antibiotic resistant profiles, 26 isolates were selected for MLST.
Results: Almost all MRSA showed a multidrug resistant phenotype. The prevalence of antibiotic resistant was moderately high: Kanamycin (96.8%), Tobramycin (85.7%), Gentamicin (87.3%), Trimethoprim (85.7%), Ciprorfloxacin (59.5%), Co-Trimoxazole (55.7%), Erythromycin (58.7%) and Clarithromycin (47.6%). Of the 65 isolates showing resistance to oxacillin and cefoxitin disks, 63 were confirmed to be mecA and nuc gene positives and 69.8% of the isolates harbored PVL toxin gene as determined by multiplex PCR assay. SCCmec typing by multiplex PCR assay revealed that type V was the most predominant type at 50.8% (n = 32) followed by SCCmec type III at 44.4% (n = 28). SCCmec types I, II and IVa were found to be least at 1.6% (n = 1) each. MLST analysis resulted with 12 different Sequence Types for 26 selected MRSA, including a newly assigned ST 2129 with PVL positive, SCCmec type III. Ten strains of SCCmec type V, PVL positives were found to be ST 772 (38.5%). Three strains belonging to ST 368 (11.5%) followed by two strains each found to be STs 1137, 1713 and 217 (7.7%) and one strain each found to be the STs 571, 2129, 474, 585, 1801, 672 and 240 (3.8%).
Conclusion: This study unveils that many CA-MRSA have successfully been established themselves in hospital of South India. The results of the study also give a hint that SCCmec V ST 772, which has recently been believed to be the native of Asia, may emerge as a dominant global epidemic MRSA strain in the coming years.

R2532 Community-acquired methicillin-resistant *Staphylococcus aureus* in a university clinical hospital, Mostar – our first experience

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Objectives: Infections caused by community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) have been recognized as a major public health problem worldwide. CA-MRSA producing Panton-Valentine leukocidin (PVL) causes mostly skin and soft tissue infections, but it can also cause severe invasive diseases, including necrotizing pneumonia. Infections with PVL-positive MRSA have been described in most European countries, but no cases of CA-MRSA infections have previously been reported in University Clinical Hospital Mostar, Bosnia-Herzegovina.
Methods: During the first six months of 2010, we have collected 25 isolates of MRSA. Antibiotic susceptibility testing was performed using disk-diffusion method, according to Clinical Laboratory Standards Institute (CLSI) guidelines. Methicillin-resistance was confirmed by molecular dipstick assay GenoTipe® MRSA (Hain Lifescience, Nehren, Germany) by detection of mecA gene. At the same time, we detected a specific fragment of PVL gene. The isolates were further analyzed by spa-sequence typing, according to SeqNet.org protocol.
Results: All isolates were resistant to penicillin, all were susceptible to sulfamethoxazole/trimethoprim, amikacin, vancomycin, teicoplanin and linezolid. Two of them were uncommonly susceptible to erithromycin, azithromycin, gentamicin, clindamycin and ciprofloxacin. All isolates in the study were positive to mecA gene. Two uncommonly susceptible isolates were found positive to PVL gene, while the other 23 were

Strain	mecA	PVL	spa-type
1	positve	negative	
2	positve	negative	t008
3	positve	negative	t001
4	positve	negative	t001
5	positve	negative	
6	positve	negative	t001
7	positve	negative	
8	positve	negative	t001
9	positve	negative	t001
10	positve	negative	t001
11	positve	negative	t001
12	positve	negative	t001
13	positve	negative	t001
14	positve	negative	t001
15	positve	negative	
16	positve	negative	
17	positve	negative	t008
18	positve	positive	t008
19	positve	positive	t008
20	positve	negative	
21	positve	negative	
22	positve	negative	
23	positve	negative	t001
24	positve	negative	
25	positve	negative	

negative. MRSA isolates from 10 patients have belonged to spa type t001, from four patients to spa type t008, and from one patient to spa type t041. Above described susceptible isolates have belonged to spa type t008. The residual of 10 isolates failed to be typed by spa-typing method. (See Table 1.)

Conclusion: Community-associated MRSA isolates cannot be discriminated from hospital MRSA only by spa typing. The possession of PVL gene in two isolates, which have belonged to spa type t008, suggests that the other two in the present study might belong to the transatlantic strains. The transmission of those strains has been previously described in literature.

Acknowledgements The authors would like to thank Prof Hans-Günther Sonntag PhD and a staff of Institut für Hygiene und Molekular Biologie, Heidelberg, Germany for perfomance spa-typing.

R2533 Impact of acute kidney injury on clinical and economic burden in patients with methicillin-resistant *Staphylococcus aureus* infection: a retrospective, multicentre and observational study

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Objectives: Acute kidney injury (AKI) associated with treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) infection is considered to be a major factor for mortality and morbidity. However, the clinical outcomes and economic burden caused by MRSA and antibiotic related adverse events have not been well evaluated among hospitalized patients.

Methods: A retrospective, multicenter and observational study was performed in eight hospitals in South Korea between March 2010 and February 2011. The risk factors for mortality and kidney injury were evaluated in patients with MRSA infections. To assess resource utilization that results from MRSA infections, direct costs charged on each patient during treatment were summed up according to the respective resource category.

Results: A total of 335 patients were identified to have MRSA infections. One-hundred and thirty-five patients (40.3%) experienced AKI during 1st line antimicrobial treatment and seventy-seven (20.3%) patients died during the study period. Male gender, underlying renal disease, gastrointestinal and central-venous catheter infections and increase of Pitt bacteremia score were independently associated with development of AKI. As for mortality, solid tumor, increase in Pitt bacteremia score, and AKI were shown to be independent risk factors. The estimated mean total medical cost per patient was KRW 5 435 361 (USD 4868) ± SD 5 492 96. In the multivariate regression analyses for hospital charges, male gender, increase in Pitt bacteremia score, and AKI were related to increased hospital costs. The mean increase in hospital costs per patient when AKI occurred was KRW 2 474 600 (USD 2216) higher than that without AKI ($p < 0.001$).

Conclusion: Kidney injury occurring during treatment for MRSA infections was significantly associated with worse clinical outcomes and higher hospital costs than patients who retained baseline renal function.

R2534 Dear Director of Finance of NHS Hospital: it is possible to close beds and achieve savings from reduction in health-care associated infections!

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Objectives: According to the National Audit Office Report [January 20, 2011], NHS caters to a population of 51 million, employs more than 1.3 million and deals with a million patients every 36 hours. The Government aims to deliver £20 billion (4%) efficiency savings in the NHS by the end of 2014–2015. Blackpool Teaching Hospitals [BTH] is a large 844 bed teaching hospital with two tertiary centres. Its share of saving is approximately £50 M over 3–4 years. The trust board is committed to reducing costs by improving quality and driving efficiency, through clinical engagement, with key priority – quality and safety in patient care. We [CEO of the hospital and Microbiologist] present a case for taking costs out of the system through significant reductions in HAI and other quality initiatives within this teaching hospital.

Methods: Using reductions in HAI and other quality initiatives such as patient falls; pressure ulcers; medication errors, etc to take costs out of the system by closing a 24 bedded ward. This realised a cost saving of over £970 K.

Results: Reductions in acute trust (post 48 hour) HAI over financial years [2007/2008; 2008/2009; 2009/2010; 2010/2011; 2011/2012 (projected)]: MRSA bacteraemias – 90% [28; 5; 3; 3; 2 or 3 resp]; *C. difficile* infections 90% [323; 207; 134; 101; 64]. Reductions in total MRSA infections 48.9% [883 in 2007–2008 to 451 in 2009–2010] incl. Of 80% reduction in bacteraemias [1.33 to 0.27/10 K bed days as compared to national and regional reduction from 1.19 and 1.09 to 0.5/10 K bed days] & 39.1% reductions in wound infections.

Savings from closure of 24 bedded ward included Staffing [£960 K]; Clinical and general supplies and services [£9754].

Conclusions: The challenge facing all NHS trusts and healthcare professionals is to balance increasing demand from patients with the requirement for efficiency and productivity, whilst maintaining excellent quality. This requires dynamic leadership from CEO, commitment from trust board, clinical engagement, continuous endeavours to recognise innovative ideas of work differently, more efficiently, identifying savings and taking costs out of the system.

R2535 USA300 CA-MRSA clone responsible for an outbreak of skin and soft tissue infections in a hospital newborn nursery in Italy

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Objectives: Community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) is a well-established human pathogen that emerged in the 1990s, becoming responsible for severe infections in previously healthy people in the community in different areas of the world. CA-MRSA is able to cause outbreaks in groups with close interpersonal contacts and recently appeared in hospital causing outbreaks in USA and Europe. Here we describe an hospital outbreak of skin and soft tissue infections (SSTI)s occurred in an hospital newborn nursery caused by USA300 CA-MRSA clone.

Methods: In October 2010, SSTIs due to MRSA were observed in nine neonates born in a hospital in northern Italy. Of these three were severe infections (a mastitis and two deep abscesses) that required admission to the neonatal intensive care unit of a tertiary-care hospital. Besides, four mothers had post-partum mastitis and four family members had SSTI. To control the outbreak, a screening for MRSA nasal carriage was performed on 217 neonates and babies, six family members and 69 hospital staff-members. Identification and antibiotic susceptibility of the isolates were obtained by Vitek 2, disk-diffusion method and E-test. EUCAST breakpoints were applied. The presence of mecA, and of the genes for PVL and ACME was investigated by PCR. Molecular characterization included SCCmec typing, spa typing, PFGE and MLST.

Results: All MRSA isolates were resistant to erythromycin and ciprofloxacin besides beta-lactam antibiotics and had identical molecular characteristics: the outbreak strain was characterized by SCCmec type IV, spa type t008, ST8 and was positive for the PVL genes and ACME. PFGE revealed an identical pulsotype, highly related to that of USA300 in all the isolates. Isolates from post-partum mastitis and family members shared the same profile of the outbreak strain. Nine healthy neonates and babies, two family members and 10 hospital staff members were found colonized by the outbreak strain.

Conclusion: To our knowledge, this is the first report of an outbreak caused by the USA300 CA-MRSA clone in a hospital newborn nursery in Europe. The large number of neonates involved, the severity of some cases, and the spread to mothers and other family members confirms the high virulence, transmissibility and persistence of the USA300 clone. Hence, it is important to reinforce infection control especially in high-risk groups, such as neonates, to prevent USA300 from becoming endemic in European hospitals.

R2536 Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* nasal colonisation in a university hospital in Rio de Janeiro

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Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) is the main pathogen involved in healthcare-associated infections. Nasal colonization is the most risk factor to the development of MRSA infections. The methicillin resistance is due to presence of a Staphylococcal Chromosome Cassette (SCCmec).
Objectives: The aim of this study was to characterize MRSA isolates from nasal colonization in patients from a university hospital in Rio de Janeiro city, between October 2005 and August 2006.
Methods: The isolates were evaluated to SCCmec types, clonal profiles, ST (sequence type) and presence of PVL genes.
Results: Of a total of 83 recovered isolates, 41 (49%) carried SCCmec IV, 36 (43%) the type III, 3 (4%) the type II and 3 (4%) the type V. The 41 type IV MRSA isolates were included in five genotypes. The genotypes associated to USA400/ST1 and USA800/ST5 were related to 92.7% of type IV isolates. All MRSA isolates from dermatology were USA800, while 53% of isolates from medical clinic were USA400. Two isolates USA400 and one USA800 present the PVL genes. Type III MRSA isolates belonged to seven genotypes, but 64% of them were related to the Brazilian epidemic clone (BEC)/ST239. This clone was associated to 29% of isolates from medical clinic and 50% of isolates from infectious disease clinic. In intensive care unit, 33% of isolates belonged to SCCmecIII/clone N/ST239 lineage. The others 10 isolates recovered in ICU belonged to six different clonalities, included a type IV isolate that belonged to ST97 and that presented PVL genes. Among 14 MRSA isolates from patients who have been transferred, 6 (43%) showed genomic profiles that was never found in patients of our hospital. Horizontal transmission events involving 19 patients showed that the USA400/type IV clone was isolated from 42% of them. In addition, one specific USA400 isolate was transmitted three times in the same ward.
Conclusion: Each ward of hospital showed spread of a specific MRSA lineage. In ICU was observed a great clonal diversity of isolates, probably because this unit receives patients of all wards of hospital. SCCmec IV/USA400/ST1 was the most found lineage in our hospital at time of study. Moreover, that lineage was the main involved in horizontal transmission, indicating how easy it is the transmission of isolates of this genetic profile, justifying the gradual substitution of clonal types well established in hospitals.

R2537 Highly successful community-associated *S. aureus* resistant to methicillin and fluoroquinolones in northwestern Spain

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Objectives: The current selection of a successful *S. aureus* clone resistant to methicillin and fluoroquinolones (MRSA-FQ) is studied. Its clonal relationship with other *S. aureus* only resistant to methicillin is analysed.
Methods: A total of 750 MRSA isolated at the Pontevedra Hospital in 2009, 2010, and 2011 were studied by the WIDER system. The antimicrobials studied included: oxacillin (OX), erythromycin, clindamycin, gentamicin, tobramycin, amikacin, levofloxacin (LE), and trimethoprim-sulfamethoxazole. The MRSA strains that were only OX resistant and OX-LE resistant were selected and PFGE studied, SCC-mec classified, and spa-typed. The arcA (ACME) and PVL genes were PCR studied. The strains were classified as community-onset MRSA (CO-MRSA) when the strain was isolated in a non-hospitalized patient. In any other cases the strains were classified as healthcare associated MRSA (HA-MRSA).
Results: Thirty-one (4%) strains were OX (R) and 97 (13%) were OX-LE (R). The prevalence of the OX-LE (R) was 7% in 2009, 13% in 2010, and 22% in 2011. The 65% of the OX-LE (R) isolates were identified as CO-MRSA. By PFGE/spa-type, five clones (A/t008, B/t148, C/t002, D/t1133, and E/t024) were identified in the OX (R) strains, while in the OX-LE (R) strains only one clone (C/t002) was identified being identical to the PFGE-C/t002 identified in the OX (R) isolates. All strains were identified as SCC-mec IV (2B). The ArcA (ACME) and PVL genes were not identified in any of the strains.
Conclusions: A successful MRSA-FQ resistant clone t002 was identified. The MRSA-FQ clone has its ancestor in a t002 MRSA clone susceptible to FQ. The clone MRSA-FQ resistant t002 was isolated more frequently in the community. Surveillance studies are essential to identify new successful MRSA clones, being this information highly outstanding for clinicians and epidemiologists.

R2538 Variability of resistance and virulence genes in human isolates of MRSA ST398 in Austria

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Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) of sequence type 398 (ST398) has gained particular attention because of its association with pig farming and its ability to colonize people, when in close contact to these animals. MRSA ST398 can mainly be assigned to the specific spa-types (like t011or t034). Typically, in addition to the resistance to Methicillin, MRSA ST398 isolates are resistant to tetracycline only. There are reports that MRSA ST398 are less virulent than other known MRSA types.
Objectives: MRSA ST398 from both MRSA reference centres were studied to determine relatedness, resistance and virulence genes in human MRSA ST398 in Austria.
Methods: From 2004 to 2008 41 MRSA ST398 were detected at the two MRSA reference centres in Austria, the Elisabethinen Hospital, Linz and the Institute of Hygiene, Medical University of Graz. To determine involved resistance and virulence genes from these two different regions a chip based microarray on the IdentiBac System (Alere Technologies GmbH) was used. IdentiBac gene profiles were compared using SplitsTree4 software. Additional spa- and SCCMec Type was determined.
Results: Spa typing assigned 36 (87.8%) to spa type t011, two to t034, two to t2346 and one t1451. All strains belonged to SCCmec type V. Analysis of the resistance genes (additional to Methicillin resistance) exhibited that 40 (97.6%) MRSA ST398 were positive for blaZ ß-lactamases and tetM tetracycline resistance genes. The tetK gene was present in 28 (68.3%) strains. Genes for Erythromycin and Clindamycin resistance ermC was found in 12 (29.3%). Three strains (7.3%) harboured the vga (strepotomycin) gene, two (4.9%) the aacA-aphD gene (gentamicin, tobramycin) and one (2.4%) the flexA (chloramphenicol) gene.
Whereas different sets for hemolysines could be identified, in none of the MRSA ST398 enterotoxins, toxic shock syndrome toxin gene (TSST) and panton valentine leukocidin toxin (PVL) could be detected. SplitsTree analyses offered that although MRSA ST398 originating from two different regions clustered together, but MRSA ST398 from 2005 to 2006 were much more related than MRSA ST398 detected in 2007–2008.
Conclusion: Since 2004 a single MRSA ST398 clone (with only small variations in resistance and virulence genes) is responsible for the most

human MRSA ST398 in Austria. Only two MRSA ST398 (4.9%) differed significantly from the isolates.
Absence of TSST- and PVL genes was in accordance with the known less virulence of MRSA ST398.

R2539 Detection and characterisation of methicillin-resistant *Staphylococcus aureus* strains isolated from pigs in Lithuania – a cross-sectional study

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Objectives: To investigate methicillin-resistant *Staphylococcus aureus* (MRSA) occurrence among pigs in Lithuania and to characterize isolated strains.
Methods: Between January 2011 and December 2011, 120 nasal swabs were taken from randomly selected breeding pigs from six different farms and additionally 40 nasal swabs were obtained from finishing pigs from two other farms at two slaughter houses just before slaughtering. MRSA was screened on Brilliance™ MRSA 2 Agar (Oxoid) and chromID™ MRSA medium (bioMérieux) and confirmed by PCR for the presence of the mecA gene. MRSA isolates were subjected to spa typing, SCCmec typing, ST398 PCR, and underwent PFGE analysis with Cfr9I. Isolates were also tested for the lukF/lukS genes encoding Panton–Valentine leukocidin (PVL). Antimicrobial susceptibility testing was performed using the broth microdilution method (MicroScan® PM21; Dade Behring Siemens) and interpreted according to CLSI guidelines. The presence of tetK, tetM, ermA, ermB, ermC, vgaA, vgaC and dfrK genes was studied by PCR.
Results: Four nasal swabs out of 160 (2.5%) were MRSA-positive. The isolates were obtained from animals of the same farm at slaughterhouse. The strains were CC398, spa type t011 and SCCmec V. None of the MRSA isolates carried the PVL genes. Analysis obtained by PFGE revealed that two isolates had similar profiles, while the other two clustered differently. Susceptibility testing revealed resistance to tetracycline in all MRSA isolates, attributed to tetK and tetM genes. All tested isolates were resistant to erythromycin and clindamycin owing to the presence of ermB gene. One MRSA strain was resistant to trimethoprim/sulfamethoxazole and carried the dfrK resistance gene. All isolates were susceptible to fluoroquinolones, mupirocin, fosfomycin, fusidic acid, rifampicin, chloramphenicol, gentamicin, netilmicin, linezolid, nitrofurantoin, teicoplanin and vancomycin.
Conclusions: This study is the first report on the prevalence and characteristics of livestock-associated MRSA isolated in pigs in Lithuania. Since the MRSA strains were isolated just before slaughtering, pigs can potentially be reservoirs of bacteria that may enter the food chain. Furthermore, MRSA ST398 shows low host specificity and can colonize various animal hosts as well as humans, which raises the question of potential interspecies and zoonotic transmission.

R2540 Clinical outcome of patients with methicillin-resistant *Staphylococcus aureus* bacteraemia, 2004–2008

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Objective: Objective of this study was to compare clinical outcomes of patients diagnosed with methicillin-resistant *Staphylococcus aureus* (MRSA) bacteremia that received vancomycin from 2004 to 2008.
Methods: Single-center retrospective chart review of 93 patients diagnosed with MRSA bacteremia from 2004 to 2008 was conducted. Hospitalized patients were included if they had two positive MRSA blood cultures collected within 24 hours of each other and received intravenous vancomycin within 48 hours of drawing blood cultures. Subsequent infections were not included in the analysis. Treatment failure was defined as one of the following: addition of an antimicrobial for treatment of MRSA bacteremia, change in antimicrobial therapy, recurrence of MRSA bacteremia within 60 days of discontinuation of vancomycin, discontinuation of vancomycin ≤10 days due to adverse effect, or MRSA isolates obtained (10 days after initiation of vancomycin but before completion of therapy. Logistic regression was used to identify association of treatment failure and 30-day mortality between study years. ANOVA test was utilized to identify statistical difference of vancomycin dose and mean vancomycin concentration between study years.
Results: Number of patients with MRSA bacteremia was similar during the study period (2004: n = 15, 2005: n = 20, 2006: n = 17, 2007: n = 21, 2008: n = 20). Majority of patients included were male (59.1%), African American (54.8%), and immunocompromised (67.7%). The median age was 53 years old. Of 22.6% of patients were admitted to an intensive care unit at the time of infection. There was no difference in mean dose (mg/kg) between each study year (2004: 15.2, 2005: 13.3, 2006: 13.4, 2007: 16.1, 2008: 13.5; p = 0.25). There was no significant change in mean concentration (mg/L) of vancomycin among study years (2004: 11.8, 2005: 12.7, 2006: 17.2, 2007: 14.6, 2008: 18.1; p = 0.10). Incidence of 30-day mortality was not significantly different by study year (2004:n = 2, 2005:n = 2, 2006:n = 0, 2007:n = 1, 2008:n = 4; OR=1.13, 95% CI: 0.71–1.78). Incidence of treatment failure was not associated with study year (2004:n = 4, 2005:n = 5, 2006:n = 0, 2007:n = 2, 2008:n = 5; OR = 0.893, 95% CI: 0.61–1.32).
Conclusion: Over a five year period, there was no change in the incidence of MRSA bacteremia. The dose of vancomycin and vancomycin concentration were similar during the study period. There was no difference in 30-day mortality and treatment failure from 2004 to 2008.

R2541 Microbiological and molecular characterisation of human clinical isolates of methicillin-resistant *Staphylococcus hominis*: evidence of new SCCmec

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Objectives: To characterise biofilm formation, antibiotic resistance, SCCmec type and genetic relatedness in *Staphylococcus hominis* clinical isolates.
Methods: Fifteen clinically relevant isolates of *S. hominis* were included. Biofilm formation was evaluated using crystal violet staining. Drug susceptibility was determined by the broth microdilution method. Methicillin resistance was evaluated with the cefoxitin disk test and mecA gene was detected by PCR. Genetic relatedness was determined by pulsed-field gel electrophoresis (PFGE) and SCCmec was typed by multiplex PCR using two different methodologies described for *Staphylococcus aureus*.
Results: Of 20% (3/15) *S. hominis* isolates were categorised as strong producers of biofilm and 33.3% (5/15) as weak producers. All isolates showed high resistance to ampicillin, erythromycin and trimethoprim (>72%). Among all isolates, 80% (12/15) were methicillin resistant and mecA positive. PFGE analysis revealed 15 different profiles with homologies that ranged from 0% to 95%.
SCCmec type III was detected in one isolate with the structure of cassette assumed as described for *S. aureus*. Furthermore, two isolates amplified SCCmec type III, mec complex A, ccrAB1; two isolates typed SCCmec III, mec complex A, ccrC1; and three isolates typed SCCmec III, mec complex A, ccrAB1 + ccrC1; one isolate typed SCCmec III, mec complex B, ccrC1.
Conclusions: The *S. hominis* isolates analysed in this study showed moderate production of biofilm, a high methicillin resistance, resistance to other antimicrobials, and low clonality. The results of this study suggest that *S. hominis* harbors new SCCmec structural elements that might be a reservoir of ccrAB1, ccrC1, and mec complex A for the assembly of the SCCmec types.

R2542 Genetic lineages, resistance and virulence factors of *Staphylococcus aureus* isolates from nasal samples of healthy companion animals in Tunisia

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Objective: To study the carriage rate, resistance mechanisms, virulence traits and genetic lineages of nasal *S. aureus* of healthy companion animals in Tunisia.
Methods: Nasal swabs of healthy animals (100 dogs and 30 cats) were obtained in National School of Veterinary Medicine and several veterinary clinics that receive animals from all Tunisia (2010–2011). Samples were inoculated into Baird Parker and ORSAB plates for *S. aureus* and methicillin-resistant *S. aureus* (MRSA) recovery, respectively. Isolates were identified by biochemical methods and nuc-gene PCR. Antibiotic susceptibility profile to 18 antibiotics was determined by disk diffusion method. The presence of nine resistance genes (tetL, tetM, tetK, blaZ, ermA, ermB, ermC, msrA and ant(6)-Ia), 18 staphylococcal enterotoxin genes and lukF/lukS-PV (encoding Panton-Valentine leucocidin, PVL), lukE-lukD, lukM, eta, etb and tsst1 genes were studied by PCR. *S. aureus* isolates were typed (spa, agr, MLST and SmaI-PFGE).
Results: Six *S. aureus* were detected (one/sample) from the 130 tested samples (4.6%), representing 4% in dogs and 6.6% in cats. All *S. aureus* were methicillin-susceptible (MSSA). Four different spa-types (t189, t279, t582 and t701) and four sequence-types (ST6 [CC6], ST15 [CC15] and ST188 [CC22], including a new sequence-type named ST2121 [CC30]) were identified among our MSSA isolates. They were ascribed to agr type I (4 isolates), II (1) and III (1). MSSA isolates showed susceptibility to the tested antibiotics with the following exceptions (% of resistance, resistance gene): penicillin (100%, blaZ), tetracycline (16.6%, tetM), erythromycin (16.6%, ermA), streptomycin (16.6%, ant(6)-Ia) and ciprofloxacin (16.6%). Virulence genes carried by MSSA were (number of isolates): lukF/lukS-PV (2, from cats), hla (6), hld (6), hlb (4), lukED (5), sea (3), ser (3), sei (2), see (2), ser (1), hlg (1), hlgv(1) and a variant of egc-cluster-like [sen-sem-sei-seu-seg] (1, from dog). The remaining virulence genes tested were negative among our isolates.
Conclusions: The nares of healthy companion animals could be a reservoir of PVL-positive community-associated-MSSA with implications in public health.

Epidemiology of MDR-Gram-negatives

R2543 Characterisation of carbapenem-resistant *Acinetobacter baumannii* clinical isolates in a tertiary care hospital in Saudi Arabia

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Background: *Acinetobacter baumannii* is an opportunistic gram negative organism causing a variety of healthcare-associated infections. Carbapenems have been used widely to treat the emerging multi-resistant *A. baumannii*. However, carbapenem-resistant *A. baumannii* (CRAB) has been documented worldwide. The aim of this study was to molecularly characterize CRAB clinical isolates in a tertiary care hospital in Saudi Arabia.
Methods: *A. baumannii* isolates were obtained from August 2010 to September 2011 in the hospital. The vitek 2 system was used to detect *A. baumannii* isolates and their susceptibility to carbapenem. In addition, E-test method was used to detect the isolate susceptibility to carbapenem. The multiplex PCR was performed for the detection of bla(OXA-23), bla(OXA-24), bla(OXA-51), bla(OXA-58), bla(IMP), and bla(VIM).
Results: A total of 132 *A. baumannii* strains were detected and characterized with 90 (68%) of them were CRAB isolates. In addition to carbapenem resistance, all strains were resistant to ciprofloxacin and 105 (79.5%) strains were resistant to aminoglycosides. All strains were susceptible to colistin and 92 (69.7%) strains were susceptible to tigecycline. All CRAB isolates studied were positive for bla(OXA-51) which was used as a marker for *A. baumannii* detection. In addition, bla(OXA-23) was detected in 105 (79.5%) strains. No bla(IMP) neither bla(VIM) was detected in any strain.
Conclusions: To our knowledge, this is the first comprehensive molecular study characterizing carbapenem resistance in *A. baumannii* in the Eastern Province of Saudi Arabia. Bla(OXA-23) is the most common gene associated with CRAB in this region.

R2544 Prevalence and antimicrobial susceptibility of extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in a tertiary care hospital

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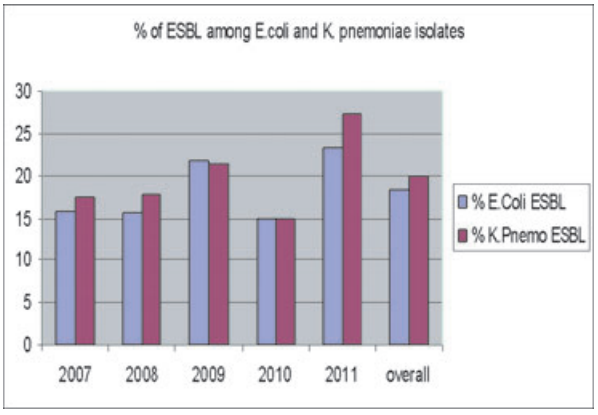
Background: The prevalence of extended-spectrum β-lactamases (ESBLs) varies between countries and institutions. We studied the prevalence of ESBL among clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* and analyzed patterns of susceptibilities to different antimicrobial agents at King AbdulAziz Medical City in Riyadh, Saudi Arabia between July 2007 and July 2011
Methods: Between July 2007 and October 2011; a total of 20268 clinical isolates of *E. coli* and 9126 *K. pneumoniae* were screened and confirmed for ESBL production by MicroScan walkaway 960 system. All isolates were from inpatients and outpatients who attended King AbdulAziz Medical City. The samples collected from all age groups. The minimum inhibitory concentration to imipenem, meropenem, piperacillin-tazobactam, cefepime, ciprofloxacin, gentamicin and amikacin were determined by the MicroScan MIC panels.
Results: Of the 20268 isolates of *E. coli* and 9126 of *K. pneumoniae* tested, there were –18.3% and 19.9% produced ESBL respectively. The majority of these isolates were from urine (57.5%) and wounds (17%). Only 7% of the blood culture isolates were ESBL-producing. Overall, carbapenems (imipenem and meropenem) had good activity against the ESBL-producing isolates tested (99% of *E. coli* isolates and over 92% *K. pneumoniae* of were susceptible). There was no difference in the activity of imipenem and meropenem against the ESBL-producing *E. coli* or *K. pneumoniae*. Over 86% and 68% of *E. coli* and *K. pneumoniae* respectively were susceptible to piperacillin-tazobactam. Susceptibilities of the isolates to amikacin varied, ranging from 95% for *E. coli* to 75% for *K. pneumoniae*. Gentamicin, ciprofloxacin and cefepime were active against 73%, 67% and 60% of the isolates, respectively.
Over the past 5 years the epidemiology of ESBL are start to change gradually and more ESBL producing isolates appear among *E. coli* over *K. pneumoniae*. ESBL (Table 1)



Conclusion: We conclude that there is increasing incidence of infection with ESBL-producing bacteria, and the high rates of antimicrobial resistance encountered among them. Clinicians should be familiar with the clinical importance of these enzymes and potential strategies for dealing with them.

R2545 Emergence of carbapenem-resistant *Klebsiella pneumoniae* infection among patients hospitalised in intensive care unit of a Warsaw infectious diseases hospital

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Objective: The spread of multidrug-resistant Gram-negative pathogens like carbapenemase producing *Kl. pneumoniae* is one of the major hazards for patients requiring long-term hospitalization or hospitalization in intensive care units (ICU).
Methods: Of the 12 patients hospitalized in ICU of Warsaw Infectious Diseases Hospital from June 2009 to April, 2010, infected with *Kl. pneumoniae* epidemic strain that coproduced carbapenemases were included in the study. Identification of isolated bacteria and MIC determination were performed using an automated system (Vitec 2 Compact, BioMérieux) or standard microbiological plates. Results were interpreted according to Clinical Laboratory Standards Institute. Clinical data were retrospectively collected from medical documentation.
Results: The infection with KPCs producing *Kl. pneumoniae* was detected in 12 out of 107 (11%) studied patients. Seven of them (7/12) were severely immunocompromised (lymphoma in one case, HIV infection in two cases, chronic immunosuppressive treatment in four cases) comparing with 19/95 patients without KPC + *Kl. pneumoniae* infecton ($p < 0.05$). KPCs producing strains were detected in urine obtained from seven patients, in bronchial fluid obtained from three patients and in blood of four patients. The first biological material from which the pathogen was cultured appeared urine in seven cases, bronchial fluid in three cases and blood in two cases. In two patients with KPCs producing *Kl. pneumoniae* present in blood, urinary tract could be assumed as the source of invasive infection. In one of this patients the infection was fatal. In one patient emergence of resistance to tigecyline following treatment with this antibiotic was observed.
Conclusions: Immunocompromised patients can be more likely to inquire infection with carbapenems resistant *Kl. pneumoniae*. Urinary tract seems to be the most frequent site of KPCs *Kl. pneumoniae* infection and can be the source of invasive infection.

R2546 Molecular detection and antibacterial susceptibility of enteropathogenic *Escherichia coli* and shigatoxigenic *Escherichia coli* strains isolated from healthy and diarrhoeic dogs

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Animal contacts have been regarded as an emerging rout of Shiga toxin-producing *Escherichia coli* (STEC) infection in humans. Diarrhoeic and asymptomatic dogs have been recognised as a reservoir of atypical enteropathogenic *Escherichia coli* (EPEC), and STEC in some investigations. In this study *E. coli* isolates from 100 faecal samples of healthy (n = 50) and diarrhoeic (n = 50) dogs were screened by polymerase chain reaction (PCR) for the presence of determining virulence genes of STEC and EPEC pathotypes including stx and eaeA. The confirmed virulence-positive strains were subjected to antimicrobial susceptibility testing against 12 antibacterial using disc diffusion method. Resistance profiles were also determined for the STEC and EPEC strains. Ten isolates from 10 dogs (10%) were shown to possess at least one of the tested virulence genes. Six of these isolates (6%) harboured only the eaeA gene and were considered as EPEC. Four isolates (4%) were stx+ and regarded as STEC, of which two were stx+/eae+. The resistance was specially observed against penicillin, ampicillin, sulfomethoxazole, streptomycin and oxytetracyclin. Altogether, nine resistance profiles were observed among 10 isolates. In conclusion, dogs can act as a reservoir for EPEC and STEC strains, and close contacts of children with companion animals can be a potential risk factor in development of diarrhoea and haemolytic uremic syndrome. In rural areas shepherd dogs can also be a transient carrier of STEC strains that they may acquire from ruminants. To our knowledge this is the first study which reports the faecal shedding of STEC and EPEC from dogs in Iran.

R2547 Multidrug-resistant *Acinetobacter baumannii* carrying the carbapenemase NDM-1 encoding gene introduced into the Czech Republic

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Objective: Carbapenem-hydrolysing ß-lactamases have emerged as the most clinically significant mechanism responsible for carbapenem resistance in *Acinetobacter baumannii*. The aim of this study was to assess the mechanism of high-level carbapenem resistance in an *A. baumannii* isolate imported to a Czech hospital.
Methods: Strain ANC 4097, which had been imported from Egypt, was recovered from the sputum of a patient hospitalized in the Czech Republic in July 2011. Antimicrobial susceptibility was determined by disc diffusion while the production of metallo-ß-lactamases was assessed using Etest MBL strips. Resistance genes were characterized by PCR and sequencing. The location and genetic background of blaNDM-1 were analyzed by hybridization of genomic DNA with a blaNDM-like probe and primer walking strategy. The strain was genotyped by multilocus sequence typing (MLST).
Results: ANC 4097 belonged to sequence type (ST) 1 which is typical of the ''European clone I'' epidemic lineage. It showed high level resistance to all ß-lactams and other antimicrobial agents except colistin, tobramycin, netilmicin, doxycycline and tigecycline. The positive MBL test suggested the production of a metallo-ß-lactamase. The strain harboured the blaNDM-1, blaOXA-23 and intrinsic blaOXA-51-like carbapenemase genes. Insertion sequence ISAba1 was identified upstream of the blaOXA-23 and blaOXA-51-like genes. The blaNDM-1 gene was located onto the chromosome of ANC 4097 and inside composite transposon Tn125.
Conclusions: This study reports on the first blaNDM-1 positive *A. baumannii* strain in the Czech Republic. The high level of carbapenem resistance in this strain is likely to result from the production of chromosomally encoded NDM-1 and overproduction of OXA-23 and OXA-51-like.

R2548 Multidrug-resistant Gram-negative rods in ophthalmological patients

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Objectives: The aim of the study was to assess the frequency of isolation of multidrug-resistant (MDR) pathogens from eye infections in hospitalised ophthalmological patients.
Antimicrobial therapy of such infections poses a challenge as many agents do not penetrate into the eye in sufficient concentration to inhibit bacterial growth.
Methods: Specimens for bacteriological culture were obtained from patients hospitalized in ophthalmological hospital (2005–2010). Samples were cultured and isolates identified according to standard microbiological methods.
Results: In total 1988 specimens were cultured, including swabs from conjunctiva, cornea, throat, nose, wounds and cornea conservation medium. The samples yielded 377 isolates, comprising 343 strains of bacteria and yeast-like fungi. Enteric rods comprised 11.31% (38/336) of all strains, while non-fermenting rods – (24/336) (7.14%). Among alarm pathogens there were extended-spectrum beta-lactamase producing – ESBL(+) enteric rods – 5.26% (2/38), and ESBL(+) Gram-negative nonfermenting rods – 16.67% (4/24). Among enteric rods the most numerous were strains of *S. marcescens* and *Klebsiella* spp. (27.5% each), *E. cloacae* and *E. coli* (12.5% each), *M. morganii* (10.0%) and *P. mirabilis* and *C. koserii* (5.0% each). ESBL(+) strains were *S. marcescens* (1) and *E. cloacae* (1). Among Gram-negative nonfermenting rods predominated strains of *P. aeruginosa* – 57.69%, followed by *S. maltophilia* – 15.38%, *Acinetobacter* spp. – 7.70%, *A.*

faecalis – 7.69%, *A. denitrificans* – 7.69% and *S. paucimobilis* – 3.85%. Three out of four strains of *S. maltophilia* were ESBL(+).
Conclusion: In this study there was a high percentage (16.67%) of ESBL(+) Gram-negative nonfermenting rods. Three out of four strains of *S. maltophilia* were ESBL(+). Multidrug-resistant strains represented opportunistic pathogens, belonging to the genus Stenotrophomonas, Pseudomonas, Serratia and Enterobacter.

R2549 Bloodstream infections due to carbapenem-resistant *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* clinical isolates in a tertiary hospital: epidemiology and outcome

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Objectives: Dissemination of carbapenemase-producing Gram-negative bacteria is a major public health concern. The aim of this study was to evaluate the epidemiology and outcome of infections associated with carbapenem-resistant *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolates derived from blood cultures in a tertiary hospital.
Methods: A total of 74 strains of *K. pneumoniae*, 38 of *P. aeruginosa* and 72 of *A. baumannii* recovered from blood cultures were studied. Isolates derived from 94 hospitalized patients (ICUs and surgical and medical wards), during one year, from November 1, 2010 to October 31, 2011. All isolates had meropenem and/or imipenem MICs > 1 mg/mL. The identification and susceptibility testing were performed via the Vitek II automated system (Biomerieux, France), and when necessary susceptibility results were confirmed with the use of E-test strips (AB Biodisc, Sweden) according to CLSI guidelines. Metallo-beta - lactamase (MBL) production was evaluated using disks containing meropenem with and without EDTA and KPC-production with boronic acid combined-disk tests, using disks containing meropenem with and without boronic acid.
Results: The mean age of patients was 56.5 years, ranging from 18 to 95 years. The majority of them were male (65%). Most of the patients (66%) were hospitalized at ICUs, while the rest were hospitalized mostly at surgical departments. With reference to diagnosis at admission, 37% were trauma patients, 8% had various infections, 7% had subarachnoid hemorrhage, 5% had burns and the rest several other conditions. The mortality rate was 52%. All isolates were multidrug resistant. *K. pneumoniae*, *P. aeruginosa* and *A. baumannii* isolates revealed 7%, 68% and 49% resistance to gentamicin, 5%, 100% and 39% resistance to tigecycline and 46%, 3% and 3% to colistin respectively. All *K. pneumoniae* isolates were producing KPC and 95% *P. aeruginosa* isolates were producing MBL.
Conclusion: It is crucial to monitor the emergence and prevalence of carbapenem resistant Gram-negative bacteria depending not only on susceptibility testing but on phenotypic detection of carbapenemase production as well, especially in critically ill patients, because in combination with other risk factors, it is associated with high mortality.

R2550 Characterisation of outbreak CTX-M plasmid from Enterobacteriaceae

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Aims: The CTX-M family of β-lactamases confers resistance to most beta-lactams and is often carried within large promiscuous plasmids. These CTX-M plasmids are increasingly prevalent within animal and human bacterial isolates. Our aim was to characterise the spread of CTX-M plasmids amongst Enterobacteriaceae strains isolated from an outbreak of cephalosporin resistant Salmonella in pigs. Furthermore, we aimed to assess the phenotypic effect of plasmid carriage on the outbreak strains.
Methods: Six *E. coli*, one Klebsiella and two Salmonella strains were selected from a well characterised group of isolates. CTX-M plasmids from these strains were extracted and transformed into NEB10B (*E. coli* K12) and characterised by plasmid profiling, PCR based replicon typing (PBRT), conjugation studies, antibiotic resistance profiling and miniaturised DNA strip array (virulence and antimicrobial resistance genes). To assess relatedness among the characterised plasmids restriction analysis was also performed.
Results: The first type of plasmid identified was confined to two *E. coli* strains, was approximately 50 kb and harboured CTX-M-14, but could not be typed by PBRT. The second type of plasmid, present in all three genera, was approximately 100 kb, harboured CTX-M-1. These plasmids were typed by PBRT and found to belong to the IncI plasmid group. A representative CTX-M-1 plasmid was selected for full sequence analysis and found to encode additional resistance genes (chloramphenicol and sulphonamide resistance) as well as genetic addiction systems. All the plasmids studied were shown to readily conjugate into other Enterobacteriaceae strains.
Conclusions: Most of the resistance to beta-lactams found in this study was conferred by the spread of a plasmid encoding CTX-M-1. The presence of additional antibiotic resistance genes in the plasmid may indicate that co-selection played a role in its spread. Furthermore, genetic addiction systems that prevent plasmid loss even in the absence of selection may have also influenced its prevalence. The studies reported here demonstrate that three related, but distinct genera of Enterobacteriaceae harboured the same IncI CTX-M-1 plasmid. The presence of a CTX-M gene in such promiscuous plasmids magnifies the risk of widespread beta-lactam resistance. Future work will include the construction of plasmid-less derivatives that will be compared to the plasmid carrying strains using a number of phenotypic studies.

R2551 Intestinal Colonisation with multidrug-resistant Enterobacteriaceae in travellers, immigrants and 'visiting friends and relatives': dominance of *E. coli* producing CTX-M enzymes

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Objective: To study the faecal carriage of ESBL and carbapenemases producing Enterobacteriaceae among travellers, inmigrants and ''visiting friends and relatives'' (VFR) that include people who had travelled to their country of origin to visit their friends and family.
Methods: Forty two faecal samples from 36 travellers, two inmigrants and four VFR were studied (September–October 2011). One faecal sample per individual was requested on return or arrival to our country. Samples were seeded in selective media (MacConkey-ceftazidime and MacConkey-cefotaxime, 2 mg/L). One isolate per morphotype was selected for further studies. Positive isolates were confirmed for ESBL (double disk synergy test) and carbapenemase (Hodge test) production. Bacterial identification and susceptibility patterns were determined using the MALDI-TOF MS (Bruker) and the semi-automated WIDER system (Fco. Soria Melguizo). blaESBLs were characterized by PCR and sequencing. Population structure was characterized by XbaI-PFGE and phylogrouping. Presence of qnr genes was detected by PCR assay.
Results: Individuals enrolled in this study travelled to or from countries of Africa (47.6%), Latin America (28.6%) and Asia (23.8%). Of 28.6% (12/36) of them (11 travellers and one immigrant) were faecal carriers of ESBL-producing Enterobacteriaceae. No fecal carriage with carbapenemases producing organisms was detected. A total of 17 isolates (16 *E. coli* and one *K. pneumoniae*) was further studied. *E. coli* isolates belonged to phylogroups A0 (37.5%), A1 (25%), D1 (25%) and D2 (12.5%). Of 64.7% (11/17) of the ESBLs were CTX-M-types and 81.8% (9/11) of them belonged to the CTX-M-1 cluster, most of them being CTX-M-15 (66.6%). Presence of qnr genes was not detected. High resistance rates to different antimicrobials were detected: 94.1% to ciprofloxacin; 88.2% to cotrimoxazol and 82.3% of the isolates were resistant to one or more aminoglycosides.

Conclusions: Individuals with contact with foreign countries had high rates of intestinal colonization by Enterobacteriaceae producing CTX-M-types, especially those of the CTX-M-1 cluster. Our study points out the importance of ecological surveillance studies of faecal carriers of multi-drug resistance organisms to establish epidemiological measures that would allow controlling the dissemination of these organisms.

R2552 Detection of blaIMP-22 in a *Klebsiella pneumoniae* clinical isolate in an acute care hospital in Portugal

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Objectives: Carbapenem resistance in Enterobacteriaceae is one of the actual major therapeutic concerns. Routine procedures of the hospital clinical microbiology laboratory, alerted for the need of extensive study of some Enterobacteriaceae isolates in order to better understand the resistance profile, namely in isolates that showed reduced susceptibility or resistance to, at least, one type of carbapenem. With that purpose, isolates were selected for genotypic approach by PCR and sequencing.
Methods: In a set of 13 Enterobacteriaceae showing reduced susceptibility to carbapenems, collected since September 2010 till September 2011, a *Klebsiella pneumoniae* blood culture isolate, showed resistance to oxiimino-beta-lactams, resistance to meropenem, reduced susceptibility to ertapenem, and susceptibility to imipenem, with MICs of 16, 4 and 2 μg/mL respectively, and susceptibility to aminoglycosides and fluoroquinolones. Identification and susceptibility testing were performed by Vitek2 (bioMérieux) and WalkAway (Dadebehring) systems. Total DNA of this *Klebsiella pneumoniae*, was subjected to amplification by multiplex PCR using primers for blaVIM, blaIMP and blaKPC and sequencing of the amplified fragment, using ABI-PRISM 3100 automatic genetic analyzer, showing the presence of blaIMP-22.
Results: In this study, we detected blaIMP-22, in a clinical isolate of *Klebsiella pneumoniae*. According to the CLSI guidelines, extended-spectrum β-lactamases production was not detected. Metalo-β-lactamases detection, by Etest MIC determination for imipenem and imipenem plus EDTA, was negative, highlighting the need of adequate phenotypic detection of this particular resistance threats. Genotypic determination of this resistance mechanism, seems useful in these situations of Imipenem susceptibility, masking carbapenemase production. This is an important issue to interpret hospital resistance epidemiology and to guide infection control procedures, to avoid outbreak installation.
Conclusions: As far as it seems, this is the first report of blaIMP-22 in a *Klebsiella pneumoniae* clinical isolate. The acquired metallo-β-lactamases represent a significant clinical threat due to their hydrolysis spectrum and infection control challenge. These atypical carbapenemase-producers may be overlooked in routine clinical microbiology laboratory testing, emphasizing the importance to survey and control the spread of such resistance determinants in nosocomial pathogens.

R2553 Characterisation of antibiotic resistance determinants in multidrug-resistant Moraxella osleonsis, an opportunisitic human pathogen

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Objectives: To characterise the antimicrobial resistance determinants present in *Moraxella osloensis*, isolated from human clinical trial studies.
Methods: Four *M. osloensis* isolates from the skin of two participants, one a placebo and one part of a clinical trial study undergoing antimicrobial treatment of Minocycline (EU ANTIRESDEV Project) were characterized using an antimicrobial resistance (AMR) gene microarray chip (Clondiag TM GmbH). Phenotypic characterisations were determined by antimicrobial disk diffusion assays and Phenotype Micro (PM) arrays from Biolog.
Results: Three of the four *M. osloensis* isolates from the skin trunk of human volunteers showed multiple resistance, both geno- and phenotypically to sulphonamide, beta- lactamase and tetracycline and produced positive signals for antibiotic resistance genes sul2, sul3, blaCMY, blaPER, blaOXA2 and tet37 with the microarray. The fourth strain was sensitive to all antibiotics tested and did not produce any positive signals with the AMR gene array. Biochemical characteristics determined using the PM arrays showed differences between the AMR resistance and sensitive strains.
Conclusion: *Moraxella osloensis* is an opportunistic human pathogen known to cause diseases such as endocarditis, osteomyelitits, septic arthritis, catheter infection and meningitis. At present all diseases have been treatable and currently there is no record in the literature of *M. osloensis* with multiple antibiotic resistances. The present discovery of multiple drug resistance in isolates of human origin is worrying and follows a growing trend of multiple resistances acquired in bacterial pathogens.

R2554 Screening of antibiotic-resistant Gram-negative organisms in hospital settings from Bucharest, Romania

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The purpose of this study was to establish the main resistance phenotypes and their genetic determinants in Gram-negative bacilli (GNB) isolated from intensive care units (ICU).
Material and Methods: A number of 531 GNB strains (334 Enterobacteriaceae and 197 Pseudomonadaceae) were isolated from 1166 positive clinical samples collected from patients hospitalized during 2011, in the ICUs of two big hospital from Buhcarest, Romania. Their resistance phenotypes were established using: disk diffusion test, double-disk diffusion test (DDST) with amoxicillin-clavulanic acid (AMC), cefotaxime (CTX) and ceftazidime (CAZ), DDST with AMC plus EDTA, imipenem (IPM) and IMP plus EDTA, Modified Hodge Test (MHT) and E-test ESBL, MBL and AmpC. The genetic support of the antibiotic resistance was investigated by simple and multiplex PCR reactions for class A Ser- β-lactamases (PSE, CARB, TEM genes families), class B – metallo-b-lactamases -MBL (IMP, VIM, SPM gene families), class C – AmpC and ESBL, as well as ciprofloxacin resistance genes (gyrA, parC) as well as the presence of mexB, mexD, mexF and mexY genes, encoding for multi-drug efflux pumps. In accordance with the recommended definitions of the degree of multidrug resistance, 32% of the GNB exhibited a multi-drug resistance (MDR) phenotype (*Escherichia* sp., *Klebsiella* sp., *Serratia* sp., *Acinetobacter* sp., *Pseudomonas* sp.), 13.2% were extended-drug resistant (XDR) (*Klebsiella* sp., *Acinetobacter* sp.) and 5.6% pan-drug resistant (PDR) (*Enterobacter* sp. and *Klebsiella* sp.). The gyrA gene, as well as the mexB, mexD, mexF si mexY genes, encoding for the efflux pumps mexAB-oprM, mexEF-oprN10 si mexCD-oprJ were detected in ciprofloxacin resistant strains and also correlated with the phenotypic resistance to aminoglycosides and carbapenems. The ESBL phenotype was correlated with the presence of blaSHV, blaTEM and blaPSE genes, while the MBL phenotype with the presence of blaVIM gene.
Conclusion: The increasing resistance in GNR provides an important signal that we need to improve our understanding of the genetic and biochemical basis of resistance mechanisms in the bacterial strains circulating in our geographical area, by using phenotypic and resistance genotyping tools.

R2555 Clinical impact and cost analysis of multidrug-resistant nosocomial *Acinetobacter baumannii* bacteraemia: a case-control study

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Objectives: *Acinetobacter baumannii* is an important nosocomial pathogen that causes high mortality, morbidity and medical cost because of its increasing resistance to antimicrobial agents. The aim of this study was to investigate clinical impact and cost analysis of multidrug resistant nosocomial A. Baumanni bacteremia.
Methods: A case-control study was carried out in the intensive care unit of the Atatürk Education and Research Hospital of Ankara, Turkey, from January 2007 through December 2010. Risk factors associated with multidrug resistance (MDR) and mortality and cost analysis were evaluated in patients with *A. baumannii* bacteremia. We selected patients who had bacteremia caused by the other gram-negative microorganisms during the study period as control group.
Results: Of the 86 patients were included in the study. Forty-one patients were in the case group, and 45 patients were in the control group. In the univariate analysis we found that arterial line ($p = 0.01$), higher SAPS II score ($p = 0.02$), lower albumin level ($p = 0.02$), the previous use of carbapenem ($p < 0.001$), quinolone ($p = 0.04$), glycopeptide ($p = 0.02$), aminoglycosid ($p = 0.001$), and metronidazole ($p = 0.01$) were risk factors for MDR *A. baumannii* bacteremia. In the multivariate analysis it was found that previous use of carbapenem ($p < 0.001$, OR: 11.9, 95% CI: 3.3–43.3), quinolone ($p < 0.02$, OR: 6.7, 95% CI: 1.3–34.4) and metronidazole ($p = 0.007$, OR: 31.8, 95% CI: 2.6–391.2, and high SAPS II score ($p = 0.01$, OR: 1.1, 95% CI: 1.0–1.1) were independent risk factors. The length of time spent at hospital, hospitalization costs and expense of antimicrobial therapy were not found statistically different between the two groups (case and control). There was no statistical significance in 14-day mortality, 28-day mortality or infection related mortality between the two groups in multivariate analysis, immunosuppression ($p = 0.027$, OR: 4.7, 95% CI: 1.2–18.4) and high SAPS II score ($p < 0.001$, OR: 1.1, 95% CI: 1.0–1.2) were independently associated with mortality in the case group.
Conclusion: The rationale use of the antibiotics is particularly important to prevent bacteremia caused by MDR *A. baumannii*.

R2556 Carbapenem resistant *Klebsiella pneumoniae* at the Lagos University teaching hospital, Lagos, Nigeria

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Objective: Carbapenem use is still low in Nigeria because they are expensive, not readily available and are reserved for life threatening gram negative infections. While carbapenem resistant *Klebsiella pneumoniae* is increasingly reported worldwide, there is limited data from Africa. The Lagos University Teaching hospital is a 761 bed referral hospital with an inpatient population of about 11 000 annually. *Klebsiella pneumoniae* has been identified as a major health care associated pathogen with reports of increasing resistance to various antibiotics. This study was undertaken to identify the risk factors for infection with carbapenem resistant *K. pneumoniae* (CRKP).
Methods: The study was conducted over a 6 month period and all in-patients with microbiological confirmation of carbapenem resistant *Klebsiella pneumoniae* infection were recruited as cases. Controls were in-patients with carbapenem susceptible *Klebsiella pneumoniae* infections. Carbapenem resistance was determined using disc diffusion tests and carbapenemase production was confirmed using the modified Hodge test. Patient data as well as data on antibiotic use and hospital stay were collected using a simple questionnaire. Bivariate and multivariate analyses were carried out to determine the association between potential risk factors and carbapenem resistance.
Results: *Klebsiella pneumoniae* was isolated from 153 in-patients of which eight were carbapenem resistant. Bivariate analysis showed that the use of three or more antibiotics ($p = 0.03$) and previous hospital admission ($p = 0.05$) were associated with CRKP infections. The use of three or more antibiotics (odds ratio [OR], 9.0 95% confidence interval [CI], 1.12–90.25) was an independent risk factor for the development of CRKP infections using multivariate analysis. Only four out of the eight CRKP isolates could be identified by the modified Hodge test (MHT) as carbapenemase producers. Resistance rates to other commonly used antibiotics were > 50% except for amikacin to which resistance rate was 25%.
Conclusion: Infections with CRKP are an emerging clinical threat and these findings highlight the need to institute antibiotic stewardship to promote rational antibiotic use as well as good infection control programmes to limit their transmission within the hospital.

Antibiotic usage

R2557 Drug-specific indicators assessing outpatient antibiotic use: a cross-sectional study of French general practitioners

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Objectives: Quality indicators assessing the use of antibiotics among General Practitioners (GPs) would be useful to target antibiotic stewardship interventions. We wanted to: (i) adapt at an individual physician level a set of 12 drug-specific quality indicators of outpatient antibiotic use developed at the European level to compare data between countries; (ii) describe the differences in antibiotic prescriptions between GPs in South-Eastern France; and (iii) study the factors associated with total antibiotic prescriptions at the GP level.
Methods: We performed a cross-sectional study analysing antibiotic prescriptions made by GPs in adults in South-Eastern France in 2009, using data from the outpatient reimbursement database of the General Health Insurance Fund.
Results: The 4971 included GPs practicing in South-Eastern France prescribed 19.1 million DDD of antibiotics to 997 437 adult patients in 2009. The 12 indicators showed wide variations of antibiotic prescriptions between GPs. The factors associated with total antibiotic use in multivariate analysis were: (i) GP's age; (ii) proportion of patients (70 yo, of patients exempt from copayments and of patients covered by the public supplementary health insurance program, total annual patient encounters and total pharmaceutical expenses; and (iii) other drug-specific indicators assessing prescribing of penicillins' combinations and of fluoroquinolones, and seasonal variation of quinolone prescriptions. A high amount of pharmaceutical expenses was one of the factors that influenced the most the quantity of antibiotics prescribed.
Conclusion: A set of 12 indicators could be calculated using reimbursement data to describe outpatient antibiotic use at the individual level. The observed heterogeneity in antibiotic prescriptions among French GPs could justify feeding back their results to each GP to try to change their prescribing behaviour.

R2558 Design of a set of quality indicators evaluating general practitioner's antibiotic use: a cross-sectional study

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Objectives: Our objective was to design quality indicators of outpatient antibiotic use that could be calculated at the individual GP level using reimbursement data only and to describe the differences in antibiotic prescriptions between GPs regarding these indicators in South-Eastern France.
Methods: Based on a literature review, we designed a set of quality indicators, assessing the quality of antibiotic prescriptions. We performed a cross-sectional study of antibiotic prescriptions in adults in South-Eastern France in 2009, using data from the outpatient reimbursement database of the General Health Insurance Fund. We carried out a cluster analysis to group GPs according to their antibiotic prescribing behaviour.

Results: The 4971 included GPs practicing in South-Eastern France prescribed 19.1 million DDD of antibiotics to 997 437 adult patients in 2009. Seven quality indicators were calculated at the GP level despite the lack of diagnosis reported in the information system. We noted wide variations in practice regarding all these indicators. High pharmaceutical expenses, high quinolone use and high seasonal variation of quinolone use were associated with antibiotic misuse in the cluster analysis.
Conclusion: The observed heterogeneity in antibiotic use among French GPs could justify feeding back their results to each GP to try to change their prescribing practices.

R2559 General practitioner's habits on antibiotic prescriptions, do they follow the recommendations?

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Objectives: In France, general practitioners (GP) deliver about 80% of all the antibiotic prescriptions. However, due to the amount of knowledge needed in such a speciality, the physician could not be up to date even with continuous medical education and the publication of several guidelines. We wanted with this study to make a snapshot of GP's habits concerning the prescription of antibiotics.
Methods: We performed a prospective study from December 2010 through March 2011. Rather than a poll, the point of call was the prescriptions that we retrieved from pharmacies. When antibiotics were prescribed, we called on the phone or interviewed directly, as soon as possible, the GP to ask him the reason of the prescription. Several data about the patient, the suspected infection and the GP's activity were collected.
Results: Our study included 100 prescriptions done by 22 GPs. A majority of GP were more than 50 years old and the mean delay of practice was 23 years. All the GPs said that they are aware of the guidelines and of their content. They also are confident when they prescribe antibiotics. Infections were as follow: ENT: 42%, lower respiratory tract: 31%, UTI: 16% and skin structure: 11%. The comparison with the French guidelines for these kinds of infection, and taking into account the molecule, the dosage and the length of treatment led to the following rate of adequacy: 36.6% for skin infections, 56.2% for UTI, 6.4% for lower respiratory tract and 28.5% for ENT infections. One of the major mistake was a lower dosage compared to the one proposed particularly for skin and respiratory tract infections (in up to 80%). Another discrepancy was a lower length of treatment mainly in ENT (85%) and respiratory tract infections (61%). There was also a lack of knowledge of bacteria usually in cause of the suspected disease. This is of importance mostly in empirical prescription, which is the case for GPs. Another practice, which is not recommended, is the use of corticosteroids in association with antibiotics mainly in lung infections (51%).
Conclusion: Even if GPs tell that they know the recommendations and follow them, it seems that they do not widely stick to them. Among the wrong uses, there are still a lot of viral infections that are treated with antibiotics. There are still a lot of things to propose to increase the knowledge of antibiotherapy to preserve its efficacy and to limit the emergence of resistant bacteria, due to inappropriate treatments.

R2560 *Clostridium difficile* associated diarrhea in cancer patients as a result of antibiotic and/or cytostatic therapy

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Objective: To analyse the isolation rate of toxigenic strains of *Clostridium difficile*, causing diarrhea and/or *Clostridium difficile* associated diarrhea (CDAD) in cancer patients treated with cytostatics and/or antimicrobials.
Materials and Methods: From September 2007 till October 2011, 205 patients with grade I-III diarrhea were examined to detect toxins A and B of *C. difficile* with an analyzer ''MiniVidas''.
Results: In 24 (11.7%) of 205 patients (female – 67%, male – 33%, mean age – 45 years [14–73 years]), toxins A and B were positive. According to NCI CTC criteria 14 (58%) patients had grade I diarrhea, 5 (21%) patients – grade II and 5 (21%) patients – grade III diarrhea. Diarrhea was acute in all cases. Mild form of CDAD was observed in 18 (75%) patients, moderate form – in 5 (21%) patients and severe form in 1 (4%) patient. Moderate/severe cases were accompanied by fever and/or leukocytosis >9000/μL and/or abdominal pains. Three patients received only cytostatic therapy and 21 patients – antibiotic therapy with or without cytostatics. Antimicrobials contributing to CDAD development were: cefepime (17%), cefoperazone/sulbactam (13%), imipenem (11%), metronidazole (10%), moxifloxacin (8%), ceftazidime (6%), levofloxacin (6%), meropenem (5%), amikacin (5%), sulfamethoxazole/trimethoprim (5%), ceftriaxone (3%), ciprofloxacin (3%), doxycycline (3%), linezolid (3%), piperacillin/tazobactam (2%). Thus, cephalosporins accounted for 39% of diarheas, fluoroquinolones – 17%, carbapenems -16%, other – 28%. Some of them were administered in combinations. Total of 20 patients received chemotherapy with etoposide, doxorubicin, cyclophosphamide, cisplatin, cytosine arabinoside, 5-fluorouracil, paclitaxel, irinotecan, ifosfamide, methotrexate, vincristine, rituximab prior to CDAD development. Treatment of CDAD included vancomycin PO 1.5–2.0 g/day in 17 patients or metronidazole PO 1.5 g/day – seven patients. The duration of treatment was 10 days. Clinical response and negative controls for fecal toxins A and B of *C. difficile* were seen in all cases. Further long-term probiotic therapy was administered. No recurrences were seen.
Conclusion: CDAD due to antimicrobials and/or cytotoxics use was revealed in 11.7% of hospitalized patients with diarrhea. Cephalosporins caused CDAD most frequently. Oral treatment of CDAD with vancomycin or metronidazole was equivalent and clinical and microbiological effect was achieved in all patients.

R2561 An observation study of antimicrobial prescribing in general paediatric patients at Birmingham Children's Hospital

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Objective: To identify changes that may be made to current paediatric practice to optimise antibiotic prescribing.
Method: Fifty consecutive emergency General Paediatric admissions during July and August 2011, from whom a blood culture was requested, were followed daily by an antimicrobial pharmacist and/or medical microbiologist, who did not intervene in patient management. Antibacterial treatment, laboratory results and clinical observations were reviewed retrospectively, and a consensus view on the appropriateness of each patient's antibacterial management was determined.
Results: Twenty-eight (56%) patients were aged <12 months, 5 (10%) aged 12–24 months, 7 (14%) aged 25–36 months and 10 (20%) aged >36 months. The commonest indications for empiric therapy were generalised sepsis (n = 25), respiratory tract infection (n = 9), meningitis (n = 5) and urinary tract infection (n = 4). Twenty-nine (58%) patients had a final diagnosis of confirmed/probable bacterial infection. Eleven (22%) had a positive culture: six *Escherichia coli* (urine), two Neisseria meningitidis (1 blood, 1 blood + CSF), one *Staphylococcus aureus* (wound), one Group A streptococcus (sputum) and one Bordetella pertussis (nasal). Hospital empiric antibiotic prescribing guidelines were followed in all cases. However, treatment was inappropriate in some way in 26/50 (52%) patients: treatment not indicated (n = 1); treatment could have been discontinued within 48 hour on the basis of laboratory results (n = 13); treatment could have been de-escalated based upon susceptibility testing (n = 8); treatment duration inadequate (n = 4). In 15/50 (30%) patients the instruction to await culture results prior to stopping antibiotics was

documented. This includes 7/13 (54%) patients whose therapy could potentially have been discontinued earlier. In 5/13 (38.5%) patients whose therapy could have been discontinued earlier a repeat C-reactive protein may have aided the decision to stop therapy.
Conclusions: Empirical broad spectrum antibiotics were appropriately prescribed. However, opportunities for timely reappraisal of therapy were frequently missed resulting in over-treatment. Measures such as repeating CRP measurement after 18–24 hour and consideration of the negative predictive value of negative blood cultures at 24–36 hour could substantially reduce antibacterial usage in hospitalised children.

R2562 The probable impact of antibiotic use on nosocomial *Pseudomonas aeruginosa* resistance

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Objectives: Emergence of resistance in nosocomial bacteria is known to be influenced by antimicrobial use. The aim of the study was to evaluate the probable impact of previous antibiotic use on beta lactam resistant *Pseudomonas aeruginosa* infections at intensive care units (ICUs).
Methods: A retrospective case-control study was performed between January 2008 and July 2011. Our hospital is a 670-bed tertiary-care teaching hospital. The patients with nosocomial infection caused by *P. aeruginosa* at three different ICUS (Neurology, Neurosurgery and Anesthesia-Reanimation) were included to the study. The patients with *P. aeruginosa* resistant to selected antibiotics were defined as case groups and the patients with *P. aeruginosa* sensitive to the related antibiotic were defined as control groups. The selection of the control patients was constituted regardless of whether the isolate is sensitive to other beta lactams or not. The case and control groups were made for each of imipenem, meropenem, ceftazidime and piperacillin-tazobactam. Previous antibiotic use was defined as at least 48 hours antibiotic usage in the period of 30 days before the isolation of *P. aeruginosa.* The investigated antibiotics to be used were cefazolin, vancomycin, teicoplanin, ciprofloxacin, amikacin and forementioned beta lactams. Case and control groups were compared and univariate and multivariate logistic regression analysis was carried out using SPSS v.15.0 software.
Results: Total of 120 patients (30 at Neurology, 34 at Neurosurgery and 56 at Anesthesia-Reanimation ICU) with *P. aeruginosa* infection were evaluated. The mean age was 58.4 ± 19.2 years, 50.8% were male. The results of univariate analysis of antibiotic use are shown in Table. Multivariate analysis revealed previous cefazolin or meropenem use was found as an independent risk factor for the development of imipenem resistance (OR = 3.31, 95% CI = 1.20–9.14, p = 0.021; OR = 4.60, 95% CI = 1.62–13.00, p = 0.004, respectively). Previous meropenem use was found as an independent risk factor for the development of meropenem resistance (OR = 8.04, 95% CI = 2.84–22.74, p < 0.001).

Table. The results of univariate analysis of antibiotic use

Previous antibiotic use	Imipenem		Meropenem		Ceftazidime		Piperacillin-tazobactam	
	Case R (n=56)	Control S (n=64)	Case R (n=32)	Control S (n=68)	Case R (n=38)	Control S (n=82)	Case R (n=44)	Control S (n=76)
Imipenem	4-7.1	7-10.9	3-5.8	8-11.8	2-5.3	9-11	4-9.1	7-9.2
Meropenem	**42-75**	25-39.1	**42-80.8**	25-36.8	26-68.4	41-50	**31-70.5**	36-47.4
Cefazolin	**19-33.9**	10-15.6	14-26.9	15-22.1	5-13.2	24-29.3	7-15.9	22-28.9
Ceftazidime	**8-14.3**	1-1.6	**7-13.5**	2-2.9	2-5.3	7-8.5	2-4.5	7-9.2
Pip-tazo	26-46.4	24-37.5	23-36.8	23-48.1	20-52.6	30-36.6	**24-54.5**	26-34.2
Teicoplanin	**25-44.6**	13-20.3	20-38.5	18-26.5	2-5.3	7-8.5	16-36.4	22-28.9
Vancomycin	17-30.4	13-20.3	17-32.7	13-19.1	9-23.7	21-25.6	11-25	19-25
Amikacin	**28-50**	16-25	**26-50**	18-26.5	16-42.1	28-34.1	18-40.9	26-34.2
Ciprofloxacin	4-7.1	6-9.4	7-13.5	3-4.4	**6-15.8**	4-4.9	**7-15.9**	3-3.9

Pip-tazo: Piperacillin-tazobactam. Bold numbers show statistically significant difference (p < 0.05)

Conclusion: Previous antibiotic use has a probable effect on emergence of resistance in *P. aeruginosa.* In case of empirical therapy of nosocomial pseudomonal infections, history of previous antibiotic use should be taken into account.

R2563 Antibiotics: over-the-counter dispensing vs. prescriptions

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Objectives: To study the pattern of dispensing antibiotics (with and/or without prescription OTC)
intermsof defined daily dose DDD, frequency, costs and indications reasons for dispensing.
Methodology: Cross-sectional study conducted in n = 24 randomly selected community pharmacies out of n = 240 located in Abu Dhabi city during the study period. A structured-closed developed to examine the impact of demographic characteristics age, gender and years of practice experience of the pharmacist with respect to dispensing antibiotics with or without prescription in terms of legalization rationality and safety. Questionnaire permitted studying the DDD frequency costs and reasons. Data were entered and analyzed by using SPSS version 17. Descriptive statistics. Odds ratios (OR) significance and 95% CI were calculated. Logistic regression conducted.
Results: Participated pharmacists dealt with a total of n = 1645 transactions with antibiotics, dispensed with prescriptions n = 1211 (73.6%) as OTC n = 434 (26.4%) customers. Only one pharmacist has not dispensed OTC antibiotic during the study period. Male pharmacists are dispensing more of OTC antibiotics. Gender of the Individuals p = 0.012 and their socioeconomic status p = 0.001 significantly affect the Individuals' manner in acquiring OTC antibiotics hence females and individuals with low socioeconomic status tend to get OTC antibiotics.co-amixoclav in addition with cefuroxime and clarithromycin, were the most dispensed antibiotics with prescription (66.4%, 91.3%, and 91.5% respectively) coamoxiclav amoxicillin and ceftriaxone were the frequent OTC dispensed antibiotics 33.6, 47.8 and 53.3% respectively. Prescribed antibiotics frequently dispensed with duration 7.5 and 10 days; while the OTC are frequently dispensed with duration 7.5 and 3 days. coamoxiclav was prescribed for sore throat as OTC Results reveal that ceftriaxone is mostly prescribed by doctors for Sexually Transmitted Diseases (STD) and also shows a threefold increase in dispensing as OTC. The total of dispensed DDD's is 16468.31 within this result 12771.68 (77.6%) were been dispensed with prescriptions and 3696.64 (22.4%) were dispensed as OTC.
Conclusion: Prevalence of dispensing of antibiotic over the counter is alarming such research and patients interviews will allow the collection of additional information on patient perception, knowledge and prescribing behavior which may be useful to develop interventions to change behavior of both prescribers and dispensers.

R2564 Use of tigecycline in intensive care: a French prospective observational study

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Objectives: Little information is available on tigecycline activity in patients (pts) with serious underlying disease or organ failure. This prospective observational study aimed at describing tigecycline prescribing patterns and pt outcomes in French ICUs.
Methods: Data of all adult pts treated with tigecycline alone or in combination for suspected or documented infection in 26 ICUs were collected over 7 days. Response to treatment was classified as cure (no other treatment or surgery), failure (persistent/relapsing infection, infection-related death >48 hour after tigecycline start, discontinuation due to adverse effects), or undetermined (death <48 hour, tigecycline <4 days due to de-escalation, antibiotics for another infection). We distinguished the less (SOFA ≤ 7) from the most (SOFA>7) severely ill pts.
Results: Of the 156 pts were included (September 2008 to April 2010): 64% male, age 60 ± 15 year, SAPS II on admission 42 ± 16. At tigecycline start, 45% had a SOFA>7 (median 11 [8–24]); 34% had fatal underlying disease, 10% chronic renal failure, 33% were

immunosuppressed and 19% diabetic. Of 93% had received antibiotics in the past 30 days.
Tigecycline was given in first-line in 47% of pts, mostly in combination (67%), for intra-abdominal (IAI, 56%), skin and soft tissue (SSTI, 19%), or other infections (36%, mainly pulmonary 24%), and for 10 ± 9 days in average. Of 84% of infections were hospital-acquired and 12% of pts had bacteremia. Tigecycline was stopped prematurely in 52% of pts, whatever the severity of illness, mainly due to resistant strain (n = 13), clinical failure (n = 14), de-escalation (n = 20), death (n = 14) or new infection (n = 4). Response to treatment is shown in the table. The cure rate was 60% at the end of treatment and 53% at 7 days (SSTI 63%, IAI 54%, other infections 46%). Failure at the end of treatment was due to persistent infection (n = 12), infection-related death >48 hour (n = 4) or clinical failure (n = 12), and at day 7 to relapse (n = 32). At both time points, the cure rate was similar with tigecycline alone or in combination; in Gram-positive, Gram-negative and anaerobic infections; and in mono or polymicrobial infections. It was similar in the less and the most severely ill patients at the end of treatment but not 7 days later (Table).

Response to treatment	Total	SOFA<=7	SOFA>7	p-value
At the end of tigecycline:	N=156	N=86	N=70	
•Cure	93 (60%)	55 (64%)	38 (54%)	0.079
•Failure	28 (18%)	17 (20%)	11 (16%)	
•Undetermined	35 (22%)	14 (16%)	21 (30%)	
7 days later (or at discharge):	N=145	N=82	N=63	
•Cure	77 (53%)	49 (60%)	28 (44%)	0.044
•Failure	32 (22%)	19 (23%)	13 (21%)	
•Undetermined	36 (25%)	14 (17%)	22 (35%)	

Conclusion: In this severe ICU population, the success rates were comparable to those obtained in clinical studies using other antibiotics in ICU. Tigecycline is a valuable alternative for the management of serious infections in ICU.

R2567 Pharmacologic assessment of guidelines on gentamicin use in infective endocarditis

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Objectives: To assess the relevance of guidelines on aminoglycosides use in the treatment of infective endocarditis.
Methods: Patients with a *Streptococcus* spp., *Staphylococcus* spp. or *Enterococcus* spp. bacteraemia were included, excluding critically ill patients. Endocarditis treatment was initiated as recommended by European guidelines (gentamicin at 3 mg/kg/day, once daily dosing for *Streptococcus* spp., twice daily dosing for *Staphylococcus* spp. or *Enterococcus* spp.). If endocarditis wasn't confirmed, gentamicin was discontinued. Peak (Cmax), Trough (Cmin) and Minimum Inhibitory Concentration (MIC) were measured as well as creatinine level. Gentamicin regimen was considered efficient when inhibitory quotient (Cmax/MIC) was superior or equal to 12.
Results: Aminoglycosides were administered during [median (min - max)] 10 (1–15) days. Among the 20 included patients, 50 peaks and 136 troughs were obtained. Modification of the initial regimens was required in 13/20 patients, because of insufficient peak (n = 8) or excessive trough (n = 5). At 3 mg/kg/day, 17.9% (n = 5/28) of measured peaks (2/18 of patients) allowed an inhibitory quotient (12 [median (min - max) = 4 (0–79)]. At 5 mg/kg/day, 40% (8/20) of measured peaks (2/6 of patients) allowed an inhibitory quotient (12 [median (min - max) = 10.5 (2–12.5)]. The only peak measured at 7 mg/kg/day had an inhibitory quotient <12. There was no significant association between once or two daily dosing and obtaining an inhibitory quotient (12. Of 19/136 (eight patients) troughs were superior to 1. There was no significant association between once or two daily dosing and obtaining a trough superior to one. Age, sex, weight or severity status weren't associated with inhibitory quotient (12 or elevated trough. Three patients had a worsening of their renal function, and all three had nephrotoxic medication concomitant to gentamicin.
Conclusion: Because of effective inhibitory quotients rarely obtained, recommended guidelines for gentamicin dosing regimens in infective endocarditis are inappropriate, as an effective inhibitory quotient is rarely obtained. Higher doses seem to be more appropriate to obtain efficiency from gentamicin. Dosing (3, 5 or 7 mg/kg) may be more important than frequency of administration (once or twice daily). Studies with sufficient number of patients are required to improve gentamicin prescribing recommendations.

R2568 Systematic post prescription review of carbapenem use in hospitals: impact on the duration of therapy

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Objectives: To describe the impact of an early review of carbapenems prescriptions in hospital using unsolicited infectious disease physician counselling (IDPC).
Methods: Prescriptions of four carbapenems from ICU, medical (M) or surgical (S) wards of two university hospitals were screened daily by the pharmacy using antimicrobial order forms or a computer-generated alert system. After inital delivery of the drug for up to 3 days, prescriptions where then reviewed by IDPs during a 5 month period to identify those likely needing counselling. Improved antibiotic use was sought by encouraging wards physicians to withdraw or de-escalate therapy.
Results: Of 327 prescriptions (adults = 254; 77.7%) were included (ICU = 131, 40.1%; M = 148, 45.2%; S = 48, 14.7%). Imipenem (n = 257; 78.6%) was the most carbapenem used while meropenem (n = 28, 8.6%), ertapenem (n = 23, 7.0%) or doripenem (n = 19, 5.8%) were less frequently used. The most common types of infection treated were urinary tract infection (n = 83, 25.4%), pneumonia (n = 74, 22.6%), gastrointestinal infections (n = 40, 12.2%) or primary bacteraemia (n = 31, 9.5%). Microbiologically documented infections and extended-spectrum béta-lactamase producing Enterobacteriacae accounted for 67.3% (n = 220) and 41.9% (n = 137) of prescriptions, respectively. Median time between delivery of the drug and review by the IDP was 2 days (IQR, 2–4 days). Overall, 142 prescriptions (43.4%) were stopped or de-escalated. The overall duration of carbapenem therapy was short (median, 2 days; IQR, 2–4 days).
Conclusions: A strategy using antimicrobial order form followed by an unsolicited antibiotic review of carbapenem prescriptions by IDP was successfully implemented in our hospitals and was associated with high rates of counselling and physicians' compliance, resulting in short courses of carbapemen therapy. The impact of this strategy on sparing hospital carbapenem use will require a longer follow-up.

R2569 Linezolid in the treatment of methicillin-resistant Staphylococcal post-neurosurgical meningitis

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Background: Linezolid is a bacteriostatic antibiotic with good cerebrospinal fluid penetration. The aim of this study was to evaluate the efficacy of linezolid in methicillin-resistant staphylococcal (methicillin-resistant *Staphylococcus aureus* [MRSA] and methicillin-resistant coagulase-negative *Staphylococcus* [MRCoNS]) meningitis.
Methods: All adult patients (age >18 years) with culture-proven MRSA or MRCoNS meningitis treated with linezolid (600 mg/12 hours intravenous) between January 2006 and September 2011 were retrospectively reviewed. Nosocomial meningitis was defined according to the CDC definitions. Samples of CSF were obtained through an intraventricular catheter if present and by lumbar puncture in the rest. The identification of *Staphylocccus* spp was confirmed using a commercial identification system. Antimicrobial susceptibilities were tested using a microdilution commercial system. An empirical antimicrobial therapy was considered as adequate if it included at least an effective antibiotic. Cure was achieved when two successive

cultures were negative and clinical signs of infection were absent. To assess survival, patients were followed-up until they died in the hospital or were discharged.

Results: Of the 11 cases (9 MRCoNS, 2 MRSA) fulfilled the inclusion criteria (82% males, mean age 66 years, limits 51–80). All patients had hospital-acquired meningitis and had undergone neurosurgery. The most frequent underlying diseases were brain haemorrhage (82%), and brain neoplasms and head trauma (both 10%). The mean time that elapsed between the surgery and the onset of the infection was 19.60 days (limits 6–49). All cases had a intraventricular catheter. The mean permanence of intraventricular catheter before the diagnosis of the meningeal infection was 16.7 days (limits 6–27) All the patients had received empirical antibiotic treatments that were adequate in 100% of the cases. Five patients received linezolid monotherapy . In three cases, a combined parenteral therapy was used with linezolid and rifampicina. Three patients received a rescue therapy with iv linezolid due to inefficay vancomicin treatment. In 91% cases, treatment was associated with the removal of the intraventricular catheter. Cure of the meningeal infection was reported in all cases. No patients died. There were no severe adverse events.

Conclusions: Our experience with linezolid suggests that it can be useful and safe the treatment of MRCoNS- and MRSA-related meningitis.

R2570 Antimycotic consumption in VINCat hospitals, Catalonia: stratified data by hospital size

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Objectives: VINCat is a nosocomial infection surveillance program in Catalonia (7.5 million of population), Spain. The aim of the study is to assess the evolution of the antimycotic consumption in 49 acute care hospitals stratified by hospital size.

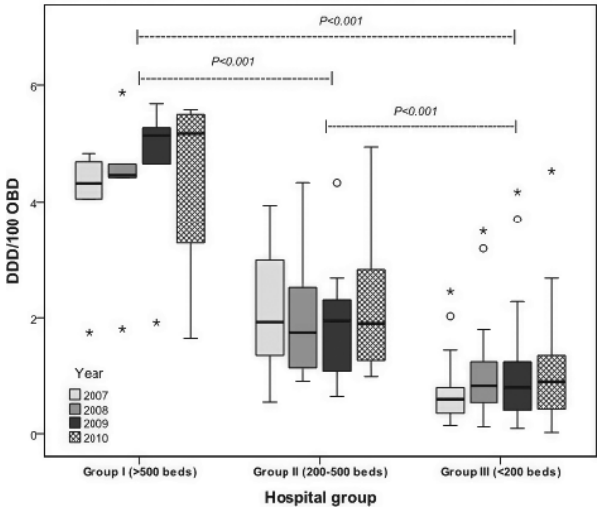
Methods: Annual analysis on antimycotic use from 2007 to 2010. Hospitals were stratified in three groups: I (more than 500 beds; N = 7), II (200–500 beds; N = 13) and III (<200 beds; N [2007] = 19, N [2010] = 29). Defined daily doses per 100 occupied bed-days (DDD/100 OBDs) were used to calculate the average of antimycotic consumption rate among all hospitals. Data in intensive care units (ICU), medical and surgical wards were analyzed separately. Trends of consumption and their differences between hospital groups were statistically analyzed using a linear mixed model with hospital as a cofactor. p Values <0.05 were considered statistically significant.

Results: Differences of antimycotic consumption between hospital size were statistically significant ($p < 0.001$). Global hospital DDD/100 OBDs 2007, 2008, 2009, 2010; group I: 3.78, 4.10, 4.30, 4.03 (increase of 6.61%; p = 0.558); Group II: 1.96, 1.98, 1.98, 2.20 (+12.24%; p = 0.887); Group III: 0.84, 1.28, 1.29, 1.14 (+35.71%; p = 0.204). ICU DDD/100 OBDs 2007–2010; group I: 19.32, 15.67, 14.52, 15.23 (-21.17%; p = 0.467); Group II: 15.05, 12.04, 12.01, 14.26 (-5.25%; p = 0.795); Group III (2008–2010): 12.64, 14.35, 16.02 (+26.74%; p = 0.809). Medical wards DDD/100 OBDs 2008–2010; group I: 4.97, 4.92, 4.84 (-2.62%; p = 0.643); Group II: 2.65, 2.33, 2.22 (-16.23%; p = 0.661); Group III: 1.95, 1.95, 1.26 (-35.38%; p = 0.843). Surgical wards DDD/100 OBDs 2008–2010; group I: 1.80, 2.16, 1.79 (−0.56%; p = 0.274); Group II: 0.55, 0.71, 1.14 (+107.27%; p = 0.840); Group III: 0.47, 0.49, 0.49 (+4.26%; p = 0.913). Higher consumption in large hospitals was observed when global data was compared by hospital size ($p < 0.001$) (Figure 1). These differences mainly came from medical and surgical wards.

Higher consumption increases were observed in voriconazole (p = 0.001), micafungin ($p < 0.001$) and anidulafungin ($p < 0.001$) mainly due to ICU.

Conclusions: (i) A different profile in antimycotic consumption was observed by hospital size. The larger hospital size was, the bigger antimycotic consumption was. (ii) A high and sustained antifungal consumption in large hospitals was observed, with a non significant increase along the years. (iii) In all size groups, the antifungal consumption came mainly from ICU.

Figure 1. Trends in Antimycotic Consumption by Hospital Size



R2571 Contrasted effect of an antimicrobial stewardship programme on antimicrobial use and on antimicrobial resistance in the intensive care unit setting

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Objectives: To improve healthcare quality and trying to reduce broad spectrum antimicrobial use, an infectious diseases team started in January 2005 quality rounds twice a week and, a close monitoring of antibiotic consumption. The present study evaluates the impact of this intervention between 2007 and 2010 in the intensive care units (ICU) setting with 24 beds of mixed medical and surgical intensive care.

Methods: The antibiotic consumption in Defined Daily Doses (DDD)/1000 patientdays was collected by the pharmacy. The antimicrobial resistance data of the Enterobacteriaceae and Pseudomonas aeuginosa were obtained from the laboratory survey. Bacterial resistances were given as percentages of resistant and intermediate isolates.

Results: The overall antibiotic use decreased in the ICU setting from 2136 in 2007 to 1092 DDD/1000 patientdays in 2010, being lower than the national mean consumption. The most significant reduction was observed with broad spectrum antibiotics, particularly with cefepime (decreased by 89%), aminoglycosides (decreased by 64%) and piperacillin/tazobactam (decreased by 61%). Cefuroxime remained stable whereas ceftazidime increased from 54 in 2008 to 88 DDD/1000 patientdays in 2010. A decrease in antibiotic resistance for Enterobacteriaceae was observed in 2010 only for cefuroxime and cefepime. Unfortunately, the piperacillin/tazobactam resistance rate increased for Enterobacteriaceae as for *P. aeruginosa*, respectively from 11% in 2007 to 24% in 2010, and from 13% in 2007 to 27% in 2010. The most significant reduction was observed in the aminoglycosides *P. aeruginosa* resistance from 26% in 2007 to 7% in 2010.

Conclusions: The intervention of the infectious diseases team resulted in a significant decrease in total antibiotic consumption but was associated with limited benefits in overall rate of resistance. Antibiotic resistant in ICU remains complex.

R2572 Vancomycin vs. linezolid in the treatment of methicillin-resistant *Staphylococcus aureus* meningitis: a retrospective cohort study

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Objectives: In this study it was aimed to compare vancomycin and linezolid in the treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) meningitis.

Methods: This study was performed at a tertiary-care general teaching hospital with an active neurosurgery ward containing 78 beds, 16 of which are in an intensive care unit. We extracted data and outcomes for all adult patients (age > 18 year) with culture-proven MRSA meningitis who received vancomycin or linezolid between January 2006 and July 2011. Demographic, clinical, and laboratory findings and predisposing factors, as well as information on response to treatment and outcome were obtained prospectively. A definite diagnosis of meningitis was based on the isolation of MRSA in at least one CSF culture. Typical CSF findings included a leukocytosis with a predominance of polymorphonuclear cells and classic clinical manifestations of meningitis. Samples were routinely centrifuged and the pellet was Gram-stained. *S. aureus* isolates were identified using routine microbiological methods. Antibacterial susceptibility tests were performed using the Kirby – Bauer disk diffusion method, as described by the Clinical and Laboratory Standards Institute (CLSI). Linezolid was given as 600 mg ×2 and vancomycin as 500 mg ×4 intravenously.

Results: There were a total of eight cases (five male, three female, aged 61.6 ± 13.2) received linezolid and nine cases (seven male, two female, aged 59.1 ± 15.6) received vancomycin, who fulfilled the inclusion criteria. One case in the linezolid group had MRSA and methicillin-resistant coagulase-negative staphylococci coinfection. All strains were susceptible to vancomycin and linezolid. All patients had hospital-acquired meningitis and had undergone neurosurgery. Microbiological success (Clearance of MRSA from CSF on day 5) with linezolid or vancomycin were 7/9 and 2/8 (p: 0.044 Fisher Exact Test). There were no severe adverse events in both arms. One month survival in the microbiologically successfully treated cases were 1/2 in vancomycin group (one died due to another nosocomial infection) and 5/7 in linezolid group in which two cases died due to other reasons (one additional nosocomial infection, one sudden cardiac death). In four of six failures with vancomycin, vancomycin minimum inhibitory concentration data was available and 2 mg/L (Etest; AB BIODISK, Solna, Sweden).

Conclusion: Although this is a retrospective cohort study, these data suggest that linezolid may be a good option in the treatment of MRSA meningitis.

R2573 Surgical prophylaxis compliance at a West London district general hospital

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Prophylactic antimicrobial use has an important part to play in the prevention of post-operative wound and deep site infections. However, the key principle in this use is to have a high concentration of the antimicrobial agent(s) in the relevant tissues at the time of the operation, when bacteria may contaminate the tissues. For most operations, this requires only a single dose of the antimicrobial(s) at induction of anaesthesia1. The aim of the audit is to determine the hospital compliance to the Surgical Prophylaxis Policy, to investigate outcomes and to agree on the necessary actions. A total of 196 patients were identified (172 included in the compliance study due to cancelations and inability to obtain the medical notes or required information). The plan was to gather information over one calendar week with provision to extend if a minimum of 100 patients was not achieved. All audited cases were reviewed by consultant microbiologist to assign compliance. Reasons for non-compliance are listed in table I. The audit has provided a snapshot of the surgical prophylaxis prescribing. The following are recommended; staff education, guidelines review, user friendly guidelines presentation, laminated wall poster for theatres, and monitoring initiatives.

Table I: Reasons for non-compliance

• Surgical prophylaxis not indicated and given	36 (39%) cases
• Surgical prophylaxis indicated and given	
○ Incorrect choice (guidelines available)	18 (19%) cases
○ Incorrect choice (guidelines not available)	1 (1%) case
○ A combination is recommended but a single agent used	3 (3%) cases
○ Incorrect dose	8 (9%) cases
○ Incorrect timing of the dose	3 (3%) cases
○ Following department guidelines	8 (9%) cases
○ 3 surgical prophylaxis doses recommended but one given	1 (1%) case
○ 1 dose surgical prophylaxis recommended but three given	2 (2%) cases
• Surgical prophylaxis indicated but not given	
○ On regular antibiotics (surgical prophylaxis required)	6 (6%) cases
○ Not on regular antibiotics	7 (8%) cases

1. Department of Health. Saving Lives: reducing infection, delivering clean and safe care, Antimicrobial Prescribing. Department of Health. 2007. Available at http://www.safetypatient.com/documentos/DH_076587buenaspracticasprescripcionantibioticos.pdf

R2574 Results from an observational study: daptomycin is effective in the treatment of catheter-related bacteraemia

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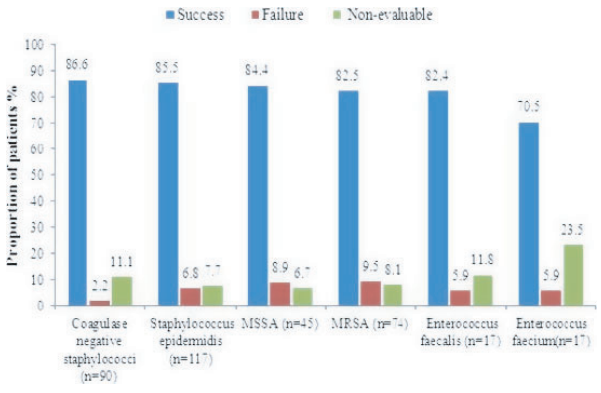
Objective: Catheter-related bloodstream infections are frequently caused by Gram-positive organisms, many of which are resistant to commonly used antibiotics. Daptomycin has rapid in vitro bactericidal activity against Staphylococci independent of methicillin susceptibility and against vancomycin susceptible or resistant Enterococci. The aim of this analysis was to assess the efficacy and safety of daptomycin in this indication.

Methods: The European Cubicin® Outcomes Registry and Experience (EU-CORE) is a retrospective non-interventional multicenter study to assess characteristics and clinical outcomes of patients receiving daptomycin. Patients with catheter-related bacteraemia who were treated with at least one dose of daptomycin were evaluated in this cohort. Treatment success was assigned by investigators using protocol criteria and defined as cured, improved, failure or non-evaluable following daptomycin therapy.

Results: The EU-CORE registry study included 487 patients with catheter-related bacteremia (of 4592 enrolled from January 2006 to June 2011). In this cohort 64% patients were male and 41% aged 65 years or more. Positive cultures of pathogens were reported in 415 patients (94% Gram positive, 6% Gram negative) and the most common pathogens



were coagulase-negative staphylococci (50%) and *S. aureus* (30% of which 59% were MRSA). The most frequently used initial dose of daptomycin was 6 mg/kg (66.3%; range <4–10 mg/kg). The median duration of daptomycin therapy was 10 days (range: 1–173 days). Of 348 (72%) patients received concomitant antibiotics, most frequently carbapenem (32%), penicillin (21%) and aminoglycoside (13%). The clinical success with daptomycin was achieved in 92% of patients (65% cured and 27% improved) and only 9% experienced failure. Daptomycin treatment was associated with high success rate against most of the pathogens including MRSA (82%) *S. epidermidis* (86%) and other coagulase-negative staphylococci (87%) (Fig. 1). Mild to moderate adverse effects (AEs) were observed in 27 (7%) patients. Five (1%) patients developed serious AEs classified as possibly related to daptomycin therapy by the investigator.

Conclusion: Daptomycin exhibited good efficacy and safety results confirming its role as a therapeutic agent for the treatment of catheter-related bacteraemia caused by Gram-positive pathogens.

R2575 Patterns of antibiotic usage in pregnant women in Russia

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Background: In pregnancy antibiotic (AB) present a special concern due to the threat of potential harmful effects on fetus and neonate. Although studies of AB usage are not uncommon in Russia there is a serious lack of comprehensive and valid data concerning the patterns of their prescription during pregnancy.

Objectives: To investigate the patterns of AB usage among pregnant women.

Methods: A population-based cohort study was conducted among obstetrician-gynecologists in two Russian cities. They were asked to recruit women with a pregnancy of (35 gestation weeks regularly attending prenatal clinics. AB exposure (patterns of AB use, indications, duration of treatment) throughout the pregnancy was recorded and evaluated with regard to teratogenic properties of drugs.

Results: Overall, 150 women aged from 16 to 39 years, mean age 25.7 ± 4.9 years were enrolled. Complicated course of pregnancy was registered in 71/150 (47%) of cases. Concomitant diseases were observed in 60/150 (40%) of women. Average number of AB prescriptions was 1.1 ± 0.3 per woman, length of treatment varied from 1 to 20 days; 16% of drugs were administered in the first trimester of pregnancy. The most frequently prescribed AB were macrolides (47%) and penicillins (41%), followed by cephalosporins (6%) and fosfomycin (4%); fluoroquinolones and nitroimidazoles were given in 2/163 and 1/163 of cases, respectively. AB were prescribed mostly by obstetrician-gynecologists (65%) and general practitioners (30%); indications to AB use were as follows: urogenital (47%), urinary tract (36%), respiratory tract (16%) and skin and soft tissue infections (1%). A total of 59% AB prescriptions belonged to Category B of the FDA pregnancy risk classification, 3% – to Category C; 39% of them were unclassified due to lack of data. Admitted possibility of additional AB use for self-medication was reported by 17% of pregnant women.

Conclusions: In general, majority of prescribed AB belonged to category B. The main concern causes the high rate of usage of drugs with undefined teratogenic risk and common practice of AB self-prescription.

R2576 Outcomes of antimicrobial stewardship intervention in a Spanish university hospital

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Background: Antimicrobial stewardship includes several strategies to improve antimicrobial use and prevent emergence of antibiotic resistance. Computer-assisted programs have been related to achieve these goals. Antibiotic Committee team designed a new computer program integrated in the computerized order entry. The objective is to assess the impact of this implementation by comparing characteristics, infections and clinical evolution of patients who receive interventions in their antimicrobial therapy vs. those in whom interventions were not considered.

Methods: Prospective cohort study at a 400-bed hospital in Barcelona, Spain between June and September 2011. Monitorized antimicrobials (MA): carbapenemics, linezolid, daptomycin, linezolid, tigecycline and voriconazole, echinocandins and amphotericins. Patients receiving a MA were included. All consecutive MA prescribed were assessed. A first assessment was done the initial day of therapy and a second assessment 72 hour later. Prescriptions were classified: with intervention and without intervention. Data collected: demographics, SAPS-II at admission (SAPS-II-A) and at antibiotic prescription (SAPS-II-AP), MA, infection related to MA, clinical evolution, microbiological data, length of hospital stay (LOS) and global and attributable mortality. Chi square and Fischer exact test for dichotomic variables and t-Student and ''U'' Mann–Whitney test for continuous variables were employed.

Results: Patients included: 100, man 61, mean age 66.8 years. UTI 26, Pneumonia 24, IAI 23, CNS 6, unknown origin sepsis 6, other 7. Main interventions: other alternatives with narrow spectrum of activity (43%) and changes in dosage schedule (27%). Intervention vs. non-intervention treatments: neither differences were found in origin and type of infection nor in cause of treatment discontinuation.

	Total (n=100)	No Intervention (n=57)		Intervention (n=43)		Value p
	Nº	Nº	%	Nº	%	
Men- N (%)	61	36	63,2	25	58,1	ns
Mean age (SD)	66,8	65.1 (±17,2)		68.9 (± 16)		ns
LOS (SD)	27,4	28.58 (± 23,3)		18.15 (± 13,1)		< 0.05
SAPS-II-A (SD)	35,01	34.67 (± 10,04)		36.63 (± 10,9)		ns
SAPS-II-AP (SD)	36,66	36.29 (± 11,58)		38.09 (± 11,9)		ns
Days with AM (range)		8.96 (±8.2)		5.21 (1-18)		<0.01
Positive cultures	82	42	73.7	40	93	<0.05
Clinical outcome						
Cure	76	42	73.7	34	79.1	ns
Failure	13	7	12.3	6	14	ns
Indeterminate	11	8	14	3	7	ns
Global mortality	25	16	28.1	9	20.9	ns
Attributable mortality	12	7	12.3	5	11.6	ns

Conclusions: Computer program has shown to be a good tool for improving antimicrobial prescription. Almost 50% of prescriptions were related to an intervention carried out by Antibiotic Committee. Although interventions were performed in patients with higher severity, they showed a reduction in lenght of hospital stay and in the days of antimicrobial therapy without impairment in clinical outcomes.

R2577 Antibiotic consumption among six general hospitals: results from a Greek surveillance network

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Objectives: To analyze data on antimicrobial consumption (AC) originated from Internal Medicine (IM), Surgical (S) and Intensive Care Units (ICU) departments of six general hospitals of Greece (four

from the region of Athens and two from the Western Greece) in order to provide a clear picture of the profile of hospital antibiotic usage.
Methods: Data on AC of systemic antibiotics in Anatomic Therapeutic Chemical (ATC) class J01 were obtained from the hospital pharmacy department records during the first six months of 2011 and were expresses in defined daily doses (DDDs) per 100 patient days (PDs) (ABC calc. vs. 3.1). Differences between compared hospital departments were accepted as statistically significant when $p < 0.05$, counting Pearson's $\times 2$ or Fisher exact tests for proportions (GraphPad Prism vs. 5.04).
Results: A higher AC was noticed among the three big hospitals of 400–550 beds (Laiko, Red Cross and Sismanoglio General Hospitals), especially in the IM departments, with mean AC (MAC): 87.2 DDDs/100 PDs and in the ICU departments with MAC: 128.4 DDDs/100 PDs). The comparison with the MAC of the other three hospitals of 120–150 beds (Polycliniki, Agrinio and Messologi General Hospitals) was statistically significant between the corresponding groups of IM and ICU departments. The highest AC was observed among the classes of carbapenems (J01DH), penicillins with b-lactamase inhibitor (J01CR) and fluroquinolones (J01MA), especially among the three big hospitals, while the AC in the other antibiotic classes as was significantly lower. The AC in S departments was generally limited among all hospitals.
Conclusion: A higher AC was noticed in the hospitals with more than 400 beds especially in IM and ICU departments. The carbapenem consumption in these hospitals is worrying. The collaboration among the six hospitals was an attempt for the creation of a surveillance network for AC among the hospital pharmacy departments in national level, aiming to collect reliable and comparable data of AC in Greece.

Molecular bacteriology

R2578 Prevalence of plasmid-mediated quinolone resistance determinants in Enterobacteriaceae

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Objectives: The presence of plasmid-mediated quinolone resistance determinants was investigated in quinolone-resistant enterobacterial isolates.
Methods: We tested a total of 136 norfloxacin, ciprofloxacin-resistant isolates of Enterobacteriaceae which were collected at the GATA from January to December 2009. The presence and identity of ESBL and the qnrA, qnrB, qnrS, aac(6′)-Ib-cr and qepA genes genes were screened by a multiplex PCR-based technique. Plasmid analysis was carried out by Kado-Lui method and the genetic relationships among the strains were determined by ERIC-PCR.
Results: All isolates were resistant to norfloxacine and ciprofloxacine by disk diffusion test. Of the 136 collected isolates, 106 isolates were *E. coli*, 29 isolates were *K. pneumoniae* and two isolates were *K. oytoca*. Prevalence of ESBLs was 42.4% of *E. coli*, 65.5% of *K. pneumoniae* and 50% of *K. oxytoca*. Ten percent of those isolates carried qnr genes encoding the qnrA, qnrB, qnrS or aac(6′)-Ib-cr determinants. Most aac(6′)-Ib-cr-positive isolates (n = 6) were ESBL producers, Among these isolates, qnrB were detected in two *K. pneumoniae* and one *E. coli* isolates, whereas qnrA was detected only one *K. pneumoniae* isolate. qnrS was detected in two isolates; one *K. pneumoniae* and one *K. oxytoca*. aac(6′)-Ib-cr was detected seven isolates of *E. coli*, whereas no qepA-positive isolates were detected. Two qnrB-positive *K. pneumoniae* isolates also had blaTEM and blaSHV genes. Plasmid DNA fingerprinting revealed the presence of approximately 80 and 130 kb in *K. pneumoniae* isolates; 80, 130 and >200 kb plasmids were detected in qnrB gene harboured *E. coli*. ERIC-PCR results revealed that the strains carried qnr genes were not clonally related. Two qnrB-positive *K. pneumoniae* isolates also had blaTEM and blaSHV genes.
Conclusion: This is the first study of qnr genes in Enterobacteriaceae isolates from our hospital. qnrB was the most frequently encountered gene in *K. pneumoniae* and *E. coli*, aac(6′)-Ib-cr gene among ESBL-producing *E. coli* was identified.

R2579 MALDI-TOF mass spectrometry in a paediatric clinic's microbiology laboratory everyday work

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Objectives: Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been recently introduced in microbiological laboratories for the identification of microorganisms with proteomics approaches. Hereby, we report the performance of MALDI-TOF MS identification of clinical microbial isolates from the microbiology laboratory serving for a pediatric hospital in comparison to conventional microbiological methods.
Methods: We performed MALDI-TOF MS by means of a Microflex mass spectrometer (Bruker Daltonics, Germany). Colonies from the culture dishes were directly deposited on the target plate without any pretreatment. Conventional identification was performed by routine phenotypic and immunological methods.
Results: In total, by means of MALDI-TOF MS we analyzed 1810 isolates obtained from pediatric patients. Score values of (1.700 that were used as a minimum cut-off for genus identification were observed for 1447 isolates (79.9%). These included 30 bacterial species from 16 genus and two fungi species from Candida genus. For isolates with score (1.700, MALDI-TOF MS genus identification was in complete agreement with conventional methods (100%), whereas correct species identification was found for 1428 isolates (98.7%). All discordant results were associated with *Streptococcus pneumoniae* identification. MALDI-TOF MS correctly identified *S. pneumoniae* in 20 from 39 isolates (51%), the rest of isolates was misidentified according to results of specific pneumococcal latex agglutination.
Conclusion: Thus, MALDI-TOF MS is a reliable and rapid method for identification of microbes isolated from clinical samples that may in many instances substitute classical laboratory methods. Moreover, accurate MALDI-TOF MS identification at the species level of all microbes having score (1.700 (except *S. pneumoniae*) substantiates the use of the score value (1.700 as a reliable cut-off for species identification of the microbes studied in the present work.

R2580 Comparison of four commercial molecular methods for the diagnosis of *Clostridium difficile* infection

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Objectives: *Clostridium difficile* infection (CDI) is the major cause of health care-associated diarrhoea. Rapid and accurate microbiological diagnosis of CDI is urgently needed. Toxigenic *C. difficile* detection by the cell culture neutralization assay (CCNA) is considered to be the gold standard. However, this procedure is time and labour intensive. The purpose of the present study was to compare four different commercial molecular methods (Cepheid XpertTM *C. difficile* assay, Illumigene *C. difficile* test, RIDA®GENE method and Duplica alpha) using the CCNA as the reference method.
Methods: The Cepheid method was tested with 220 faecal samples. The Illumigene with 263, the RIDA®GENE as well as the Duplica alpha with 181 samples each. In all investigations consecutive, non-repetitive fresh unformed stool specimens from patients older than 2 years of age were analysed. All four methods were compared with the standard laboratory diagnosis method i.e. CCNA and culture.
Results: In a comparison with the CCNA reference method the sensitivities ranged from 90% to 97% and specificities from 93% to 99%. The methods except Illumigene are based on RT-PCR. The Illumigene uses a Loop-mediated isothermal amplification (LAMP) technology. The major difference between the methods was the pre-analyses time and the reaction time. The fastest method was that from Cepheid. In average it took 65 minute from the arrival of the sample until the final result. For the Illumigene method, the corresponding time was 75 minute. Both the RIDA®GENE as well as the Duplica alpha methods the overall time was 2.5 hour. The tested methods, except Cepheid, were run in batches. The Cepheid method has been run

continuously since every sample could be run independently of each other. The Cepheid method targets the genes for the toxin B, the binary toxin as well as the deletion in the regulatory TcdC gene. The Illumigene targets a conservative region in the toxin A gene. The RIDA®GENE targets both the toxin A and toxin B genes and the Duplica alpha targets the toxin B gene.

Conclusion: The four methods have high sensitivities and specificities. The methods except the Cepheid XpertTM *C. difficile* assay, required a separate step of DNA extraction and were run in batches. The Cepheid method has the fastest turn around time of 65 minute followed by Illumigene (75 minute). With the Cepheid Xpert TM *C. difficile* assay it is possible to run each sample independently.

R2581 GeneXpert MTB/RIF system in pulmonary and extrapulmonary specimens: comparison with other nucleic acid technologies

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Objectives: New techniques for rapid diagnosis of TB and drug resistance have been used in hospitals to facilitate critical decisions. One of the latest systems, the GeneXpertTM MTB/RIF (Xpert). The Xpert assay integrates DNA extraction, genomic amplification, semiquantitative detection of *M. tuberculosis* complex, and rifampin (RIF) resistance determination in a single cartridge, for DNA extraction and amplification of a 192-bp segment of the rpoB gene. The aim of the present study was to evaluated the effectiveness of the Xpert system for the detection of TB in respiratory and non-respiratory specimens and to compare with conventional culture methods and reverse hybridation assay.

Methods: Between February 2010 and October 2011, 90 specimens ordered for routine mycobacterial testing were prospectively studied. Samples were processed by standard procedures: microscopic examination for acid-fast organisms (Ziehl-Neelsen stain) and mycobacterial culture using Lowenstein-Jensen and BacT/ALERT (bioMérieux). Mycobacterium isolates were identified by AccuProbe (Gen-Probe Inc./bioMérieux) and sensibility were tested using Genotype MTBDR plus (Hain Lifescience). The GeneXpert assay was run according to the manufacturer's instructions on all 90 samples.

Results: Of the 90 specimens tested 50 were respiratory and 40 non-respiratory samples (11 acid-fast smear positive and 79 smear negative). The Xpert assay detected 100% smear-positive, culture-positive cases and smear-negative culture-positive cases, as determined by growth on solid medium or on both solid and liquid media. *M. tuberculosis* was not detected in all of the culture-negative samples. One specimen yielded false positive result with Xpert MTB/RIF when compared to the reference method. No resistance were detected for the tested isolates. No crossed-contamination was detected with other mycobacteria in any sample. No uninterpretable results were described in this study.

Conclusion: Xpert is a useful and easy-to-apply test for the rapid diagnostic of TB. In this study we demonstrate that Xpert is highly sensitive and specific for TB detection.

The MTB/RIF assay is simple to perform with minimal training, is not prone to facilities, and has a high sensitivity in smear-negative tuberculosis. Xpert is a platform that detects a relatively small number of mutations and in population with high prevalence of RIF resistance more studies are required in order to assess the performance of this test.

R2582 Evaluation of a triple molecular genetic test for the diagnosis of *Helicobacter pylori* infection and its resistance to clarithromycin and fluoroquinolones in clinical practice

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Objectives: To assess the applicability, efficacy and accuracy of a triple molecular genetic test for identification of *Helicobacter pylori* (HP) infection and its resistance to clarithromycin and fluoroquinolones in every day clinical practice.

Patients and Methods: Forty consecutive patients undergone upper gastrointestinal (Gl) endoscopy due to various upper GI tract symptoms. HP infection was assessed by a RUT test (CLO) and/or histology of gastric biopsies (two antral and two corpus specimens) and one sample for the molecular genetic test. (GenoType Helico DR test- Hain). It was analyzed into three steps. Multiplex PCR and DNA strip hybridization were performed for identification of HP. The determination of its resistance to clarithromycin was made by the detection of the most significant mutations of 23S gene (positions 2146 and 2147) and its resistance to fluoroquinolones from the examination of gyrA gene (codons 87 and 91).

Results: There was concordance in positivity of HP, with at least one of the applicable HP detection tests (CLO and/or histology) in all patients (100%) found positive for HP (19/40, 47.5%). In four patients CLO test was positive while histology and the molecular genetic test were negative, so concordance to negativity of HP was 81%(4/21). Resistance to clarithromycin was detected in 6/19 (31.5%) patients but 1/19 (5.2%) was found resistant to fluoroquinolones. Total resistance to antibiotics was high enough reaching 36.7% of the HP+ population. Eradication HP regimes, selected according to resistance, succeeded in 100% of HP+ patients, as confirmed with one month later, breath test.

Conclusion: The use of the molecular genetic test is proved easily applicable and highly sensitive in the detection of HP infection and it defines the resistance of clarithromycin and/or fluoroquinolones. The progressive increase of antibiotics resistance should be controlled with targeted treatment, so that clarithromycin will continue to be a useful tool.

R2583 Determination of bacterial DNA in clinical blood samples: volume matters

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Objectives: The molecular detection of bacteria in blood may provide a rapid tool for diagnosis of bloodstream infections since this omits culturing. A major drawback to date is the limited clinical sensitivity, mainly caused by the limited volume of blood used. In this study we investigated the influence on clinical sensitivity of increased blood volume from patients with proven bloodstream infection. For this purpose we used a new rapid method (Polaris®) that is in development within Biocartis which claims to selectively remove human DNA prior to the extraction of bacterial DNA enabling higher blood volume input in the PCR. Preclinical results revealed a sensitivity of up to 1 CFU/mL. This method was compared to a reference method in patients with bacteremia & endocarditis.

Methods: Whole blood samples (6 mL EDTA) from patients (n = 9) with positive blood cultures with *Enterococcus faecalis* (n = 3) or *Staphylococcus aureus* (n = 6) were analysed. Samples were processed using the Polaris method (5 mL) and the reference method (0.2 mL). The reference method achieves bacterial lysis with Triton-Tris-EDTA for *E. faecalis* and lysostaphine for *S. aureus*. DNA was extracted with the EasyMag® system and quantified with a specific real-time PCR assay (Lightcycler 480®). Additionally, serial measurements during treatment for culture-proven endocarditis were performed in three patients.

Results: Bacterial DNA was detected in 8/9 patients with the Polaris method and in 6/9 with the reference method; the signal in the other samples did not cross the detection limit. The Polaris method had a median cycle threshold (CT) value of 35.2 (range 29.7–>42) compared to 36.7 (range 32.7–>42) with the reference method. A Wilcoxon signed rank test showed a significant difference between both methods ($p = 0.04$). In three endocarditis patients of whom follow up samples were available, bacterial DNA remained longer detectable with Polaris than with the reference method.

Conclusion: The Polaris method has a higher sensitivity than the reference method in clinical samples and provides more possibilities for serial measurements in clinical blood specimen during treatment. The larger volume that can be processed with this method is likely an important factor for clinical usage. This method may provide the step that increases sensitivity required to make the molecular detection of bloodstream infections clinically applicable.

R2584 Development of rapid point-of-care tests for the detection of bacteria implicated in surgical site infections and the determination of their antibiotic resistance profile

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Objectives: Surgical site infections (SSI), particularly those complicated by antibiotic resistance can be a problem in human and veterinary medicine. In view of the wellbeing and economic cost of SSIs on the patient and practice involved, it is imperative that the causative agent(s) of an SSI and their antibiotic resistance profile are rapidly identified to aid rapid and targeted treatment. With the advent of rapid isothermal DNA amplification technology, the opportunity exists to develop rapid in-practice diagnostic tests to facilitate pre and post operative diagnostic microbiology and subsequent treatment regimes. The work described here aimed to develop rapid diagnostic tests to evaluate the type of organisms involved in surgical site infections and their antibiotic resistance gene profiles.
Methods: The isothermal amplification technique – Loop mediated Isothermal Amplification (LAMP) originally described by Notomi et al. was used as the basis of the rapid diagnostic tests in the work reported here. For each of the designed assays, a set of six primers specific to an organism specific gene or antibiotic resistance gene were designed, using – LAMP designer software i.e. PrimerExplorer V4 by Eiken Chemical Co. ltd. or Manually under specified conditions.
Results: To date, LAMP assays have been developed for the identification of Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli/Shigella, Enterococcus faecalis, Enterococcus faecium and Staphylococcus pseudintermedius, as well as the detection of the Chloramphenicol AcetylTransferase (cat) gene in Gram negative bacteria. These assays have been validated using crude DNA lysates from positive control strains, and detection has been determined within 15 minute in all assays. Upon testing against a panel of 28 different bacterial species, the majority of the assays have shown no cross-reactivity.
Conclusion: The preliminary results obtained hitherto for the assays show that they are both specific and rapid in detecting their target. In addition to this, the relative inexpensive nature (no thermocycler required) and simplicity (can be used with simple laboratory heating equipment such as a heat block) of carrying out the reactions, makes LAMP a suitable alternative to conventional PCR and culture-based methods of bacteria and antimicrobial resistance identification, as well as a prime candidate for the development of rapid and cost efficient diagnostic tests.

R2585 Evaluation of the Cepheid GeneXpert® vanA/vanB assay

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Objectives: Rapid identification of vancomycin resistant enterococci (VRE) carriage may be useful in the clinical setting, with faster identification of VRE positive patients allowing prompt infection control strategies to be implemented. As such, we investigated the turn-around-time (TAT), sensitivity and specificity of the Cepheid GeneXpert® vanA/vanB rapid PCR assay, when compared to culture using selective chromogenic agar, for the detection of VRE.
Methods: Patients (n = 89) from two wards were screened for VRE carriage by two commercial methods. Rectal and perianal swabs were taken simultaneously. Rectal swabs were cultured on Brilliance™ VRE agar. Perianal swabs were subjected to PCR using the vanA/vanB assay according to the manufacturer's instructions. Patient samples yielding discrepant results were investigated further using Enterococcosel™ broth enrichment of the perianal swab, followed by culture on Brilliance™ VRE agar. TAT was noted for each sample. The sensitivity and specificity of the PCR assay was calculated through comparison with the results obtained by culture (±enrichment).
Results: In our hands, samples processed by PCR had a mean TAT of 26 hours 30 minutes, representing an average reduction in TAT of 44 hours 18 minutes when compared to culture. The sensitivity and specificity of the vanA/vanB PCR assay was 94% and 70% respectively. Taken alone, analysis of the vanA component of the PCR reaction resulted in a sensitivity of 94% and specificity of 89%. The sensitivity and specificity of the vanB PCR was 11% and 80% respectively.
Conclusion: The Cepheid GeneXpert® vanA/vanB assay is a sensitive test for the detection of VRE, yielding results faster than culture on chromogenic agar. A number of false positive results were observed using the assay. A large proportion of the false positives were attributed to the presence of the vanB gene, which is found in a number of non-enterococcal species. In our hands, the relatively low specificity of the PCR assay would require all samples positive by PCR to be confirmed by culture or an alternative method. Nevertheless, the assay could provide a useful tool for the rapid screening out of negative patients.

R2586 Assessment of Neisseria gonorrhoeae Chlamydia trachomatis simultaneous detection by real-time PCR according to the care unit

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Objectives: Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (NG) are the two most important agents of sexually transmitted bacterial infections (STI). These infections if untreated can be at the origin of serious complications. The objective of our study was to estimate the relevance of the systematic simultaneous detection of these two bacteria by real time PCR on urogenital specimens colleted from women or men consulting in different care units either for a screening test or based on symptomatic suspicion.
Method: From November 1st, 2010 to August 31st, 2011, Abbott Real Time CT/NG assay was systematically performed on all urogenital specimens sent to diagnose STI. Endocervical swabs were collected from female of the Family Planning clinic (FP) and the Department of Obstetrics and Gynecology (OG), self-collected vaginal swab was collected from female subjects of STI Screening Center (SC) and urine sample was collected from male subjects of SC.
Results: A total of 1861 CT/NG PCR were performed during this period (sex-ratio M/F: 0.25). Of 58.8% were prescribed by FP, 28.5% (SC) and 10.1% by OG. Of 161 specimens were positive for CT (M/F: 0.15) and 32 for NG (M/F: 0.23). Of 20 specimens were positive for both (M/F: 0.18). The prevalence of CT was 8.2% in FP, 8.5% in SC and 11.7% in OG. Medium age was 22 years for women and 25 years for men. CT infections were asymptomatic in 81% of cases. The prevalence of NG was 1.5% in FP, 0.7% in SC and 4.2% in OG. Medium age was 23 years for women and 24 years for men. A total of 7 NG salpingitidis were diagnosed of which 4 were co-infected with CT.
Conclusion: CT/NG multiplex PCR is a fast, practical technique, easily completed on urines and more sensitive than culture. It allowed us to increase the number of NG biological diagnosis especially on woman specimens in all care units. CT/NG PCR is a useful tool for STI diagnosis and treatment especially asymptomatic patients.

R2587 DNA extraction from broad range of micro-organisms for molecular diagnosis

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Molecular analysis has proven as a valuable tool in the timely diagnosis of the aetiological agents of infectious diseases such as sepsis, endocarditis and meningitis. DNA extraction from a wide variety of microorganisms is a key issue for their detection at high analytical sensitivity and precise sequence-based identification. The aim was to test the broad-range lysing capability of a reagent included in the diagnostic kit, UMD™ Universal, using a variety of clinical material coming in for routine molecular analysis.

Methods: Clinical material from patients under suspect of an infection included liquid and tissue specimens from different body sites. The specimens were extracted according to protocols supplied with UMD™ Universal (Molzym, Bremen, Germany). This kit includes universal rRNA gene PCR assays for the detection of bacteria and fungi. If the assay was positive, amplicons were sequenced and analysed using NCBI BLAST and SepsiTest-BLAST to identify the organisms.

Results: In total, 404 clinical specimens were extracted and PCR-analysed, including whole blood, synovial fluid, swabs taken from wounds, prostheses, pus and bones, liquor, and heart valve and aortal tissues. The organisms identified included typical pathogens and strains which are commensal as well as aetiological (e.g. CoNS, viridans streptococci). Among typical pathogens, *S. aureus*, *E. faecalis*, *E. coli*, and *C. albicans* were the most prominent. Rare aetiologies comprised taxonomically diverse strains, including, among others, *Bacteroides fragilis*, Bartonella quintana, Borrelia graninii, Coxiella burnetii, Listeria monocytogenes, *Providencia stuartii*, and *Tropheryma whippleii*. One blood sample was positive for the aetiological agent of malaria, Plasmodium falciparum (specific assay).

Conclusion: During the pre-analytical processing of clinical material by UMD™ Universal, a reagent is applied for the destruction of microbial cell walls. The chemical composition and thus the rigidity of cell walls is very different among the microbiota. The data from randomly collected, diverse material indicated that the reagent can lyse a broad range of microorganisms, including Gram-positive and Gram-negative bacteria, yeasts, other fungi and a protist. This character of the reagent greatly supports the precise molecular identification of common and rare aetiologies.

R2588 Multiplex-PCR for the rapid detection of clinically relevant antibiotic resistance genes

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Objectives: Spreading of antibiotic resistance determinants in clinical isolates is worrisome, limiting antibiotic usage and their effectiveness. Early detection of these genes in such isolates is therefore crucial, not only for the choice of an effective antibiotic therapy, but also for to establish appropriate infection control strategies. Here we describe a novel multiplex-PCR (mP) method for the rapid detection of a number of clinically relevant resistance genes directly from bacterial colonies.

Methods: Allelic variants of selected gene families of beta-lactams, aminoglycoside and glycopeptide resistant genes were retrieved from the REDDB database and used to identify primer sequences. Oligos were synthesized by MWG Biotech and used for the optimization of six distinct mP reactions. Inoculated agar plates were grown O/N at 37°C and used to prepare a standardized bacterial suspension (SBS). An aliquot of the obtained suspension was centrifuged and the pellet was resuspended in a previously described lysis buffer. After an incubation of 2 hours at 60°C, the sample was heated at 95°C for 15 minutes then pelleted by centrifugation. 1 μL of the obtained sample was used as template for mP reactions in a total volume of 12.5 μL using the GoTaq polymerase (Promega) and a $MgCl_2$ final concentrations of 50 mM. Cycling conditions were as follows: 5 minute at 94°C, 35 cycles of 30 seconds at 94°C, 40 seconds at 54°C, and 1 minute at 72°C, and a final extension step of 5 minute at 72°C. Amplification products were resolved by gel electrophoresis and eventually analysed by enzymatic restriction for the discrimination of amplicons.

Results: Positive control strains (n = 30) (used separately or mixed) yielded expected amplicons, thus validating the mP protocol. The use of SBSs gave highly reproducible results, even when used 4 weeks after preparation. Specificity of each mP was excellent, with any unattended amplicons in the range of expected products. Restriction of ambiguous fragments allowed the discrimination of amplified product.

Conclusions: In this work a mP based approach for the detection of resistance determinants starting from isolated colonies was set-up and optimized. Amplification from SBS instead of purified genomic DNA allows a considerable reduction of time necessary for the detection of resistance genes.

R2589 Quantification on the BD MAX™ System with BD MAX™ MRSA assay

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Introduction: The BD MAX™ System is a next-generation automated workstation for molecular testing. BD MAX™ System is designed to standardize and streamline workflow and offer full walk-away automation, the system accommodates varying workloads for enhanced laboratory efficiency and flexibility.

Objectives: (i) Determine if the BD MAX™ System could be used to perform quantitative analysis. (ii) Determine the Sample Preparation efficiency of the BD MAX™ System.

Methods: BD MAX™ MRSA Assay[1] has been used as the model assay. A simulated MRSA target has been genetically inserted into the genome of a Bacillus subtilis strain (BSgm). Purified genomic DNA (gDNA) and fixed BSgm cells were used to evaluate the performances of the BD MAX™ System using the MRSA Assay. Serial dilutions of BSgm gDNA were analyzed in PCR-only mode on the BD MAX™ System with the BD MAX™ MRSA Assay. Fixed BSgm cells were analyzed in full-process mode (automated Sample preparation and real-time PCR) on the BD MAX™ System with the BD MAX™ MRSA Assay. The statistical analyses were focused on the second derivative peak apex (SDPA) PCR parameter.

Results: The statistical analysis showed a dynamic range over six orders of magnitude with the BD MAX™ MRSA Assay on the BD MAX™ System. Analysis comparing the PCR only to the full process results with the BD MAX™ MRSA Assay on the BD MAX™ System showed efficient Sample Preparation with the automated in vitro diagnostic system. The statistical analysis showed that the BD MAX™ MRSA Assay and System could be effective in quantification studies for concentration of cells higher or equal to 1.23 cells/mL .

Conclusion: Quantification studies can be performed on the BD MAXTM System using BD MAX™ MRSA Assay for MRSA infection levels higher than 1.23 cells/mL.

[1]The BD MAX™ MRSA Assay is not available for sale or use in the U.S.

R2590 Carbapenem-resistant *Serratia marcescens* and *Enterobacter cloacae* isolates harbouring OXA-48 carbapenemase, Turkey

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Objectives: The frequency of carbapenem resistance due to class-D OXA-48 type beta-lactamase in Enterobacteriaceae is increasing. We report the identification and characterization of three carbapenem-resistant isolates (two *Serratia marcescens* and one *Enterobacter cloacae*)

Methods: These isolates recovered from three patients hospitalized in two different hospitals in Istanbul in 2010, *S. marcescens* isolates recovered from blood samples and *E. cloacae* recovered from rectal

swab. Minimum inhibitory concentrations were determined using agar dilution and E-test, beta-lactamase production by phenotypic tests (E-test MBL, ESBL and MHT) PCR and sequencing were used to search for bla(VIM), bla(IMP), bla(SPM), bla (KPC), bla(CTX-M-1 group), bla(OXA-48), bla(TEM), bla(SHV), bla (PER), plasmid encoded AmpC and the quinolone resistance genes Qnr A, B, S.

Results: The three isolates were resistant ((4 mgl/L) to imipenem, meropenem and ertapenem all harbored the blaOXA-48 gene. The *E. cloacae* isolate also harbored blaIMP-1 and qnrA genes. *S. marcescens* isolates were also resistant to tigecycline (3 mg/L). *S. marcescens* 2 and *E. cloacae* were resistant to ceftazidime, cefotaxime, piperacillin/tazobactam, cefoperazone/sulbactam, and gentamicin; *S. marcescens* 1 was resistant to piperacillin/tazobactam and cefoperazone/sulbactam.

Conclusion: This is the first report of OXA-48 – producing *S. marcescens* and it is important that surveillance studies be undertaken in hospitals to limit the nosocomial spread of these strains.

R2591 Evaluation of four commercial assays for rapid detection of *Clostridium difficile* toxin in stool samples

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Objective: The aim of this study was to evaluate four available commercial assays for *C. difficile* toxin detection: Vidas® *C. difficile* Tox A/B (bioMérieux, Clinical Diagnostics), Xpert® *C. difficile* (Cepheid), Illumigene® *C. difficile* (Meridian Bioscience, Inc), and Simplexa™ *C. difficile* Universal Direct (Focus Diagnostics)

Methods: A total of 48 clinical stool samples were tested by selected methodologies, with the exception of Simplexa ™ *C. difficile* used to test only 36 of these samples. Vidas® and Xpert® were performed on fresh stool samples. Illumigene® and Simplexa™ were executed in stored samples (-20°C). The true positives and negatives were established in accordance with a consensus result, based on the results of at least two methods. An in-house PCR was used for confirmation and identification of toxin A and B Clostridium genes.

Results: Consensus results were positive in 60% (n = 29) of samples and negative in 40% (n = 19). Equivocal results were found in 27% (n = 13) of Vidas® and 4% (n = 2) of Illumigene® results. Sensitivity, specificity, PPV and NPV were as follows: Vidas® – 72%, 89%, 91%, 68%; Xpert® – 100%, 84%, 90%, 100%; Illumigene® – 79%, 100%, 100%, 76%; Simplexa™ – 82%, 100%, 100%, 76%. Binary toxin was identified by Xpert® in 6% samples (n = 3), always in association with toxin B. QCMD 2011 *Clostridium difficile* DNA EQA samples were tested with Simplexa™, correctly identifying the complete panel.

Conclusion: We found the high percentage of equivocal results by Vidas® to be a problem, together with its low sensitivity. Xpert® showed the best sensitivity, however, Illumigene® and Simplexa™ had a better specificity and NPV, but were executed after sample storage, which can contribute to the poorer performance, as confirmed by QCMD results.

R2592 Detection of *Staphylococcus aureus* multiclonality in screening samples using spa gene denaturing gradient gel electrophoresis

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Objectives: *Staphylococcus aureus* is responsible for a wide range of infections. The understanding of the biological nature of *S. aureus* colonization is still limited, and virtually all studies of the nasal carriage presume that individuals are colonized by a single isolate. However, the issue of multiclonality is clinically important since screening of a single isolate from patients infected with *S. aureus* may identify an antibiotic-susceptible strain rather than a second, more resistant strain. Furthermore, data regarding colonization is also based on culture of a low number (1–3) of colonies from each sampling site. Recently, a molecular method based on the *S. aureus* protein A gene (spa) was developed to characterize multiclonality. In this study, we investigated screening samples from 31 individuals hospitalized at the intensive care unit (ICU) in Jönköping, Sweden, for possible multiple colonization of *S. aureus* using spa-DGGE.

Methods: Swabs from throat, nares and in some cases groin were cultured on blood agar plates. Confirmation of *S. aureus* was performed by DNase testing and growth of pink colonies on BD BBL™ CHROMagar™ Staph aureus plates (Becton Dickinson). Prior to DNA extraction total growth of each agar plate was harvested in 700 µl 0.9% NaCl. Bacterial DNA was extracted from the suspension using the EZ1 DNA Tissue Kit on BioRobot EZ1 (Qiagen, Hilden, Germany). Extracted DNA was amplified in 50 µl reactions and PCR-products were analyzed by DGGE using a Bio-Rad Dcode Universal Mutation Detection System (Bio-Rad Laboratories, Hercules, CA).

Results: In total 15 out of 31 patients were colonized with *S. aureus* at least once during the ICU stay. This revealed twentyfive *S. aureus* positive samples out of in total 181 collected samples. Using spa-DGGE two samples from one patient revealed colonization with two different spa-types. Hence, one out of 15 patients (7%) showed dual colonization with *S. aureus* and two out of 25 samples (8%) contained two strains, respectively.

Conclusions: In this study, we detected dual colonization of *S. aureus* in screening samples from a patient at the local ICU using spa-DGGE. This powerful molecular method can separate strains differing only in one base in the PCR-product obtained, and colonization and possible infections caused by more than one isolate can be revealed. New insights into *S. aureus* epidemiology using spa-DGGE may optimize infection control measures and treatment options for infected patients.

Molecular virology

R2593 High occurrence of Epstein-Barr virus infection during exacerbations of inflammatory bowel disease

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Background: Although Epstein-Barr (EBV) infection has been linked to the pathogenesis of inflammatory bowel disease (IBD), the virus participation as an exacerbating factor remains unclear. The aim of this study was to clarify the clinical significance of EBV infection complicating IBD exacerbation and to correlate EBV detection with various clinical characteristics in IBD patients.

Methods: The study was performed in a cohort of 94 IBD patients (63 with ulcerative colitis and 31 with Crohn's disease) (mean age 47.7 ± 18.3) selected from two adult gastrointestinal referral centers in Athens, Greece. The presence of EBV was examined in blood and intestinal tissue samples by molecular assays and comparisons were made among patients with disease exacerbation and those with remission. Demographic and clinical characteristics of all participants were also recorded.

Results: Among IBD patients, 67 and 27 were in a disease state of exacerbation and remission respectively. The mean age of groups of IBD exacerbation and IBD remission were 46.4 ± 18.7 and 51.52 ± 18 years respectively. Six (8.9%) patients with exacerbation and 2 (7.4%) patients with remission had EBV genome in both the intestinal tissue and blood detected by PCR. In addition, 32 (47.7%) patients with exacerbation and 4 (14.8%) patients with remission had detectable EBV genome only in their intestinal mucosa, while 9 (13.4%) patients with exacerbation and 7 (25.9%) patients with remission had positive EBV PCR only in blood. Detectable EBV genome in the intestine was identified in 38 (56.7%) patients with exacerbation and in 6 (22.2%) patients with remission ($p = 0.001$). No difference in prevalence of EBV genome in blood was indicated among the groups.

Conclusion: Patients with IBD exacerbation had higher frequency of EBV infection in their inflamed intestine when compared with patients

with IBD remission, implying a potential involvement of the virus to the onset or the severity of IBD exacerbation. Further research is required to elucidate possible underlying mechanisms of pathogenesis.

R2594 Dobrava virus detection in a patient with haemorrhagic fever with renal syndrome: a case report and review

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Objectives: Haemorrhagic fever with renal syndrome (HFRS) is an acute infectious disease, caused by different viruses from the Bunyaviridae family. In the Balkan region, some of the cases emerge with severe general status, haemorrhagic syndrome, and particular damage of kidney function. In Bulgaria, HFRS is registered since 1953. Prevalence of different hantaviruses causing HFRS in Bulgaria is not clear.
Case report: A man residing in a town near the northeastern city of Bulgaria, Shumen, reported to have a direct contact with rats in his farm. After sudden onset of illness (temperature, headache, nausea, vomiting, abdominal pain and myalgia) he was admitted to the regional hospital. Physical examination revealed oliguria, haematuria, acute renal dysfunction, bradycardia and petechiae on the body. Blood samples from the patient were used to perform real-time RT-PCR, while serum samples were tested by ELISA for antibodies against hantaviruses. Dobrava virus was detected in this patient confirmed by Taqman and SYBR Green I real-time assays and serologically by high titer of IgM specific antibodies.
Conclusion: Distribution of different hantaviruses in Bulgaria needs elucidation. By mapping hantavirus infections using molecular methods, this severe viral hemorrhagic fever will presumably be detected in new areas.

R2595 Evaluation of two new commercial cytomegalovirus real-time PCR quantitation assays

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Automated real-time PCR systems have become the most common method in the quantitation of viral load during cytomegalovirus (CMV) infection in immuno-compromised patients. The aim of this study was to evaluate the clinical application of two news commercial quantitative CMV PCR assays (COBAS® Ampliprep/COBAS® Taqman® CMV Test, Roche Diagnostics, Germany and REALQUALITY RQ-CMV, AB Analitica, Italy), in solid organ and stem cell transplant patients. CMV R-geneTM (Argene, France) was compared to the COBAS® Ampliprep/COBAS® Taqman® CMV Test and REALQUALITY RQ-CMV for the detection and quantitation of CMV load in plasma samples. A total of 56 plasma specimens collected into potassium EDTA tubes were quantified. DNA was automatically isolated from 200 µL of plasma using a NucliSENS easyMAG system (Biomerieux, France) and the Generic 2.0.1 protocol according to the manufacturer's instructions. Total DNA was eluted in a final volume of 50 µL. All three tests were carried out according to the manufacturer's instructions. Inhibitors of PCR were detected in DNA extracts thanks to the use of an inhibition control included in the amplification premix. Fifty six samples of plasma were tested in parallel with CMV R-gene and with one of the two commercial kits. Results are summarised in Table 1. Eleven (19.6%) discordant results were obtained. All discrepant samples had a low viral load. All samples that gave positive results in both tests were analysed by the Spearman's rank test (n: 12 for COBAS®, n: 17 for RQ-CMV). A good correlation between the two commercial kits and the CMV R-gene was obtained. The Spearman′s correlation coefficient was 0.90 and 0.89, respectively. Our study showed that COBAS® Ampliprep/COBAS® Taqman® CMV Test and REALQUALITY RQ-CMV are accurate, efficient, reliable and versatile tools for rapid diagnosis and monitoring of CMV disease in transplantation recipients.

Table 1. CMV detection by ™ Ampliprep/COBAS™ Taqman™ CMV Test and by REALQUALITY RQ-CMV assays in plasma samples

		RQ-CMV (a)		COBAS®	
		Positive	Negative	Positive	Negative
CMV R-gene	Positive	17	10	19	8
	Negative	1	23	3	26

(a) 5 samples inhibited

R2596 Standardisation of a PCR assay for the detection of Bocavirus using a synthetic control

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Introduction: Acute Respiratory Infections (ARI) are respiratory tract pathologies which constitute a major public health problem because they cause high morbidity and mortality rates in the population worldwide. In 2005, Swedish scientists first detected in nasopharyngeal aspirates, a virus that had not previously been reported. Given its relatively recent discovery, little is known about this agent, so its role as a possible causative agent in ARIs is uncertain. The Polymerase Chain Reaction (PCR) has been proposed as an alternative to conventional diagnostic methods because it provides better results in terms of speed, simplicity, sensitivity and specificity. Despite this, its main disadvantage is its high costs in viral positive controls. For this reason, to standardize a PCR assay for detection of Bocavirus using a synthetic control was the aim of this project.
Objective: Standardization of a PCR assay for the detection of Bocavirus using an in vitro synthesized control.
Methods: We designed a primer (INTF) that together with the primer 542R reported by Allander et al. (2005), amplified a 100 bp sequence specific to Bocavirus. NCBI bioinformatics tools were used to approximate prior PCR results and to confirm that this sequence corresponded to the desired region. A PCR temperature gradient from 50 to 60°C was done to determine an appropriate annealing temperature. Also, a titration of the synthetic control between 2 and 4 µL per reaction was performed, as well as a modification of the final concentration of this sequence, carrying out serial dilutions of the stock. Similarly, a titration of magnesium chloride concentration was done between 1 and 4 mM. Finally, a comparison between the protocol with no modifications and a reaction mixture ready to use suggested by a commercial manufacturer was done.
Results: After performing various tests, it was determined that the conditions that could be modified were the primers concentration to 0.1 pmol/µL each, the magnesium chloride concentration to 3 mM and the annealing temperature to 52°C. The final assay was carried out with all the proposed modifications, which showed a brighter and clearer band compared to the band obtained in the first PCR assay.
Conclusions: Implementing all the variations made to the protocol, it was possible to affirm that these modifications were useful for an adequate standardization of the test and that the implementation of this synthetic control is a possible solution to the lack of controls for PCR.

Molecular mycology

R2597 Initial treament as consequence of using PNA-FISH for yeast in blood cultures

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Objectives: The clinical guidance from microbiologists following blood cultures (BC) with yeast depends on patient history and the pathogen retrieved. *C. albicans* has a high grade of susceptibility to fluconazole. Other species have a low grade of susceptibility to fluconazole and should be treated with other antifungals. PNA-FISH (peptide nucleic acid fluorescence in situ hybridization) identifies pathogens within 90 minute. The clinical consequences of early species detection were studied.

Methods: In November 2009, PNA-FISH was implemented for examination of yeast directly from positive BC. Records of BC containing yeast from January 2010–September 2011 were reviewed, and the guidance given was recorded.
Results: We identified 103 patients having BC with yeast (51 *C. albicans* and 52 non-*C. albicans*). PNA-FISH was used to identify the pathogen of 53 cases; 28 *C. albicans* and 25 non-*C. albicans*. Patients with *C. albicans*: of 28 cases identified PNA-FISH, 5 (18%) patients were dead or transferred to other hospitals. Of the 23 patients available for evaluation, 15 (65%) were treated with fluconazole and 8 (35%) received caspofungin or amphotericine B. For patients with C albicans fungaemia, where PNA-FISH was not used, 8 (42%) patients received fluconazole and 11 (58%) got either caspofungin or amphotericine B. Patients with non-*C. albicans*: of 25 cases identified with PNA-FISH, 19 were available for evaluation. Of 16 (84%) received caspofungin or amphotericine B and 3 (16%) got fluconazole. Of 17 cases not identified with PNA-FISH, 10 (59%) received caspofungin or amphotericine B and 7 (41%) got fluconazole. Given that fluconazole is the right treatment for *C. albicans*, and for other yeasts caspofungin or amphotericine B should be used, 31 of 41 (74%) patients got correct treatment in the PNA-FISH group compared to 18 of 36 (50%) patients in the group not examined by PNA-FISH ($p = 0.037$).
Conclusions: PNA-FISH was able to improve guidance from clinical microbiologist to clinical doctors. The benefits were early diagnosis and early appropriate treatment. Obviously, these improvements in patients care also hold economic benefits.

R2598 Upregulation of the ERG11 gene in *Candida krusei* by Azoles

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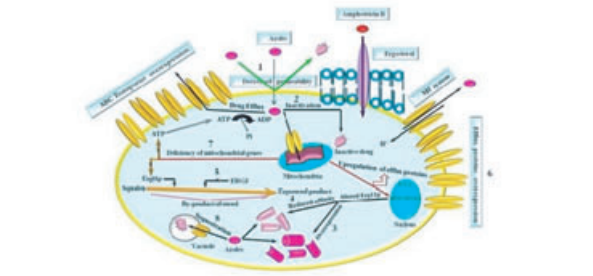
Background and the purpose of the study: *Candida* species (spp.) are the agents of local and systemic opportunistic infections have become a major cause of morbidity and mortality in the last few decades. Azole resistance in *Candida krusei* (*C. krusei*) species appears to be the result of gene alterations in relation to the ergosterol biosynthetic pathway, as well as efflux pumps. The main aim of this study was to examine the RNA expression of ERG11 in *C. krusei* which had been identified to be resistance to azoles.
Method: The ERG11 mRNA expression was investigated in four Iranian clinical isolates of *C. krusei*, which were resistant to fluconazole and itraconazole by a semiquantitative RT-PCR.
Results: The mRNA expression levels were observed in four out of four isolates by this technique. Furthermore, we found the variation in ERG11 expression levels among four representative isolates of *C. krusei*. Although DNA sequencing revealed no significant genetic alteration in the ERG11 gene, one heterozygous polymorphism was observed in two isolates, but not in others. This polymorphism was found in the third base of codon 313 for Thr (ACT>ACC).



Major conclusion: Even though such a polymorphism creates a new Ear1 restriction site, we considered it to have no significant effect on the resistance of *C. krusei* to azoles. Our results are consistent with previous studies and may provide further evidence for the genetic heterogeneity and complexity of the ergosterol biosynthetic pathway or efflux pumps.

R2599 Outbreak of neonatal invasive candidiasis due to *Candida albicans* invetigated by molecular typing

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Objective: Invasive candidiasis (IC) has emerged as a major problem in neonatal intensive care units (NICUs). We investigated herein the temporal clustering of six cases of neonatal IC due to *Candida albicans* in the NICU of Farhat Hached teaching Hospital at Sousse City, Tunisia.
Methods: Eighteen isolates obtained from six infected neonates (eleven isolates were collected from blood and deep-site samples, six isolates from implanted medical devices and one isolate from a urine sample) and two isolates from two nurses working at the same unit and suffering from fingers' onychomycosis were genotyped by electrophoretic karyotyping (EK) and restriction endonuclease analysis of genomic DNA by using Sfi I (PFGE-Sfi I).
Results: The 20 tested isolates generated nine different PFGE-Sfi I patterns but only four different karyotypes. PFGE-Sfi I was more effective in discriminating between the temporally related isolates. It showed that: (i) Both HCWs had specific strains excluding them as source of infections in neonates. (ii) Isolates collected from three neonates were identical providing evidence of their clonal origin and the occurrence of a horizontal transmission of *C. albicans* in the unit. (iii) The three remaining neonates had specific strains confirming that the IC cases were coincidental. (iv) Microevolution occurred in one catheter-related candidemia case.
Conclusion: Our results illustrate the relevance of the molecular approach to investigate suspected outbreaks in hospital surveys and the effectiveness of PFGE-Sfi I for the typing of epidemiologically related *C. albicans* isolates.

Molecular typing

R2600 MIRU-VNTR genotyping of human *Mycobacterium bovis* strains in Tunisia

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Introduction: Advances in molecular typing, particularly with the developing MIRU-VNTR technique, have improved the ability to distinguish among strains of *Mycobacterium bovis*.
Objective: The discriminatory power of MIRU-VNTR to typing based on the standardized 15 loci was assessed for 23 human strains of *M. bovis* collected at the laboratory of microbiology of the Pneumology Hospital of Tunis
Materials and Methods: From 2000 to 2010, 23 human *M. bovis* strains were isolated, 13 were isolated from extrapulmonary samples and two from sputa. Genotyping was performed using the 15 MIRU-VNTR set. PCR products were analyzed by gel electrophoresis. The allelic diversity (h) was calculated.
Results: Molecular evidence demonstrated that *M. bovis* isolates are not related even though between cases occurring in the same area and the same place. Only two of the 23 *M. bovis* strains were clustered. Allelic diversity was calculated (h) and we defined: 12 loci as highly discriminative (h (0, 5): MIRU 4, MIRU 10, Miru26, Miru 31, ETR C Mtub 21Mtub 4, QUB11b, ETR A, Mtub39 QUB 26 and QUB4156. Three loci as moderately discriminative ($0.2 < h \leq 0.5$): MIRU 16, MIRU 40, Mtub39

With the nine most polymorphic loci, we obtained the same initial result that with the 15. We consider that these nine loci are distinctive enough for initial epidemiological studies and allow us to save time and minimize expenses.

Conclusion: Analysis of allelic diversity is a powerful tool to define a first line set that will be discriminative for *M. bovis* strains in Tunisia. Improved discrimination between isolates is needed for better tracing of epidemiological links and identifying the source of contamination.

R2601 Comparison of AMOS, MLVA and Bruce-Ladder assays for typing of *Brucella* spp.

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Brucellae are intracellular bacteria that are pathogenic for humans and animals. The infection generally results from transmission via the gastrointestinal route by the consumption of unpasteurised dairy products. A genus-specific PCR assay is sufficient in diagnosis of human brucellosis or contamination of food products. However, the species-specific differential assays are needed for epidemiological investigation and finding the source of infection. The first assay, based on the polymorphism of IS711 region was AMOS PCR. Then, Bruce-ladder which allowed molecular typing of all *Brucella* species was proposed as official in OIE Manual. Parallely, a new ''fingerprinting'' approach, multiple-locus variable-number tandem repeat (VNTR) analysis (MLVA) was described. The aim of the study was to perform a comparisonal study of AMOS, Bruce-ladder and MLVA for typing of *Brucella* spp. The *Brucella* reference strains: *B. abortus* bv. 1, 2, 3, 4, 5, 6, 9, *B. melitensis* bv. 1, 2, 3, *B. suis* bv. 1, 2, 3, 4, 5, *B. ovis*, *B. canis* and *B. neotomae* strain were used. Moreover, 138 the *Brucella* clinical isolates were examined. We observed that AMOS PCR could correctly identify only *B. abortus* bv1, bv2, and bv4, *B. suis* bv1, three biovars of *B. melitensis* (bv1, bv2, and bv3) and *B. ovis*. A major advantage of the Bruce-ladder PCR assay was that it could identify and differentiate for the first time all of the *Brucella* species and the vaccine strains in the same test. In contrast to AMOS PCR, Bruce-ladder PCR was also able to detect DNA from *B. canis*, *B. neotomae*, *B. abortus* biovars 3, 5, 6, and 9, and *B. suis* biovars 2, 3, 4, and 5. The only one disadvantage of this assay is that the high quality DNA polymerase should be used. MLVA analysis has been developed for molecular typing of *Brucella*. In our study we could correctly recognise the species among *B. aborus*, *B. melitensis*, *B. ovis* and *B. neotomae* using VNTR6, VNTR8, VNTR11, VNTR12, VNTR42, VNTR43, VNTR 45 and VNTR55. Using the reference strains of *B. abortus* bv5 we observed the same VNTR profile as *B. abortus* bv9. Using MLVA we could not differentiate *B. canis* from *B. suis* bv4. Only Bruce-ladder correctly identified the species of all *Brucella* strains tested. Moreover, this assay is performed in one step, comparing to the MLVA which needs at least eight separated PCR amplifications. In conclusion, Bruce-ladder PCR can be a useful tool for the rapid species identification of *Brucella* strains.

R2602 An outbreak of multidrug-resistant *Acinetobacter baumannii* producing the carbapenem hydrolysing oxacillinases in an intensive are unit in Turkey

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Objectives: We aimed to analyse the spread of *Acinetobacter baumannii* in the intensive care unit of Gulhane Military Medical Academy (GMMA) Hospital from march to may 2010. Outbreak investigation based on a prospective ICU surveillance.

Methods: GMMA Hospital is a 1500-bed teaching hospital in Ankara, Turkey. Two doctors, five nurses work in the 11-bed Intensive Care Unit. Twelve clinical *A. baumannii* isolates from seven patients and eight *A. baumannii* isolates from 51 environmental specimens were investigated. Identification and antimicrobial susceptibility testing were performed by both conventional methods and Phoenix System. The presence of carbapenemase was tested by Modified Hodge test (MHT). To determine the cause of carbapenem resistance, four groups of OXA carbapenemase genes (OXA-23, OXA-24, OXA-51 and OXA-58) were searched by multiplex PCR, metallo beta -lactamase (MBL) presence was investigated by MBL E-test. Pulsed-field gel electrophoresis (PFGE) using ApaI restriction endonuclease was performed for genotyping.

Results: Twenty multidrug resistant *A. baumannii* isolates were recovered: six from catheter, four surgical wounds, one blood, one pleural effusion and eight from environmental samples. All *A. baumannii* isolates were resistant to gentamicin, cefotaxime, ceftazidime, cefepime, imipenem, meropenem, piperacillin-tazobactam, and cotrimoxazole. Resistance rate to amikacin was 70%. All isolates were susceptible to colistin. MHT was positive for all strains. There was no MBL activity. All of the isolates had blaOXA-51, 15 isolates had blaOXA-23, and five isolates had blaOXA-58 genes. PFGE analysis of outbreak-isolates indicated the presence of two clones. Nine isolates from four patients and six environmental strains had similar PFGE patterns and three clinical isolates and two environmental strains had another clone. The first clone was first isolated from pleural effusion of patient transfered to ICU. This clone was also isolated from the emergency trolley and from drug trolley of the second patients. The second clone was spread from medicine trolley.

Conclusion: OXA-23 and OXA-58 were the source of carbapenem resistance in our *A. baumannii* isolates. The clonal dissemination of *A. baumannii* strains in ICU was confirmed by molecular method. Environmental contamination, close contact patients and health care workers, and widespread imipenem use were the important factors that lead rapid clonal spread of multi-drug resistant *A. baumannii.*

R2603 Phylogenetic analysis of β-lactamases

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Objectives: Recently, the number of annually identified β-lactamases with extended spectrum activity (ESBL) against cephalosporins (ESBL) or activity against carbapenems (etc. NDM-1, VIM) has been dynamically increasing. In contrast to MRSA, an overall rapid and reliable PCR-based test to detect the presence of all ESBL- and other clinically relevant beta-lactamase (i.e. KPC, VIM etc.) in clinical samples is difficult to develop because of the high sequence diversity of different beta-lactamase-subtypes. In preparation to develop such a test we analyzed all amino acid sequences of β-lactamases available from NCBI data base by performing an overall phylogenetic analysis and correlation to substrate-inhibitor profiles.

Methods: In total, 643 sequences with at least 200 residues could be aligned and analyzed using DS Gene 1.5 software (Accelrys Ltd) with the phylogenetic method by neighbor joininging. The resulting phylogenetic tree was correlated to the functional properties proposed by Bush and Jacoby in 2010 [1] and analyzed in detail.

Results: As expected, the sequence alignment reflected the differences in substrate patterns of the β-lactamases. Closer relationships were found for AmpC and OXA types, whereas GES, CTX-M, IMI and KPC formed another phylogenetic group. Moreover, we indentified mutation hot spots, which are responsible for specific changes of the phenotype.

Conclusion: We identified some unique amino acid substitutions that cause specific changes in the phenotype of TEM indicating that better understanding of substitution's dynamics within the types might simplify the prediction of the phenotype by SNP typing. We focus on the validation of such unique substitutions within the other molecular classes that exhibit much higher sequence variation compared to TEM.

1. Bush, K. and G.A. Jacoby, Updated functional classification of β-lactamases. Antimicrob Agents Chemother, 2010 54(3): 969–76.

R2604 Phylogenetic analysis with Multi Locus sequence typing reveals a potential new clade of *Clostridium difficile*

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Objectives: *Clostridium difficile* is the most frequent cause of nosocomial diarrhoea worldwide. Since 2004, the reported incidence of this disease has increased significantly, particularly of the notorious ''hypervirulent'' PCR ribotypes 027 (RT027) and RT078. Recently, PCR ribotypes that are closely related to RT 027 and RT 078 have been identified, representing a 027 lineage and a 078 lineage, respectively. However, little is known about their phylogenetic classification. Currently, a large variety of typing techniques is in use for *C. difficile*. This can be confusing, especially when typing techniques contradict each other. For example one PCR RT122 isolate belonged to the above mentioned 027 lineage, while several other PCR RT122 isolates did not. Since Multi Locus Sequence Typing (MLST) is a technique suitable for studying the phylogenetic relationships, this technique was applied to analyze potential relationships of various *C. difficile* RTs within the 027 lineage and the 078 lineage as well as several controversies of *C. difficile* strain typing.
Methods: MLST was performed using the method described by Griffiths et al. The concatenated MLST sequences were used to construct a phylogenetic neighbour-joining tree with MAFFT. All *C. difficile* isolates included in the study are derived from the National Reference Laboratory (RIVM, LUMC)
Results: In total, 94 *C. difficile* isolates belonging to 75 different PCR ribotypes were investigated using MLST. In addition to the already described five evident clades of *C. difficile*, a potential sixth clade was discovered. This clade comprises of one sequence type, ST-122 associated with RT131. This type is found in humans, although infrequently. Furthermore, it was observed that the hypervirulent PCR ribotypes 027 and 078 are present within two distinct clades, which also harbour several other PCR ribotypes that are highly related to RT027 and RT078. Analysis of the posed controversy showed that *C. difficile* RT122 with the 027 marker is typed as a different sequence type located in a divergent clade as RT122 not containing the marker.
Conclusion: PCR ribotyping is a less effective typing method for studying close phylogenetic relations. MLST can accurately link groups of isolates and thereby appears to be an appropriate method for recognition and classification of potential hypervirulent strains. Furthermore, a new sixth clade is identified, of which the clinical and epidemiological relevance is under study.

R2605 Culture-independent detection of *Mycobacterium marinum*: the cause of cutaneous infections in humans

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Objectives: The low frequency of infection caused by non-tuberculous mycobacteria (NTM), non specific symptoms and requirements of specific cultivation approaches could detract from a correct diagnosis. Infections caused by NTMs are increasing nowadays because of living style, environment and other factors affecting human' susceptibility to diseases. In the Czech Republic is reported each year around 100 cases of confirmed NTM infections (source: ÚZIS). The most frequently isolated agents are: MAC (50%), *M. kansasii* (20%), *M. xenopi* (12%) and other PPM (18%, *M. marinum*, *M. malmoense* and others). *M. marinum*, the cause of chronic systemic infections in fish, occasionally causes granulomatous skin lesions involving the hands, forearms, elbows and knees in humans reporting contact with water environment.
Methods: Rapid, sensitive and specific method for the detection of *M. marinum* in human and animal samples was set up in this study. The duplex real-time PCR assays (erp) described here provides a fast, sensitive and specific diagnosis of *M. marinum*, in comparison to more time-consuming conventional PCR and DNA hybridization methods.
Results: We developed a quick and sufficiently sensitive system for the detection of *M. marinum* in tissue samples. Developed qPCR system was successfully used for detection of *M. marinum* infection in four humans. Interestingly, one of the patients was previously misdiagnosed and treated with methylprednisolone for period of 3 month, which resulted in a rare systemic spread of *M. marinum* into the testis and epidimidis. Simultaneously, the screening for *M. marinum* presence in all patients' aquaria revealed positive fish.
Conclusion: The protocol used enabled us to complete analysis of sample, including controls, in approximately 6 hour; including extraction and assay time, and is suitable for implementation in routine laboratory diagnostics. To our best knowledge, this is the first report of detection and quantification of *M. marinum* directly from infected human tissue. This approach could also be beneficial for detection of mycobacterioses in fish aquaculture, where *M. marinum* infections results in economic losses.
This work was supported by Grants Nos. MZE0002716202 and QH91240 from the Ministry of Agriculture and Grant ''AdmireVet'' No. CZ 1.05/2.1.00/01.0006-ED0006/01/01 from the Ministry of Education, Youth and Sports of the Czech Republic.

R2606 Concordance between pulsed field gel electrophoresis and sequence-based typing techniques in the study of legionnaires' disease outbreaks

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Objectives: To evaluate the concordance between the Pulsed Field Gel Electrophoresis (PFGE) and Sequence-Based Typing (SBT) techniques in the study of Legionnaires'disease (LD) outbreaks.
Methods: We included six outbreaks of LD according to epidemiological surveillance and PFGE results that occurred in the Catalan Autonomous Community. We analyzed nine clinical and 39 environmental *Legionella pneumophila* isolates. The strains were typed by Sfi I-PFGE and by SBT method according to the European Working Group for Legionella Infections (EWGLI) standard protocol (7 genes) and database.
Results: SBT method showed an 83.3% (5/6) concordance with the PFGE method in identifying the source of the outbreak. In the non-concordant outbreak clinical and environmental strains showed the same ST profile and one band difference in the PFGE profile. Other data should be noted: (i) Some environmental epidemiologically related strains with minor molecular differences such as only 1–2 bands in the fingerprinting PFGE profile were indistinguishable by SBT method, although different from the epidemic strain, and (ii) In one case, two molecularly different (four bands) environmental isolates corresponding to the same outbreak were identical by SBT.
Conclusions: Although preliminary, the results of this study show that SBT has a good concordance with PFGE method in identifying the source of a LD outbreak. Nevertheless, some discordances observed between both methods, lead us to consider SBT, if confirmed in a larger number of outbreaks, as a screening technique in epidemiological molecular studies of LD outbreaks. In order to achieve a cost-effective screening tool, a proposal would be to analyze some of the seven genes. Currently, we are analyzing more isolates to know the true role of SBT technique in the investigation of the source of LD outbreaks.
Funded by FIS 10/0191.

Molecular biology – others

R2607 From manual to automated processes in molecular diagnostics of infectious diseases

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In a therapy-based setting fast reporting of diagnostic results is key. The laboratory equipment must support this ''need for speed''. But what criteria does this exactly include? We run a multitude of laboratory-

developed and commercial assays, plus we receive samples throughout the day from many sources. Also the kind of biological material varies, as well as the tube formats. All these factors are quite a challenge when it comes to quick reporting of the results. Therefore, we decided to evaluate our workflow, identify potential for improvement, and define the ''must haves'' for new equipment accordingly.
It appeared that the reduction of administrative tasks was crucial for a workflow improvement in our lab. If the workflow involves creating the plate setup, looking at controls manually, typing results into LIS, or analyzing PCR curves, then all of this is much too time-consuming and also prone to errors, which could put patient results at risk.
So any new equipment would need to provide automated solutions for the test setup and reporting. It should follow lean principles like less waste and one-piece flow. A connection with the LIS would be utmost important.
Here, we evaluate our new automated workflow solution, following the criteria defined earlier. For many criteria the newly purchased QIAsymphony RGQ provided what we were looking for — flexible throughput, in process loading, no reagent waste, and the possibility to load different input and output tubes. The only thing we were missing was the integration with our existing LIS, but we solved this by developing the middleware in cooperation with a Dutch company (Bodégro) specializing in software development for laboratory automation. Here we present the results of the evaluation of the old workflow, the steps we took to improve it and the middleware solution we developed.

R2608 The protective effect of specific blocking TREM-1 on endotoxin-induced acute lung injury in mice

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Objective: To explore the protective effect and its mechanism of LP17 for specific blocking TREM-1 on endotoxin-induced acute lung injury in mice.
Methods: 36 BALB/C mice were randomly divided into three groups: control group, ALI group and LP17 group, intraperitoneal injection of lipopolysaccharide (LPS) to establish a mouse model of ALI, intravenous injection of LP17 (3.5 mg/kg) to establish LP17 group, comparing the groups 6, 12, 24, 48 hours lung pathology Smith score using enzyme-linked immunosorbent assay (ELISA) determination of the point in the lung tissue and serum tumor necrosis factor-a (TNF-a), interleukin -10 (IL-10), TREM-1, sTREM-1 levels. At the same time, using fluorescence quantitative RT-PCR to detect the expression of TREM-1mRNA point.
Results: (i) The lung pathology showed, LP17 makes 12, 24, 48 hours lung pathology Smith score decreased significantly ($p < 0.05$). (ii) TNF-a of lung tissue homogenate showed, LP17 group's TNF-a levels decreased gradually with time changing, and TNF-a of 12, 24 hours point lower than the same time point of ALI group ($p < 0.05$); TNF-a of serum showed, TNF-a of 24, 48 hours-point lower than the same time point of ALI group ($p < 0.05$). IL-10 concentrations of lung homogenates and serum have been maintaining at a high level, 48 hours was significantly higher than ALI group's ($p < 0.05$). (ii) TREM-1 of lung tissue homogenate showed, TREM-1 concentrations of 12, 24 hours point lower than the same time point of ALI group ($p < 0.05$); Blood and lung tissue expression of TREM-1mRNA results showed, TREM-1mRNA expression in blood of LP17 group in the 12, 48 hours were lower than ALI group at the samt time point ($p < 0.05$), TREM-1mRNA expression in lung tissue of LP17 group in the 12, 48 hours were lower than ALI group at the samt time point ($p < 0.05$).
Conclusion: LP17 lung tissue by blocking TREM-1 significantly reduced LPS-induced ALI in mice lung tissue inflammation, improved inflammatory imbalance, the protection does not depend on the gene level.

R2609 Infections as factors of genome destabilisation and manifestation of hereditary diseases

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The importance of methylation in the etiopathogenesis of hereditary diseases can open new possibilities of their therapy. It is known that in human cells viral DNA sequences can be methylated, which leads to blockage of transcription and subsequent elimination of the virus. But in a genetically determined deficiency of enzymes folate cycle (DEFC) is an infringement of these processes. Therefore, to study the role of DNA methylation as a component of the cell ''immune system'' in DEFC is an urgent problem.
Objective: To investigate the relationship DEFC with carriage TORCH and urogenital infections (UGI) in manifistetion of hereditary diseases.
Methods: Molecular study of 62 patients with various forms of hereditary diseases and clinical manifestations of DEFC. By PCR method investigated polymorphisms of folate cycle genes C677T MTHFR and A66G MTRR, DNA study of UGI and TORCH: Herpes simplex virus I, II (HSV), Cytomegalovirus (CMV), Chlamydia trachomatis (Chl.tr.), Toxoplasma gondii (Toxo), Mycoplasma hominis (M.h.), Mycoplasma genitalium (M.g.), Ureaplasma urealiticum (Ur.ur.).
Results: In 58 (93.5%) of patients identified the presence of polymorphisms C677T MTHFR and/or the A66G MTRR. Compounds alleles MTHFR/MTRR were follows: hmzg/hmzg – 6 (9.7%); htrzg/htrzg – 11 (17.7%); htrzg/hmzg – 13 (21.0%); hmzg/htrzg 2 (3.2%); N/hmzg – 15 (24.2%); N/htrzg – 6 (9.7%); hmzg/N – 0; htrzg/N – 5 (8.1%); N/N – 4 (6.4%). In 51 (82.3%) patients were identified carrier of UGI and/or TORCH. The most frequently identified carrier of CMV – 18 (29%) and Ur.ur. – 17 (27.4%) patients. The analysis of infections combination with the presence of MTHFR/MTRR polymorphisms: most common carriers of HSV were detected by a genetic compound htrzg/hmzg – in 30.8%, CMV – with htrzg/htrzg in 36.4% and htrzg/hmzg in 23.1%, Chl.tr. – with htrzg/htrzg in 27.3%, M.h. and M.g. – with N hmzg in 20%, Ur.ur. – with htrzg/htrzg in 36.4% and htrzg/hmzg – in 30.8%.
Conclusions: The most frequently carrier TORCH and UGI associated with compound heterozygous MTHFR/MTRR, wich corresponded with severe hereditary diseases clinic. Therefore we can assume that with a decrease in methylation in the cells can be observed an increased activation of infectious factors leading to the launch mechanism of genome destabilization and manifestation of hereditary diseases. Diet and cofactor therapy are an integral part of the pathogenetic therapy of these disorders, wich can improve the condition of patients with DEFC.

R2610 The contribution of a multiplex real-time PCR to detect bacterial and fungal bloodstream infections in a cohort of thoracic allograft recipients

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Objectives: Bloodstream infections (BSIs) are a major cause of morbidity and mortality in thoracic allograft recipients. The early and accurate identification of the responsible pathogen and the administration of the appropriate treatment are of critical importance for improved patient survival. In the early post-transplant (Tx) period (0–2 months post-Tx) when recipients are receiving empirical antibiotic treatment, detection of pathogens by the conventional blood culture (BC) methodology may be hindered by the empirical broad-spectrum antibiotic therapy administered for preventing post-transplant infections. In the present study we investigated the potential clinical utility of a multiplex real-time PCR system (SeptiFast®, Roche Diagnostics) to detect BSIs infections in a cohort of thoracic allograft.
Methods: The study included the analysis of 130 blood samples from 30 thoracic allograft recipients (23 heart and seven lung) using SeptiFast® in parallel with BC. Samples were drawn when there were

clinical and laboratory signs of a BSI. The applied multiplex real-time PCR assay allowed the detection of a wide panel of Gram-positive and Gram-negative bacteria and fungi in 6 hours and was performed according to manufacturer's guidelines.
Results: Real-time PCR yielded concurrent negative and positive results with BC methodology in 113 (86.9%) and 5 (3.9%) samples, respectively, with 100% concordance in species identification. Diverging results were obtained in 9 (6.9%) samples, where only real-time PCR was positive and in 3 (2.3%) samples, where only blood cultures were positive. Eight of the PCR-only positive samples were drawn from patients during the early post-transplant period when they were under empirical antibiotic treatment. The combined use of SeptiFast® and BC increased the number of positive samples detected to 17 (13.1%) from 14 (10.8%) detected with SeptiFast® alone or from 8 (6.1%) detected with BC alone. In cases of concurrent positivity, SeptiFast® results were available on average 1.5 days earlier than BC results.
Conclusion: The PCR-based SeptiFast® test is a valuable add-on to the traditional BC method for the rapid etiological diagnosis of BSIs in thoracic transplant recipients, especially during the immediate post-transplant period when empirical antimicrobial therapy is also administered to the recipients.

R2611 Association of tumour necrosis factor–alpha gene polymorphisms with susceptibility to respiratory virus infection

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Objectives: Tumor necrosis factor-alpha (TNF-a) is a proinflammatory cytokine that is important in the innate host defense and thus in the defense of infectious agents. It has been known that several TNF-alpha gene polymorphisms in a promoter region are related to TNF-alpha production. Among them, the G to A substitution at the position 308 (TNF-alpha 308 A) and at the position 238 (TNF-alpha 238 A) in the TNF-alpha promoter are associated with the high or low production of TNF-alpha .The aim of this study was to investigate whether these genetic variants of TNF-a were associated with susceptibility to respiratory virus infection.
Methods: This study included 183 children hospitalized as a result of respiratory symptom. DNA was extracted from nasopharyngeal aspirates and tested several times (more than two times) with Seeplex TM RV detection kit. Susceptible group consisted of 137 patients with more than 50% positive ratio in test and non-susceptible group consisted of 46 patients without any positive results. We used five primers and two separate polymerase chain reaction (PCR) to detect the TNF-polymorphism by the multiplex amplification refractory mutation system (ARMS) technique.
Results: No statistically significant difference in the -308 and -238 A allele frequencies was found between two group.
Conclusions: These findings suggest that TNF-alpha polymorphism did not show genetic predisposition with regard to susceptibility to respiratory virus infection.

R2612 Performance of a commercial real-time PCR test in the diagnosis of toxoplasmosis

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Objectives: PCR test is widely employed in the diagnosis of congenital, ocular and in immunosuppressed patients toxoplasmosis. Most laboratories use in house PCR either conventional or Real Time. In the last report of Quality Control for Molecular Diagnostics 2010 External quality assessment programme (QCMD2010 Toxoplasma gondii EQA Programme) 77% of the data came from in house PCR which continues to be the dominant technology. We aimed evaluating the performance of TOXOPLASMA Q-PCR Alert Kit (Nanogen Advanced Diagnostics S.p.A Buttigliera Alta, Italy) a commercial Real Time PCR assay.
Methods: From October 2009 to October 2011 we tested 309 samples:173 amniotic fluid (AF) from treated mothers with toxoplasmic infection,69 peripheral blood (PB), 57 cerebrospinal fluid (CSF), seven aqueous (HA) and one Vitreous humor from immunocompromised patients (HIV positive and transplanted. DNA was extracted from all the samples with NucliSENS EasyMAG (BIOMERIEUX Marcy l'Etoile, France) according to manufacturer's instruction and kept frozen until used. Real Time PCR were performed with the TOXOPLASMA Q-PCR Alert Kit onan ABIPRISM 7300 (Applied Biosystem Carlsbad, USA) following the manufacturer's instructions. The gene target is rep 529. All the samples were run in triplicate and an internal control (beta Globin gene) and positive and negative samples were included.
Results: Three out of the 173 amniotic sample tested positive and the newborn resulted infected at birth in two cases; the third one is on follow up. One hundred and three newborns completed follow up and the negativity of PCR test was confirmed by anti Toxoplasma antibody absence at 1 year. The two positive PCR on CSF, (one of these patients was positive also for PB) became negative after 1 month treatment. We had also a PCR positive result in a vitreous sample. All the patients improved after treatment. We detected with this kit all the positive samples, even at the lowest concentration, of QCMD Toxoplasma gondii EQA Programme.
Conclusions: All the 103 newborns from mother whose AF was negative that completed the follow-up resulted not infected, the other two with positive PCR had congenital infection. In immunosuppressed patients diagnosis was quicker and made an early therapy possible. Control 1 month later showed the therapy efficacy. Sensitivity compared to other in house methods seems to be the same. Real Time Alert Q-PCR test seems to be very useful in the diagnosis of toxoplasmosis on any biological sample.

R2613 Evaluation of the xTAG® stool sample pretreatment pack using the xTAG® gastrointestinal pathogen panel

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Objectives: To achieve optimal nucleic acid extraction efficacy, especially for parasites, pretreating stool samples prior to nucleic acid extraction is recommended when using the xTAG® Gastrointestinal Pathogen Panel (xTAG GPP). Here we describe the use and performance of the xTAG® Stool Sample Pretreatment Pack under development for the pretreatment of stool samples prior to nucleic acid extraction and subsequent testing with xTAG GPP.
Methods: Eighteen stool samples (from de-identified subjects with gastroenteritis and asymptomatic subjects in which a cultured pathogen was added to the stool) were used for a side-by-side comparison study. One aliquot of each sample was pretreated with the procedure as outlined in the xTAG GPP package insert using Bertin SK38 Soil Kit bead tubes with NucliSENS® easyMAG® Lysis Buffer. The other aliquot from the same sample was pretreated with xTAG® Stool Sample Pretreatment Pack under development which contains pretreatment bead tubes and pretreatment buffer. All pretreated samples were extracted with easyMAG. Aliquots from additional four samples that were pretreated with xTAG Stool Sample Pretreatment Pack were used to assess the compatibility of the pretreated material with four different nucleic acid extraction platforms including QIAamp® MinElute® Virus spin kits, the QIAsymphony®, the Roche® MagNA pure and BioMerieux® easyMAG systems. All extracted nucleic acid was analyzed with xTAG GPP on a Luminex® 200TM system running xPONENT® 3.1 software.
Results: All 18 samples prepared with the xTAG Stool Sample Pretreatment Pack gave the correct calls when assessed with xTAG GPP. Specific MFIs were mostly comparable between samples treated with the xTAG Stool Sample Pretreatment Pack and the NucliSENS buffer and Bertin beads. Interestingly, samples treated with the xTAG Stool Sample Pretreatment Pack showed relatively lower rate of PCR inhibition (0/45 for 15 samples in triplicate) than samples treated with NucliSENS easyMAG Lysis Buffer (3/45) as indicated by failure of

internal control detection. In addition, samples processed with the xTAG Stool Sample Pretreatment Pack were compatible with the four nucleic acid extraction platforms evaluated.
Conclusions: The study showed that xTAG Stool Sample pretreatment pack under development is a promising solution for stool sample preparation prior to nucleic acid extraction and molecular testing. Further evaluation of this pretreatment pack with a larger sample set is warranted.

Diagnostic/laboratory methods (other than molecular)

R2614 Comparison of cattle blood with sheep, rabbit and human blood in blood supplemented media

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Objective: Isolation of bacterial pathogens from clinical specimens is still the ''Gold Standard'' in diagnosing infectious diseases. Isolation of fastidious groups such as *Streptococci*, *Neisseria* and *Haemophilus* species require blood supplemented media for their growth. Due to lack of availability of recommended type of animal blood such as sheep and horse blood, expired banked human blood is widely used in developing countries. According to our experience and the experience of previous workers, human blood agar shows poor haemolysis pattern causing difficulty in identification. Considering the wide availability and dispersion of cattle throughout Sri Lanka, we are assessing the overall suitability of cattle blood as an enrichment substance in agar media in this study. We could not find any published studies determining the suitability of cattle blood for the above purpose.
Methods: We performed a descriptive, laboratory based study from 1st January to 30th April 2010. In this study, we tested the suitability of cattle blood enriched media for growth, identification, isolation from clinical specimens and antibiotic sensitivity testing for a selected group of bacteria. At the same time, we compared sheep, rabbit and human blood which are being used in microbiology laboratories in Sri Lanka currently for isolation of bacteria.
Results: Both defibrinated and citrated forms of cattle blood gave similar results for growth, identification, isolation of bacteria from clinical specimens and antibiotic sensitivity testing. Cattle blood gives synergistic haemolysis in CAMP test for identification of Group B streptococci. Haemolysis patterns and degree of haemolysis is very similar in sheep and cattle blood Haemolysis produced in human blood agar was minimal when compared to animal blood agar. Results for the disk diffusion antibiotic sensitivity testing were similar on sheep blood enriched Mueller Hinton agar and cattle blood enriched Mueller Hinton agar for the strains tested in the study.
Conclusion: Either defibrinated or citrated cattle blood can be used to enrich bacterial culture media for growth, identification and isolation of bacteria from clinical specimens. Further studies are necessary to recommend cattle blood as an enrichment substance in antibiotic sensitivity testing.

R2615 Gray-scale and Doppler ultrasonographic evaluation of chronic osteomyelitis in clinical practice

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Background: Chronic osteomyelitis (COM) in adults remains a difficult to treat disease. The key to successful management is early diagnosis as well as targeted and long lasting antimicrobial therapy. Our study aimed to carry out a useful framework for planning treatment of the patients with COM using ultrasonography. Furthermore, we evaluated how Color Doppler ultrasonography might be useful in detecting and monitoring resolution of COM.
Methods: Patients with COM were evaluated over a 2 years period (2009–2011). All patients were examined clinically for signs of infection (pain, erythema, edema and present of a fistula) at baseline and after 3, 6 and 12 months. In parallel, a gray-scale and color Doppler ultrasonography was performed for evaluation of progression of treatment.
Results: A total of 45 patients (28 male, 17 female) with an average period from initial diagnosis of COM of 23.3 months were included. There were 11 infections regarding the upper body and 34 involving the lower body, with the knee joint and the tibia representing the most affected sites. Fifteen COM were post-traumatic, 24 were post-operative, two were related to diabetic foot and four were primary. In addition, 26 patients had opthopaedic devices and 29 were free of foreign bodies. In 36 patients, a positive culture was identified (gram positive in 18, gram negative in 10 and polymicrobial in 8. Upon baseline evaluation, 39 patients had signs of infection and 18 had a fistula. Regarding ultrasonography, 35 patients had increased periosteal vascularity, low-resistance arterial flow, periosteal thickening, discontinuity of the cortex with or without a fistulous tract at baseline, findings consistent with COM. After 3, 6 and 12 months of treatment, 43% (15), 20% (7) and 26% (9) patients, respectively, had shown improvement. Four patients were lost during follow-up. Finally, a correlation between clinical and imaging findings was observed.
Conclusion: A gray-scale and color Doppler ultrasonography is a useful, rapid and cheap tool for the guidance of treatment in chronic osteomyelitis and of particular value in patients with metallic implants.

R2616 Serum procalcitonin increase in earthquake victims associated with sepsis

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Objective: Procalcitonin (PCT) as the biologically active precursor of calcitonin, had shown closely correlation with outcome of critically ill patients. Serum concentration of PCT was rapid rising during bacterial infections.
Methods: The PCT was assessed with C-reactive protein (CRP), Acute Physiology and Chronic Health Evaluation II (APACHE II) and result of blood cluture in rescuing earthquake vitims after Wenchuan earthquake.
Results: A prospective, population-based investigation of sepsis was conducted over a 7-month period. The trial was a prospective clinical study with sepsis group and control group. The changing of serum PCT were detected with first, third and fifth day after patients admitted in our hospital. The sersum PCT was a marked increase in death group, while CRP was slightly decline. In invalid group and improve group, sersum PCT was rising with illness progresses and decreasing with improvement, but CRP value was changed unconspicuously. The mean value of PCT was significantly different in gram-positive cocci, gram negative bacilli and candida infection ($p < 0.05$). But in terms of level of CRP and APACHE-II, no difference was found between patients with sepsis ($p = 0.551$ and 0.733).
Conclusions: PCT is a very valuable diagnostic indicator of infection and the advantage of PCT is supporting rapid diagnosis. Furthermore, serum PCT levels increase more in patients with sepsis by GN than the GP of fungi.

R2617 Evaluation of the new Vidas® Lyme IgM and Vidas® Lyme IgG kits as screening test for the serological diagnosis of Lyme borreliosis

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Objectives: CDC currently recommends a two-tier testing algorithm for Lyme disease: an enzyme immunoassay as a screening test, followed by an immunoblot if the result is positive. We evaluated the performance of the new generation Vidas® Lyme IgM and Vidas®

Lyme IgG kits (Vidas® LYM and Vidas® LYG) as a screening test for Lyme disease and compared it to the former Vidas® Lyme test (Vidas® LYT – total antibodies). A commercial immunoblot (Borrelia Europe plus TpN17, Virotech) was used as a confirmation test.
Methods: A total of 143 frozen retrospective serum samples were tested. All samples were already tested by the Vidas® LYT assay using an immunoblot to confirm all positive results. The samples were divided into four categories. Cat. I: 41 patients: Vidas® LYT positive – blot negative (false-positives)
Cat. II: 30 patients: Vidas® LYT positive – blot positive (true-positives) Cat. III: 21 patients: Vidas® LYT positive – blot undetermined Cat. IV: 51 patients: Vidas® LYT negative. All sera were then tested with Vidas® LYM and LYG assays.
Results: Cat. I: of the 41 samples, only nine had a positive Vidas® LYM and/or Vidas® LYG result (78% less false positive results). Cat. II: of the 30 samples, 28 were positive with the Vidas® LYM and/or Vidas® LYG test. We noted a very good correlation between the IgM and IgG results of the Vidas® test and the IgM and IgG results of the immunoblot (96% and 93% respectively). Cat. III: of the 21 samples, 11 were negative with the Vidas® LYM and/or Vidas® LYG test. In seven of these 11 cases, we noted a discordance between the results of the immunoblot and the clinical picture (erythema migrans-like vs. IgG blot undetermined/IgM blot negative), so we can't exclude the possibility of false positive IgG immunoblots. Cat. IV: of the 51 samples, two were positive with the Vidas® LYG test (index 0.21/0.31). Both samples were tested with immunoblot: one sample was negative; the other sample had an undetermined blot IgG. No clinical information was available.
Conclusion: The new generation Vidas® LYM and Vidas® LYG test, based on recombinant protein technology, demonstrated a significant better specificity (78% less false positives) compared to the former Vidas® LYT test which is based on the native antigen. The sensitivity seemed comparable between the two tests.

R2618 Controlling quality of blood culture's preanalytical phase: a benchmark of KPI's

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Objectives and Methods: Blood cultures remain the most important diagnostic tool for the detection of blood stream infections (BSI). Blood culture yield can be improved by optimizing several factors in the analytical phase but depends also on the quality of the preanalytical phase. Quality control on blood cultures preanalytical phase can be performed by measuring key performance indicators (KPI) and setting thresholds for acceptable proficiency. Corrective actions should be ensued to improve diagnostic accuracy when these limits are exceeded. In five Belgian hospitals, a benchmark of the quality of blood cultures preanalytical phase was performed by comparing the results of the following KPI's: the true positive rate, the contamination rate, and the collected blood volume.
Results: In all five hospitals, the true positive blood culture rate fell within the predefined acceptation criterion of 5–15% (Baron et al., 2005), whereas the contamination rate exceeded the target value (of 3%) in four locations. Most unexpected, more than one third of the blood culture bottles was incorrectly filled, and this irrespective of the manufacturer of the blood culture vials (Becton Dickinson® [BD] and bioMérieux®). The main reason of this unexpected result is the lack of correlation between the vacuum in the vials and the optimal blood volume that needs to be collected. Therefore, it should be mandatory for a laboratory to check the filling of their blood culture bottles regularly and to provide feedback to their clinical staff. As a consequence of this shortcoming, one manufacturer recently developed an automatic blood volume monitoring system.
Conclusion: This study shows that quality control of blood cultures preanalytical phase can reveal critical shortcomings in the preanalytical process and ultimately leads to diagnostic improvement.

R2619 Stable combination discs of imipenem and dipicolinic acid, for phenotypic detection of metallo-beta-lactamases in *Pseudomonas aeruginosa* and *Acinetobacter* spp

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Objectives: Inhibitor-based methods are used to detect metallo-beta-lactamases (MBL) in Enterobacteriaceae, *P. aeruginosa* and *Acinetobacter* spp. Combined discs with EDTA may show false positive results with *Acinetobacter* and *P. aeruginosa*, due to the intrinsic antibacterial activity of EDTA against these microorganisms. Dipicolinic acid a potent inhibitor of IMP-1, VIM-1, VIM-2 and SIM-1 MBL has no intrinsic antibacterial effect against *P. aeruginosa* or *Acinetobacter* spp. Imipenem + Dipicolinic acid combined disc is expected to detect MBL in *P. aeruginosa* and *Acinetobacter* spp. A comparative study was performed against Meropenem 10 μg + dipicolinic acid (DPA), which has shown its effectivity in detecting MBL in isolates of Enterobacteriaceae.
Methods: Ten isolates of *P. aeruginosa* possessing either IMP-1 or VIM-2 and 10 isolates of *Acinetobacter* spp. possessing either IMP-1, VIM-2 or SIM-1 MBL were tested against Imipenem 10 μg and Imipenem 10 μg + Dipicolinic acid as well as Meropenem 10 μg and Meropenem 10 μg + Dipicolinic acid on MH agar with Mc Farland 0.5 inoculum. Zones of inhibition ≥5 mm larger with the combination discs compared to the single discs, indicates the presence of a MBL.
Results: (i) *Pseudomonas aeruginosa*: 10 out of 10 MBL were detected using Imipenem + DPA, while the Meropenem + DPA combination detected 90% of the VIM-2, but only 40% of the IMP-1 producing isolates. (ii) *Acinetobacter* spp. The Imipenem 10 μg + DPA combined disc detected 10 out of 10 MBL, while the Meropenem + DPA disc detected nine out of 10 MBL.
Conclusion: Combination discs of Imipenem 10 μg and Dipicolinic acid are very effective in detecting MBL in both *Pseudomonas aeruginosa* and *Acinetobacter* spp. Sensitivity and specificity were 100%. nd specificity were 100%.

R2620 Diagnosis of intestinal parasitic infections using fluorescence microscopy

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Objective: Intestinal parasites are a real public health problem in developing countries. They are generally responsible for many symptoms among which malabsorption, anemia, abdominal pain and diarrhea. The fight against intestinal parasites requires multifaceted approaches with prior identification of the parasite species involved. Diagnostic methods based on microscopic identification of parasites remain common in developing countries, despite their low sensitivity. Recently, new fluorescent microscopes with light emitting diodes have improved the diagnosis of other protozoan parasites such as malaria using a DNA-specific dye DAPI (4′,6-diamidino-2-phenylindole. This study was designed to compare a rapid fluorescence microscopy – based method for diagnosis of intestinal parasites to classical microscopy and to collect epidemiological data in rural and urban settings to Cameroon.
Methods: From september 2009 to march 2010, 583 stool samples from outclinic patients were analyzed, including 300 in the city of Douala and 283 in the rural area of Njombe. Each sample was submitted to direct microscopic examination and formalin-ether concentration technique. The observation under fluorescence after staining with DAPI and white light was made using a fluorescence microscope CyScope® (Partec GmbH, Görlitz, Germany). Statistical analysis were done on SPSS statistics version 17 (SPSS Science Inc, USA).
Results: Stool samples had less visible artifacts under fluorescence and helminth eggs were very clearly observed. In opposite, protozoa were better distinguished using white light. The search for parasites was positive in 155 (26.6%) of the 583 patients in the study. The prevalence in Njombe was significantly higher than Douala (39.2% against 14.7%,

$p < 0.001$). The most common prevalent species in Douala was *Entamoeba histolytica* (10.3%), while in Njombe, *Schistosoma mansoni* dominated 13.1%.

Conclusion: This work has confirmed a high prevalence of intestinal parasites in a rural area of Cameroon and has also shown that the simultaneous use of white and fluorescence lights for stool exams could help to better observe parasites. Thus, the use of fluorescence microscopy for routine diagnosis of intestinal parasites deserves further investigation.

R2621 Comparison of different methods for the detection of *Clostridium difficile* and its toxins A/B in stool samples

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Objectives: *Clostridium difficile* associated diarrhea (CDAD) is the most prevalent reason for nosocomial diarrhea and the incidence of CDAD is increasing worldwide. Rapid and effective testing is mandatory for optimal patient care and to stop transmission. We evaluated the performance of various currently available ELISA and rapid tests in comparison to the cell cytotoxicity neutralization assay (CCNA) and culture.

Methods: Five hundred and two stool specimens from hospitalized patients suspected of having CDI were included. All samples were homogenized and tested by CCNA and culture, all further tests were performed in parallel from the same sample. The following tests were performed: ELISA: *C. difficile* Toxin A+B® (Seramun), Premier Toxin A&B® (Meridian), ProSpect *C. difficile* Toxin A/B® (Oxoid) and RIDASCREEN C. difficile Toxin A/B® (R-Biopharm); rapid tests: RIDA QUICK® *C. difficile* Toxin A/B (R-Biopharm) RIDA QUICK® *C. difficile* Toxin A/B (R-Biopharm) and Xpect C. diff ToxA/B® (Oxoid). All tests were performed according to manufacturer's instructions.

Results: Among the 502 stool samples 108 (21.5%) were positive by culture, 68 (14%) were positive by CCNA and 51 (12%) were positive by CCNA and culture. For the ELISAs, we found a sensitivity, specificity, positive and negative predictive value of 66%, 97%, 74% 95% respectively for *C. difficile* Toxin A+B® (Seramun), 82%, 98%, 85%, 97% for C. DIFF QUIK CHEK COMPLETE® (Wampole), 81%, 98%, 85% and 95% for Premier Toxin A&B® (Meridian), 87%, 96%, 78%, 98% for ProSpect *C. difficile* Toxin A/B® (Oxoid) and 90%, 97%, 81% and 98% for RIDASCREEN *C. difficile* Toxin A/B® (R-Biopharm), respectively. The rapid tests revealed sensitivities, specificities, PPVs and NPVs 85%, 99%, 92%, 98% for C. diff. QUICK CHEK COMPLETE (Wampole), 94%, 97%, 83% and 99% for RIDA QUICK® *C. difficile* Toxin A/B (R-Biopharm) and 72%, 94%, 66%, 96% for Xpect C. diff ToxA/B® (Oxoid).

Conclusions: In contrast to CCNA, ELISA based tests can be performed easily and render reliable results within a few hours. They have become the method of choice for routine testing in many laboratories. The RIDA QUICK® C. difficile Toxin A/B (R-Biopharm) detecting toxin A/B reached the highest combined value (sensitivity + specificity) and therefore performed even better than C. diff. QUICK CHEK COMPLETE® (Wampole) test, which consists of combined GDH (glutamate dehydrogenase) + toxin A/B detection.

R2622 Validation of new liquid faecal swab for the detection of *Clostridium difficile* from faecal specimens with multiple diagnostic assays

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Objective: Pathology labs are being faced with the challenge of doing more with less money. This has led to a dramatic surge in innovative ideas and products. The pre-analytical process has been targeted particularly as significant improvements in efficiencies can be made here. The majority of front-end solutions require liquid base approach to the collection of samples which has led to the marketing of various forms of liquid swabs. The latest foray into liquid based microbiology comes from MW&E and its new liquid medium faecal swab. The goal was an innovative specimen collection device that would automatically transform the sample into liquid in standardized containers containing medium specific for enteric pathogens. This study was carried out to determine the compatibility of the liquid faecal swab with assays which detect C. difficile toxins A/B via an ELISA approach, GDH (Glutamate Dehyrogenase) and a molecular test.

Methods:

- Fifty faecal specimens, of which 49 had tested positive previously for *C. difficile* direct from a faecal pot were used to validate the liquid faecal swab device.
- The foam tipped swab of the collection kit was used to sample the clinical material.
- The commercial kits used in the validation were: C.DIFF QUIK CHEK® by TechLab (A rapid test for the detection of *C. difficile* Glutamate Dehyrogenase); VIDAS® *C. difficile* Toxin A & B by Biomerieux and the BD GeneOhm™ real time PCR assay by Becton Dickinson.
- Testing of the commercial kits where according to manufacturer's protocols.
- A negative faecal liquid swab was tested for each kit as a control and to determine if there was any interference from the liquid medium (modified Cary Blair).

Results:

- Of the 50 samples tested 49 were positive utilizing all the above named kits whether the sample was taken from the liquid faecal swab or direct from a traditional stool container.
- The foam swab collected on average 136 mg of faecal material.

Conclusion:

- The dilution factor introduced by the liquid medium had no effect on the result. There was a 100% correlation from doing the assay direct from the stool or performing it from the faecal liquid medium.
- The Cary Blair medium had no effect on the PCR method. There was a 100% correlation between doing the PCR direct on the stool sample or straight from the inoculated Cary Blair.

R2623 Evaluation of a new chromogenic medium for isolation of *Clostridium difficile*

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Objective: Rapid and effective methods for the isolation of *Clostridium difficile* are necessary to obtain isolates for typing and to facilitate diagnosis of *C. difficile* infections (CDI). chromID *C. difficile* agar (bioMérieux) is a new chromogenic medium for the isolation of *C. difficile* in 24 hours which recently came to the market. The objective of the study was to assess the performances of this new selective medium. chromID *C. difficile* agar was compared to the CLO (bioMérieux) and TCCA (Taurocholate Cycloserine Cefoxitin agar) media.

Methods: Four hundred and six untreated diarrheic stools from patients suspected of CDI were plated on the three media and incubated anaerobically at 37°C. Cultures were read after 24 and 48 hours of incubation for chromID *C. difficile* agar medium and after 48 hours for CLO and TCCA media. Suspensions of stools were inoculated on media according to a standardized procedure in order to perform a semi-quantification of the endogenous flora and *C. difficile* colonies. Identification of *C. difficile* was based on morphological criteria (black and irregular colonies on chromID *C. difficile* agar), Gram stain and RapID 32 A galleries (bioMérieux).

Results: *C. difficile* was recovered from 55 stools (13.5%) using a combination of all the media. Forty (72.7%), 47 (85.5%), 46 (83.6%) and 39 (70.9%) stools were positive on chromID *C. difficile* agar in 24 and 48 hours, CLO and TCCA, respectively. Endogenous flora was absent in 67.7% 30.8%, 16.5% and 3.9% on chromID *C. difficile* agar 24, 48 hours, CLO and TCCA respectively. Conversely, 3.9% of chromID *C. difficile* agar 24 hours, 15% of chromID *C. difficile* agar 48 hours, 15.3% of CLO and 59.1% of TCCA showed abondant endogenous flora.

Conclusion: Identification of *C. difficile* on chromID *C. difficile* agar in 24 hours is easy and sensitive. Moreover, this new chromogenic culture medium appears to be highly selective.

R2624 Evaluation a chromogenic culture medium for isolation of *Clostridium difficile* from stool samples

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Introduction: Culture of *Clostridium difficile* from stool specimens remains a gold standard in the diagnosis of *C. difficile* infections (CDI). By comparison with other rapid tests the delay to obtain a result is a major drawback. CHROM ID CD TM (bio-Mérieux, Lyon, France) is a transparent chromogenic medium for the detection of C. difficile. Colonies grow black within 24 hours. We evaluated this medium against our homemade CCFA medium and the commercial CLO-medium TM (bio-Mérieux, Lyon, France).
Materials and Methods: Stools were from adult inpatients of the St Luc-UCL University hospital (890 beds) suffering from diarrhoea. Stool samples, diluted in Cary-Blair medium (Copan) were inoculated on CCFA, CLO and CHROM ID CD using a 30 µL loop. The CCFA and CLO media were read after 48 hours, the CHROM ID CD after 24 hours. Identification was confirmed by MALDI-TOF (Bruker) mass spectrometer. Cell cytotoxicity assay on MRC5 cells (CTA) and the combined GDH-TOX A&B immunoassay (C. diff Quik Chek Complete TM) were performed for toxin detection. PCR-ribotyping was performed on all isolates using capillary gel electrophoresis. Toxin detection on colonies was performed using CTA and a molecular assay, illumigene® (Meridian).
Results: A total of 86 stool specimens from 70 patients (36 F/50 M) collected in May 2011 were tested. *C. difficile* was isolated in 23 samples (27%). The strain was toxigenic in 15/23 cases. Results are given in the Table: Culture was positive in 21/23 samples on CHROM ID CD TM (against 18 on CCFA and 20 on CLO) and all isolates were identified within 24 hours. However, in three cases, colonies did not appear black but were easily recognized by binocular examination. Two of these three isolates were toxigenic and belonged to the same ribotype. The toxigenic status of the isolates was correctly identified by illumigene® applied on colonies from 24 hours cultures. GDH correlated very well with positive cultures. Toxin EIA was positive in 8/15 stools with toxigenic isolates and CTA in 9/15.

N	CHROM ID CD	CCFA	CLO	TOXIGENIC ISOLATE	GDH +	TOX EIA +	CTA +
17	+	+	+	11/17	17	7	8
1	+	–	+	0/1	1		
1	–	+	+	1/1	1	1	1
3	+	–	–	2/3	1		
1	–	–	+	1/2			
63	–	–	–		1		

Conclusion: The CHROM ID CD is a very sensitive medium for the isolation of *C. difficile* in stools in 24 hours.

R2625 Comparative study of Access® immunoassay for the detection of HIV antibody and antigen

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Objective: Access HIV combo is a new diagnostic paramagnetic–particle chemiluminiscent immunoassay for the qualitative detection of anti-HIV antibodies and antigen in human serum or plasma. The aim of this study was to evaluate the performance of this assay in terms of sensitivity, specificity and precision in our routine laboratory.

Method: Access HIV combo assay was evaluated on UniCel DxI 800 immunoassay system and compared to the Architect HIV Ab/Ag assay. For the performance studies, we used 122 unselected serum samples from blood donors, pregnant women and hospital patients. We used 55 reactive patient samples and seven quality control samples.
Results: Unselected serum samples tested on both Access and Architect assays were all negative. The agreement between Access and Architect assays was 100%. Among the 55 samples previously reactive in the Architect assay, three tested negative in the Access assay. Samples from these patients, and one sample that was reactive in both assays, were negative when tested with a HIV RNA PCR and Western Blot. The clinical specificity for Access HIV combo was 99.2% and the sensitivity was 100% in this study. For the two assays precision was equivocal.
Conclusions: Access HIV combo assay performance was very close to the Architect assay. The performance obtained was excellent in terms of clinical specificity and clinical sensitivity. Access HIV combo assay used on UniCel DxI 800 is well suited for routine use in a hospital laboratory with a high volume of HIV screening tests.

R2626 The Lean Six Sigma approach to improving patient management cycle

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Objectives: In challenging times where the containment of costs is becoming a critical success factor, microbiology laboratories are under pressure to continue delivering high quality results with an increased focus on timings while optimizing the available resources. San Raffaele Microbiology Lab had to reallocate 1 Full Time Equivalent employee (FTE) to manage the increasing workload in the Mycobacteria and Moleculare Biology Services. The main challenge was to make the most of already available automated solutions in the lab by reorganizing the current workflow.
Methods: Lean and Six Sigma are the two most powerful strategies to achieve operational and service excellence in any organization today. The Lab Performance Assessment was performed by two ''Green belt Lean Six Sigma'' specialists and a Microbiology Application Specialist in two main phases. The first phase was performed in May 2011 with the collection of all necessary indicators, the second phase, performed in July 2011, was based on a 4-day observation at the lab with final recommendations. The roadmap obtained outlined how the lab could improve procedures such as reducing time waste and enhance efficiency, while simultaneously increasing results quality and reducing errors.
Results: The current routine in the microbiology lab is performed within 40 working hours per day with the existing dedicated resources. The analysis has shown the possibility to save 10 working hours which are equivalent to either 37% of productivity gained, or the reallocation of 1.4 FTE to different activities. It was highlighted the management of urines, which is 38% of the total number of specimens and 40% of the total workload, resulted to be the main area for improvement. TAT was 70 hours which could be reduced by 50% (35, 5 hours) through the new suggested workflow. These results were achievable through the full integration of automated solutions, PREVI™ Isola, PREVI™ Color Gram, VITEK® 2, VITEK® MS, together with the reorganization of the workflow.
Conclusion: The purpose of the new workflow for the management of the urines greatly improves the TAT and allows for the reallocation of 1.4 FTE to the Services with a high added value for patients. These potential improvements will enable the lab to optimize the workload/capacity ratio and manage the increasing activity in the near future.

R2627 Evaluation of Sigma VCM™ for detection of *Bordetella pertussis* and H1N1 (2009) influenza virus by real-time PCR

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Laboratory testing of pathogens involved in respiratory tract infections require an appropriate sampling and transport medium to preserve microorganisms. Nasopharyngeal swabs are usually collected for the investigation of respiratory viruses and *Bordetella pertussis* infection. Sigma VCM™ (SVCM) is a universal medium combined with swabs, for collection and transport of viruses, *Mycoplasma*, *Chlamydia*, *Ureaplasma* and *Neisseria gonorrhoeae*.

Objective: We evaluated the utility of SVCM for detection of *B. pertussis* (BP) and H1N1 (2009) influenza virus (Flu-H1N1), compared with conventional/routine transport media.

Methods: A total of 50 nasopharyngeal aspirates previously positive for BP ($n = 25$) or Flu-H1N1 ($n = 25$) in 2011 (real-time PCR) were used. Samples had been frozen at -80°C. For BP investigation, two aliquots of 200 µL of each thawed sample were placed in a sterile tube and Sigma swabs and conventional swabs were introduced in each tube and subsequently in SVCM or Amies transport (A) medium, respectively. The same procedure was carried out for Flu-H1N1 investigation, SVCM was compared with in-house viral transport (B) medium (Eagle's MEM plus 1% bovine albumin and antibiotics). Tubes were refrigerated at 4°C for 24 hours. A-swabs were resuspended in 1 mL of sterile saline solution prior to molecular testing. Nucleic acids were extracted from 200 µL of each sample, and specific real-time PCR was carried out in parallel.

Results: BP DNA was detected in 23 (92%) cases in SVCM and B medium (100% concordant), although increased recovery of DNA was observed with SVCM (lower Cp in 21 samples, 91.3%). Cp difference between the SVCM and the fresh sample (FS) ranged from -0.7 to 1.76, and from 0.36 to 3.08 between A and FS ($p < 0.05$). SVCM was better than B, qualitative and quantitatively, for Flu-H1N1 detection. Positive results were obtained in 19 (76%) SVCM and in 17 (68%) B samples, respectively; and increased recovery of viral RNA was observed with SVCM (lower Cp in 15 samples, 79%). Cp difference between SVCM and FS ranged from 2.79 to 5.53, and from 4.29 to 7.31 between B and FS ($p < 0.05$).

Conclusion: SVCM is better than conventional transport media for the investigation of Flu-H1N1 and BP in nasopharyngeal specimens. SVCM avoids the need of several sampling from the same anatomic site, since bacteria and viruses preserve efficiently in the same transport medium.

R2628 Compare HBsAg cutoff index values of initially borderline or weakly reactive samples with neutralisation results for electrochemiluminescence immunoassay in China

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Objective: Hepatitis B surface antigen (HBsAg) is an important serological marker for diagnosis of hepatitis B virus (HBV) infection. The usual criteria for analysis of HBsAg are detection of HBsAg and result confirmation by antibody neutralization. We observed a relatively high proportion of weakly reactive samples were false positive with electrochemiluminescence immunoassay (ECLIA). In this study, we compared cutoff index (COI) values of the borderline or initially reactive samples (COI < 7.0) with HBsAg confirmation test in order to set up modified cutoff with an expectation of a decrease in complexity, improved turnaround time, and decreased expense.

Methods: Two hundred and thirty-eight HBsAg initially borderline or reactive sera or plasma with COI <7 were collected in West China Hospital of Sichuan University. Each sample must be re-determined in duplicate with Elecsys HBsAg II assay according to manufacturers' instructions. All repeated reactive samples were confirmed with HBsAg neutralization assay.

Results: 48.7% (116 out of 238) samples, deemed as weakly reactive (WR) samples, were found COIs < 2. Up to 12.1% (11 out of 91) repeated WR samples didn't pass confirmation. Statistical analysis indicated that more weakly reactive (WR) samples (100%) were found in HBsAg negative group than those (42.7%) in repeatedly reactive group ($p < 0.05$). There were 25 HBsAg negative samples and 11 samples which didn't pass the neutralization. And one of them was found HBV DNA existed. All these samples were found with anti-HBc and anti-HBe. In our study, we could improve the PPV from 95.2% to 99.4% and to 100% by setting up mortified cutoff values of 1.455 and 1.785.

Conclusion: It was the first report of evaluation of ECLIA with HBsAg confirmatory test in lower COIs samples. Evaluation of HBsAg prevalence through Elecsys HBsAg II will overestimate the true positive, especially in WR samples. When interpreting WR samples, one should look at the whole profile of HBV tests and viral loads, in addition to HBsAg neutralization test. Besides, arbitrary revised COIs may decreased the need of HBsAg neutralization test.

R2629 Streptococcal Lancefield grouping using Thermo Scientific PathoDxtra strep grouping kit

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Objectives: Thermo Scientific PathoDxtra™ Strep Grouping Kit (Thermo Fisher Scientific) and Prolex™ Streptococcal Grouping Latex Kit (Pro-Lab Diagnostics) were evaluated to compare the ability of the kits to accurately identify Lancefield grouped streptococci.

Methods: Three hundred and eleven Lancefield grouped streptococci (56 group A [GAS], 85 group B [GBS], 30 group C [GCS], 93 group D [GDS], 10 group F [GFS] and 37 group G [GGS]), 79 non-groupable streptococci/enterococci and 29 non-streptococci isolates (including *Staphylococcus* species) were inoculated onto Columbia Horse Blood agar and incubated at 36 ± 1°C overnight. Colonies were tested using PathoDxtra Strep Grouping Kit and Prolex Streptococcal Grouping Latex Kit according to manufacturer's instructions for use. All isolates had previously been identified using MALDI-TOF mass spectrometry and sodA sequencing.

Results: Sensitivity of PathoDxtra Strep Grouping Kit when testing individual streptococcal Lancefield groups was comparable to or greater than Prolex Streptococcal Grouping Latex Kit: GAS 100% and 98.2%, GBS 96.5% and 97.6%, GCS 100% and 86.7%, GDS 66.7% and 59.1%, GFS 100% and 90%, GGS 100% and 100% for PathoDxtra and Prolex kits, respectively. Overall, sensitivity of PathoDxtra Strep Grouping Kit (89.1%) was higher than Prolex Streptococcal Grouping Latex Kit (85.2%). Specificities of PathoDxtra Strep Grouping Kit and Prolex Streptococcal Grouping Latex Kit were comparable: 97.8% and 97.5%, respectively.

Conclusion: Overall, performance of PathoDxtra Strep Grouping Kit was better than Prolex Streptococcal Grouping Latex Kit, especially in detection of GCS and GDS. PathoDxtra Strep Grouping Kit showed fewer cross reactions from both Lancefield groupable and non-groupable streptococci isolates compared to Prolex Streptococcal Grouping Latex Kit. Incorporating a vivid blue latex, PathoDxtra Strep Grouping Kit gave clear, easily readable agglutination reactions within 60 seconds.

R2630 Performance evaluation of multianalyte mariPOC® respiratory infection test for adenovirus types 1–8, 14, 19 and 21

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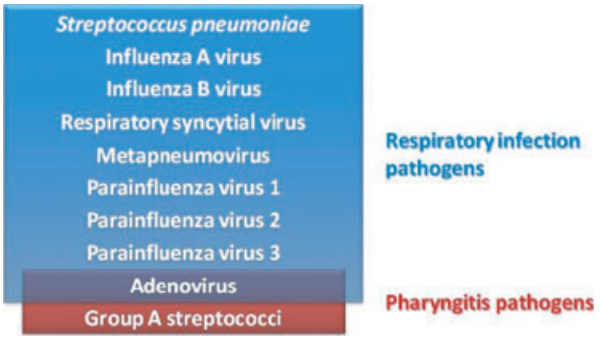
Objective: Multianalyte mariPOC® test system allows efficient point-of-care testing of respiratory tract infections (CE marked). It applies a fully automated random-access fluorescent analyser and antigen

detection. The coverage exceeds the test selection available as lateral flow rapid tests or immunofluorescence assay kits (Figure). Both pharyngitis, upper, and lower respiratory infections can be detected. Sampling is done by a swab either from the nasopharynx or from the throat. The test is extremely simple to operate and hands-on-time is less than a minute per sample. It reports positive samples after 20 minutes, while low positive and negative samples are reported in 2 hours. The objective was to study mariPOC's performance for the detection of adenovirus types 1–8, 14, 19 and 21. A highly sensitive laboratory method based on DELFIA® technique was used as a reference test.
Methods: Heat inactivated adenovirus preparations of types 1, 3, 4, 5, 6 and 7 were purchased from the Department of Virology, Turku, Finland as solutions of similar viral titres. Purified adenovirus hexon protein of type 2 was from the same source. *Adenovirus* preparations of types 14, 19 and 21, and positive clinical samples of types 2 and 8 were from HUSLAB, Finland. The viral preparations were diluted with mariPOC RTI sample buffer and analysed with the automated analyser.
Results: The analytical detection sensitivity of the mariPOC adenovirus-specific method is 2 pM (0.7 ng/mL of antigen). This level of sensitivity is among the best in the current mariPOC menu of tested pathogens. The mariPOC detected all studied adenovirus types. Preparations of types 1, and 3–7 had been produced by a similar process and they were of similar titre. Preparations of types 2, 8, 14, 19 and 21 were produced by varying processes or were clinical samples, and thus they were of varying viral titres. There was a high correlation of signals between the mariPOC and the reference DELFIA method ($p = 0.84$).



Conclusions: mariPOC detected all studied adenovirus types with high sensitivity. These types cause most respiratory tract infections associated with adenoviruses. The mariPOC is expected to be well suited for rapid diagnostic testing of adenovirus infections. It has potentially superior clinical specificity in etiological diagnosis compared to PCR. Antigen testing finds better clinically meaningful infections while PCR (especially of DNA) will also detect subclinical infections, contamination and carriership.

R2631 The Modified Hodge Test negative predictive value among carbapenem-resistant isolates in a Brazilian university hospital

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Objectives: Evaluate the Modified Hodge Test (MHT) performance to rule out KPC in a Brazilian University Hospital.
Methods: From April 2009 to July 2011, 1521 Enterobacteriaceae isolates not susceptible to ertapenem (ENSE), i.e. MIC > 0.5 mg/L, were submitted to MHT. All the positive MHT isolates and 36% of the negative MHT were also PCR tested for blaKPC2. Susceptibility Tests were done by Vitek2 ® (AST-105 and 104) and the carbapenens drugs were also tested by disk-diffusion and interpretation according to CLSI M100-S11.
Results: Sixty-four percentage of the ENSE isolates were MHT negative, 35.6% MHT positive and 0.4% MHT undetermined. Among 541 MHT positive 84% were KPC and among 354 MHT negative 100% was not KPC. Overall 30% (456) of the ENSE were positive for gene blaKPC2 being 93% *K. pneumoniae*; 2% *E. cloacae*; 1.8% *E. coli*, 1.1% *Serratia* spp. and 2.1% other species. Of those 99.8% were MHT positive and only one isolate was undetermined. KPC isolates antibiogram showed very high resistance rates among distinct classes but aminoglycosides (amikacin 5%, gentamicin 12% susceptible) and tygacil (25%). Colistin had an MIC50/90 of 0.5 and 16 mg/L respectively. KPC isolates had 69.8% resistant rate to imipenem and 49.6% to meropenem by Vitek2 ®. Meropenem had a 30% of Very Major Error by the Vitek2®. Among ENSE isolates the MHT had 100% Sensitivity, 80% Specificity, 84% Positive Predictive Value and 100% Negative Predictive Value (NPV).
Conclusion: MHT results had very good correlation with molecular KPC and NPV was excellent. MHT is a good alternative phenotypic tool for routine and low resource laboratories to easily rule out carbapenemase isolates.

R2632 An investigation of the suitability of liquid transport medium for recovery of enteric pathogens from faecal specimens

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In recent years a number of automated processing systems have been introduced into clinical microbiology laboratories. These systems require a liquid specimen such as blood or urine as the matrix for processing. Transport swabs are also available with liquid medium for respiratory, urogenital, and wound specimens. Faecal specimens, however, which account for a considerable proportion of specimens in a routine clinical laboratory, could not be processed unless first emulsified and suspended in a broth. Recently a transport swab for faecal specimens (Faecal Transwab®) has been developed which at the time of collection converts faecal specimens into a liquid specimens; suitable for direct processing on automated platforms. The present study was devised to investigate the performance over typical transport periods of this new device with a range of important enteric pathogens.
Methods: The Clinical and Laboratory Standards Institute standard M40-A describes methods for assessing the ability of transport devices to maintain various microorganisms in a viable condition for up to 48 hours during transport at ambient or refrigerated temperatures. The standard, however, does not include any enteric pathogens. The present study used the principles and methods of CLSI M40-A to evaluate the new device, adapted for the enteric microorganisms which are the target for faecal swabs. Stock Test suspensions were prepared for Eschericia coli 0157, *Campylobacter* spp., *Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii*, *Shigella sonni*, *Vibrio cholerae*, *Salmonella typhi*, *Salmonella typhimurium*, and *Salmonella enteritidis*. Swabs in triplicate were inoculated with dilutions of each microorganism, and held for 0, 24 and 48 hours at ambient temperature and refrigerated temperature. After the holding period aliquots of the transport medium were inoculated on to plates of the appropriate agar medium, incubated, and any colonies were counted.
Results: Acceptable recoveries within the parameters for CLSI M40-A were recorded for all organisms at refrigerated temperature, at the ambient temperature over the 48 hours period a decline in the number of colonies was observed.
Conclusion: This investigation has shown that Fecal Transwab® device can efficiently recover a range of enteric pathogens at refrigerated temperature during simulated transport conditions over a 48 hours period, in compliance with the principles of CLSI standard M40-A.

R2633 Group B streptococcus carriage screening using direct latex agglutination on selective broth

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Objectives: Group B streptococcus (GBS) is a leading bacterial cause of neonatal disease worldwide. CDC recommends a method to detect GBS carriage in all pregnant women. The aim of the study was to evaluate direct latex agglutination on incubated selective broth as a screening method.

Methods: Two hundred and forty-five vaginorectal swabs submitted to our laboratory during 1 month were tested by both methods. They were immersed in SBM broth (Todd-Hewitt with gentamicin, nalidixic acid, 5% blood; Biomedics®) and incubated at 35°C overnight. Subcultures onto 5% blood agar were incubated 24 hours at 35°C with 5% CO2. GBS suspected colonies were confirmed by catalase reaction, Gram stain and latex assay (also hippurate hydrolysis test when colonies were non-hemolytic). Negative plates were reincubated and examined on the following day. In addition, groups B and D latex testing (Slidex® Strepto Plus, bioMérieux®) was performed on all SBM adapting the protocol to broth. Samples were considered positive when B+D-, negative when B-D+ or B-D- and undetermined when B+D+.

Results: Results are shown in Table 1. We found 40 specimens positive and 134 negative by both methods. Culture method gave nine more positive specimens which tested B+D+. We also found four samples B+D- but negative with CDC method. Repeated subcultures in those cases confirmed the presence of GBS, giving a total of 53 true positive specimens (21.6%). Another 58 B+D+ samples were negative by standard method. As we lack a molecular method to further investigate the B+D+ samples in those 67 (27.3%) cases subculture remains necessary. For the positive and negative results the method had a sensitivity, specificity and predictive values of 100%.

Table 1. Results of CDC culture and latex detection methods.

Culture	Latex agglutination	Nº
+	B+D-	40
+	B-D-	0
-	B+D-	4
-	B-D-/B-D+	134
-	B+D+	58
+	B+D+	9
49	44	245

Conclusions: In a previous study using only group B latex test on incubated broth we found some positive results in samples which contained polyagglutinating strains (usually non-hemolytic streptococci) and were negative for GBS by CDC method. It is possible however that some of them were true positive as GBS could have been masked in subcultures. Unfortunately we could not confirm this. Using both B and D groups it is possible to give reliable positive and negative results on the first day and select which samples need to be subcultured. Direct latex agglutination on overnight incubated selective broth is a good first step screening method for GBS carriage detection and it decreases the turnaround time and the laboratory workload.

R2634 Evaluation of phenotypic methods to detect ESBL-producing Enterobacteriaceae, in Coimbra, Portugal

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The increase of resistance to antibiotics caused by the production of Extended Spectrum beta-lactamases (ESBL) among Enterobacteriaceae can be a serious problem. The aim of the present study was to compare different methodologies to screen for ESBL-producers among Enterobacteriaceae isolates recovered from patients in Centro Hospitalar de Coimbra-Portugal.

Methods: From September 2011 until October 2011 ESBL-producing isolates were collected in the Centro Hospitalar de Coimbra, EPE, central Portugal. Isolates were identified by Walkaway Systems (Siemens, USA). ESBL producers were confirmed using the following methodologies: Etest® (BioMérieux, France) ESBL with Cefotaxime/Cefotaxime + Clavulanic acid, Ceftazidime/Ceftazidime + Clavulanic acid and Cefepime/Cefepime + Clavulanic acid strips; CicaBeta test (MAST, UK) and Disc test (MAST, UK) according to manufacturer's instructions.

Results: From total of 498 Enterobacteriaceae isolates, the Walkaway system identified 50 isolates with an ESBL-positive phenotype, which were included in this study. The isolates belonged to *Escherichia coli* (25), *Klebsiella pneumoniae* (24) and *Klebsiella oxytoca* (1) species and were collected mainly from urine, but also sputum and blood cultures. Thirty-two isolates were isolated from samples collected from patients in the Emergency Room (ER) and 18 were from inpatients. These results were compared to the CicaBeta test results and it failed to identify five isolates as ESBL-producers. The same isolates were used to compare the performance of the Etest strips and Disc test (MAST). Etest strips identified 48 isolates as ESBL producers and two gave an inconclusive result, whereas 47 isolates were identified by the Disc test as ESBL producers and three were negative.

Conclusions: The number of ESBL producing isolates collected from the ER is considerable and requires surveillance since it indicates that ESBL producers are spreading to the community settings. These findings highlight the importance of routine detection of ESBL producers. Although phenotypic methods for determination of ESBL production have been widely used in routine microbiology laboratories, they have certain limitations. The results in this study favour the use of automated antimicrobial susceptibility system for this purpose, nevertheless CLSI phenotypic confirmation increase the reliability.

R2635 MALDI-TOF – to early goal-directed antibiotic treatment

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Objectives: Without loss of specificity, MALDI-TOF (Matrix-assisted laser desorption/ionization – time of flight) mass spectrometry provides faster species determination in the microbiological routine lab than do conventional methods. The aim of this study was to evaluate whether the introduction of Maldi-Tof in septic patients would shorten the duration of empiric broad-spectrum antibiotics and favour a faster shift to antibiotics with a more narrow spectrum.

Methods: We compared data concerning the antibiotic regimen in inpatients with positive blood cultures (BacT/ALERTR, bioMérieux) from two periods, November-December 2010 and May-August 2011, before and after the introduction of MALDI-TOF (MALDI Biotyper 2.0, Bruker Daltronics) in the laboratory routine. During the first period, 60 positive blood cultures from 42 patients were analysed with Gram stain followed by biochemical tests and susceptibility testing. During the second period, 80 positive blood cultures from 57 patients were analyzed with the same methods together with MALDI-TOF.

Results: Most patients started empiric treatment with a broad-spectrum antibiotic. The time from positive blood culture to the switch from empiric to goal-directed antibiotic treatment was several hours longer before compared to after introduction of MALDI-TOF in the routine. With MALDI-TOF, species identification was completed already 4–6 hours after a positive blood culture, and this often lead to a change of antibiotic regimen. MALDI-TOF seemed to shorten the time with broad-spectrum antibiotic treatment in patients with gram-positive bacteria but not with gram-negatives. In infections with gram-positive bacteria there was a reduction from 18.6 to 10.5 hours. MALDI-TOF also led to a quicker decision whether or not a gram-positive bacteraemia was a contamination, sometimes leading to earlier termination of treatment.

Conclusion: MALDI-TOF in routine diagnostics of positive blood cultures may lead to an earlier switch from empiric to directed antibiotic treatment, and sometimes termination of treatment, thereby reducing the amount of broad-spectrum antibiotics used, especially for gram-positive bacteraemia.

R2636 Validation of Eswab for the detection of urogenital mycoplasma from endocervical specimens using the Mycoplasma Duo test

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Objectives: *Ureaplasma urealyticum* (Uu) and *Mycoplasma hominis* (Mh) are commensal organisms which colonize the urogenital tract mucosa and under some circumstances multiply excessively giving rise to a number of diseases including: prostatitis, epididymitis, chorioamniotis, endomitritis, salpingitis and vaginitis. A liquid and universal transport medium storable at room temperature would be beneficial. This study compares endocervical specimens collected by Eswab to specimens preserved in Mycoplasma Duo suspension Medium (MDSM) for the detection of Uu and Mh.
Methods: Eswab is a tube with 2 mL Liquid Amies transport medium and a flocked swab for sample collection and transportation. Endocervical specimens ($n = 30$) were used for this evaluation. Innoculation of both Eswab and MDSM was freshly carried out of after sample collection and Mycoplasma Duo microplate was seeded according to the manufacturer procedure either with Eswab liquid or MDSM. Final results were given after 48 hours of incubation at 37°C and expressed as follow: negative: Uu and Mh <10 exp.4 positive: Uu or Mh >10 exp.4.
Results: Eswab collected samples showed 92% positive concordance ($n = 10$ Uu and $n = 2$ Mh + Uu) and 94% negative concordance with MDSM collected samples ($n = 18$). One Eswab collected samples was positive for Uu but was found negative with the MDSM sample. At 24 hours, 11 Eswab samples were already positive after 24 hours vs. 8 using the MDSM.
Conclusion: Eswab is compatible with the performance of Mycoplasma Duo. Using Eswab liquid instead of MDSM leads to the detection of more than 92% of both negative or positive samples tested with MDSM, with however the ability to detect earlier positivity for Uu/Mh and to detect positive samples undected using MDSM. Therefore, due to some discrepancies, further validation should be carried out including more Mh positive samples.

R2637 Rapid and accurate identification of alpha haemolytic streptococci using the Vitek MS® MALDI-TOF system

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Objectives: The alpha haemolytic streptococci are an important group of clinically significant bacteria and their correct identification can prove problematic, inaccurate and time consuming when traditional phenotypic methods are used. The objective of this study was to show the easy, rapid and accurate identification of alpha haemolytic streptococci including Streptococcus pneumoniae the recently launched Vitek MS® (Biomerieux, France) matrix assisted laser desorption/ionisation time of flight (MALDI-TOF) mass spectrometry system.
Method: One hundred and forty-eight previously characterised strains of alpha haemolytic streptococci were cultured onto Columbia Horse Blood Agar. Disposable target slides were inoculated with a small amount of a single bacterial colony to provide a thin layer of bacterial growth. This was then overlaid with 1 μL of matrix solution and air dried. The resulting slide was then processed in the Vitek MS® instrument with automatic database analysis of resulting mass spectra. A second target spot was analysed if no spectra or identification were obtained. Discordant isolates were subsequently identified using the GP –ID Card on a Vitek 2 (Biomerieux, France) system and were considered to be the reference identification.
Results: Of 148 strains of alpha haemolytic streptococci tested by the Vitek MS®, 34/35 (97.1%) of *Streptococcus pneumoniae* isolates identified correctly to species. 90/113 (79.6%) of other alpha haemolytic streptococci strains gave good identification to species level. 112/113 (99.1%) of other alpha haemolytic streptococci strains identified to the correct group. 147/148 (98.6%) of the total isolates provided an identification correct to genus level. One Streptococcus pneumoniae strain failed to give any identification using the Vitek MS system.
Conclusions: The Vitek MS® MALDI-TOF system provides an easy, specific, accurate and importantly rapid identification of alpha haemolytic streptococci and significantly *Streptococcus pneumoniae*. Although the Vitek MS® is unable to differentiate between strains of *S. mitis* and *S. oralis*, all isolates were correctly identified into the *S. mitis* group.

R2638 Typing of *Propionibacterium acnes* by MALDI-TOF MS

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Objectives: *P. acnes* has been considered traditionally as a non-pathogenic member of the skin flora, however today it is accepted as an opportunistic pathogen associated with several diseases such as severe acne vulgaris, corneal ulceration, endophtalmitis, endocarditis, late-stage prostatic joint infection, and shunt-associated central nervous system infection. The aim of the present study was to evaluate the possibility to use MS-based typing for this important anaerobic species after routine identification by MALDI-TOF MS.
Methods: *P. acnes* strains obtained from patients with severe acne, blood, joint infection or skin, were identified by conventional methods and by MALDI-TOF MS. After acquisition the mass spectra of P. acnes strains (MS Bruker Biotyper 2.0), the type of which were determined earlier as type I, II and III, mass spectrum sets were imported in the ClinProTools 2.2 software. Spectra were normalized and recalibrated using the respective functionalities of the software. Type characteristic peaks and peak shifts were searched. In addition, peak variations between the different types of *P. acnes* were investigated by using our FlexAnalysis 3.3 software. Out of the main spectra (MSP) of strains with known types, differentiating library was created for *P. acnes* type I (IA and IB), II and III and this library was challenged with spectra of further 60 *P. acnes* isolates with different origins. MLST analysis of the strains was also carried out.
Results: By careful analyses of the mass spectra of seven strains with known types of *P. acnes*, specific peaks and peak shifts were found in the range of 6800 and 7400 Da, which could be selected as characteristic peaks for type I, II and III. Even differentiation of types IA and IB was possible with a characteristic peak with 9950 Da present in type 1 A *P. acnes* and missing in type IB strain. Using this approach out of the further 60 clinical *P. acnes* isolates 52 (86%) could be typed after identification by MALDI-TOF MS. MLST results were compared with the MS-based typing results.
Conclusion: Since the introduction of MALDI-TOF MS for microbiological application, it has evolved from an experimental tool to a technology with significant benefit for routine microbiological laboratories first of all for the identification of bacteria and fungi. However careful evaluation of the MSP of well known bacteria may give a possibility to use this technique also for typing of bacteria such as presented here.

R2639 Tuberculosis laboratory diagnosis: compliance with UK national guidance

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Objectives: The UK Department of Health (DH) has provided guidance for laboratories that provide diagnostic services for tuberculosis (TB). A retrospective study was carried out to examine laboratory practices and turnaround times, supported by the reference laboratory, for processing respiratory specimens only.

Method: Data on respiratory specimens for TB investigations for a period of 1 year (January 2010–December 2010) was collected from the laboratory information system (LIMS). Turnaround times were determined for reporting acid-alcohol fast bacilli (AAFB) microscopy. All specimens were cultured using a liquid culture analyser and all positive cultures were referred to the reference laboratory. Turnaround times for culture identification and susceptibility results were determined.
Results: A total of 1037 respiratory specimens were received. 749 (72.2%) of these specimens had AAFB microscopy reported within the recommended 1 day period. 112 (10.8%) specimens were reported within 2 days, 107 (10.3%) within 3 days and 53 (5.1%) within 4 days. The average time to result of AAFB microscopy was 1.39 days. Of the 1037 respiratory specimens, 77 (7%) were culture positive but only eight grew Mycobacterium tuberculosis. The average time for the reference laboratory to report identification was 13.30 days but the average time for reporting identification of the 77 positive samples was 38.86 days. Only five specimens were reported within the target 21 days (6.5%) which included one of 6 (16.6%) isolates identified as *M. tuberculosis* for whom the average time was 31.5 days. The average time to return susceptibility results on these isolates to first-line agents was 55.7 days.
Discussion & Conclusion: This laboratory provides a 5 days service for routine requests. Investigation of AAFB microscopy revealed that the non-compliance with the 24 hours target is because of samples arriving late in the laboratory, out of hours or over the weekend .The study shows that compliance to meet the 21 days guideline for reporting identification is poor. Again factors contributing to this included delays in setting up the culture, taking off a positive culture from the machine, transport to the reference laboratory and time taken by the reference laboratory to report identification and need for repeat specimen due to contamination. These factors also contribute to delayed reporting of susceptibility results and thus time taken for final reporting is well outside the recommended 30 days.

R2640 Evaluation of optimal neutrophil gelatinase-associated lipocalin value as a screening biomarker for urinary tract infections in children

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Objectives: Neutrophil gelatinase-associated lipocalin (NGAL) is identified as a 25-kDa protein that is a promising biomarker for detecting kidney injury. Urinary tract infection (UTI) is one of the most common bacterial infections in children. Although urine culture is the gold standard for the diagnosis of UTI, positive culture results require 2–3 days for complete bacterial identification. Currently, urine pH, leukocyte esterase, nitrite levels, etc., are used to screen for UTI, but these tests have sensitivities of about 70–80%. Recently, several studies have suggested that urine NGAL (uNGAL) may be used as a novel biomarker to predict UTI in children. The aim of this study was to assess whether uNGAL and serum NGAL (sNGAL) represent reliable markers of UTI in children, and to evaluate the appropriate levels of NGAL for the screening of UTI.
Methods: We analyzed 375 and 760 samples from UTI and non-UTI patients, respectively. If the culture showed more than 105 CFU/mL of a single pathogen, or if the child was symptomatic with the culture showing more than 104 CFU/mL, the child was diagnosed as having a UTI. sNGAL and uNGAL levels were measured by using enzyme-linked immunosorbent assay (Bioporto, Denmark). The sensitivity, specificity, and optimal cut-off values of NGAL for UTI screening were calculated using the receiver operating characteristic (ROC) curves formed using NGAL values and the number of UTI and non-UTI cases.
Results: sNGAL and uNGAL levels were more elevated in the UTI cases than in the non-UTI cases, but the differences in levels were not statistically significant ($p > 0.005$). The optimal cut-off values of sNGAL and uNGAL for UTI screening were 65.25 ng/mL and 5.65 ng/mL, respectively, with sensitivities of 70% each, which was similar to the corresponding sensitivities of other conventional screening tests. These values were lower than the proposed cut-off values of sNGAL and uNGAL by the manufacturers, which were 106 ng/mL and 9.8 ng/mL, respectively.
Conclusions: sNGAL and uNGAL are not the only markers for an early prediction of UTI. If NGAL levels are considered for the screening of UTI, the cut-off levels lower than those recommended by the manufacturer will have to be used. Therefore, further investigations, for example, comparison of NGALs with other markers for the screening of UTI, are required to evaluate the exact cut-off values of NGAL for UTI in children.

R2641 Comparison of EUCAST and CLSI cephalosporin discs to detect ESBL phenotype in Enterobacteriaceae at a university hospital in Poland

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Objectives: The aim of the study was to compare low cephalosporin content discs (EUCAST) to high cephalosporin content discs (CLSI) with addtition of cefepime disc in detecting ESBL phenotype in Enterobacteriaceae. EUCAST was introduced in Poland in 2011 however ESBL detection is still recommended to be done with CLSI cephalosporin discs.
Methods: Thirty one consecutive one per patient isolates of Enterobacteriaceae with ESBL phenotype were subjected to additional testing with cefotaxime (5 and 30 μg), ceftazidime (10 and 30 μg), cefepime (30 μg) and amoxicillin/clavulanate (20/10 μg) discs. Existence of zone enlargement between different cephalosporins and clavulanate containing discs was recorded.
Results: There were 12 *Klebsiella paneumoniae*, seven *Enterobacter cloacae*, six *Escherichia coli*, five *K. oxytoca* and one *Citrobacter koseri* isolates identified by conventional and WalkAway Microscan (Siemens) method. All isolates demonstrated presence of inhibition zone enlargement between cephalosporin and clavulanate containing discs with exception of two *K. oxytoca* and their both ceftazidime containing discs, one *K. oxytoca* and ceftazidime 10 μg and cefepime 30 μg discs and another *K. pneumoniae* ceftazidime 10 μg.
Conclusion: Low (EUCAST) and high (CLSI) cephalosporin content discs were equally good at detecting ESBL phenotype in our isolates. This could result in using only EUCAST discs for both susceptibility testing and ESBL phenotype detection. Combination of cefotaxime (5 μg) and cefepime (30 ug) seems most appropriate in our setting as it ensures ESBL observation even in AmpC derepressed isolates sometimes encountered in our patients.

R2642 EBV tests performance on DiaSorin's newly launched LIAISON® XL

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Objective: The aim of the present study was to evaluate the EBV (EBNA, VCA-IGG and IGM) kits panel on the LIAISON® XL, DiaSorin's next generation analyzer, against the performance of the well established LIAISON® platform.
Methods: One hundred and twenty routine samples were collected and used in the evaluation for the EBNA performance while 112 and 98 were assayed also for VCA-IGG and IGM. A proportion of 40%, 20% and 60% the samples chosen were below or around EBNA, VCA-IGG and IGM cut-offs, respectively. Samples were run on the LIAISON® and LIAISON® XL analyzers, on the same day. We checked the concordance between the two analyzers, and discrepant samples were rechecked in another lab or confirmed by blotting.
Results: The overall correlation between the two analyzers results was 96.9%. The concordance in the EBNA assay was 94.6% Among the seven discrepant results it seems that the LIAISON XL had two FP and four FN and one result was inconclusive. The concordance in the VCA-IGG assay was 98.2%. Among the three discrepant results it seems that

the LIAISON had one FP, one FN and one result was inconclusive. The concordance in the IGM assay was 96.9%. Among the three discrepant results it seems that the LIAISON XL had three FP. In view of the algorithm adopted at our institution (initially performing EBNA and only if negative continuing to perform VCA-IGG and IGM), most of the discrepant results (borderline levels) will not affect the diagnosis. However, adjusting the lower cut-off of the EBNA assay to 6 U/mL instead of 5 U/mL renders similar clinical outcome between the analyzers. Using fine tuning of cut-offs, the overall correlation between the two analyzers was 98.8%.

Conclusion: The LIAISON® XL offers higher throughput, long walk-away time and improved instrument reliability. The performance of the EBV panel was comparable between the two platforms.

R2643 Evaluation of an anti-listeriolysin O ELISA on the diagnosis of intra-uterine infections with *Listeria monocytogenes*

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Objectives: *Listeria monocytogenes* is a foodborne pathogen with mortality up to 30%. Listeriosis has particular predilection for pregnant women and can cause severe complications like spontaneous abortion, stillbirth and preterm labour. According to literature, detection of anti-listeriolysin O (LLO) might be useful for serodiagnosis of listeriosis, especially when cultures stay negative. The aims of our study were to investigate the seroprevalence of anti-LLO in women of childbearing age and examine if detection of anti-LLO IgG in serum could contribute to diagnosis of listeriosis in pregnancy.

Methods: We used a commercially available ELISA for the detection of anti-LLO IgG in human serum and plasma (Diatheva). A collection of 176 sera from 83 admitted pregnant women (nine spontaneous abortions, 39 stillbirths and 35 preterm labours, respectively), nine culture positive listeriosis patients and 84 control sera (women visiting our fertility clinic) was tested.

Results: An optical density (OD) of 0.650 was determined as optimal cutoff value. The results showed that 5.6% (5/84) of the controls had IgG antibodies toward LLO. Positive serology was present in one pregnant woman (1/83, 0.8%) with a stillbirth due to massive feto-maternal transfusion. Cultures from this patient were negative for Listeria. All nine Listeria culture-positive patients tested negative for anti-LLO antibodies.

Conclusion: Production of anti-LLO in the control group indicates the possibility of previous or current (subclinical) listeriosis in this group. Lower prevalence of anti-LLO in pregnant women may be due to nutritional restrictions during pregnancy. None of the nine culture positive listeriosis patients showed an anti-LLO response. Seroprevalence of anti-LLO in women of childbearing age is very low. This test is not suitable for detecting culture-negative listeriosis.

R2644 Added value of selective broth enrichment for the detection of rectal carriage of extended-spectrum beta-lactamase producing Enterobacteriaceae in hospitalised patients

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Objectives: Adequate laboratory methods for the detection of extended-spectrum beta-lactamase producing Enterobacteriaceae (ESBL-E) are crucial in the prevention of nosocomial transmission of ESBL-E and appropriate antimicrobial therapy for ESBL-E infections. The use of broth enrichment in the laboratory detection of ESBL-E has been an unresolved issue. This study aimed to evaluate the added value of selective broth enrichment for the detection of rectal carriage of ESBL-E in hospitalised patients.

Methods: In October 2011 an ESBL-E prevalence survey was performed in a Dutch teaching hospital. Rectal swabs were taken from all patients hospitalised on the day of the survey. Swabs were directly plated on a selective ESBL screening agar plate (EbSA, Cepheid), and subsequently placed in a selective tryptic soy broth, containing cefotaxime (0.25 mg/L) and vancomycin (8 mg/L) (TSB-VC). After 18–24 hours incubation (35–37°C) the EbSA agar plate was read and the TSB-VC was subcultured on an EbSA agar plate that was read after 18–24 hours incubation (35–37°C). Species identification and susceptibility testing was performed for all isolates that grew on either one of the EbSA agar plates using VITEK 2 (bioMérieux). For suspected isolates (MIC ceftazidime and/or MIC cefotaxime >1 mg/L) the presence of ESBL was phenotypically confirmed with the combination disk diffusion method for cefotaxime, ceftazidime, and cefepime, both alone and with clavulanic acid (Rosco). Test results were considered positive if the inhibition zone around the disk was ≥5 mm increased for the combination with clavulanic acid.

Results: Rectal swabs were obtained from 556 patients. ESBL-E was cultured in 38 (6.8%) patients. Direct EbSA culture detected ESBL-E in swabs from 22 (4.0%) patients. TSB-VC subculture increased the number of ESBL-E positive cultures to 37 (6.7%) (McNemar Chisquare=10.56, $p = 0.0012$). Only one of 38 (2.6%) ESBL-E positive patients was not detected after TSB-VC enrichment. Escherichia coli was the predominant ESBL-positive species identified (26/38; 68%)

Conclusions: The use of selective broth enrichment resulted in a substantial and statistically significant increase in the yield of ESBL-E screening in hospitalised patients. Broth enrichment is, therefore, considered indispensable for the reliable detection of ESBL-E.

R2645 PNA FISH® (AdvanDx, Vedbaek, DK) for rapid identification of *Staphylococcus aureus* and Coagulase-negative staphylococci directly from positive blood cultures

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Objectives: *Staphylococcus aureus* bacteremia is associated with high mortality and requires prompt and aggressive antibiotic therapy whereas the presence of coagulase-negative staphylococci (CNS) in a blood culture often is due to contamination. Routine pathogen identification in positive blood cultures showing ''Gram-positive cocci in clusters'' is unavailable on a same-day basis. This could lead to patients with *S. aureus* bacteraemia receiving inadequate therapy and/or patients with CNS getting unnecessary antibiotics until standard, next-day species identification. The *S. aureus*/CNS PNA FISH (Peptide nucleic acid fluorescence in-situ hybridization- AdvanDx, Vedbaek, DK) system claims to differentiate between *S. aureus* and CNS in 90 minutes which could have immediate clinical and therapeutic implications. In addition, the system should permit identification of each in mixed cultures of S. aureus and CNS that can be challenging by conventional methods. We studied the performance of the *S. aureus*/CNS PNA FISH system compared to our routine culture-based method for pathogen identification in positive blood cultures.

Methods: PNA FISH was performed directly on smears from 75 blood cultures flagged as being positive by the Bactec 9240 (Becton Dickinson) during a 14-week validation period. The assay uses fluorophore-labeled dual probes which stain S. aureus green and CNS red under UV light microscopy. Pathogen identification by PNA FISH and our routine culture-based method were compared.

Results: Among 54 showing Gram positive cocci in clusters (GPCCL) 20 *S. aureus*, 31 CNS and two mixed S. aureus/CNS cultures were identified by both PNA FISH and standard culture and identification. However, routine culture missed the CNS that was covered with a heavy growth of *S. aureus* in one of the mixed cultures. One GPCCL identified as Micrococcus spp by culture was appropriately negative by PNA FISH. As expected, PNA FISH was also negative when performed on 16 Gram negative rods (GNR) and five other Gram positive bacterial species. Overall test sensitivity and specificity were 100% (53 of 53) and 100% (22 of 22), respectively.

Conclusions: PNA FISH accurately identified *S. aureus* and CNS in blood cultures with gram stain showing GPCCL including two with mixed *S. aureus* and CNS cultures. Reporting PNA FISH results soon after the Gram stain may have positive clinical, therapeutic and economic implications.

R2646 Evaluation of Britania® kit based on novel meropenem/inhibitor combination disks for the detection of contemporary carbapenemases in Enterobacteriaceae: a reliable approach for KPC, MBLs and OXA-48/163 recognition

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Phenotypic detection of carbapenemase producers remains a challenge for the routine microbiology lab. and is important to guide the appropriate antimicrobial therapy and to prevent nosocomial spread. Combinations of meropenem (MER) with boronic acid (APB), EDTA and cloxacillin (CLO) may be used to detect KPC, MBL or AmpC hyperproducers, respectively. The major limitation of these combinations is that they did not include strategies for the detection of other carbapenemases as oxacillinases or for the discrimination of other frequent interfering mechanism as CTX-M plus porins loss.

Aim: Here we tested a kit produced by Laboratorios Britania, Argentina, based on reported MER (10 μg) disk combinations (APB 600 μg/disk, CLO 3000 μg/disk, EDTA 750 μg/disk) and the innovative incorporation of tazobactam (TZ 100 μg/disk) designed for the detection of carbapenem-resistance strains with ESBLs plus porins loss.

Methods: A panel of 132 Enterobacteriaceae was assessed (*n*): KPC, 20; MBL (IMP-1, IMP-8, VIM-2, NDM-1) 16; OXA-48/163, 15; Sme, 8; NMC-A/IMI, 2; GES-5, 2; CTX-M plus porin loss, 26; AmpC hyperproducers, 24; other mechanisms, 19. Disk diffusion was carried out following CLSI recommendations.

Results: Cutoff values were selected to provide the best sensitivity (Sn) and specificity (Sp) (increments in mm): APB ≥4; TZ and EDTA ≥5: CLOX ≥7. The Sn and Sp for predicting KPC (APB +ve, CLO -ve) was 95% and 98%, respectively (one *E. coli* KPC was miss detected). All KPCs tested negative for TZ. The remaining class A carbapenemases were detected with a Sn and Sp of 92% and 97%, respectively. The Sn and Sp for predicting MBL (EDTA +ve) was 91% and 90%, respectively. Interfering AmpC strains (APB +ve, CLO +ve, TZ -ve) were differentiated from carbapenemase producers with 92% and 97% of Sn and Sp. The Sn and Sp for predicting the other interfering mechanism, CTX-M plus porin loss (APB -ve, CLO -ve, TZ +ve), was achieved with a Sn of 91% and Sp of 90%. Those strains with APB -ve, CLO -ve and TZ -ve results were considered suspicious of OXA production. With this strategy OXA-48/163 was inferred with a high Sn (90%).

Conclusions: The kit was able to identify class A and B carbapenemases and also to differentiate all the interfering mechanisms, allowing the inclusion for the first time, of a phenotypic approach for oxacillinases detection. The MER/Inhibitor combinations kit permitted the differentiation of all major carbapenemases KPC, MBL or OXA-48/163 with high performance.

R2647 Evaluation of the vaginal ecosystem status in women with pre-term delivery

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Objectives: To determine the significance of the state of the vaginal ecosystem in women with preterm delivery.

Methods: Ninety three pregnant women were examined – 60 women with preterm delivery/I – group/and 33 pregnant women at term/II-group/. Vaginal smears were examinated by Nugent. Score 0–3 normal, 4–6 intermediate/clue cells not present/ – smear not consistent with BV/ bacterial vaginosis/. Score 4–6/clue cells are present/ and score ≥7– smear consistent with BV. HP /+/ hydrogen peroxide producing *Lactobacillus* spp., *U. urealyticum*, *Candida* spp. and other associated with genital infections aerobic microorganisms/*S. agalactiae* GBS, *S. aureus*, *E. coli*/were determined by cultural methods.

Results: In the group I with preterm delivery 43% were with normal score, 54% with intermediate smears – 31% clue cells not present – smears not consistent with BV and 23% clue cells are present – smears consistent with BV; 3% with bacterial vaginosis. In the group II of pregnant women at term vaginal smears were determined as normal flora in 84%, 16% intermediate – 7% of them with clue cells – smears consistent with BV. HP/+/*Lactobacillus* spp. were isolated in 13% group I and 81% in group II. *U. urealyticum* were positive in 17% group I and 12% in group II. *Candida* spp. were isolated in 10% group I and 33% group II. GBS /17% group I and 7% group II, *S. aureus* 3% group I-5% group II, *E. coli* 14% group I-6% group II.

Conclusion: More than 50% of the preterm delivery connected with desordered vaginal ecosystem. Twenty six percent of vaginal smears were determined as consistent with BV. HP/+/*Lactobacillus* spp. were isolated only in 13% in group I, while in group II they were predominant – 81%. *U. urealyticum* and other associated with genital infections aerobic microorganisms / GBS, *S. aureus*, *E. coli*/more often been isolated in group I, while *Candida* spp. prevalent in women at term.

R2648 Comparison of the the Copan LIM broth to the BHI broth for the isolation of GBS-colonised women prior delivery

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Objective: Currently vaginal specimens for the detection of antenatal colonization status of B Streptococcus (GBS) in women are collected with traditional transystem gel and swabs are incubated in BHI (Brain Heart Infusion) enrichment broth. Since the implementation of the WASP automation (Copan Italia) in our laboratory, traditional swabs in gel transystem were replaced with the Copan liquid base microbiology (LBM) device for the collection and transport of all clinical specimens. Copan LIM broth (Todd Hewitt with CNA), is a tube with 2 mL of selective enrichment medium specific for the incubation of vaginal swabs from pregnant women prior delivery for the detection of GBS. The objective of this study is to compare vaginal specimens, collected with Amies Gel transystem in primary culture and after incubation in BHI broth, to vaginal specimen's primary culture and after incubation in Copan LIM broth for detection of GBS for prenatal screening.

Methods: For this validation 252 consecutive combined vaginal specimens were collected from women for GBS prenatal screening using a Copan dual swab Amies Gel Transystem. One swab was first used for primary culture in a Columbia Agar plate then incubated inside a tube of BHI (Oxoid); the other swab plated as per current laboratory procedure, but incubated in a tube of LIM broth. Columbia Agar plates and both broths were incubated 35–37°C. After 18–24 hours BHI and the LIM broths were checked for turbidity and an aliquot was seeded on Columbia agar. All culture results from original and broths inoculations for both swabs were recorded.

Results: In the 252 patients 68 (26.9%) were positive for GBS and 184 were negative. Forty patients were positive in both primary culture and both BHI and LIM broths. 28 patients were positive only after inoculation from the enrichment broths, 18 were positive in both broth, nine were positive only in the LIM broth and one only in the BHI broth. Turbidity was present in all positive broths.

Conclusions: The Copan LIM broth detected more Streptococcus B positive patients compared to the BHI broth. The LIM broth, a liquid base microbiology device, facilitates the implementation of the WASP automation in our laboratory and is improving the detection of GBS colonized pre-labor women allowing treatment for the prevention of neonatal GBS disease.

R2649 Can we refer to previous isolates with acceptable confidence? A first evidence-based approach

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Objectives: When a micro-organism is repeatedly isolated in the same patient from the same sample source within a limited time frame, this repeated isolation might be identical to the prior isolation, especially when similarity can be confirmed by morphological and enzymatic characteristics. The term 'identical' here covers identification as well as susceptibility features. Prominent institutions e.g. CLSI, Garcia et al. have suggested the use of a referral strategy for the work up of such repetitive isolations that consent with specific conditions for the referring procedure. In spite of these guidelines, no evidence-based studies have been published yet to justify this strategy which could imply faster turn-around-times and lower the work load for laboratory staff.

Materials and Methods: In a prospective study, 100 gram-negative isolates from urinary, respiratory and other human sample sources were included. At the time of primary assessment of culture media, these isolations showed a presumptive identification similar to a recently (<1 week) isolated micro-organism and also met the other inclusion criteria (originating from the same patient andthe same sample source). These repetitive isolations were subjected to fully automated analysis (ID and AST) by means of Phoenix instrument (BD, Eucast vs. 2010). Based on FDA criteria for comparison of automatic ID and AST test instruments, acceptance criteria were preconceived. For identification, the aim was an agreement of at least 95%. Susceptibility results were compared based on categorical (dis) agreements. Criteria for major errors were <3%, very major errors <1.5%.

Results: Regarding identification, an agreement of 97% was achieved. For the AST results, a total of 1094 categorical results were compared (restraining all antibiotics subject to intrinsic resistances as summarized by EUCAST [versie…]). Categorical agreement was 97.27%: 1.37% minor errors were observed, 0.27% major errors, 1.09% very major errors. In the subgroup ''categorical results obtained from cultures isolated under antibiotic therapy (n = 426)'', the increase of categorical disagreements was statistically significant compared to those in the subgroup without antibiotic therapy. (McNemar, $p < 0.01$) However, these results still met our criteria.

Disagreement	Interpretation	Sample Type		Total (n=1094)
		Urinary (n=604)	Other (n=490)	
Minor Error	Repeat AST result: S/R Prior AST result : I or vice versa	1,49%	1,22%	1,37%
Major Error	Repeat AST result: R Primary AST result: S	0,82%	1,42%	1,09%
Very Major Error	Repeat AST result: S Primary AST result: R	0,33%	0,20%	0,27%

Conclusion: Our strategy for referring duplicate isolates meets the FDA based preconceived criteria and therefore supports expert-opinions (CLSI, Garcia et al).

R2650 Ability of BacT/ALERT® FAN plus blood-culture bottles to detect bacterial pathogens in samples containing therapeutic levels of antimicrobials

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Objectives: BacT/ALERT Microbial Detection System is an automated blood culture system that allows for the growth and detection of microorganisms commonly encountered in patients with bacteremia and or fungemia. This system contains the BacT/ALERT incubator and culture media. In order to maximize recovery, antimicrobial agents in the blood sample must be reduced below inhibitory concentrations. BacT/ALERT Aerobic FA Plus, Aerobic PF Plus, and Anaerobic FN Plus blood culture bottles contain polymeric adsorbent beads that adsorb and neutralize many antimicrobial agents. This study was designed to evaluate the performance of the beads for recovery of susceptible organisms in the presence of inhibitory levels of antimicrobial agents.

Methods: Organism recovery was evaluated by combining the test organism, along with 10 mL of buffer or whole blood containing 100–120% peak serum levels (PSL) of the target antimicrobial agent, in FA Plus or FN Plus culture bottles. Test organisms included a panel of susceptible ATCC strains. Inoculated bottles were then incubated in BacT/ALERT® 3D instruments and examined for growth over a 5 days incubation cycle.

Results: FA Plus bottles yielded 100% recovery of test organisms when evaluated with 120% PSL of vancomycin, piperacillin, piperacillin-tazobactam, amikacin, fluconazole, and amphotericin B. One hundred percent recovery was also achieved at 100% PSL ampicillin. FN Plus bottles yielded 100% recovery when tested with 120% peak PSL of clindamycin, cefoxitin, oxacillin, and imipenem. One hundred percent recovery was also achieved at 100% PSL vancomycin, moxifloxacin, and piperacillin-tazobactam. Recovery in the presence of ertapenem and meropenem is organism dependent.

Conclusion: This study demonstrates that the presence of adsorbent polymer beads in BacT/ALERT FAN Plus blood culture bottles allows for the recovery of susceptible test organisms in the presence of effective antimicrobial agents at elevated PSL.

R2651 Comparison of pre-treatment of sputum and endotracheal aspirate samples with SL-Solution (Copan®) or Digest-EUR (Eurobio®)

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Objectives: Lower respiratory tract infections are frequent. Some respiratory samples require fluidification before culture. SL-Solution and Digest'Eur are two commercially available pre-treatment reagents for liquefying thick or mucous specimens. The aim of this study was to compare them in terms of efficiency.

Methods: A total of 56 consecutive sputum and tracheal aspirates was collected from daily routine in our university hospital laboratory and treated as follows. All were fluidified by both SL-solution and Digest-Eur, according to the manufacturer's instructions. Each digested sample was 1000-fold diluted and 10 µL were inoculated on four culture agar media: Columbia blood1, nalidixic acid-colistin2, Drigalski2 and Haemophilus medium1 (BioMérieux) for 24 hours at 37°C under aerobic conditions with one or without two 10% CO2.

Microscopic examination after Gram staining was also compared for 10 samples before and after SL-solution pre-treatment.

Results: Cultures were qualitatively and quantitatively equal in 66% of the cases (37/56). No culture was negative with one reagent and positive with the other. Culture after SL-solution pre-treatment was more powerful in 29% of cases (16/56), with a higher colony count (9/56, up to 10-fold higher), or due to detection of an additional bacterial species (7/56). In this latter situation, potential pathogens (*Enterobacteria* or *S. maltophilia*) were observed in four samples, while oropharyngeal flora bacteria, coagulase negative *Staphylococci* or *Candida* were detected in the three others. Culture after Digest'Eur pre-treatment was more relevant in 5% of the cases (3/56). One sample showed a difference with a *H. influenzae* count higher than the significative threshold. The two other discrepancies were qualitative (growth of beta-hemolytic *Streptococcus* or *Enterobacteria*) but culture counts were under the significative threshold in both cases. Microscopic examination after fluidification by SL-Solution was the same as without in six out of 10 cases.

Conclusion: SL-solution is at least as good as Digest'Eur for sputum pre-treatment. Fluidification by SL-Solution was found equivalent or more relevant (Gram negative bacteria) in 95% of the cases. When less performing, differences were minimal and with no consequences. SL-

solution pre-treatment is not adequate for microscopic examination that must be made directly on the sample. SL-solution is a rapid, practical and ready-to-use system to calibrate and fluidify lower respiratory samples.

R2652 An investigation into the use of Kiestra Lab Automation and Vision technology for the analysis of disc diffusion antimicrobial susceptibility testing plates

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Objective: To evaluate the results produced by Vision analysis of digital susceptibility plate images when compared to the results recorded by HPC registered Biomedical Scientists (BMS) at the Lister Hospital, Stevenage, UK.
Method: Routine plates cultured in the laboratory, according to the British Society of Antimicrobial Chemotherapy (BSAC) guidelines for organisms grown on Iso-sensitest agar (Oxoid) at 35°C in atmospheric air, were used in this investigation. For analysis of these images, several pre-defined tools within Vision software were linked. By adjusting light and shutter speed settings, a true representation of each plate was captured. Images were then processed through the Vision method. Each tool within the Vision method was then adjusted to detect a specific area of the plate, i.e. the antimicrobial discs or the zone of inhibition. The images created by each tool were then merged to produce one image, displaying all three components. Results were then compared to those recorded by BMS. This process was repeated for the different genus of organisms; Staphylococcus species, Enterococci species, *Pseudomonas* species and the family, Enterobacteriaceae.
Results: In total 238 images were analysed, 44 *Staphylococcus* spp., 50 *Pseudomonas* spp., 45 *Enterococci* spp. and 99 Enterobacteriaceae from urine, blood and swab cultures. 1428 antimicrobial discs were present and Vision detected 1424 discs, 99.7% detected. All four missed discs had two discs present, overlaying each other resulting in an irregular, larger shape. Vision results showed 12% minor errors, the different zone size is reported as the same result, 2.5% false resistance and 0 false sensitive results when compared to BMS results.
Conclusion: This new technology produces accurate, reproducible results for measuring zones of inhibition on disc diffusion antimicrobial susceptibility plates. This technology has no associated additional reagent cost and reduces the amount of time required by BMS staff to interpret these images. Additional technology can recognise the antimicrobial discs, and combined these results could be linked to software capable of interpretation according to published zone sizes and rules for the identification of resistance mechanisms. At this time BMS are still required to validate the Vision results before reporting.

Methods for antibacterial susceptibility testing

R2653 Comparative evaluation of three automated systems for detection of different mechanisms of resistance in bacteria: results gathered in an ESCMID Postgraduate Technical Workshop in Izmir, Turkey

Z. Gulay, M. Bicmen, A. Sari on behalf of invited faculty for practical sessions and participants at the ESCMID PGTW (Izmir, Turkey)*

Comparative evaluation of the results for microorganisms with special resistance mechanisms analysed by different commercial systems in PGTW on ''Clinical Implications of Antimicrobial Susceptibility Testing'' which took place at the Department of Medical Microbiology of DEU, Faculty of Medicine (Izmir, Turkey) between March 11 and 13, 2011 was intended.
Four Gram negative clinical isolates (Amp-C positive *E. cloacae*, GSBL positive *E. coli*, OXA-48 and GSBL positive *K. pneumoniae* and NDM-1 positive *K. pneumoniae*) and three Gram positive cocci (a penicillin resistant *S. pneumoniae* clinical isolate, a vanB positive *E. faecium* and heteroVISA Mu3) of which resistance mechanisms were defined completely by disk diffusion, E test, and molecular methods, were studied by two different groups of participants and by three different automated systems (BD Phoenix, VITEK 2 and MicroScan). The results obtained by Vitek 2 were the same in both of the groups whereas reproducibility for BD Phoenix and MicroScan was found as 96.9% and 95.8%, respectively. Categorical agreement for Vitek 2, BD Phoenix and MicroScan was calculated as 95.8%, 90.6% and 88.5% and the error rates in the same order were 1.1%, 4.2% and 5.2%, for very major errors, 1.1%, 2.1% and 2.1%, for major errors, and 2.1%, 3.1% and 4.2% for minor errors, respectively.
All resistance mechanisms were determined by Vitek 2 accurately. Phoenix failed to detect vancomycin heteroresistance in *S. aureus* and MicroScan both Va heteroresistance and AmpC production in Enterobacter. For the two carbapenemase positive isolates, the results for the carbapenems (imipenem and meropenem) were in a total inagreement (S vs. R; very major error) by the disk diffusion and the Vitek 2 system. Nevertheless, the strains were reported as resistant by Vitek 2 as detected a carbapenemase ''KPC or Metallo-beta-lactamase''.
In conclusion, it was seen that Vitek-2 was the most successful system in comparison with the other systems in reproducibility and detection of the resistance mechanisms present. However, in spite of changing susceptible results to resistant for IMP and MEM (MIC ≤1 μg/mL) in the two carbapenemase positive strains which were obviously resistant by disk diffusion (inhibition zone=6 mm), has caused suspicions about the reliability of this system as current recommendations by EUCAST and CLSI do not include changing susceptibility results according to the resistance mechanism. This was also criticised by the participants.

R2654 Standardising a procedure for the evaluation of antimicrobial activity of wound dressings and assessment of three wound dressings

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Objectives: A wide selection of wound dressings is available on the market with varying claims of antimicrobial efficacy. A valid standard method for evaluation of their antimicrobial activity has not been established. In this study we suggest a standardised time kill assay procedure for antimicrobial activity evaluation of wound dressings in order to make studies more comparable and reproducible. We also tested two silver-containing and one propolis-containing dressing against *S. aureus*, *S. pyogenes*, *E. coli* and *P. aeruginosa* using our proposed procedure.
Methods: The following dressings were tested: silver-containing dressings Aquacel Ag (Conva Tec Inc., Uxbridge UK), Acticoat (Smith & Nephew, Hull, UK) and a propolis-containing dressing (PCD) that is in development. Sterile gauze was used as a negative control. Squares of 1 cm^2 of each dressing were placed in a suspension of each organism in the peptone water and either Brain Heart Infusion broth or Mueller-Hinton broth (Oxoid Ltd., Basingstoke, UK) was added to each dressing. After incubation at 37°C for 48 hours aliquots of bacterial broth were taken from each vial at specific time intervals and spread on to plates with Iso Sensitivity agar (Oxoid Ltd.). The plates were incubated overnight at 37°C and bacterial counts (CFU/mL) were performed.
Results: Acticoat exhibited the most potent antibacterial activity against all organisms. Aquacel Ag also demonstrated good antibacterial activity although inferior to Acticoat. Propolis-containing dressing did not show bactericidal effect, only marginally slowing down growth of *S. aureus* and *S. pyogenes* compared to the sterile gauze.
Conclusion: We suggest a standardised time kill assay for evaluation of the antimicrobial activity of wound dressings. The dressings should be exposed to a standardised inoculum of approximately $1–1.5 \times 10^6$ CFU/mL which is consistent with that used for antibiotic sensitivity testing in laboratories. Mueller-Hinton broth is the most appropriate medium for this assay as it supports the growth of most

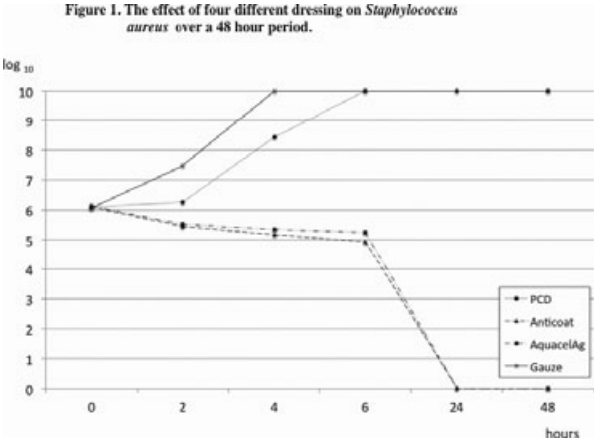

Figure 1. The effect of four different dressing on *Staphylococcus aureus* over a 48 hour period.

common wound pathogens and does not contain inhibitors. A 1 cm^2 is the most suitable and practical size of dressing. Acticoat was the most active dressing of those tested using this method.

Table 1: AST results fermenters		S	I	R	%S	%I	%R
Ampicillin	EUCAST	N/R	N/R	112			70,89
	CLSI	47	0	111	29,75	0,00	70,25
Amoxicillin-clavulanic acid	EUCAST	N/R	N/R	58			36,94
	CLSI	99	16	42	63,06	10,19	26,75
Piperacillin-Tazobactam	EUCAST	143	5	6	92,86	3,25	3,90
	CLSI	148	2	4	96,10	1,30	2,60
Ceftriaxone	EUCAST	129	1	16	88,36	0,68	10,96
	CLSI	130	3	13	89,04	2,05	8,90
Ceftazidime	EUCAST	130	0	21	86,09	0,00	13,91
	CLSI	131	3	17	86,75	1,99	11,26
Cefepime	EUCAST	144	7	7	91,14	4,43	4,43
	CLSI	150	0	8	94,94	0,00	5,06
Meropenem	EUCAST	157	2	0	98,74	1,26	0,00
	CLSI	159	0	0	100,00	0,00	0,00
Amikacin	EUCAST	149	2	7	94,30	1,27	4,43
	CLSI	151	1	6	95,57	0,63	3,80
Levofloxacin	EUCAST	127	5	25	80,89	3,18	15,92
	CLSI	131	3	23	83,44	1,91	14,65
Trimethoprim	EUCAST	119	2	36	75,80	1,27	22,93
	CLSI	120	0	37	76,43	0,00	23,57
Nitrofurantoin	EUCAST	89	0	22	80,18	0,00	19,82
	CLSI	84	5	22	75,68	4,50	19,82
Tigecycline	EUCAST	130	4	4	94,20	2,90	2,90
	CLSI	130	5	3	94,20	3,62	2,17

R2655 Evaluation of a Gram-negative EUCAST panel (NBC46) on the Siemens MicroScan® WalkAway®-96 Plus system

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Objectives: The aim of this study was to evaluate the gram negative EUCAST panel (NBC46) for identification (ID) and antimicrobial susceptibility testing (AST) of gram negative rods on the Siemens MicroScan® WalkAway®-96 Plus System (Microscan) and to investigate the impact on susceptibility reporting due to the shift of MIC breakpoints from CLSI to EUCAST.

Methods: During 1 month, all gram negative rods isolated from clinical samples in daily routine were examined using both the CLSI panel (NBC42) and the EUCAST panel (NBC46) on Microscan for ID as well as for AST.

Results: Of the 173 isolates (159 fermenters and 14 non-fermenters) all identifications were identical at species level, using the two different panels. The identification of fermenters resulted in 62% *Escherichia coli*, 11% *Proteus* sp., 9% *Enterobacter* sp., 7% *Klebsiella* sp., 4% *Citrobacter* sp., 4% *Morganella morganii* and 2% *Serratia marcescens*. All non-fermenters were *Pseudomonas aeruginosa*. In order to emphasize true interpretation differences between CLSI and EUCAST, 34 of 1919 (1.8%) AST results with significant discrepant MIC values were discarded. A discrepant MIC value was defined as having a difference of more than one dilution. In the group of fermenters the major differences in interpretation were found with Amoxicillin-clavulanic acid (AMC): 37% were resistant using EUCAST and 27% using CLSI. An impact on susceptibility was also observed for Cefepime, Piperacillin-Tazobactam, Levofloxacin, Meropenem and Amikacin. These antibiotics showed a reduction in susceptibility from respectively 3.8%, 3.3%, 2.5%, 1.3% and 1.3% (see results table 1).

Keeping in mind the low total number of non-fermenters, the greatest differences in AST interpretation using EUCAST were found for the following antibiotics (less susceptible): Piperacillin-Tazobactam (14.3%), Levofloxacin (14.3%) and Amikacin (7.1%).

Conclusion: We evaluated a new EUCAST panel on Microscan for ID and AST of gram negative rods. Ten percent of our routine cultured Enterobacteriaceae now reported non-resistant for AMC, would be reported resistant using EUCAST. These findings combined with the facts that AMC is the most prescribed antibiotic in our hospital and that EUCAST offers no breakpoints for S or I for this antibiotic, highlights the necessity to inform our medical staff of the impact of implementation of EUCAST guidelines.

R2656 Antimicrobial combination testing of MDR cystic fibrosis isolates of *B. cepacia*: comparison between FICI and SBPI

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Objectives: The Cystic Fibrosis Antibiotic Susceptibility Testing Service (CFASS) based at Aberdeen Royal Infirmary provides an extended susceptibility testing service for multidrug-resistant non-fermenting Gram negative bacterial isolates from the respiratory secretions of Scottish individuals with cystic fibrosis. CFASS aims to offer useful antimicrobial combinations to assist clinicians in making appropriate treatment decisions in problematic individuals.

Methods: Two hundred and thirty-three isolates which were phenotypically identified as *Burkholderia cepacia*/*B. cepacia* complex were submitted to CFASS over a 10 year period. E test was used to facilitate MIC and combination testing of the isolates. MICs of up to 21 antimicrobials were determined and interpreted as per CLSI guidelines. Usually six antimicrobial pairs were tested in combination per isolate and the results interpreted by fractional inhibitory concentration index (FICI) and also by Susceptible Breakpoint Index (SBPI) which allows easy ranking of the antimicrobial combinations by their in-vitro effectiveness.

Results: Number of isolates: 233 MIC: Number 4259% Susceptible 15.7% Intermediate 4.8% Resistant 79.5. Most susceptible co-trimoxazole (54.5%) minocycline (48.1%) doxycycline (47.2%).

Antimicrobial Combinations: Number 1595% Synergy 9.2% Non-interactive 89.3% Antagonism 1.5 Median SBPI 2.7 FICI for combinations tested ≥10 times (N = 35): Most synergy tobramycin + ceftazidime (33.3%) tobramycin + meropenem (28.6%) ciprofloxacin + piperacillin/tazobactam (25.0%). Most antagonism piperacillin/tazobactam + co-trimoxazole (11.1%) piperacillin/tazobactam + ciprofloxacin (10.0%) meropenem + co-trimoxazole (4.5%). Median SBPI for combinations tested ≥10 times (N = 35): Best combination doxycycline + meropenem (9.3) doxycycline + piperacillin/tazobactam (9.3) levofloxacin + minocycline (7.6). Worst combination ticarcillin/clavulanate + co-trimoxazole (0.5) ciprofloxacin + imipenem (0.8) chloramphenicol + levofloxacin (0.8).

Conclusions: The antimicrobial combinations which were most effective by SBPI in comparison to the ones which were most synergistic by FICI were altogether different. The SBPI is calculated based on the MIC of the antimicrobials in combination to their susceptible breakpoints and, therefore, might possibly be more discriminatory than the FICI in determining the effectiveness of the antimicrobial combinations in vitro and likely to have some clinical relevance in vivo.

R2657 Comparison of antimicrobial susceptibility testing by Etest, Kirby-Bauer disc method and Microscan® for *Pseudomonas aeruginosa* isolates from cystic fibrosis patients

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Background: *P. aeruginosa* is a major pathogen in cystic fibrosis (CF), contributing to CF exacerbations, pneumonia and ultimately respiratory failure. Treatment is frequently complicated by development of antibiotic resistance which limits therapeutic options and often restricts treatment to intravenous antibiotics; susceptibility results provide valuable information used to tailor empiric therapy to targeted antimicrobial therapy. However, regarding *P. aeruginosa* isolates from CF patients, there are many conflicting data concerning the consistency and accuracy of susceptibility results obtained from commonly used laboratory methods of susceptibility testing.
Methods: *P. aeruginosa* isolates (mucoid and non-mucoid) from CF surveillance sputum cultures were identified and tested using three different methods of susceptibility testing: E-test (ET) and Kirby-Bauer (KB) disk methods using cation adjusted Mueller-Hinton agar; and Microscan® (MS) microdilutional broth method. For each method, each isolate was tested against a pre-specified panel of antibiotics including antibiotics from the fluoroquinolone, aminoglycoside and beta-lactam classes. Susceptibilities were compared by error analysis as defined by the Clinical and Laboratory Standards Institute (CLSI) to identify and classify discrepancies between the three methods of susceptibility testing.
Results: Twenty-four *P. aeruginosa* isolates were tested. Among susceptibilities reported for ET vs. KB and ET vs. MS, all major errors observed were in the aminoglycoside group with one exception occurring in the piperacillin-tazobactam group; however, no very major errors were identified. Overall discrepancy rates between all tests were highest for aminoglycosides (21–37.5%) followed by beta-lactam antibiotics (4–16%). Overall, mucoid isolates of *P. aeruginosa* contributed to three major errors.
Conclusions: Discrepancies between susceptibility results obtained by commonly used testing methods varied widely, with the highest rate of discrepancies seen among aminoglycosides. In contrast to previous data, mucoid isolates were not associated with a greater number of major errors in this study. These discrepancies in susceptibility results have the potential to significantly impact therapeutic drug and dosing decisions, especially in the CF population. Further study is needed to characterize if a lower MIC among susceptible isolates is associated with better clinical outcomes in CF patients.

R2658 Comparison of antibiotic resistance patterns of group G streptococci in Austria using disc diffusion and the new AST-ST01 card on the Vitek II instrument

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Objectives: There is an increase of severe infections caused by group G steptococci (GGS). Therefore antibiotic resistance patterns of GGS are of interest. Aim of this study was to investigate the antimicrobial susceptibility and to compare the disc diffusion method with the new AST-ST01 card on the VITEK II instrument (bioMérieux).
Methods: From July 2008 to July 2010, 108 nonduplicate GGS isolates were collected at the Institute of Hygiene, Medical University of Graz. 24 GGS were isolated from the respiratory tract and 84 from other body sites (e.g. wounds, blood, genital tract). The isolates were characterised according their morphology and with the Streptex® GGS-specific antiserum (remel). For species identification the ID-GP card (bioMérieux) was used; resistance testing was performed by disc diffusion and with the new AST-ST01 card (bioMérieux). The AST-ST01 card includes ampicilin, benzylpenicillin, cefotaxime, ceftriaxone, clindamycin, erythromycin, levofloxacin, linezolid, tetracycline, trimethoprim/sulfamethoxazole, vancomycin and detection of inducible MLSB resistance. The antibiotic susceptibility results were interpreted according to EUCAST guidelines.
Results: All GGS isolates were identified on species level as *Streptococcus dysgalactiae* subspecies equisimilis. For susceptibility testing disc diffusion method and AST-ST01 card showed 100% consistence. All 108 GGS isolates were susceptible to ampicilin, benzylpenicillin, cefotaxime, ceftriaxone, linezolid, trimethoprim/sulfamethoxazole and vancomycin. Resistance rates were highest for tetracycline with 63% (68/108). Resistance rate for erythromycin was 14% (15/108) with only one isolate showing an M phenotype, six isolates with a consistent MLSB resistance and eight isolates with an inducible MLSB resistance leading to a resistance rate for clindamycin of 13% (14/108). Resistance to levofloxacin was found in a single GGS isolate only.
Discussion: For resistance testing of GGS the disc diffusion test and the AST-ST01 card gave identical results. The GGS isolates from Austria shows a high resistance rate to tetracycline, followed by a low resistance to erythromycin and clindamycin. Resistance to chinolones is rare.

R2659 CLSI and EUCAST: comparison of clinical breakpoints in bacterial strains isolated in paediatric haematological patients

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Objectives: It is important to analyse the impact of changes in breakpoints suggested by CLSI and EUCAST because it could be significant for the surveillance of antimicrobial resistance.
In our institute CLSI breakpoints have been used until the beginning of 2011, then we passed to EUCAST. We made a retrospective study (from 2004) to estimate how the antibiotic resistance incidence would have been if we had used EUCAST breakpoints in gram negative bacteria isolated from bloodcultures of pediatric haematological patients.
Methods: We evaluated 97 antimicrobial reports performed from 2004 to 2010 analysing MIC values of ceftazidime, meropenem, ciprofloxacin and amikacin got with automatised system Phoenix 100 (Becton Dickinson) in 30 Escherichia coli, 29 *Pseudomonas aeruginosa* and 27 KES group (*Klebsiella*, *Enterobacter*, *Serratia*) isolated from bloodcultures. We compared the interpretation by CLSI 2010 and by EUCAST.
Results: The data obtained are summarized in the Table1.

Table 1.

		Escherichia coli			*Pseudomonas aeruginosa*			KES group		
	MIC	N°	CLSI	EUCAST	N°	CLSI	EUCAST	N°	CLSI	EUCAST
Ceftazidime	<=1	27	S	S	2	S	S	18	S	S
	2				14	S	S			
	4				6	S	S			
	8				2	S	S			
	>=16	3	R	R	1	R	R	9	R	R
Ciprofloxacin	<=0.5	23	S	S	28	S	S	25	S	S
	1	1	S	R	1	S	R			
	>2	6	R	R				2	R	R
Meropenem	<=1	30	S	S	26	S	S	26	S	S
	2	1	S	S						
	4	2	S	S						
	8							1	I	R
Amikacin	<=8	27	S	S	29	S	S	25	S	S
	16							2	S	R
	>32	3	R	R						

Conclusion: From the evaluation of our results there is not any discrepancies regarding the interpretation of the MIC of ceftazidime for all bacteria groups. We had changes for ciprofloxacin in only one strain of *E. coli* and one of *P. aeruginosa*. In KES group we had one discrepancy regarding meropenem and two for amikacin. Applying EUCAST breakpoints we had a little increase of antibiotic resistance incidence and this fact could have protected by therapeutic failures.

R2660 Emerging *Klebsiella pneumoniae* isolates producing KPC-2 and VIM-1 carbapenemases in a Greek hospital

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Worldwide increase in the occurrence and dissemination of KPC beta-lactamases among Gram (-) pathogens makes critical the early detection of these enzymes.
Objectives: Accurate detection of production or co-production of class A KPC and class B metallo –beta-lactamase (MBL) *Klebsiella pneumoniae* clinical isolates.
Methods: From November 2010 – November 2011, 30 *K. pneumoniae* ICU blood isolates with reduced susceptibility to carbapenemes (MIC > 1 μg/mL) were detected. Antibiotic susceptibility to several antibiotics was estimated by Kirby-Bauer method, MIC determination by automated system (Phoenix, BD) and E-test (Biomerieux SA, France), according to CLSI standards. The isolates were submitted to phenotypic combined disk test by both EDTA and boronic acid – meropenem for the differentiation of KPC and MBL enzymes. Genotypic confirmation for the presence of carbapenemase genes was carried out by PCR and molecular typing was performed by PFGE in 11 representative isolates.
Results: The clinical isolates were mulltidrug resistant with an MIC range to meropenem (<1 to ≥2), while colistin (≤0.5–≥4), gentamicin (2–8), tigecycline (≤2–≥8) and fosfomycin (≤16–128) were the most active agents. Twenty isolates produced KPC carbapenemases, seven isolates only VIM while four produced both KPC and VIM carbapenemases. Five isolates produced ESBL additionally. Pulsed field typing gathered five isolates into subtype A1 and one in subtype A2, which is the most common type producing KPC-2 in Greece. Three isolates were included in sybtype B, the second most common type of KPC in Greece which produced both KPC and VIM . Two isolates were not genetically related.
Conclusion: Dissemination of bla KPC is mainly due to certain *K. pneumoniae* types. Fosfomycin, colistin and tigecyclin remain among the therapeutic options for KPC infections. Phenotypic and molecular tests were in accordance for detection of KPC, MBL producers and coproducers in the majority of isolates. They are proved useful tools in the clinical laboratory especially in regions were MBL and KPC represent a potential source of clinical failure in patients treated with carbapenemes.

Public health and community-acquired infections

R2661 Vaginal trichomoniasis among HIV patients attending primary health care centres of Jos, Nigeria

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Aims/Objectives: To determine the prevalence of trichomonal infection in HIV/AIDS and non-HIV control group of patients in a population of women with abnormal vaginal discharge.
Study Design: We conducted a simple cross-sectional study.
Place and Duration of Study: Primary health care centres in Jos metropolis and Jos University Teaching Hospital, during December 2006 to December 2007.
Methods: Seven hundred high vaginal swabs were collected; 350 from HIV positive and another 350 from HIV-negative control group of patients with abnormal vaginal discharge attending primary health care centres in Jos metropolis and analysed for microscopy and culture in Jos University Teaching Hospital. Data on epidemiologic indices from the patients, using structured interviewer-administered questionnaires were collected.
Results: The rate of trichomoniasis among all participants in the study was17% (n = 120/700). The prevalence rate of trichomoniasis among persons with HIV was 24% while it was found to be 10.3% among HIV negative controls. The difference was statistically significant ($\times 2 = 23.172$; df = 1; $p < 0.05$). The rate of co-infection of *T. vaginalis* in Bacterial vaginosis was 42%(n = 50/120), while it was 24%(n = 29/120) in candidiasis. The singles had a 35% high rate of trichomonal infection. The infected women had a median age of 26 years, and a median number of 3 intra-vaginal sex partners per week.
Conclusion: There was a significant statistical difference in prevalence of *Trichomonas vaginalis* between HIV/AIDS group and non-HIV (control) group of patients in the study ($p < 0.05$). Local HIV prevention strategies should target such women with trichomonal infection for intervention efforts, especially in HIV endemic area of sub-continent of Africa to further reduce the burden of HIV in the population.

R2662 Detection of influenza virus A and B in stools of adult patients reporting acute diarrhoea in general practice

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Background: Little is known about presence of influenza in gastroenteric tract of patients suffering from acute diarrhea (AD) and during a period when enteric and respiratory viruses are circulating.
Objectives: The aim of this study was to determine the presence of influenza virus A (A/H1N1 2009, A/H3N2) and B in stools of patients consulting their GP for AD.
Methods: A case-control study was performed from December 2010 to April 2011. French Sentinel GPs collected a stool sample from adult patients consulting for AD and from control patients matched on sex and age. All stool specimens were tested for influenza virus A (A/H1N1 2009 and A/H3N2) and influenza B, and also for five enteric viruses usually associated with AD (Astrovirus, Group A Rotavirus, Enteric Adenovirus 40 and 41, Norovirus-GI and Norovirus-GII).
Results: GPs enrolled 138 cases and 93 controls. Ten (7.2%, CI95%=[2.9; 11.6]) cases tested positive for influenza viruses: eight for influenza virus B, and two for influenza virus A (1 A/H1N12009 and 1 A/H3N2). Among the 10 patients, five were positive only for an influenza virus while five were co-infected with influenza and enteric viruses. None of the 93 controls tested positive for influenza or enteric viruses.
Conclusions: This study showed that the presence of influenza viruses in stools, especially influenza virus B, is not unusual among GPs' patients suffering from AD. Simultaneous investigations of enteric and respiratory viruses in patients complaining of AD, could be useful for next studies to better identify the agents responsible for AD and to understand the potential mode of transmission and interaction of these viruses during epidemic Influenza Like Illness and AD outbreaks.

R2663 Mortality and associated risk factors of *Escherichia coli* bacteraemia in a prospective cohort of young, old and very old patients

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Few data are available about the characteristics and risk factors for death of *E. coli* bacteremia in the elderly.
Objectives: To compare demographic, clinical, microbiological characteristics and risk factors for in-hospital death of *E. coli* bloodstream infections between young (18–64 years), old (65–79 years) and very old (≥80 years) patients.
Methods: French prospective multicenter cohort study (n = 1051 patients). Comorbidities (immunosuppression, organ insufficiencies and cancer), clinical (nosocomial/health care-related, portal of entry, treatment) and microbiological (drug resistance, 18 different virulence factors, phylogenetic groups) characteristics and outcome were compared between the three age groups. Chi-squared or Fisher test were used to compare qualitative variable when appropriate and

quantitative variables were compared with t test when ANOVA was significant. Host and bacterial factors associated with in-hospital death (up to 28 days) were identified by a binary logistic regression for multivariate analysis for each age group.

Results: Six hundred fifty six (62%) patients were over 65 years (372 old and 284 very old). Overall, 43.5% of young and 42.5% of old patients were immunocompromised and heart failure concerned 25.1% of the very old patients. Young and old patients' comorbidities scores were higher than that of very old patients (1.49 ± 1.26 and 1.55 ± 1.22 vs. 1.15 ± 1.15, $p < 0.0001$). Very old patients infections were more often health care associated ($p = 0.013$), of pulmonary origin ($p = 0.027$), and caused by less resistant isolates ($p = 0.011$). Presence of E. coli virulence factors did not differ between groups, except for iroN, more prevalent in the young group ($p = 0.007$). Mortality was higher in old patients than in the other two groups (10.4% vs. 16.4% and 12.0%, respectively; $p = 0.039$). Multivariate analysis of risk factors for death according to the age is shown in Table 1.

Risk factors	Survivors n (%)	Non survivors n (%)	Multivariate analysis	
			OR [95% CI]	p
Young (18-64 years)				
Cirrhosis	18(5.4)	11(27.5)	10 [3.8-27]	<0.0001
Immunocompromised	144(40.7)	27(65.9)	2.7 [1.2-6.2]	0.016
Urinary portal of entry	211(59.6)	11(26.8)	0.37 [0.16-0.82]	0.015
Polymicrobial bacteremia	12(3.4)	10(24.4)	6.3 [2.4-18]	<0.0001
Old (65-79 years)				
Cirrhosis	13(4.4)	7(11.7)	3.4 [1.2-9.4]	0.017
Immunocompromised	120(38.6)	38(62.3)	2.4 [1.3-4.5]	0.004
Nosocomial infection	58(18.6)	25(41.0)	2.6 [1.4-4.8]	0.003
ireA	90(29.0)	6(9.8)	0.27 [0.11-0.67]	0.005
Cefotaxime resistance	8(2.3)	5(12.2)	4.0 [1.0-16]	0.036
Very old (≥80 years)				
Chronic renal insufficiency	36(14.5)	12(35.3)	3.3 [1.5-7.4]	0.003
hra	169(67.6)	16(47.1)	0.42 [0.20-0.87]	0.020

Discussion: *E. coli* bacteremia is a frequent disease in the elderly population. Prognosis is poorer in old patients (65–79 years), as a consequence of the age itself and numerous comorbidities. Risk factors for death are age group specific.

R2664 Stroke-associated pneumonia in a rural general hospital

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Objectives: Stroke is the main cause of disability in high-income countries and ranks second as a cause of death worldwide. Stroke-associated pneumonia (SAP) constitutes a clinically relevant complication of stroke, because it increases the mortality and has a negative impact on the neurological prognosis of the patient. The aim of this study was to determine the incident of SAP and the microbiological data and outcome of patients with SAP in our Internal Medicine Ward.

Methods: We retrospectively investigated the medical files of all patients admitted to our Internal Medicine Ward, over a period of 5 years, with acute stroke. Patients who developed pneumonia were identified and divided into two groups: group 1 patients who developed pneumonia within the first 72 hours of admission and group 2 patients who developed pneumonia after 72 of admission. The demographical, laboratory, radiological, microbiological data and outcome at discharge of patients with SAP were registered and analyzed. Patients who had dysphagia and fever before stroke onset and those who required mechanical ventilation during hospitalization, were excluded.

Results: Three hundred and eighty-nine patients with stroke were registered, 112 (28.8%) had SAP and 64 (57.1%) of them registered at the group1. Sixty-nine (61.6%) patients were male and 43 (38.4%) female with mean age of 64 ± 13.4 years. Fifty-one (45.5%) patients had positive cultures of tracheal aspirates and 32 (28.7%) had infiltrates on their chest radiographs. The microorganisms identified in group 1 and group 2 were: *Staphylococcus aureus* 10 (15.6%) vs. 6 (12.5%), *Pseudomonas aeruginosa* 9 (14.1%) vs. 8 (16.7%), *Klebsiella pneumoniae* 0 (0%) vs. 4 (8.4%), *Streptococcus pneumoniae* 6 (9.4%) vs. 3 (6.25%), *Candida albicans* 1 (1.6%) vs. 4 (8.4%), respectively. Median length of stay was 12 days compared to 6 days for all stroke patients and the mortality rate was 36.6%. However, the mortality rate was not affected by age, gender, time of onset and results of cultures. Patients in group 2 tended to be older and had higher frequency of positive cultures of tracheal aspirate and chest radiography with infiltrates, as compared to patients in group1.

Conclusions: Pneumonia is a common complication after acute stroke and it is associated with a high mortality and prolongs the hospital stay. *Pseudomonas aeruginosa* and *Staphylococcus aureus* are most common organisms in stroke-associated pneumonia both of groups.

R2665 Evaluation of epidemiological, clinical and laboratory characteristics of patients with spondylodiscitis in a tertiary care hospital in Turkey

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Objectives: Spondylodiscitis is the infection of the intervertebral disc and the adjacent vertebrae and is hematogenous in origin in most cases. It can also occur postoperatively. In this report, we present epidemiological, clinical and laboratory data of patients with spondylodiscitis admitted in our clinic.

Methods: Epidemiologic, clinical and laboratory data of patients with spondylodiscitis were evaluated retrospectively according to hospital records.

Results: Totally 24 patients followed due to spondylodiscitis were evaluated. Two patients who had cancer metastasis to lumber vertebrae were excluded. Of the study patients, 16 were male (72.7%) and mean age of patients was 54 ± 16.3 years. There was history of fresh cheese consumption in eight patients (36.4%) and animal husbandry in 7 (31.8%). None of patients had trauma, steroid therapy or tuberculosis history. Eight patients (36.4%) had concomitant systemic diseases and three (13.6%) had malignancy. Of patients, 17 (77.3%) had undergo various operations; among them, in nine patients the operation region was vertebrae and intervertebral disc. On admission, most common symptoms were backache, difficulty in walking, pain in legs, sweating, fever and numbness in toes (100%, 40.9%, 27.3%, 22.7%, 18.2% and 18.2%, respectively). In the laboratory investigations; WBC, CRP and sedimentation rate were high in 22.7%, 63.6% and 77.3% of patients, respectively. Brucella agglutination test was positive in eight patients (36.4%). Blood and tuberculosis cultures were remained negative but ARB was found positive in the abscess material of one patient. *Escherichia coli* and *Bacillus pumilus* were isolated from cultures of abscess material in two other patients. Radiological investigations including vertebral tomography, magnetic resonance imaging and sintigraphy were compatible with spondylodiscitis, one patient's findings were resemble to tuberculosis. So, eight patients were diagnosed as brucellar spondylodiscitis, two as tuberculous and nine as postoperative spondylodiscitis. Eleven patients (50%) received ampicillin sulbactam, eight patients (36.4%) received treatment for brucellosis, two (9.1%) for tuberculosis and one (4.5%) meropenem for ESBL positive *E. coli*. Duration of the therapy was 6 weeks in 16 patients, 3 months in four and 6 months in two.

Conclusion: Operation is the main cause of spondylodiscitis. But tuberculosis and brucellosis should always be in mind as a cause of spondylodiscitis in endemic regions.

R2666 Community-acquired urinary tract infection: prevalence and resistence – a 1 year experience

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Objectives: The aim of this study was to evaluate the prevalence of the community-acquired UTI and the antibiotic resistance of the bacteria involved.
Methods: Eight thousand eight hundred and twenty-eight urine samples were analyzed from January 2010 until December 2010. Urine samples were cultured in CPS ID3 and incubated at 37°C for 24 hours. Identification and antibiotic susceptibility tests were made by the automated system Vitek 2 Compact (Biomérieux). Susceptibility to 16 antibiotics commonly used in UTIs was evaluated: amoxicillin, amoxicillin/clavulanic acid, cephalothin, cefuroxime, ceftazidime, ceftriaxone, cefoxitin, cefepime, norfloxacin, ciprofloxacin, fosfomycin, gentamicin, tobramycin, amikacin, trimethoprim/sulfamethoxazole and nitrofurantoin.
Results: According to guidelines 1594 urines (18.1%) were associated to UTI. Urinary isolates were collected from patients ≤50 years 37.8%, (602/1594), >50 years 62.2%, (992/1594). The prevalence of *Escherichia coli* was 64.9% (1034/1594) and for other Gram negative bacteria 23.6% (376/1594). *Klebsiella pneumoniae* were identified from 168 urines (10.5%) while for *Proteus mirabilis* 145 urines (9.1%). Among the Gram positive bacteria 10.4% (166/1594), *Enterococcus faecalis* was the predominant species 6.3% (101/1594) followed by *Streptococcus agalactiae* 2% (32/1594) and *Staphylococcus saprophyticus* 1% (16/1594). For *E. coli*, *K. pneumoniae*, *P. mirabilis* the antibiotics with higher frequency of resistance were ciprofloxacin (10%, 11.3%, 10.3%) in association to trimethoprim/sulfamethoxazole (27.3%, 19.6%, 29.7%), respectively. Fosfomycin showed more efficacy than others antibiotics with frequency of 1.5% (*E. coli*), 2.4% (*K. pneumoniae*) and 2.8% (*P. mirabilis*). Among *E. coli* isolates the frequency of resistant strains were less than for *K. pneumoniae* and *P. mirabilis* isolates.
Conclusion: This study shows that *E. coli* is involved in 64.9% of the community-acquired UTI, a number lower than expected, followed by other Gram negative bacteria like *K. pneumoniae* and *P. mirabilis*. *S. saprophyticus* represents only 1% of the UTI, much lower than the value described in literature where it is considered the second main agent of UTI. High resistance rates were obtained for amoxicillin, quinolones and trimethoprim-sulfamethoxazol. These antibiotics should not be used for the empiric treatment of community-acquired UTI. Low resistance rates are shown for fosfomycin and nitrofurantoin. These two antibiotics may be a good option for the empiric treatment of UTI.

R2667 Prevalence and sensitivity of viral and bacterial enteropathogens in a local hospital in Spain (2004–2010)

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Objectives: The aim of this study was to establish the prevalence and sensitivity of isolated bacterial and viral enteric pathogens in the Health Care Area of Manzanares (Ciudad Real, Spain) from 2004 to 2010.
Materials and Methods: We studied all stool samples received from patients with suspected gastroenteritis and were processed for *Salmonella* spp., *Shigella* spp., *Yersinia* spp., *Aeromonas* spp. and *Campylobacter* spp. Suspected cases of *Campylobacter* spp. were identificated using API Campy® (bioMérieux, France). In other cases, identification and susceptibility testing were carried out in the WIDER® automated system (Soria Melguizo, Spain). We used specific antisera (Bio-Rad, Spain) for the serotyping of Salmonella spp. All samples from patients younger than 5 years were tested for viral pathogens (*Rotavirus* and *Adenovirus* species) by inmunocromatographic methods (Rota/Adenoscreen® Dipstick, Microgen Bioproducts, UK).
Results: We isolated 764 bacterial pathogens from 3287 stool samples (23.2%). We observed 437 (21%) positive samples to *Rotavirus* and 96 (5%) to *Adenovirus* from 2069 and 1870 stool samples analyzed respectively. From bacterial pathogens, 326 isolates were *Salmonella* spp. (42.7%), 297 *Campylobacter* spp. (38.9%), 101 *Aeromonas* spp. (13.2%), 28 *Yersinia enterocolitica* (3.7%), 11 *Shigella* spp. (1.4%) and 10 *Vibrio* spp. (1.3%). The most prevalent *Salmonella* spp. serogroups were B (43.6%) and D (41.7%). *Campylobacter jejuni* was identified in the majority of *Campylobacter* spp. cultures. We found 73 bacterial mixed infections, 71 double and two triple and 67 (91.8%) occurred in children under 12 years. The sensitivity percentage of *Salmonella* spp. to nalidixic acid and ciprofloxacin was 61.9% and 91.9%. In *Salmonella* spp. serogroup B, the sensitivity to amoxicillin and nalidixic acid was 34.4% and 92.6% and in serogroup D of 72.6% and 52.2% respectively. We isolated three ESBL producing *Salmonella* spp. As for *Campylobacter* spp. 98.2% of isolates were sensitive to erythromycin and 12.2% to ciprofloxacin.
Conclusions: (i) The most prevalent enteropathogens in our area were *Rotavirus*, *Salmonella* spp. and *Campylobacter* spp. among the investigated pathogens. (ii) *Salmonella* spp., *Aeromonas* spp. and *Y. enterocolitica* had high sensitivity to ciprofloxacin and 3rd generation cephalosporins. (iii) The frequency of bacterial mixed infections was high in our Area, and were more common in children. (iv) Rotavirus infection was more prevalent than Adenovirus infection.

R2668 Improvement of CRB-65 as a prognostic scoring system in adult patients with community-acquired pneumonia

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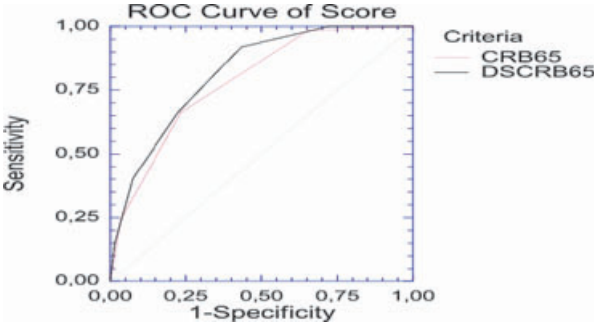
Objectives: Community-acquired pneumonia (CAP) is the leading cause of hospitalisation among infectious diseases. In accordance with the work by Dwyer et al. (Scand J. Infect. Dis. 2011; 43: 448–455) modifications were tested to improve the accuracy of CRB-65 (DS CRB-65) as a simple, but useful bedside scoring system, and to compare it with three established severity scoring systems (PSI, CURB-65 and CRB-65) to estimate risk of death within 30 days of ER assessment in patients with CAP. DS CRB-65 implies adding coexisting conditions defined according to the pneumonia severity index (PSI) rule (malignancy, liver, cerebrovascular, and renal disease and congestive heart failure), as well as peripherally measured oxygen saturation (SpO2) < 90% to the four parameters of CRB-65.
Methods: For the period December 2008 through March 2010 patients, 18 years or older with CAP, were retrospectively included from an inner city hospital. Age, gender, ICU-treatment, underlying conditions, and 30-day mortality were recorded. Patients had not been admitted to hospital during the preceding 2 weeks before enrolment. PSI, CURB-65, CRB-65 and DS CRB-65 were calculated from information given by the patient, the patient files, and laboratory databases on day of admission. Receiver operating characteristic (ROC) curves were constructed and the area under the curve (AUC) was measured to compare accuracy of the scoring systems prediction of 30-day mortality.
Results: A total of 1077 patients were included (67.9% admitted to hospital, 32.1% treated on an outpatient basis), mean age was 64.3 years, overall mortality was 5.8%, and 50.7% were male. Of the admitted patients 11.4% received ICU-treatment. The AUC, standard error, Z-value, and 95% confidence interval according to the ROC-curves of the different scoring systems were as follows – PSI (0.82, 0.02, 15.6, 0.77–0.85), CURB-65 (0.80, 0.02, 12.5, 0.75–0.85), CRB-65 (0.78, 0.03, 11.3, 0.73–0.83), DS CRB-65 (0.82, 0.02, 14.1, 0.77–0.86). There was a significant statistical difference between the ROC curve AUCs of CRB-65 and DS CRB-65 ($p = 0.004$).
Conclusion: Modification of CRB-65 (DS CRB-65), according to the PSI rule, with addition of one point for the presence of any underlying disease, and with one point if SpO2 was <90%, increased its prognostic accuracy in CAP with retained independence of laboratory data. The



modified CRB-65 may have potential to be used in ERs and outpatient clinics for assessment of prognosis in patients with CAP.

R2669 Identification of a human strain of *Streptococcus dysgalactiae* subsp. equisimilis in a dog: a new zoonosis?

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Objectives: *Streptococcus dysgalactiae* subsp. equisimilis (SDSE) is an important and sometimes lethal pathogen in humans, while it has been considered mainly as opportunistic in animals. Very limited cases of SDSE diseases have been described in dogs so far. Several zoonoses are caused by a close contact between humans and animals. Recently, a PCR protocol for distinction between SDSE of human (hSDSE) and equine origin has been optimised. The objective of this work was to evaluate if dogs may harbour human SDSE, with consequent potential impact on human health.
Methods: Forty-seven beta-haemolytic streptococci isolated from different clinical specimens from dogs have been identified by PCR. The four strains identified as *S. dysgalactiae* have been tested by PCR for identifying the subspecies and for detecting a sequence of the streptokinase (skc) precursor gene of hSDSE.
Results: Forty-one out of 47 streptococci strains have been identified as *Streptococcus canis* and four strains were *S. dysgalactiae*. One *S. dysgalactiae* strain resulted positive for the skc precursor gene specific of hSDSE, while the other three strains were negative. The dog had fever, was lacking appetite, had enlarged and painful retro-pharyngeal lymph nodes and significant increase of the tonsillar volume
Conclusion: This is the first detection of a SDSE strain of human origin in dogs. Dogs infected by hSDSE might represent a potential risk for human health. This risk should be better investigated because of the close contact between pets and humans, including children. Species identification of beta-haemolytic streptococci isolated from dogs is usually not requested in veterinary clinical microbiology for economic reasons. If SDSE transmission between dogs and humans will be confirmed, a rapid identification of hSDSE in infected dogs could be useful for preventing the bacterial transmission to humans, with particular attention to children and immunocompromised people.

R2670 Commonality among CTX-M-15-producing ST131-O25b uropathogenic *Escherichia coli* isolates from companion animals and humans in Portugal

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Objectives: To assess similarities by PFGE analysis of E. coli isolates from the sequence type 131 serotype O25-variant b worldwide pandemic clone among companion animal and human urinary tract infection in Portugal and its association with fluoroquinolone-resistance and ESBL production.
Methods: All isolates were previously identified as ST131-O25b by PCR. The veterinary community *E. coli* isolates (n = 44, 36 from dogs and eight from cats), were collected from 2004 until 2009 at the Veterinary Teaching Hospital of the FVM and at private practices in the Lisbon area. Human strains (n = 41) were isolated in hospitals and in a community Diagnostic Laboratory in the Lisbon area, during 2005 and 2006. Of these, 15 were from hospitalized and 26 from ambulatory patients. The subset of ST131-O25b isolates underwent susceptibility testing by disk diffusion, ESBL phenotyping and genotyping and PFGE analysis. PFGE digital images were analyzed using Bionumerics software version 6.6. Similarities were calculated using the Dice coefficient, with 0.5% optimization, a maximum position tolerance of 1.0%, and clustered by UPGMA.
Results: Thirteen (31.7%) ST131 human isolates were CTX-M-15 beta-lactamase producers (seven *E. coli* isolates from community-associated UTI and six from hospitalized patients). Five (11%) CTX-M producer E. coli isolates were isolated from three dogs (2 CTX-M-15 and 1 CTX-M-32) and two cats respectively (1 CTX-M-15 and 1 CTX-M-32). All human and animal ESBL-producer isolates were also ciprofloxacin-resistant. The ESBL-producer isolates also harboured simultaneously the blaTEM and blaOXA-1 genes (nine human and two animal isolates). PFGE analysis showed 100% similarity between one human nosocomial and a dog community both CTX-M-15-producer isolates and these clustered (>85% similarity) with two other human nosocomial and cat CTX-M-15-producer isolates.
Conclusions: We had previously demonstrated that the ST131-O25b pandemic *E. coli* clone is a prevalent clone in the Lisbon area in Portugal and that the majority of these isolates lack ESBL genes. Nevertheless, the present study shows the similarity of CTX-M-15-producer *E. coli* ST131-O25b clone across host species. Our findings confirm that the transfer of resistance markers and resistance isolates between animals and owners/caretakers is a strong possibility either by infection or direct contact. Companion animals may play an important role in the dissemination of the ST131-O25b pandemic *E. coli* clone in the community.

R2671 Community-aquired urinary tract infections: spectrum of causative agents and susceptibility to common antimicrobials. A 4-year study

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Objectives: This study was performed to investigate the spectrum of bacteria associated with community-acquired bacteriuria and their resistance patterns to antibiotics commonly used as empirical therapy.
Methods: During a 4 years period (September 2007–September 2011) a total of 3.126 urine samples from outpatients with urinary tract infection (UTI) symptoms were submitted in our laboratory and cultured by conventional methods. Species identification and susceptibility testing was performed with the Wider system (Soria), while the phenotypic detection of the production of extended spectrum b-lactamases (ESBL) was performed by the double disk synergy test, the combined disk test and the two-sided E-test, when necessary, on M. H. agar according to CLSI criteria.
Results: Bacterial pathogens were identified in 1.236 samples (39.5%). *E. coli* encountered more frequently (73%), followed by *P. mirabilis* (6.4%), *K. pneumoniae* (5.3%), several Gram negative bacteria (5.7%), *E. faecalis* (4.5%) and *S. saprophyticus* (1.9%). ESBL were detected in 15.3% of *K. pneumoniae* strains, 6.3% of *P. mirabilis* and 2.1% of *E. coli*. Susceptibility testing of Gram (–) isolates to most commonly used per-os antimicrobials Ampicillin, Amoxicillin/clavulanic, Cefuroxime, Trimethoprime/sulfamethoxazole and Norfloxacin revealed resistance 44.6%, 9.5%, 9.2%, 26.7% and 8.7%, respectively. *Enterococci* exhibited resistance to Norfloxacin 8.9%, while all *S. saprophyticus* strains were susceptible at Trimethoprime/sulfamethoxazole and Norfloxacin.
Conclusions: *E. coli* remains the most prevalent causative agent of community-acquired UTI (73%). Resistance rates to quinolones, amoxiccilin/clavulanic and per-os cephalosporines remained low and

it seems that these agents can still be used in empirical antimicrobial therapy of uncomplicated UTI. On the other hand, high resistance rates to trimethoprime/sulfamethoxazole and ampicillin indicates that their use in therapy of UTI may lead to treatment failure.

R2672 Absence of faecal shedding of Listeria monocytogenes in the Netherlands

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Objectives: In the Netherlands, *Listeria monocytogenes* faecal carriage of up to 70% has been described. *Listeria* is usually acquired by consuming food contaminated with *L. monocytogenes*. Because of its high mortality (up to 30%), listeriosis is a major public health concern and the Community legislation lays down food safety criteria for *Listeria* in risk products.
The aim of this study was to determine faecal shedding of *L. monocytogenes* using culture and PCR.
Methods: A total of 437 stool specimens was collected from four different patient groups (267 gastro-enteritis patients, 91 immunocompromised patients, 45 pregnant women and 35 chronic bowel disease patients, respectively). Culture was performed using cold enrichment and PALCAM selective agar plates. PCR was performed using a primer-probe combination as described by Oravcová et al., targeting the actA gene.
Results: None of the faecal samples was *L. monocytogenes* positive, neither culture nor PCR positive. 4.8% (20/437) of the samples could not be assessed using PCR due to inhibition of the PCR reaction.
Conclusion: Compared to previous Dutch reports in the seventies of the last century, faecal shedding of Listeria has dramatically declined in the Netherlands. Since publication of these reports there have been major changes in regulations on food safety. Our data suggest that the current food safety policy in the Netherlands regarding *L. monocytogenes* control is highly successful.

R2673 Prevalence and risk factors of HIV, hepatitis B virus and syphilis among pregnant women on Mayotte Island, Indian Ocean, 2008–2009

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Objectives: Indian Ocean's Islands have been described as areas with a high prevalence of sexually-transmitted infections (STIs) except human immunodeficiency virus (HIV) infection. The objective was to assess the prevalence of infection by HIV, hepatitis B virus (HBV) and syphilis and their risk factors among pregnant women (PW) of Mayotte Island, Indian Ocean.
Methods: A cross-sectional study was conducted at 11 antenatal clinic centers of Mayotte Island's from September 2008 to September 2009. All PW attending for their pregnancies in the participating centers were eligible if they conformed to the following inclusion criteria: a screening at consultation done for HIV, HBV, and syphilis, and provide written informed consent. Among the 13 antenatal clinic centre, two were not included for practical reason. One because of a very low activity and the other because it was located on another island. Socio-demographic and behavioral characteristics were collected by interviewer-administered questionnaire. Blood samples were obtained for testing HIV, HBV, and syphilis. Risk factors were analyzed by logistic regression.
Results: Of the 708 eligible PW, 671 (94.5%) consented to participate. No prevalent case of HIV infection was detected. The prevalences of hepatitis B surface antigen (HBsAg), active syphilis (RPR+TPHA+) and any sexually transmitted infection (STIs) (at least one selected STIs marker) were 3.4%, 2.1%, and 5.4%, respectively. HBsAg positive test was associated with being born in Madagascar, and having a casual partner. Lack of education, partner's refusal to use condom and history of STIs were associated with active syphilis. STI pathogens were associated with lack of education, age 15 years or over at first sexual intercourse, having a casual partner and a history of STI.
Conclusions: The continuing low prevalence of HIV and high incidence of STIs confirmed the Indian Ocean situation with HIV/STIs. Identifying the factors associated with this phenomenon could help to prevent an HIV epidemic in low HIV transmission areas such as Mayotte Island.

R2674 Impact of changes in the surveillance system on the epidemiology of pertussis over 20 years in Catalonia, Spain

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Objectives: Pertussis is an infectious disease that causes important morbidity in developed countries. In Catalonia, a region in the Northeast of Spain with 7.5 million inhabitants, organized in 41 counties, pertussis surveillance began in 1980, when notification was by numerical report. Since then, the surveillance system has undergone various changes. In 1997, there was a change from numerical to individual report, with physicians being required to complete a report form for each case. In 2003, a program aimed at increasing laboratory diagnosis of pertussis was begun. The aim of this study was to analyse the changes in pertussis surveillance in Catalonia and their impact on reported disease incidence in rural and urban areas.
Methods: The 20-year study period (1990–2009) was divided into three periods: 1990–1996; 1997–2002; and 2003–2009. Rural and urban counties were defined according to population density. The mean population density of Catalonia was calculated. Counties with a density above the mean (≥201 inhab/km^2) were considered as urban areas and counties below the mean (<201 inhab/km^2) as rural. The incidence rate of pertussis was estimated for each period. The RR between rural and urban areas and the 95% CI were calculated, with rural areas taken as the reference value. Statistical significance was established at an alpha level of 0.05.
Results: A total of 7251 cases were reported to the Department of Health during 1990–2009. In 1990–1996, 5064 cases were reported with an incidence rate of 11.92×10.5 persons-year. In 1997–2002, 425 cases were reported with an incidence rate of 1.13×10.5 persons-year. In 2003–2009, 1762 cases were reported with an incidence rate of 3.52×10.5 persons-year. The RR obtained comparing the rates of urban and rural counties in 1990–1996 was 1.43 (95% CI 1.34–1.54; $p < 0.001$). In 1997–2002, there were no differences between urban and rural counties (RR:1.10; 95% CI 0.89–1.37, $p < 0.358$). Rates were again higher in urban compared to rural counties in 2003–2009 (RR:1.36; 95% CI 1.20–1.53; $p < 0.001$).
Conclusions: The change from numerical to individual report was associated with a reduction reported cases. However, when laboratory facilities were introduced, the detection of incidence increased. In the periods when high incidence was observed, urban areas showed higher rates than rural areas. This underlines the importance of taking into account the surveillance system when changes in the epidemiology of pertussis are observed.

R2675 Laboratory surveillance of polio and other enteroviruses circulation in Latvia

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Objectives: Latvia had been recognized by WHO as polio free country in 2002, but the possibility to import wild polio virus from endemic countries or vaccine-derived poliovirus (VDPV) with regained neurovirulence, can become new challenge for the eradication of polio. Laboratory surveillance revealing of enteroviruses in clinical materials, especially from patient with acute flaccid paralysis as well as virus findings in environmental samples is background for completing

WHO programme and the main control procedure for polio circulation in Latvia.

Methods: Clinical materials as faeces, CSF, pharyngeal swabs, post mortem collected specimens, sewage were collected and analysed in according to the WHO recommendation. Three different cell lines: L-20B, Hep-2C, RD (A) were used for enterovirus isolation. Isolated polio and enteroviruses strains were characterized for serotype on the base of neutralization reaction with specific antisera. Intratyping diagnostics have been completed at the National Institute of Health and Welfare, Helsinki-Collaborating Center of WHO Global Polio Laboratory Net.

Results: The last wild polio virus in Latvia had been detected in 1965. It was WPV1 isolated from sewage. Since this year only polio vaccine associated viruses have been detected in Latvia. From 2000 to 2008 years 11569 samples were laboratory examined. Polioviruses were found in 152 samples of sewage water (Sabin PV1- 54, PV2-48, PV3-50) and in 29 ones of faeces (Sabin PV1-14, PV2-5, PV3-10). The last polio vaccine associated viruses were detected in 2008. No one *Poliovirus* was found in 2009 and 2010 years. In September of 2011 PV1 has been isolated from RIGA international airport's sewage water and characterized as polio vaccine associated virus in WHO CC laboratory. Intensified sewage sampling and diagnostics weekly from the airport and main Riga city water refine buildings no one poliovirus was detected. Active circulation of others enteroviruses was detected in all years. From last events, in 2010 dominated Echo 30 (104 cases) and Coxackie A9 (70 cases), that was the main cause of the big aseptic meningitis outbreak in south-eastern Latvia.

Conclusions: During 2 years period, after introducing inactivated polio vaccine in Latvia in 2006, laboratory detected only sporadic polio vaccine associated viruses and no one in 2009–10 years. The source of PV1 Sabin, isolated in 2011 from the airport's sewage, is unknown; rather it was imported from countries, using oral polio vaccine.

R2676 The evaluation of 46 cases presenting with rare adherence and complications due to brucellosis

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Objectives: The study was established in order to take care of the physicians from all departments rather than Infection Diseases about diffential diagnosis of brucellosis that can present atypical adherence and complications, also imitate other infectious and non-infectious diseases.

Methods: Between January 2002 and October 2011, totally 487 cases with brucellosis were followed and treated prospectively in our clinics. Forty-six of these cases who did not have spesific sign, symptoms and/or laboratuary findings about brucellosis and also presented unexpected adherence and complications were included to study.

Table 1. The distribution patern of the patients due to affected systems and organs with symptoms at application time

Organs/Systems	Rare adherence and complications	Case number	Symptoms at application time
Muscle-Skleton System	Cervical spondylodiscitis	3	Neck pain
	Cervical + toracal spondylodiscitis	1	Neck and back pain
	Sternoclavicular osteomyelitis and abcess	1	Sternal swelling and pain
		4	Shoulder pain, swelling, fever
	Tendinitis, Tenosynovitis	3	Abdominal pain, fever
	Psoas abcess	1	Knee swelling, redness, pain
	Knee prothesis infection		
Cardiovascular System	Native aortic and mytral valv endocarditis	1	Fever, dyspnea
		1	Fever and chest pain
	Native mytral valv endocarditis in a case with aortic valv replacement		
Hematologic Adherence	Disseminе intravascular coagulation	1	Gingival and nasal hemorragia
	Cause of painful crisis in cases with sickle cell anemia	3	Fever, prevalent severe bone pain
Nerve System	Transverse myelitis	1	Unable to walk, urinary retention
	Miller Fisher Syndrome	1	Diplopia, unable to walk Unbalanced walking, vertigo
	Cerebellar abcess	1	Cranial palcik
	Progressive spastic paraparesis	1	Muddy vision and diplopia
	Optic neuritis and vi abducence nerve paralysis	1	Sightless, headache, fever
	Ophthalmoparesis, papillitis, pancardiac hemorragia, endocarditis	1	Muddy vision, fever, diplopia
	Supranuclear ophthalmoplegia	1	Quadriplegia, diplopia
Genitourinary System	Urinary tract infection	2	Dizuria
	Ovarian abscess	2	Abdominal pain
	Testicular abscess	1	Testicular swelling
Skin and Mucosal Adherence	Maculopapuler eruptions	2	Total body eruption
	Erythema nodosum	2	Painfull crural swelling
	Frontoflum lesion	1	Knee cruel
	Palmar erythema	1	Palmar redness and pain
	Molar eruption	1	Buccal mucosal hyperemia
	Palmar eczema	1	Palmar redness, pruritus
Several Tissue and Organs Adherence	Fibrotic-peritonitis	1	Abdominal pain, cough
	Orchitis-peritonitis septs	1	Abdominal pain, fever
	Pneumonias and pleural effusion	1	Dyspnea, cough
	Splenic abscess	1	Fever
Other	Seronegative brucellosis	2	Fever
	Cause of febrile neutropenia	1	Fever

Results: Seventeen of the 46 cases were female and 29 of them were male. The mean age was calculated as 40.8 years. Thirty-six of the cases were detected as acute, while nine of them subacute and one of them chronical brucellosis. *Brucella melitensis* was isolated in blood samples of 17 cases, urine sample of one case, both urine and blood samples of one case, abcess samples of four cases, blood sample and mitral valv of one case, pleural and peritoneal fluid samples of one case. The distribution patern of the patients due to affected systems and organs with symptoms at application time was presented in Table 1. Also, it was detected that 37 (80.4%) of the cases were initially evaluated by different clinics rather than Infection Diseases.

Conclusion: As seen in our study, an important proportion that can not be underestimated (9.4%) of brucellosis might progress with atypical clinics and be evaluated by different clinics rather than Infection Diseases. The treatable disease can be easily overlooked if it is not mentioned in differantial diagnosis in endemic regions. Therefore, we think that the differantial diagnosis of brucellosis might be kept in mind by not only the specialists of Infection Diseases but also the physicians of other branches, too.

R2677 Evaluation of 89 cases of *Brucellar spondylodiscitis*

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Objective: Spondylodiscitis due to brucellosis as well as longer duration of treatment required during the diagnosis and computed tomography with magnetic resonance imaging examinations such as the use of advanced disease with focal organ involvement is one of the most troublesome. In our study, we followed the patients were evaluated with brucellar spondylodiscitis.

Methods: Our clinic between March 2004-October 2011 total of 487 cases of brucellosis were monitored prospectively, and these patients 89 (18.2%) cases were identified spondylodiscitis The diagnosis of spondilodiscitis was established by physical examination and radiological findings obtained by diagnosting imaging tools.

Results: Total 89 cases, while 49 men and 40 women mean age 58.02 (age range:26–86 years), respectively. Fifty one cases (57.3%) acute, subacute, 34 (38.2%) and 4 (4.4%) were considered chronic. Three patients (3.3%) relapsed with a spondylodiscitis is determined. The most common site of lumbar spine involvement in 64 (71.9%), respectively, while the thoracic and cervical spine involvement was found to be 25.8% and 2.2%. Thirty-six cases of blood culture both yielded *Brucella* spp. In addition to the twenty-eight cases of paravertebral abscess, spondylodiscitis, 20 cases of epidural abscess and one patient was found intraosseous abscess. In two patients with neurological deficits due to surgical treatment is applied to identify other medical treatment was performed in all cases. Medical treatment was performed at least 4 months. All patients had healed without sequelae.

Conclusion: In our study, approximately one fifth of spondylodiscitis seen almost all cases of brucellosis with the most common cause of focal involvemed organ. Contrary to expectations, a large portion of patients diagnosed with acute or subacute period, and again a large part of the paravertebral and epidural abscess has been identified as a serious involvemed. Spondylodiscitis due to other factors in different cases of epidural and paravertebral abscess is improved with medical treatment without the need for surgical treatment. This is so serious, especially in endemic areas, brucellosis should be considered in the differential diagnosis of vertebral involvement in cases of spondylodiscitis.

R2678 Measles epidemic in Istanbul – a measles-free metropolitan area for the past 5 years

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Objectives: Annual average of measles cases reported in Istanbul with a total population of 13 million people was 500 cases whereas approximately 3000 cases were reported in the epidemic years. A

pleasant successful point has been achieved in the elimination of measles disease due to the continuous vaccination process that has been performed in the children between ages of 0–14 years and has reached the rates over 95%. Only seven imported cases have been reported in this notifiable disease during the 5-years term since 2006 in Istanbul. However, a measles epidemic has been observed in Istanbul at the beginning of the year 2011 that has affected the adults around 30-ages. The confirmed 42 (including nine medical staff), 14 and 38 cases were between 19–35 ages interval, below 1-year of age and in childhood, respectively.

Cases: The six cases followed in our hospital ranged between 22–32 ages and four cases were female (including one case with 33 weeks gestational age). All of the patients were clustered in the same region however there was no association. Their application dates were between 19th February and 25th March. While all the patients were found to have typical maculopapular rash, fever, measles IgM positivity, elevated ALT and AST values; Koplik's spots were found in four patients. Of the patients, three of the patients were evaluated to have allergic eruption at the first hospital admission. While pneumonia was detected clinically and radiologically in four patients, *H. influenzae* was isolated from sputum in one patient. Of the three cases that were medical staff, one has shown disease symptoms 7 days after the vaccination and bilateral viral pneumonia developed in the patient.

Conclusions: Measles is a vaccine-preventable disease. However, some problems have been experienced in the diagnosis of the recent cases in Istanbul and the patients could have been diagnosed only after they have received several treatments for different diseases since awareness and familiarness level of the disease decreased between people and medical staff by the years. The presence of the susceptible cohorts of the people complicates the elimination of the disease.

Emerging infectious diseases

R2679 Ongoing circulation of a novel sandfly fever virus variant, sandfly fever *Turkish virus* in Ankara province, Turkey

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Objectives: Sandfly Fever Turkish Virus (SFTV), a variant of Sandfly Fever Virus (SFV) Sicilian serotype, was identified in Turkey during an investigation of an outbreak of a febrile disease of unknown origin. SFTV is associated with sandfly fever, a self-limited febrile disease common in Mediterranean basin, but with a severe clinical picture with elevated liver enzymes and thrombocytopenia and requiring up to 30 days for complete recovery. The objective of this study was to identify the acute infections with SFTV at a research and training hospital in Ankara Province, Turkey, where cases had been observed previously between 2007 and 2009. West Nile virus (WNV) infections, which was responsible for an outbreak in Turkey in 2010 and has to be considered in the differential diagnosis of sandfly fever, were also investigated.

Methods: A total of 16 serum and plasma pairs from patients with suspected SFTV during July-August 2011 were included in the study with informed consent. Inclusion criteria were defined as fever, accompanied by any of the following: malaise, abdominal discomfort, photophobia, headache plus leukopenia, thrombocytopenia and elevated liver enzymes. For the detection of SFTV and other SFVs, a nested PCR with *Phlebovirus* consensus primers, targeting the viral polymerase (L segment) were employed. Amplicons from the PCR were subjected to cycle sequencing for identification. SFV IgM antibodies were detected via a commercial immunofluorescence assay (IFA, Sandfly fever virus IgM mosaic I, EuroImmun, Germany). All sera were also evaluated in parallel for WNV using a commercial assay (West Nile virus IgM IIFT, EuroImmun, Germany) for antibodies and an in-house reverse transcription real-time PCR for viral RNA.

Results: All samples were negative for WNV RNA or IgM. A total of four samples (4/16, 25%) were reactive in Phlebovirus consensus PCR. Sequencing of the amplicons revealed sequences similar to previously published SFTV L segment, distinct from other SFV types. In eight sera, SFV IgMs were identified. In three PCR positive samples (3/4,75%), IgM antibodies to Sicilian, Cyprus or Toscana serotypes were detected via the commercial IFA.

Conclusions: Symptomatic cases due to acute SFTV infections were demonstrated in this study via generic PCR and cycle sequencing. These findings confirm the ongoing circulation of this novel SFV in the region where it was originally identified.

R2680 *Burkholderia cepacia* complex in adult cystic fibrosis patients: species, sensibility and other micro-organisms associated

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Objectives: The *Burkholdderia cepacia complex* (Bcc) species are important opportunistic pathogens with intrinsic antibiotic resistance. They cause devastating infections in patients with cystic fibrosis (CF) and other vulnerable individuals. The aim of this study was to describe the distribution of species, Bcc antimicrobial susceptibility profiles and its relation with other microorganisms isolated in sputum sample isolated from adult CF patients examined in a cystic fibrosis unit at Hospital Universitario de La Princesa (Madrid).

Methods: From March 2009 to June 2011, all sputum samples from CF patients were cultivated following standard procedures for these samples. Bcc was isolated in the BCSA (B cepacia selective agar) medium. The identification and susceptibility was performed by Walk away (SIEMENS). The determination of the species status of *B. cepacia* complex strains was carried in a reference laboratory (Carlos III, Majadahonda) by sequentiation ribosomal 16S. The susceptibility break points cosidered were according to the Clinical Laboratory Estandar Institute guideless (CLSI).

Results: Bcc was isolated from eight out of 70 patients (11.4%), one of them Bcc was eradicated. 62.5% were women and 37.5% were men. The average age was 26 years old and pulmonary function expressed in FEV1 in average was 64.6%. Four patients (50%) had *B. cenocepacia*, one patient had *B. cepacia*, other one had *B. multivorans*, other one had *B. vietnamiensis*, other one had *B. stabilis* and other one patient had *B. contaminans*. One of them had two different species (cenocepacia and contaminans). 40% of Bcc was susceptible to ceftazidime, 20% to levofloxacin, 87.5% to meropenem and 72.5% to cotrimoxazol. Three patients who were colonized by Bcc were also colonized by *Staphylococcus aureus*.

Conclusions: We found a high rate of patients colonized by Bcc. The B. cenocepacia was the most prevalent among the Bcc isolated in CF adult patients. Meropenem and cotrimoxazol showed the best activity and levofloxacin was the less active one against our strains. In this study, we concluded that there is a certain grade of co-colonization by Bcc and *S. aureus*.

R2681 Six cases of human intestinal spirochetosis

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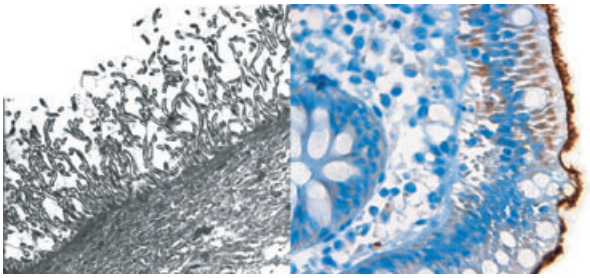
Objective: Human intestinal spirochetosis (HIS) has been associated with two species of Brachyspira: *Brachyspira aalborgi* and *Brachyspira pilosicoli*. B aalborgi has been isolated mostly from human and B pilosicoli from animals. Prevalence rates of HIS are variable, being higher in poorly developed areas or in patients infected with HIV. Although HIS is usually an incidental finding there is strong evidence to be a cause of diarrhea, diffuse abdominal pain or gastrointestinal bleeding. Our aim in this work was to identify to species level the cases of HIS that were diagnosed in our Hospital by the Pathology laboratory and compare them with previous papers.

Methods: From 2002 to 2011 six cases of heavy colonization with spirochetes were diagnosed in the Pathology laboratories of our Hospitals. The stains used in the diagnosis were Hematoxylin-eosin in a

first step and Warthin-Starry for confirmation of cases of typical hematoxyphilic fringe on the brush border. The extraction of the DNA from the samples fixed in formalin and embedded in parafinn wax was performed following the protocol of the Instituto de Salud Carlos III by using QIAamp DNA mini kit. Generic primers for all Brachyspira species were designed targeting the NADH-oxidase (nox) gene for the amplification of a 776 bp fragment Positive samples were then sequenced and analyzed. Data of age, sex, HIV serology and clinical symptoms were collected from medical records.

Results: Five of the six samples were positive to the PCR and four of them identified to species level after nox gene sequencing. Three of the samples were identified as *Brachyspira aalborgi*, one as *Brachyspira pilosicoli*; the remaining sample yielded a positive result by PCR and it is being sequencing at time to writing this abstract. All the patients were older than 60 years and only one of them was female. Except for one case that was unknown HIV serology, all were negative. Four of the patients had symptoms of diarrhea, one was asymptomatic at the time of discovery IS and for the other one there was not medical record.



Conclusion: As in previous reports, most of the diagnosis of HIS were due to B aalborgi and B pilosicoli.

None of our patients were HIV infected although is the high rate of colonization in this population. Four of our cases had symptoms compatible with the disease but despite of this none of them received specific treatment. HIS is a condition with a non difficult microbiological diagnosis, although clinical awareness can be low.

R2682 Different pathogenicity among *Corynebacterium* species isolated from clinical specimens

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Objectives: To evaluate the pathogenicity among *Corynebacterium* species isolated from clinical specimens.

Methods: During a 5 years period, we recovered 252 isolates of *Corynebacterium* species (mostly from blood cultures) from 223 in-patients. All isolates were identified by phenotypic studies including API CoryneTM V2.0 and Biolog GP2TM. 16S rDNA and rpoB amplification and sequencing were also performed. Resulting sequences were BLAST compared with the deposited GeneBank sequences. Patients were grouped according with the *Corynebacterium* specie isolated. Only groups with more than five patients-same specie involved were further analysed. The clinical significance of each isolate was categorized by means of clinical and epidemiological data as definitive, probable, possible or indeterminate. Species with more than 50% of the cases involved in definitive or probable clinical infections (group A) were compared with those involved in <50% (group B). The difference between both groups was compared by the EPIDAT program.

Results: Table shows the results with eight species responsible of definitive or probable clinical significance (between 25.0 and 84.2% of the cases). In group A we have included species highly involved in true clinical infections (between 57.1 and 84.2%) while in group B species less involved in true clinical infections are included (between 25.0 and 50.0%). The difference between both groups were highly significant: $p < 0.0001$; OR: 3.71; (IC95:1.92–7.15).

Table. Different pathogenicity among Corynebacterium species isolated from clinical specimens from 92 (41.2% of total) in-patients with definitive or probable clinical significance

Corynebacterium spp.	No. in-patients* (total)	No. in-patients* with definitive or probable clinical significance	Group
C. jeikeium	19	16 (84.2%)	A
C. ureicelerivorans	6	5 (83.3%)	A
C. urealyticum	8	6 (75.0%)	A
C. striatum	28	16 (57.1%)	A
C. afermentans subsp. *afermentans*	18	9 (50.0%)	B
C. coyleae	24	10 (41.7%)	B
C. amycolatum	71	27 (38.0%)	B
C. aurimucosum	12	3 (25.0%)	B
Total	186	92	

*one isolate per patient

Conclusions: The isolation of any *Corynebacterium* species from clinical samples must be evaluated taking into account clinical and microbiological data to consider them as true responsible for an infection. However, our study suggests that some species as *C. jeikeium*, *C. ureicelerivorans*, and *C. urealyticum* are more frequently responsible of clinical infections while *C. aurimucosum*, and *C. amycolatum* are less. Studies on pathogenicity and virulence of the genus are merited. Once considered any isolate as clinical significant, antimicrobial susceptibility data must be provided as antibiotic resistance varies but it is particularly high in *C. jeikeium*, *C. urealyticum*, *C. striatum*, and *C. amycolatum*.

R2683 Infectious spondylodiscitis: clinical-epidemiological features and therapeutic management

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Objective: Spinal infections are an emerging problem, representing 3–5% of all cases of osteomyelitis. The aim of this study was the analysis of clinical and epidemiological features of a cohort of patients with infectious spondylodiscitis.

Methods: All patients with spondylodiscitis admitted at the S. M. Goretti Hospital of Latina, Italy were included and followed up for at least 6 months. The diagnosis of spondylodiscitis was made according to clinical, radiological and microbiological criteria. All cases were classified into community-acquired (CA) and hospital-acquired (HA) spondylodiscitis; the latter were subdivided into non post-operative and post-operative, when the onset of symptoms was within 1 year from spinal surgery. For every patient, the following data were recorded: demographic characteristics, comorbidities, clinical features, laboratory data, imaging, antimicrobial and surgical treatment, outcome and complications at follow-up.

Results: The study included 32 patients (66% M and 34% F, mean age of 58 years. Twenty-one patients had CA- spondylodiscitis, seven experienced post-operative HA-spondylodiscitis and four non post-operative spondylodiscitis. Five patients suffered from TB spondylitis. Spondylodiscitis affected more often the lumbar spine (78%) and four people had also endocarditis. Unremitting pain was the initial complaint of all patients, whereas fever was reported in 69% of people. At baseline blood tests showed raised ESR and CRP in all cases, while the leucocyte count was elevated in 26%. At 6 weeks follow-up, CRP returned to normal in 29 patients, whereas ESR persisted elevated in the majority of cases. Pathogens were identified in 50% of patients. Blood cultures grew bacteria in nine cases and 11 patients (34%) underwent spinal biopsy, that detected microbes in three cases. MRI was performed in all patient. The mean time to repeat MRI was 34 days and the second MRI images demonstrated worsening changes in 40% of patients, despite clinical improvement. Antimicrobial therapy was

administered parenterally for a mean of 37 days and combinations of beta-lactams and glycopeptides or beta-lactams and a quinolone were used. Clinical improvement and dropping of inflammatory markers were observed in all patients, and no recrudescence of infection occurred within 6 months.

Conclusions: Infectious spondylodiscitis represents an emerging infection at community and hospital setting. The appropriate lenght of therapy could be addressed with comparative trials.

R2684 Infective endocarditis complications during evaluation of fever of unknown origin

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Introduction: Infective endocarditis is inflammatory process localized on valves. Intracardial complications: miocarditis, myocardial abscess, valvular ring abscess, purulent pericarditis, chord rupture, papillary muscle or septum, fistulas, rhythm disorders are common. Septic embolizations with infarcts are also possible. Neurological complications: meningitis, meningoencephalitis, brain abscess are rare.

Aim: Research frequency of single complications seen in our patients, and established association of these complications with risk factors, during evaluation of fever of unknown origin.

Material and Methods: All patients admitted with presentation of fever of unknown origin in which endocarditis was established in period 2005–2010. We diagnosed 52 patients as endocarditis, age 18–73 in the Department of clinical pharmacotherapy, Clinic for infectious and tropical diseases, Belgrade.

Results: Risk factors are previous cardiology disease and congenital cardiology defects and cardiac surgical interventions at 27.08% (cardiac surgery 12.5%). The most frequent causative microorganisms are *Staphylococci* (32.3%), *Streptococci* (26.5%) and *Enterococci* (26.5%). Neurological complications was observed in 41.67% of the cases, 35% of them had headache, conscious crisis 45%, cranial nerves disorders – lesions of facial nerve 5%, disphagia 10%, 5% with speech problems. Septic thromboses in central nerve system are diagnosed at 5% of patients. Of them, meningoencephalitis had 10%, cerebritis 25%, purulent meningitis 5%, other had minor singes. Symptoms by the CNS were marked at 90% of the patients who had neurological complications, and they appeared before infective endocarditis diagnosed. Cardiology complications detected at 25% (of them mitral valves defect 25%, aorta valves defect 58.33%, thricuspid valves defect 8.33%, rhythm disorders 8.33%, chord rupture 25%, aneurysm aorta 8.33%). Spleen abscess and cardiac abscess 2.08%.

Conclusion: Major clinical manifestation was fever, without other symptoms. Patients with neurological complications had neurological manifestations before endocarditis diagnosis were established. Cardiological and other complications are developed and diagnosed later. The appearance of neurological complications is relevant for the course, treatment and the outcome of the disease.

R2685 The results of the deep tissue culture test for diabetic foot

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Objectives: Diabetes mellitus is a prevalent disease in all ages that may have long-term effects over all body organs. Diabetic foot (DF) is a clinical entity associated with orthopedic, neurologic and the vascular structures that may be frequently complicated with infection. In this study, it has been aimed to determine and to evaluate the agents isolated via deep tissue cultures from the DF and to assess the antibiotic susceptibilities.

Methods: In 6-month period, 112 samples of deep tissue cultures for DF were sent to our laboratory for investigation. The identification of the bacteria was performed via conventional biochemical methods and Vitek Compact Systems 2 (Biomerieux, France) while their sensitivity to antibiotics was assessed via disc diffusion method accordingly with the recommendations of Clinical and Laboratory Standards Institute.

Results: Of the cases followed-up during 6 months; isolation rate of responsible agents was 10% in ones below 50 years old whereas this ratio was 31% in those above 50 years old. The 42 (37.5%) of the investigated 112 deep tissue cultures were polimicrobial materials with two different isolates. Of the determined bacteria; 24% were Gram positive cocci whereas 76% were Gram negative enteric bacilli. Antibiotic resistance rates were found 5%, 22%, 12%, 11%, 2%, 3%, 2%, and 5% in Pseudomonas strains against ceftazidime, aztreonam, amikasin, ciprofloxacin, cefepime and piperacillin-tazobactam, cefoperazone-sulbactam and imipenem, respectively.

Conclusions: Antibiotic resistance rates against the commonly used antibiotics were not found high in Pseudomonas strains that have been most frequently isolated from the cases followed-up with diagnosis of DF infection. Therefore, treatment failure of DF infection infections may depend on different factors such as adaptation of the patient and foot care, regulation of diabetes, comorbid diseases, sufficient revitalization of foot with respect to vascular aspects rather than the resistance to the applied antibiotics. The treatment of DF must be performed via multidisciplinary approach.

R2686 Two oropharyngeal tularaemia cases with pregnancy

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Introduction: Tularemia cases have been reported since 1930 in Turkey. Oropharyngeal tularemia is the most common type of the disease and transmission route is generally through contact with contaminated water. The number of human tularemia cases have increased over the past 20 years. Here we discuss two cases of pregnant women diagnosed and followed as oropharyngeal tularemia in our department.

Cases: The first case was a 23 years old woman who applied to outpatient department. She was 4 weeks pregnant and complained of high fever, chills, malaise and swelling on neck for 2 months. In physical examination, the fever was 37.5°C and oropharynx was hyperemic. There was a 2 × 1 cm size adenopathy on the right cervical region of the neck. The antibody against tularemia was detected as positive 1/320 dilution in micro-agglutination test. The patient did not accept gentamicin therapy. In the follow-up, adenopathy were distinctly decreased, body fever was getting normal and malaise was recovered. The patient and the baby were normal during and after delivery. The second case was a 33 years old woman who applied with high fever, throat pain, chills, malaise, myalgia, arthralgia and swelling on submandibular region of the neck for 3 days. She was 22 weeks pregnant. In physical examination the fever was 37°C. There was a 5 × 5 cm, tender and hyperemic adenopathy on right submandibular region. The antibody against tularemia was detected as positive 1/320 dilution in micro-agglutination test. The patient did not accept gentamicin therapy either. In the follow-up, adenopathy had suppurated spontaneously, and high fever and other complaints resolved after 1 month. After delivery the baby was also normal and the patient only had a small size adenopathy.

Conclusion: There is no anti-microbial drug in group B to treat tularemia in pregnancy. Gentamicin is recommended by guidelines. World Health Organization also recommends gentamicin or ciprofloxacin. Our patients did not accept treatment. In conclusion, two cases of pregnant women with oropharyngeal tularemia recovered without specific therapy. The recovery of these two cases could have been due to the low virulence of F. *Tularensis* subspecies holarctica which is common in Turkey.

R2687 **Oral syphilis: a review of literature and the description of five unusual clinical case**

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Objectives: To review the literature about oral lesions in syphilis and to analyze and compare clinical cases followed in the recent clinical practice.
Methods: All studies were abstracted from PUBMED using key words matched in different ways. We selected the studies where oral lesions were present in the three stages (primary, secondary, tertiary). From our clinical practice in the last 3 years we retrospectively selected and analyzed clinical data of syphilis patients with oral lesions who underwent the outpatient clinic of our unit. We performed diagnostic biopsy on the oral mucosa with immunohistochemical staining for Treponema pallidum, VDRL and FTA-abs serological tests.
Results: The analysis of literature revealed twenty-one articles of oral syphilis concerning oral lesions in thirty patients from 1954 to August 2011 of which ten cases of primary stage (33%), sixteen of secondary stage (53%) and four cases of tertiary stage (13%). The primary lesions were always represented by ulcers, of which eight were asymptomatic and two were painful. The secondary lesions were represented by an heterogeneous pattern: solitary or multiple ulcerations (six cases), mucous patches (three cases), leukoplakia-like lesions (two cases), erosive lesions (one case), aphtae (one case) and tonsillar swelling (one case). All tertiary lesions are gumma, of which two were on the hard palate, one on the soft palate and one on the gingiva. Our cases had all secondary syphilis with an unusual clinical aspect: one with keratotic, bilateral plaques of the hard palate, one with heritematous, bilateral lesions of the hard palate, one with an ulcer with neoplastic aspect, one polymorph keratotic lesions with reticular lichenoid-like pattern on the buccal mucosa, one with vesciculo-bullous lesions on the gingiva and tongue. All the diagnostic tests performed were positive for the diagnosis of syphilis.
Conclusion: As shown by the review of literature, syphilis rates have increased dramatically in recent years. Data are in line with our clinical experience in the last years showing clearly the increasing of rates together with the difficulties in differential diagnosis. Because of the ability of syphilis to imitate many different diseases as oral cancer, immuno-bullous diseases, lichenoid lesions, leukoplakia and eritroplakia, clinicians must be aware, having in their mind that in case of atypical clinical presentations or poor response to therapy syphilis must be considered.

Infection control

R2688 **Barrier from patient empowerment in hand hygiene programme: from different point of views of patients/ family members, health care workers, and general population in Asian culture**

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Background: ''Patient empowerment'' is a component of hand hygiene promotion programs of the World Health Organization, but little is known about the perceptions of patients/families, health care workers' (HCW) and the general population.
Methods: To assess patients/families, HCWs and the general population, a cross-sectional survey using questionnaires was conducted in a tertiary teaching hospital in Taiwan to determine attitudes about hand hygiene.
Results: A total of 1492 questionnaires were analyzed from three groups. Responses related to patient empowerment showed that 97.1% (333/336) of patients/families agreed with the concept, but only 67.8% (232/342) stated they would remind HCWs to perform hand hygiene. Responses of HCWs indicated,553 (553/880, 62.8%) would be willing to be reminded of hand hygiene by the patient/family while 151 (151/880, 17.2%) would not. Of the general population participants, 234 (234/245, 95.5%) would be willing to remind HCWs of hand hygiene, while 3 (3/245, 1.2%) said they would not.

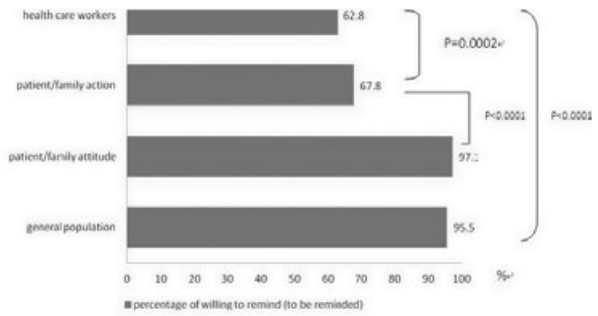

Figure 1. Different attitudes toward patient empowerment in hand hygiene among different groups

Conclusions: The attitude toward patient empowerment in hand hygiene differed among HCWs, patients/families and the general population. Further studies exploring the perception of patient empowerment from the HCWs point of view may provide benefit in designing future education programs and promotion methods related to enhanced hand hygiene.

R2689 **Faecal carriage of extended-spectrum beta-lactamase-producing Enterobacteriaceae in Korean community and hospital settings**

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Background: The production of extended-spectrum beta-lactamases (ESBLs) by Enterobacteriaceae has been a great health concern in hospitals and long-term care facilities. Recently, ESBL-producing Enterobacteriaceae (ESBL-E) were identified as important causes of community-onset infections as well as nosocomial infections. Previous studies on fecal carriage of ESBL-E were conducted in high-risk patients in the hospital setting and only a few reports evaluated fecal carriage of ESBL-E in the community setting.
Methods: A total of 384 samples from healthy individuals and high-risk patients were collected. Screening was performed using commercial chromogenic medium (ChromID ESBL agar; bioMérieux, Marcy l'Etoile, France). Identification and antimicrobial susceptibility testing were performed using the Vitek 2 system (bioMérieux). Susceptibility was determined using the new CLSI break points, and the presence of ESBLs of grown isolates were confirmed by CLSI guidelines (CLSI, 2011).
Results: The prevalence of ESBL-E carriage among high-risk patients was 55.0%, which was significantly higher than that among healthy individuals (20.3%; $p < 0.0001$). A large majority (96.6%) of the isolates from healthy individuals were *E. coli*, but *K. pneumoniae* was more commonly detected (45.0%) in high-risk patients than in healthy individuals ($p < 0.0001$). *K. pneumoniae* isolates exhibited significantly higher non-susceptibility (intermediate and resistant) than *E. coli* to CAZ (70.0% vs. 30.4%; $p = 0.002$), AMC (70.0% vs. 39.4%; $p = 0.022$), IPM (15.0% vs. 1.3%; $p = 0.025$), and MEM (10.0% vs. 0.0%; $p = 0.039$). In contrast, *E. coli* exhibited higher non-susceptibility to CTX compared to *K. pneumoniae* (100.0% vs. 85.0%; $p = 0.039$). The minimal inhibitory concentrations (MICs) for CAZ, LVX, ATM, FEP, and NN were higher for *E. coli* from high-risk patients than for those from healthy individuals. *E. coli* from high-risk patients exhibited significantly higher non-susceptibility to LVX (95.5% vs. 42.1%; $p = 0.000$) and FEP (27.3% vs. 3.5%; $p = 0.005$).
Conclusion: The prevalence of ESBL-E carriage was higher in Korea than that reported previously in other countries. The distribution and

antibiotic susceptibility of colonized ESBL-E were different between healthy-individuals and high-risk patients. Active surveillance of ESBL-E carriage is suggested for infection control and epidemiologic purposes, and the use of chromogenic agar appears to be an efficient and reliable method.

R2690 Clinical application of a molecular method based on real-time RT-PCR in epidemiological detection of the influenza A/H1N1 virus

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Objectives: Between late March and early April 2009, a swine origin of a novel influenza A/H1N1 was identified and it spread rapidly in many countries via human-to-human transmission. The next 2 years, in Italy, the diagnosis of pandemic (H1N1) 2009 was continued for epidemiological purposes. Regional Reference Centre for Microbiological Emergencies (CRREM) in Bologna compared two molecular methods based on Real Time RT-PCR. Establishment of adequate methods for the diagnosis of emerging viruses with the potential of spreading rapidly is necessary for the timely identification of cases and for implementation of public health measures to limit their spread.
Methods: In this study two Real-Time RT-PCR were compared: the PCR based kit established by the Centers for Disease Control and prevention (CDC) specific for detection and characterization of A/H1N1 that amplified four different targets (InfA, SwInfA, SwH1N1, RNAseP), considered as the reference standard, and a new kit Xpert® Flu (Cepheid) that amplified FluA, 2009 H1N1 and FluB targets.
Results: On the whole, samples from 60 potential 2009 H1N1 cases have been screened from December 2010 to March 2011 at the our center using the the two methods in parallel. Among these, 47 cases (78.3%) gave the same result for both methods: 3 (6.4%) were positive for InfA, 18 (38.3%) were positive both for InfA and for SwH1N1 and 26 (55.3%) resulted negative. Methods gave different results for 13 sample: 10 samples were positive for CDC PCR and negative for Xpert® Flu kit, two samples were positive for Xpert® Flu kit and negative for CDC PCR, one samples resulted invalid with Xpert® Flu kit and positive with CDC PCR. In addition 66 potential 2009 H1N1 cases were screened only with the new kit Xpert® Flu: 5 (7.6%) resulted positive for FluA and 2009 H1N1, 2 (3.0%) for FluA, 8 (12.1%) for Flu B and 1 (1.5%) sample was positive both for FluA and for H1N1 and for FluB.
Conclusion: The new kit Xpert® Flu (Cepheid) allows the rapid identification in 70 minutes not only of FluA and 2009 H1N1 but also of FluB and this reduces the need for additional or confirmatory testing. The novel method is suitable for the diagnosis of pandemic (H1N1) 2009 virus and, although less sensitive than PCR established by CDC, could be used as a screening test of influenza virus during the epidemic season. This can allow rapid results (TAT 3 hours) for a rapid therapeutic treatment and management of the patient and limiting the spread of the virus.

R2691 Quantitative adenosine tri-phosphate detection to aid infection control

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Objective: Time trend analysis observing the integration of quantitative adenosine tri-phosphate detection into routine infection control surveillance at an NHS University Hospital.
Background: Decontamination of environment and of equipment is central to infection control, and the framework for setting and measuring performance outcomes is derived from the National Patient Safety Agency. Audits are routinely carried out using visible contamination as an indication of cleanliness; however this does not necessarily represent absence of transmissible pathogens. Microbiological settle plates or environmental swabbing both delay audit feedback and have poor reproducibility. ATP detection uses quantitative bioluminescence to measure the levels of organic material on a surface, providing a monitoring tool that can be used at both technical and managerial levels of audit.
Methods: Internal validation and implementation of standardised sampling with a commercially available device (Systemsure Plus ®) with subsequent hospital wide roll out by the Infection Control Team. Monthly audits of fixed assets, close contact and direct contact items in all clinical areas with immediate feedback to ward managers and annual reporting to Infection Control Committee and Patient Environment Action Team (PEAT).
Results: Quantitative ATP detection identified specific areas with high levels of organic material which appeared clean at the macroscopic level. Identification of poorly decontaminated fixed assets included door plates (832 units pre targeted clean, <100 units post) and ward phones (553 units pre targeted clean, <100 units post) and of direct contact items including commodes. For this latter item use of the ATP device allowed pinpointing of subsections of the commode that had been repeatedly poorly decontaminated including the commode arms (169 units pre targeted clean, <100 units post). Targeted resources and training were then allocated to improve decontamination techniques. This resulted in a decrease in ATP detection over repeated audits.
Conclusion: Quantitative ATP detection adds a focused tool to the infection control armamentarium to identify poorly decontaminated items that may contribute to onward transmission of infective pathogens. This tool allows immediate feedback at clinician level as well as providing data allowing time trend analysis to identify particular clinical areas that need additional training or particular items that are poorly decontaminated.

R2692 An investigation into the source of a strain of *Burkholderia cepacia* (genomovar I) isolated from multiple patients and hospitals over a period of 19 months

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Objectives: Our laboratory routinely performs national surveillance of the *Burkholderia cepacia* complex (Bcc) because of the association of certain strains with poor outcome and transmissibility among Cystic Fibrosis (CF) patients. Common in the environment and intrinsically resistant to many antibacterial agents, these organisms have also been described as contaminants of various medical products, including nasal spray, nebulization solution, mouthwash, ultrasound gel, ophthalmic solutions and chlorhexidine. During 2010/2011 we received substantial numbers of isolates of *B. cepacia* (genomovar I) from non-CF patients, prompting an investigation into the nature and possible source of these cases.
Methods: Identification of the isolates to species level was by recA sequence cluster analysis and molecular comparison was conducted using pulsed-field gel electrophoresis. MICs were determined by agar dilution.
Results: Molecular analyses revealed that isolates from 28 non-CF patients from 19 hospitals across the UK, received between March 2010 and October 2011, represented a single strain. Of the eleven isolates screened all had similar antibiograms, with susceptibility to meropenem, co-trimoxazole and ceftazidime. A questionnaire, collecting clinical information including significance of infection, exposure to various products and procedures, antibiotic usage and details of specimen collection was sent to clinical microbiologists in the hospitals involved to generate a hypothesis for a potential common source. Resulting data revealed that most isolates were from blood. The clinical significance was generally uncertain, but there was evidence of at least one confirmed intra-venous line infection.
Conclusion: No potential source of this common strain has been identified, highlighting the difficulty in investigating an outbreak spread over time and place. The use of chlorhexidine-containing products in

both the disinfection of medical devices and patient's skin was the most common shared factor between hospitals, though this may reflect widespread use rather than an association with infection.

R2693 The missing link in the health-care associated infection acquisition cycle: an innovative patient hand-hygiene audit led by doctors at a tertiary cardiac centre in northwestern England

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Background: Handhygiene compliance in healthcare settings is acknowledged as the single most important infection prevention intervention to reduce healthcare associated infections [HAI]. Department of Health guidance has made HHA results a mandatory item of agenda at trust board, divisional, hospital infection control committee, care quality inspection, etc. Blackpool Teaching Hospitals [BTH], winner of HAI technology innovation team award 2009, operates a most successful HAI programme with high emphasis on antibiotic stewardship and handhygiene compliance. CEO of the trust recently led a system change of HHA within the trust from conventional dedicated staff conducting HHA, to a secret shopper style conduct of HHA by a variety of trained HC professionals visiting the clinical area. We present findings from an innovative HHA project led by junior doctors [JD]. The aim of this project was to explore the missing link in the HAI acquisition cycle – the patient HH.
Methods: Prospective audit of patient hand hygiene before meals over 2-weeks of October2011 in the Lancashire cardiac unit. The standard was the hospital policy requiring healthcare assistants [HCA] to explain HH to patients and offer HH wipes before meals. The audit was a joint microbiology – JDs in cardiac surgery project. Data was covertly collected during each meal distribution time by JDs/HCAs and analysed by JDs and microbiologist.
Results: Key findings from 76 patient included in HHA over 2-weeks included 471 observations during breakfast, lunch and dinner. Male: Female ratio of 2.5:1. Overall Hand hygiene compliance was observed in 73% (344/471). HHcompliance was not always consistent between three meals in some patients. Details of compliance variation between gender; during different meals to be presented.
Conclusions: Poor hand hygiene compliance can potentially undermine the hospital HAI program. The focus of hand hygiene has consistently been on healthcare staff specially the doctors and nursing staff. The microbiology – infection control – JD alliance at BTH are constantly working to improve the awareness and standards of HHcompliance. The Phase 1 of this project conducted in 2009/10 by JDs [supervised by microbiologist and using identical HHA tool as IC nurses] highlighted similar and poor [37%] compliance within all health professionals. This phase 2 patient HHA results will be used to inform the trust wide enhanced hand hygiene programme launched in 2011. Details of programme to be presented.

R2694 Increased risk of surgical site infection in older patients in a cohort survey: targets for quality improvement in antibiotic prophylaxis

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Objectives: Surgical site infections (SSIs) remain a major issue of patient safety despite improvements in surgical practice and infection control techniques. Although the effect of SSI on mortality, duration of hospitalization, and hospital costs has been well described in the general population, these data are limited in older operative patients. The objectives of our study were: i) to describe SSI risk in older operative patients and ii) to evaluate compliance with guidelines for antimicrobial prophylaxis.
Methods: A 12-months cohort study was performed in accordance with the methods, protocols and definitions of the HELICS. For SSIs, rates were calculated by operative procedure and risk index category. The compliance of current prophylactic antibiotic practices in the ward with the published national guidelines (SNLG 17, 2008) was assessed.
Results: A total of 250 patients were enrolled and 253 surgical procedures were included. An increase of the incidence of SSI was observed comparing the older operative patients (≥65 years) with the younger group, from 6.1 per 100 surgical procedures to 2.2 per 100 surgical procedures. Furthermore, the older patients were less likely to be subjected to laparoscopic procedures (18.2% vs. 33.3%) and more likely to undergo emergency surgery than the younger one (10.6% vs. 1.6%, $p < 0.05$). The mean length of stay and the mean duration of the surgical procedure were significantly higher in the older operative patients group (6.6 days vs. 3.5 days and 127 minutes vs. 99 minutes, $p < 0.05$). Antibiotic was administered peri-operatively for 98.8% of surgical procedures and post-operatively in 98.4% of them. The most frequently prescribed category of antibiotics was cephalosporins (79.6%). The single most frequently used drug was ceftriaxone (46.4%). Antibiotic prophylaxis was indicated in 35.2% of surgical procedures in our survey, as from national guidelines, and it was appropriately administered in 36.1% of surgical procedures.
Conclusions: Results of this study let us identify older patients at higher risk of infection and can be used to design and implement strategies directed toward the prevention of SSIs. Furthermore, our study underlines the need to develop multimodal intervention programs to improve compliance with antimicrobial prophylaxis practices and finally decrease SSI rates.

R2695 Assessment of the rate of environmental recolonisation after manual and airborne decontamination in an ICU of a tertiary hospital

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Objectives: The hospital environment is a major source of infections. In the present study the extent of the environmental contamination of a 7-bed ICU, the impact of manual and airborne decontamination as well as the rate of recolonization was investigated.
Methods: The ICU was emptied and not in use for 13 days. Sampling was conducted before any type of cleaning, after manual cleaning, after airborne decontamination and on the 3rd, 9th and 16th day after patients' admission. Samples were taken from 21 standard sites using moistened swabs. The samples were cultured directly and after incubation in nutrient broth, on blood, MacConkey and Sabouraud agar. All strains isolated were identified using microscopy, Gram staining and biochemical methods. Susceptibility testing of selected strains was performed using the VITEK II automated system (biomerieux, France). At the same time all causative agents of infections occurring in the ICU during the last year were systematically recorded.
Results: Direct plating has shown 15 sites (71%) to be contaminated before any cleaning, while the number of contaminated sites was 11 (52%) after both cleaning procedures ($p = 0.34$). The third day after patient admission the contaminated sites were found to be 19 (90%) ($p = 0.017$). After incubation in nutrient broth the contaminated sites were 90%, 81% and 95% respectively. Results are shown in the table below. The strains most commonly isolated were *Acinetobacter* spp., *Klebsiella* spp., *Pseudomonas* spp. and CN Staphylococci. These bacteria were also the causative agents of the majority of infections occurring in the ICU patients during last year.
Conclusion: Extensive contamination was demonstrated in the ICU. The majority of the contaminating bacteria was the same and had the same antimicrobial susceptibilities as the pathogens causing infections in the ICU patients' population. Airborne decontamination, in addition to manual cleaning, can reduce in some extend environmental contamination but cannot eliminate it. The almost immediate recontamination towards pre-cleaning levels is suggestive of a more frequent implementation of environmental decontamination. The compliance of the cleaning personnel to the decontamination

	Positive sites after direct plating	Positive sites after nutrient broth incubation	Negative sites after nutrient broth incubation
Before any type of cleaning	15/21 (71%)	19/21 (90%)	2/21 (10%)
After manual cleaning	12/21 (57%)	19/21 (90%)	2/21 (10%)
After airborne decontamination	11/21 (52%)	17/21 (81%)	4/21 (19%)
Day 3 after patient admission	19/21 (90%)	20/21 (95%)	1/21 (5%)
Day 9 after patient admission	16/21 (76%)	18/21 (86%)	3/21 (14%)
Day 16 after patient admission	17/21 (81%)	20/21 (95%)	1/21 (5%)

protocols is mandatory. More studies are needed in order to determine the optimal schedule of cleaning procedures.

R2696 Influenza vaccination uptake among health care workers after a multimodal educational campaign in the post pandemic year

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Background: ESCMID guidelines recommend that health care workers should be vaccinated annually against influenza. Despite this, vaccination rates have been low in our hospital and they worsened after the H1N1 pandemic in 2009. Our aim was to evaluate the impact of a multimodal educational campaign over the vaccination uptake among health care workers (HCW) in our centre.
Methods: During the 2011–2012 vaccination programme a multimodal educational and motivational campaign was applied in a University Hospital of 500 beds, serving a population circa 350 000 hours. The campaign included: management and staff commitment in order to create an institutional climate favoring vaccination practices, educational seminars, widely distributed motivational posters and informative leaflets, adaptation of vaccination schedules for all shifts with special interest in areas of high risk patients (ICU, NICU, and the onco-hematology ward). All employees of the Hospital (n = 1672) were asked to fill in anonymously a self-administered questionnaire. It inquired about attitudes, perceptions, acceptance of the vaccination and reasons of their choice. Informative leaflets were based on the most common problems detected in the questionnaire. Annual vaccination rates since 2007 were calculated.
Results: Thirty posters and 300 leaflets were distributed throughout the hospital. 428 HCW (25.6%), full filled the questionnaire. 72.05% were females, 34.6% were nurses and 30.7% were physicians. The main reason given for not being immunized was fear of the adverse effects of the influenza vaccine. Healthcare workers trivalent inactivated vaccine uptake ranged from 27.34% in 2007 to 28.4% in 2008 and 27.21% in 2009. In 2009–2010, the uptake rate for trivalent vaccine was 16.3% and for monovalent pandemic H1N1 vaccine was 12.8%. After the multimodal interventional campaign, the global rates of vaccination reached 30.6%. Vaccination rates in high-risk units were: 38.9% in ICU, 62.9% in NICU and 68.7% in the onco-hematology ward.
Conclusions: Despite the improvement in the coverage rate of influenza vaccination in our study, we would expect a higher rate because of wide vaccine availability. The results indicate the need for on-going education of influenza disease among HCWs to increase vaccination rates.

R2698 Sexually tnsmitted infections in women attending the obstetrics and gynaecologic unit of a university hospital in the north of Milan: prevalence and risk behaviours

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Objectives: Sexually transmitted infections (STIs) are a large group of human infections worldwide widespread in people of reproductive age. In a group of HIV-1 negative women the prevalence of different sexually transmitted pathogens (*Human papillomavirus*, *Neisseria gonorrhoeae*, *Chlamidia trachomatis*, *Mycoplasma hominis*/*genitalium*, *Ureaplasma urealyticum*/*parvum*, *Trichomonas vaginalis*) and their risk factors were evaluated.
Methods: A retrospective 36 months study (January 2008-December 2010) was managed with 233 women (200/233 Italians, 15/233 European, 3/233 Africans, 10/233 South Americans, 5/233 Asians) in the age range from 15 to 70 years (median age 38 years) attending the Obstetrics and Gynaecologic Unit of L. Sacco University Hospital. For each patient cervical sample was collected (PreservCyt; Thin Prep®, UK) for the detection of HPV (Inno-Lipa HPV genotyping Extra; Innogenetics, Belgium) and bacteria infections (Multiplex PCR; Seeplex® STI Master ACE detection, Seegene, Korea). Demographic (nationality, age and sex) and clinical data (age of first intercourse, multiple sexual partners, use of intrauterine device, pregnancy) were recorded in 132 out of 233 patients. Statistical analysis were evaluated with CCA-software X1Stat 4.0.
Results: The overall prevalence of STIs was 80.7% (188/233): 49% (92/188) were co-infections, 51% (96/188) were due to a single agent. The most common agent detected in total population was HPV, 68.7% (160/233), followed by *U. urealitycum*/*parvum* 44.6% (104/233), *M. hominis* 13.3% (31/233), *C. trachomatis* 2.6% (6/233), *T. vaginalis* and *N. gonorrhoeae* 1.3% each one (3/233), and *M. genytalium* 0.8% (2/233). Correspondence analysis worked out between pathogens and risk factors showed that younger women with high risk factors (early age of first intercourse, >5 partners) are most affected by *N. gonorrheae*. A significant statistically high prevalence rate with *U. urealyticum*/*parvum* or HPV were reported in all women.
Conclusion: Epidemiological studies have demonstrated the role of screening for genital infection, essential to reveal the presence of the different sexually transmitted infectious agents. The presence of HPV may have an influence in vaginal flora, but in our findings bacterial infections are not statistically associated with HPV and behavioural risks.

R2699 Filamentous fungi in hydroponic plants for decoration in a university hospital

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Objectives: Nosocomial infections caused by filamentous fungi are ill defined and thus a challenge for infection control. Commonly detected in severely immunocompromised patients these infections may be acquired ubiquitously from the environment in and outside the hospital. Decoration plants are meant to embellish the in-hospital areas and make them more homely and agreeable to long-term patients. Hydroponic plants using clay pebbles as potting medium are generally preferred in hospitals as the pebbles are thought to be clean and therefore not contaminated by spores.
Methods: After an outbreak of Fusarium infections at the burns intensive care unit involving two patients we tested hydroponic plants at sites of the hospital for the presence of filamentous fungi. Apart from the burns intensive care unit we randomly chose nine wards, three staff rooms and three public areas within the hospital. In each of the sites we collected 1–2 clay pebbles of hydroponic plants and enriched them in Sabouraud-Bouillon. Turbid turned bouillons were cultured using Sabouraud agar. Identification of the fungi was achieved by standard macro- and micromorphological criteria. Typing of Fusarium species

was done using the DiversiLab™ kit (bioMérieux). Retrospective chart review was performed to detect invasive hyphomycetal fungal infections.
Results: In hydroponics clay pebbles of 22 plants we found 11 fungal species in all specimens (Table). Scanning the hospital records for invasive fungal infections caused by these species did not reveal associated infections in the past. Comparing environmental and patients' isolates of Fusarium species by genotyping showed no similarity.

Species	Growth
Acremonium species	2
Aspergillus species*	9
Aureobasidium pullulans	1
Candida parapsilosis	1
Cladophialophora species	1
Cladosporium species	2
Fusarium solani	2
Hyphomyceten	4
Penicillium species	11
Trichoderma species	2
Verticilium species	3
11 Species	38

*including A.fumigatus, A.glaucus, A.nidulans, A.niger.

Conclusion: Hydroponic decoration plants are colonized with multiple fungal species that might cause invasive fungal infections in severely immunocompromised patients. Thus surveillance of invasive fungal infections should be done in a tertiary care hospital with patients with hemato-oncological malignancies, transplantation and immunomodulation. Hydroponic plants should not be present in these high risk areas.

R2700 Effectiveness of an educational programme to reduce catheter-related blood stream infections in a tertiary paediatric hospital

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Objectives: Central venous catheters are widely used in hospital practice. However, they are the leading source of bloodstream infections in hospitalised patients. The aim of this study was to assess the effectiveness of an educational programme to prevent catheter-related blood stream infections (CRBSI).
Methods: Between June and December 2010 the Hospital Infection Control Team of the Children's Memorial Health Institute (400-bed tertiary paediatric hospital in Warsaw) performed an educational training programme involving physicians and nurses on diagnosis and prevention of CRBSI. The programme included lectures and practical demonstrations. The standard of care of central venous catheter (CVC) was implemented in all hospital wards. To assess the effectiveness of these measures the rate of CRBSI was determined in a 6-month period before (January – June 2010) and after (January – June 2011) the intervention.
Preliminary Results: Before the intervention there were 11 CRBSI diagnosed in 427 CVC insertions (2.6%). After the intervention the rate of CRBSI increased to 6.4% (25 infections in 388 CVC insertions. The rate of CRBSI per 1000 catheter-days was higher after the intervention. Additionally, after the intervention the frequency of blood cultures performed in patients with CVCs increased by 47%.
Conclusions: The implementation of the educational programme was associated with an increased number of blood cultures performed in CVC patients and a higher frequency of CRBSI diagnosed within the first 6 months after the intervention. These partly unexpected findings result probably from better diagnosis and reporting of CBRSI after the intervention.

R2701 A junior doctor's change management and leadership project: hand-hygiene compliance audit programme at a teaching hospital in northwestern England

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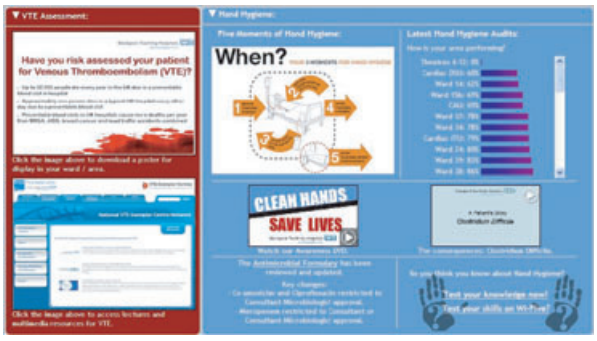
Background: According to CDC, people don't wash their hands as often they say they do or as often as they should''. Hand washing is the single most effective way to prevent spread of infections. We present a summary from a junior doctor's change management and leadership project – hand hygiene [HH] audit programme at Blackpool Teaching Hospitals.
Methods: Review of conventional HH audit rates when conducted by clipboard bearing dedicated nurses; Review of results of HH audit [2009] and re-audit [2010] conducted by JDs using identical HH audit tool as nurses, but large number of observations and un-disclosed observational audit. Presentation of results to trust executive directors, hospital infection control committee and CEO.
Results: Conventional dedicated nurse conducted HHA results above 90% compliance, JDs conducted HHAs included 3060 minutes [2009] and 1840 minutes [2010] of observations with compliance rates of 37% and 35% [$p > 0.05$] respectively. Introduction of new secret shopper style HHA programme [based on Royal Devon and Exeter risk based criteria]. Refer attached picture. Details to be presented.



Conclusions: Poor HH compliance can potentially undermine trust HAI programme. The value of findings from a conventional dedicated nurse led HHA may be limited by bias, hawthorne effect and complacent behaviour from high (>90%) HHA rates. The JD led HHA, supervised by microbiologist, was part of a change management and leadership project. This involved studying the conventional HHA system, planning the project, leading and supervising a group of JDs; analyzing and presenting findings at hospital committees and to CEO; discussing validity of findings amidst resistance; leading up to CEO led decision to change the HHA programme from the conventional dedicated nurse led HHA system to a secret shopper style HHA conducted by a variety of trained healthcare professionals visiting the clinical areas. This study has revealed a detailed picture regarding hand hygiene compliance and auditing methods. Unbiased compliance results are needed for an effective infection control programme.

R2702 Epidemiological and laboratory investigation and effective control of a nosocomial outbreak of gastroenteritis due to *Norovirus*

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Objectives: We aim to describe the epidemiological and laboratory investigation of a nosocomial outbreak of gastroenteritis and the effectiveness measures taken for its containment.
Methods: In April 3, 2011, an outbreak of gastroenteritis occurred in patients and personnel of the Orthopedic Department of Tzaneio General Hospital of Piraeus, Greece, a 450-bed, tertiary-care center. The hospital infection control committee was notified. Cases were defined as patients, visitors or hospital personnel with symptoms of vomiting and/or diarrhea. Clinical and epidemiological information was collected from cases and all patients hospitalized in the specific department. Investigation for similar cases was made to other hospital departments. Instructions for strict hand hygiene and contact precautions were given. Hospital surfaces in the vicinity of the affected patients were cleaned with hypochlorite solution and the rooms were aerated. Visits to the patients were discouraged. All affected hospital staff was asked to refrain from work until symptom resolution. Stool samples from select cases were sent for routine culture, Clostridium difficile toxin assay, and detection of viral antigens.
Results: The outbreak lasted for 10 days and it was contained within the hospital department of origin. A total of 21 cases were identified (81.0% female). Ten (47.6%) were inpatients, 3 (14.3%) were patient visitors, and 8 (38.1%) were hospital personnel. The attack rate was 10/36 (27.8%) for inpatients and 6/11 (54.5%) for the nursing staff. The 10 hospitalized case-patients were located in seven different rooms. All cases had diarrhea, 6 (28.6%) had vomiting and 1 (4.8%) had fever. Symptoms lasted for 1–3 days and were generally mild. A common food- or water-borne source was not identified. The case patient was infected from a visitor. Stool cultures were negative. The antigen immunoassays were positive for *Norovirus*. This was confirmed with polymerase chain reaction.
Conclusion: A *Norovirus* outbreak was introduced to a hospital department from the community. The outbreak quickly spread among inpatients, possibly via infected healthcare professionals, who had a high attack rate. This could compromise the implementation of proper infection control measures due to required absence from work. The prompt response to the outbreak prevented the spread to other hospital departments.

Clinical epidemiology of nosocomial infections

R2704 High mortality in bacteraemia and candidaemia in critically ill patients – report from Swedish Intensive Care Registry

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Objective: Increasing prevalence of bacteremia and candidemia with significant resistance to antimicrobial agents is an increasing concern among ICU patients. The objective of this report from Swedish Registry of Intensive care (SIR) was to study the frequency and cause of culture verified sepsis in critically ill patients and to analyse mortality in sepsis caused by Candida albicans, Candida non albicans and bacteria.
Methods: Setting: Starting 10 years ago an increasing number of ICU: s in Sweden reports each episode of care (EOC) to the Swedish Intensive care Registry (SIR). Mortality is followed weekly for all patients by link to the Swedish population registry. A specific routine for collection of microbial data directly from the laboratories connected individually to each EOC has been tested and implemented for laboratories covering 1/3 of the Swedish population.
Participants: 47 ICU: s reported 1540 EOC: s during the period January 2005 to November 2011, with a diagnosis of sepsis (ICD10: A419, R572 or R651) and a positive blood culture within 14 days before admission until discharge. For patients with more than one EOC was only the last EOC included which reduced the number of observations included in mortality calculations to 1416.
Variables: Primary outcome was 30 day mortality calculated from admission to ICU.
Results: Thousand four hundred and sixteen patients met inclusion criteria and were included in the analysis. The most common causes of sepsis were: *E. coli* (24%) followed by Coagulase Negative Staphylococci (CoNS) (21%), *Streptococcus* spp. (19%), *S. aureus* (14%), *Klebsiella* spp. (8%) and *Candida* spp. (6%) [*Candida albicans* 4% and Candida non albicans 2%]. The 30-day crude mortality was 34% for patients with sepsis caused by *S. aureus*. Correspondingly 30 days mortality was for Candida non albicans 34%, *Candida albicans* 31%, *Klebsiella* spp. 26%, CoNS 25%, *E. coli* 22%. Distribution of species in blood cultures from the 87 patients with candidemia were: *C. albicans* 62, *C. glabrata* 11, *C. krusei* 1, *C. tropicalis* 4, C. other 4, C. non specified 9.
Conclusion: The highest (>30%) crude mortality in critically ill patients with sepsis was seen in patients with *S. aureus* and Candida infections. Further analysis of independent risk factors for mortality in sepsis caused by different pathogens are warranted.

R2705 Hospital-acquired infections in patients with acute ischaemic stroke

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Objectives: Hospital-acquired infections represent a significant problem during hospitalization of patients with acute ischemic stroke and occur in 15–65% of patients. The aim of this study was to assess the rate of nosocomial infections in patients with acute ischemic stroke, their risk factors, most common pathogens and influence on patients' outcome.
Methods: We analyzed patients with the diagnosis of acute ischemic stroke who were treated in the Department for emergency neurology in the Clinic for Neurology, Clinical Center Serbia, from October the 1st 2009 till the 31st of September 2010. Patients who had fever or other signs of infection on admission and those transferred from another hospital were excluded from the study.
Results: We analyzed 133 patients, with the average age of 58.4 ± 15.5 years. Hospital-acquired infection occurred in 63 (47.4%) patients. The most frequent were urinary tract infections in 27 (20.3%) and pneumonia which was present in 19 (14.3%) patients. Nine patients had primary blood stream infection. Pneumonia was the cause of secondary blood stream infection in four patients while urinary tract was the origin of sepsis in two patients. Severe neurological deficit on admission ($p = 0.001$, adjusted odds ratio OR [CI] 4.02 [1.05–15.41]), severe movement impairment ($p = 0.000$, adjusted OR [CI] 3.41 [1.01–11.51]) and the presence of urinary catheter ($p = 0.000$, adjusted OR [CI] 8.81 [1.25–61.84]) were found to be independent predictors for infection in ischemic stroke. The most commonly isolated pathogens were *Pseudomonas* sp. in 20 (20.8%), *Klebsiella* sp. in 19 (19.8%) and *Acinetobacter* in 13 (13.6%) cases. Gram positive bacteria *Staphylococcus aureus*, Coagulase-negative *Staphylococcus* and *Enterococcus* sp. were the major causes of primary sepsis. The rate of carbapenem, cefepim and ciprofloxacin resistance of *Pseudomonas* was 67.5%, 80%, and 100%, *Acinetobacter* 69.3%, 84.6% and 92.3%, and *Klebsiella* 0%, 80% and 89.5%. The presence of infection in stroke patients was associated with longer hospital stay and was found to be an independent predictor for poor functional outcome ($p < 0.0001$, adjusted OR [CI] 7.81 [1.04–58.82]).
Conclusion: Hospital acquired infections in stroke patients are very frequent. They are caused by resistant microorganisms and are associated with poor functional outcome. Prevention and adequate

initial therapy of these infections based on the prevalent pathogens are important for final outcome of stroke patients.

R2706 Infections in patients who undergo craniotomy: a prospective study

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Objectives: To identify the prevalence and the microbiology of infections in patients who undergo craniotomy and to determine the risk factors for post-craniotomy meningitis (PCM) in Greece.
Methods: Prospective study in patients >18 years old who underwent craniotomy between 2006 and 2008 in the University Hospital of Heraklion.
Results: Three hundred and thirty-four patients were analyzed. Their median age was 51. 65.6% of the patients were men. 50% of the surgeries were emergency procedures and 18.7% were revision surgeries. Traumatic brain injury was the most common cause for craniotomy. The most common infection in this population was ventilator associated pneumonia (VAP) (22.5%). The most common VAP pathogen was *Acinetobacter* spp. (44%). They were 100% sensitive to colistin but only 30% sensitive to imipenem. The pathogens were 100% resistant to ticarcillin/clavunate, piperacillin/tazobactam, cefepime, aztreonam and ciprofloxacin. Surgical site infections (SSIs) and urine tract infections were encountered in 9% of the patients. Meningitis was noted in 4.8%. *Acinetobacter* spp. were the most common pathogens in PCM. They also were 100% sensitive to colistin but 100% resistant to piperacillin/tazobactam, cefepime, aztreonam, aminoglycosides and ciprofloxacin. Other pathogens involved in PCM included *Klebsiella* spp., *P. aeruginosa*, *S. aureus*, *E. cloacae* and *P. mirabilis* (6% each). PCM was culture-proven in 100%. Perioperative steroid use (OR 11.5), CSF leak (OR 48) and ventricular drain use (OR 70) were independently associated with PCM use. Internal Medicine Unit (ICU) admission was associated with PCM ($p < 0.001$). There was no difference in survival between patients who developed and did not develop meningitis.
Conclusion: VAP was the most common infection in people who undergo craniotomy. PCM developed in 4.8% which was similar to rates reported before. Multiresistant Acinetobacter spp were the main pathogens. Communication between CSF and environment, CSF leak and perioperative steroid administration were independently associated with PCM in this prospective study.

R2707 *Clostridium difficile* health care and community-associated infection: a cross-sectional study in a hospital for tropical diseases

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Objective: Spreading of virulent *C. difficile* by ribotype 027 infection is increasing in Europe but with an unequal distribution. Up to now, only two cases by ribotype 027 strains have been reported in Spain. The aim of this study was to determine the prevalence in hospitalized and non-hospitalized patients and the epidemiology associated to *C. difficile* infection (CDI) in a Hospital specialized on Tropical Medicine.
Methods: From October 2009 to October 2011, stool samples from patients with clinical suspicion of CDI were examined. Subjects were classified in two groups, hospitalized patients and outpatients, and a different diagnostic algorithm was performed. In the first group, detection was made by a real-time PCR which includes detection of ribotype 027 (Xpert®; *C. difficile*). In outpatients, a two-step algorithm was used: screening with a rapid test (Techlab C. difficile Quik Chek Complete®) and positive cases were confirmed by real-time PCR.
Results: A total of 690 specimens from 582 patients (279 male and 303 women) were studied. 343 corresponding to hospitalized patients, most of them natives (87.2%), and the rest immigrants (7.3%) and travellers (5.5%). Among 239 outpatients, the majority were travellers (76.6%) followed by Spaniards (19.2%) and immigrants (4.2%). A total of 40 patients were positive for CDI, 33 hospitalized patients (prevalence=9.6%) and seven outpatients (prevalence=2.9%). Most of cases detected with CDI in hospitalized patients were >70 years old (median age, 81; interquartile range [IQR], 75.5–88.5), in contrast to outpatients (median age, 40; IQR, 33–65; $p = 0.005$). Out of 33 CDI-positive inpatients, 31 were native and three immigrants. One of the immigrants was positive for 027 strain. She was a Portuguese female who had been operated in her country less than a month ago. Among seven outpatients positive for CDI, three were travelers and two of them have developed diarrhea after antibiotic treatment for traveller's diarrhea. The remaining outpatients were immigrants and natives who had previously taken antibiotics or proton pump inhibitors.
Conclusion: A high prevalence of CDI infection in hospitalized patients with detection ribotype 027 was observed. In addition, the prevalence obtained in outpatients, notably in those with antibiotic treatment after traveller's diarrhea, is far from negligible. These data suggest the need of diagnosis of ribotype 027 in hospitalized patients and the screening of CDI out of the traditional risk groups.

R2708 Mathematical modelling of health-care- associated infections – a systematic review

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Objectives: In the mid-90 seconds, the first mathematical models addressing healthcare-associated infections (HCAI) were introduced. Since then, these models have contributed to an increased understanding of hospital epidemiology. We conducted a systematic review in order to establish 1) how mathematical models have been applied in the field of HCAI, 2) how the methods and model structures have developed over time and 3) what the results of these models have shown. This was done in order to synthesise key lessons learnt from these models and provide directions for future research in the area of modelling HCAI.
Methods: We searched MEDLINE, EMBASE, CINAHL plus and the grey literature using search terms for mathematical modelling, HCAI and relevant organisms.
Results: The first mathematical models of HCAI aimed at conceptualising transmission dynamics in single wards using deterministic approaches. Following this, stochastic models began to include chance due to its importance to the transmission process (particularly in small patient populations). The dominant pathogen studied is methicillin resistant *Staphylococcus aureus*. Others include vancomycin resistant enterococci and *Clostridium difficile*. Models have explored a multitude of factors important to HCAI transmission and control, for example, antibiotic effects and the development of resistance, variability in transmission routes, effectiveness of interventions and differences in transmission between strains or settings (including community transmission). Higher awareness of the significance of HCAI has lead to improved national and international surveillance systems, leading in turn to greater availability of data to inform modelling studies. This has been coupled with the development of more sophisticated methods e.g. for model parameterisation (e.g. Markov Chain Monte Carlo methods) and characterising uncertainty, and has led to improved model validation.
Conclusion: The ecology and epidemiology of HCAI can be complex. Mathematical models have proved to be useful tools to aid our understanding of the spread of these infections and the likely impact of control measures. Improved national and international data collection initiatives have enabled development of more realistic models, however new insights and questions facing the field call for further elaboration and collaboration.

R2709 Breast implant associated-infection conservative treatment. Implant exposure is highly predictive of failure

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Objective: Breast implants are used for breast augmentation and reconstruction after mastectomy. Infection is one of the leading causes of morbidity that occurs after breast implantation, and complicates around 2% of interventions in most case series. Surgical removal of the implant is mandatory in most cases. The goal of the present study was to retrospectively analyze if a conservative treatment based on long-term antimicrobial use could be a sure and effective alternative.
Methods: Study location: Clínica Universidad de Navarra, a 300-bed University Hospital in Pamplona, Spain. Dates: January 2000 to October 2011. Study design and patients: A retrospective review of all consecutive patients diagnosed with breast implant associated infection that were treated in a conservative way was conducted. Demographic, clinical, microbiological, antimicrobial treatment and outcome data were collected. Cure was defined as clinical infection disappearance and implant retention.
Results: Eighteen patients were identified. Median age at the time of infection was 46 years (interquartilic range: 40–53). Thirteen patients had a reconstructive and five an augmentation surgery. Median implant life span from placement to removal was 307 days (interquartilic range: 20–337). Clinical picture included fever (88%), local pain (83%), erythema (77%) and swelling (50%). Implant was exposed at diagnosis in six patients. Cultures were performed in nine patients (50%). Most common isolated microorganism was methicillin-susceptible Staphylococcus aureus (44%). *E. coli*, *Corynebacterium jeikeium*, *Corynebacterium pseudodiphtericum*, *Staphylococcus epidermidis*, *Prevotella bivia* and *Abiothrofia adjacens* were involved in the other episodes. All patients were started on IV (13) or oral (5) antimicrobial therapy. Antimicrobials were administered for a mean of 89 days (interquartilic range: 48–98). Breast implants had to be surgically removed in eight patients (44%) because of treatment failure. Clinical cure with breast implant retention was achieved in 84% of the patients with a non-exposed implant, while 100% of the patients with implant exposure required removal.
Conclusion: Not all breast implant associated infections mandate implant removal. Breast implant exposure is highly predictive of failure if a conservative treatment is intended. If implant is not exposed a conservative treatment based in long-term administration of antimicrobials could be a valuable therapeutic option.

R2710 Impact of inappropriate initial empiric antibiotic therapy and antimicrobial resistance on outcome of patients with complicated intra-abdominal infections requiring surgery: a propective cohort study in Italy

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Study Design: Multicenter prospective observational cohort study.
Objective: To evaluate the impact of inappropriate empiric therapy in patients undergoing surgery for complicated intra-abdominal infections (cIAI).
Methods: Setting: accrual at two clinical centres in Italy, from October 2009 to May 2011.
Participants inclusion criteria were: (i) age >18 years, (ii) complicated intra-abdominal infection (iii) requiring surgery (iv) fever or leukocytosis. Patients with underlying immunodeficiency or with life expectancy <30 days were excluded.
Variables: the primary outcome was clinical success, defined as infection cure in response to combined initial antibiotic therapy plus surgery.
Statistical Methods: Univariable analysis was performed using the Chi square test for categorical variables and the Student's t test for continuous ones. Logistic regression models were used to identify the variables independently associated with clinical success. The chosen level of significance was 5% and the p values reported were two tailed.
Results: Participants: 107 eligible patients had a mean age of 53 years (SD + 18.8), 63% were male. Most patients had complicated appendicitis (24%), or intra-abdominal abscess (21%). Eighty-two pathogens were isolated from 38 patients (35.5%). Escherichia coli was the most frequently identified pathogen (21%).
Main Results: In 82 cases (77%) the initial antibiotic therapy was appropriate. Clinical success was observed in 74% (79/107) of the cases. Patients with appropriate initial antibiotic therapy had clinical success in 77% vs. 64% in the inappropriate therapy group (OR 1.87, 95% CI 0.64–5.41; p = 0.2). At multivariable analysis among those with initial appropriate antibiotic therapy, clinical success was significantly associated with younger patient age (OR 1.1; 95% CI 1.0–1.3; p = 0.02); localised cIAI (OR 10.0; 95% CI 1.0–50; p = 0.05); and shorter duration of first line antibiotic treatment (OR 1.43; 95% CI 1.11–2.0; p = 0.04). Patients with resistant isolates (n = 17) had a longer mean duration of antibiotic therapy (19 vs. 12, p = 0.04) and longer hospital stay compared with patients with sensitive strains (23 vs. 15, p = 0.08).
Conclusions: We report the results of the first prospective study on inappropriate antibiotic therapy and outcome of cIAI. A trend towards a better outcome in cIAI patients with appropriate initial therapy was observed. cIAI due to resistant strains required longer duration of therapy and hospital stay.

R2711 Clinical epidemiology of nosocomial candidaemia among non-neutropenic adult patients

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Objectives: To evaluate incidence, causative *Candida* species, risk factors, treatment and outcome of nosocomial candidemia.
Methods: Retrospective study of nosocomial candidemia observed from January 2008 to June 2011 at an italian tertiary care hospital with no pediatric and transplantation departments.
Results: One hundred forty-five episodes of candidemia occurred in 139 patients (52% males). Median age was 81 years (range 14–98 years). At diagnosis of candidemia, 70% of patients were hospitalized in medical wards, 17% in surgical wards, 13% in intensive care units (ICUs). Overall hospital incidence was 1.56 episodes per 10 000 patient-days (1.90 in 2008, 1.57 in 2009, 1.20 in 2010, 1.57 in 2011). The incidence was 4.28 episodes per 10 000 patient-days in ICUs, 1.69 in medical wards and 0.89 in surgical wards. The most frequent *Candida* species isolated was *C. albicans* (55%), followed by *C. parapsilosis* (24%), *C. glabrata* (10%), *C. tropicalis* (4%) and other *Candida* species (7%). Underlying diseases were: solid cancer (39%), diabetes mellitus (25%), surgery (27%), autoimmune disease (5%), hematological malignancy (3%), HIV (1%). Potential risk factors for candidemia were: antibiotic therapy (93%), urinary catheter (75%), total parenteral nutrition (TPN) (60%), surgery (27%), mechanical ventilation (18%), dialysis (9%), corticosteroid therapy (8%).
A central venous catheter (CVC) ≥48 hours before candidemia was present in 53% of patients; of them, 39% had CVC-related candidemia. CVCs were removed in 71% of patients, with median removal time of 2 days (range 0–19 days). Systemic antifungals for at least 7 days were given to 69% of patients. Considering all treated patients, fluconazole and caspofungin were administered to 85% and 12% of cases, respectively. The overall crude mortality at discharge was 52%. Mortality rate was associated to hospitalization in medical wards (p .018), CVC retention (p .003) and inadequate antifungal therapy (p < 0.001). The presence of CVC, CVC timing removal (≤3 days vs. >3 days) and Candida species were not significantly associated with crude mortality.
Conclusion: This study shows high incidence of nosocomial candidemia, especially in very elderly patients admitted in medical wards. Only about one fourth of patients had CVC-related candidemia.

Mortality rate was highest in medical wards and associated to inadequate antifungal therapy and CVC retention.

R2712 Comparison of genetic and resistance properties of Clostridium difficile isolates collected from clinical specimens from a large urban area in Germany

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Objective: The Clostridium difficile ''epidemic strain'' BNAP-1/027causing severe disease in North America and parts of Europe is not prevalent in Germany although the incidence of CDAD has greatly increased. Characteristics of a large collection of Clostridium difficile isolates from patients in a densely populated urban area may provide useful data concerning local epidemiology.
Methods: In the period from 2007 to 2011 a large number of isolates has been collected from the greater Düsseldorf area and subjected to ribotyping, PCR analysis of the presence of the toxin genes; tcdA, tcdB and cdtB and mutation causing truncation of the tcdC gene as well as resistance to erythromycin and moxifloxacin.
Results: A total of 392 isolates have been analysed to date. Most of the isolates (376 [95.9%]) possessed both tcdA and tcdB genes and 16 [4.1%] possessed neither toxin gene. No isolate carried only tcdA or tcdB. The binary toxin gene, cdtB was found in only 52 [13.3%] isolates, all of which possessed cdtA and cdtB genes. A mutation of the tcdC gene leading to the expression of a truncated protein was found in 62 [16.5%] of the toxin-gene carrying isolates. All isolates possessing the cdtB gene also carried a mutation of the tcdC gene. Ribotyping displayed a large spread of different ribotypes. The ribotype with the most isolates contained 68 members; unfortunately the classification is still unknown. Only 27 isolates were type 027. All other clusters contained <20 isolates, most <10, per cluster. A total of 201 [51.3%] isolates were resistant to both erythromycin and moxifloxacin, 66 [16.8%] sensitive to both, 108 [27.6%] sensitive to erythromycin only and 17 [4.3%] to moxifloxacin only.
Conclusion: The isolates investigated so far show a wide diversity and no single ribotype or molecular type appears to be predominant in the area studied. This correlates with the epidemiological data obtained from the largest hospital in the collection area, in which nosocomial spread of clones is rare. In addition the ribotype 027, as in other studies in Germany does not appear to be common. Ribotyping a large number of isolates is technically demanding and thus alternative methods will be included in the ongoing study. A very small number of clinical Clostridium difficile isolates (all from patients with diarrhoea) possessed no toxin gene, a fact that has relevance for the use of PCR as a diagnostic method.

R2713 High rates of carbapenem resistance and mortality in patients with nosocomial *Pseudomonas putida* bacteraemia

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Objectives: *Pseudomonas putida* belongs to the fluorescent group of *Pseudomonas* species and has been recognized as a rare cause of bacteremia. This organism was considered to be a lower virulent pathogen. But, recently, multi-drug resistant and carbapenem-resistant *P. putida* have been emerged which caused difficult-to-treat nosocomial infections in seriously ill patients. However, the clinical data on the prevalence and mortality rates of carbapenem resistant *P. putida* infections are still lacking. Here, we investigated the antibiotic resistance rates of nosocomial *P. putida* blood isolates and clinical characteristics and mortality of *P. putida* bacteremia.
Methods: From January 2006 through September 2010 the cases of nosocomial *P. putida* bacteremia were collected by review of Clinical Microbiology Laboratory Records at Chonnam National University Hospital (1000-bedded) and Chonnam National University Hwasun Hospital (700-bedded). During the study period four cases of *P. putida* bacteremia outbreak related to contaminated infusion fluid were confirmed, and this cases were excluded in this study. Medical records were retrospectively reviewed. Species identification and antibiotic susceptibility was determined by the VITEK 2 system (bioMérieux Inc., Hazelwood, MO, USA).
Results: Four (31%) and five (39%) of 13 *P. putida* isolates were resistant to imipenem and meropenem, respectively. All of 13 *P. putida* isolates were susceptible to colistimethate. Eleven (84.6%) patients had indwelling devices related to primary infections. Common primary infection sites were ventilator-related pneumonia (6.55%) and biliary tract (2.18%). Single cases of necrotizing fasciitis, surgical site infection, peritonitis and central venous catheter-related infection were observed. Thirty-day mortality in patients with *P. putida* bacteremia was 54% (7/13): 60% (3/5) in patients with carbapenem-resistant *P. putida* bacteremia, but 50% (4/8) in patients with carbapenem-susceptible isolates. Three patients who received inappropriate antibiotic treatment because of carbapenem resistance were all died.
Conclusion: Nosocomial infection of *P. putida* could show high rate of resistance to most potent beta-lactams, carbapenems, and it can cause significant morbidity and mortality for infected patients. So it is necessary to aware the fatality of nosocomia *P. putida* bacteremia and consider the early initiation of the susceptible antibiotic regimen such as colistimethate.

Travel medicine, tropical and parasitic diseases

R2714 Prevalence of intestinal parasitic infections among suspected referred patients to a reference laboratory in Ilam, Iran

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Objectives: The aim of the present study was to estimate the prevalence of intestinal parasitic infections among suspected referred patients to Reference laboratory of Ilam in the west of Iran during the period March 2010 to April 2010.
Methods: Samples were collected from stool of 1600 suspected referred patients to Reference laboratory of Ilam in the west of Iran during the period March 2010 to April 2010.
The stool samples were examined for intestinal parasites by direct microscopic.
Results: The results indicated that intestinal parasitic infections among patients in the study area are mainly water-borne. Intestinal parasites were detected by direct smear in 154 of 1600 (9.6%) suspected referred patients to the laboratory. At least one intestinal parasite was found in stool samples from 124 patients, two parasites in 27, and mixed infections with three or more parasites were seen in stool samples of three patients. The parasites were *Giardia intestinalis* 77 (50%), *Entamoeba histolytica/E. dispar* 19 (12.3%), *Entamoeba coli* 36 (23.3%).
Conclusion: The prevalence of *E. histolytica/E. dispar* in the present study to base on microscopy examination was 1.18%. Presumably, the number of *E. histolytica* positive cases would be higher if all samples were tested by Molecular methods. The ratio of *E. histolytica* to *E. dispar* found in this study was 1–3.5 is higher than the estimated global ratio of 1–10.

R2715 Case report: prolonged multiple paralysis after rattlesnake bite in Brazil

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Objectives: Report a case of a man that was bitten by a rattlesnake in Brazil with compromised the activities of several cranial nerves and muscle groups for an unusual period of time.
Methods: Review of clinical record from Hospital Vital Brazil, Butantan Institute, Brazil.
Results: In April, 2009, a 42 years old Brazilian man, previously health, natural of Santana de Parnaíba was admitted to Vital Brazil Hospital, Butantan Institute (São Paulo, SP, Brazil) 1 hour and :20 minutes after been beaten by a *Crotalus durissus terrificus* (total length 101 cm) identified by the Herpetology Laboratory of the Butantan Institute, São Paulo. The patient was drunk and tried to capture the snake and reporting many bites on both hands. The bite sites presented pain, bleeding and swelling. In the Hospital admission he was conscious and hypotense. The pacient was classified as moderate and received ten vials of anti-crotalic antivenom. Five hours later after the admission, he developed myalgia associated with change of visual acuity, bilateral ptosis. Coagulation tests was incoagulable, and a peripheral blood film revealed white blood cells: 11 200/mm^3 (4400–11 300/mm^3) with neutrophilia, urea: 41 mg/dL (10–50 mg/dL); creatinine: 1.5 mg/dL (0.4–1.3 mg/dL), creatine kinase: 1095 U/l (38–174 U/L). On the third day of hospitalization, the patient remained with blurred vision, bilateral ptosis, complete ophthalmoplegia with mild anisocoria, tearing, conjunctival hyperemia, hiccups that only improved after the use of chlorpromazine hydrochloride, salivation, difficulty chewing, swallowing food solid, refers bitter taste (change in taste), decreased olfaction and difficulty walking. He developed non dialytic acute renal failure and evolved with remission of myalgia. Kept bilateral ptosis with ophthalmoplegia, mild anisocoria and tearing for more than 2 months after the snakebite.
Conclusion: Accidents caused by rattlesnakes are responsible for approximately 9% of venomous snake bites in Brazil (in 2009 were reported 2211 cases). The South American rattlesnake venom has neurotoxic, coagulant and myotoxic activities. The myotoxic activity can cause severe rhabdomyolysis and acute renal failure. The neurotoxic activity can cause cranial nerve involvement and paralysis of skeletal muscles that usually regress within a few days. It is very unusual reports in the literature with compromise many cranial nerves and muscle groups lasting months after snake bites.

R2716 Requests for malaria prevention advice to the HPA Malaria Reference Laboratory

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Objectives: The Malaria Reference Laboratory (MRL), Health Protection Agency (HPA), UK regularly gets requests for advice about malaria prophylaxis for overseas travellers and provides a specialist advisory service for complex queries from medical practitioners. This audit was conducted to look at types of queries with an aim to improve quality of service.
Methods: All requests for advice are sent on a proforma faxed to the MRL. We reviewed all the faxed requests received over a period of 6 months in 2011. The information was collated and the number and types of queries were scrutinised.
Results: There were a total of 450 requests over a period of 6 months. 98% requests were from general practitioners. Median age of travellers was 33 years, 58% were females. 70% travellers were visiting single destinations and 75% were visiting both rural and urban areas. The commonest reason for travelling was visiting family. The most common queries were about prophylaxis for travellers to multiple destinations with a mix of high and low risk of malaria (93, 21%). Others included prophylaxis for a prolonged duration (80, 18%), antimalarial prophylaxis for the immunosuppressed (35.8%), drug interactions between antimalarials and other drugs like warfarin, oral contraceptives and antiarrhythmic drugs (26, 6%) and specific questions about prophylaxis for children (61, 14%), women who were pregnant or planning pregnancy (36, 8%). The MRL has a dedicated website with guidance for health professionals regarding antimalarial prophylaxis which could have been used for 27% of the enquiries, however a substantial number of questions were related to patients with multiple problems (100/450, 22%), eg pregnancy with epilepsy and long term stay in a high risk area.
Conclusion: Malaria prevention enquiries which are not readily resolved by consulting written guidelines can be answered by a fax back service which has the additional benefit of providing a written record of advice given.

R2717 Prevalence of *Chlamydia trachomatis* infection in male population

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Introduction: *Chlamydia trachomatis* (CT) is more common than any other male sexually transmitted disease with millions of new reported cases each year. The bacteria are found in the urethral swab, urine and sperm, and can ba transferred through genital fluids from one partner to the other. Men, especially under 25 who have had more than one partner within the last year, should have regular Chlamydia tests even there are no symptoms present. The symptoms vary in both type and severity; from discharge, irritation of orificium urethrae, painful and frequent urination, to orchitis with subsequent infertility. Asymptomatic men with chlamydial infections are under-identified and probably play an important role in sustaining the epidemic.
Aim: The objective of this study was to measure the prevalence of *Chlamydia trachomatis* infection among men using direct immunofluorescent assay.
Method: A total of 450 male samples (435 urethral swabs and 15 first void urine specimens) in a period of 2 years were enrolled in this study. The patients were on age between 17 and 69, all sexually active. A direct immunofluorescent assay – Patho DX (Oxoid, UK) was performed for diagnosis of CT.
Results: The positivity of the samples was 21.1% (95/450). The majority of positive patients were on age 21–50 years, median age 32. Symptoms with different severity were reported in half (45) of positive and 78 of Chlamydia negative patients. The most frequently reported symptom in 95 CT positive patients was white watery discharge – noted in 87%, and in only 10 of the negative patients. Risk biheiviour with multiple sex partners during the evaluated period was noted in 85 patients, 52 of them being CT positive. One month post therapy follow up was performed on 40 of the positive patients, 34 being CT negative, and the rest needed further antibiotic administration.
Conclusion: Chlamydia positivity is rather high in the evaluated population especially in the age around 30, implicating the need of regular screening. The screening for Chlamydia should enroll all sexyally active men, not only those with symptoms. CT investigations needed are as well in the evaluation of the antibiotic therapy success.

R2718 Prevalence of Strongyloidosis and Chagas' disease co-infection in Latin-American migrants in a tertiary hospital in Spain

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Objectives: Strongyloidosis (St), produced by the geo-helminth Strongyloides stercoralis, is worldwide distributed and endemic to warm and humid areas. Chagas disease (CD), caused by *Trypanosoma*

cruzi, is endemic to Latin America, and of emerging importance in non-endemic countries because of migration of people infected with *T. cruzi*. St and CD are neglected tropical diseases (NTDs) presenting common features, long-life persistance, pauci-symptoms, prolonged morbidity and potential mortaliy. The objective of this study was to determine the prevalence of St and CD co-infection in Latin-American migrants in our hospital.

Methods: One hundred sixty-four patients attending to our tropical medicine unit were screened for St in our lab between January 2010 and October 2011 by at least one of these techniques (i) microscopic examination of fresh and concentrated faeces; (ii) culture of faecal specimens or (iii) ELISA test (Strongyloides Serology Microwell ELISA kit, DRG-International). Four hundred ninety-one patients were screened for CD at the same period of time by two ELISA tests (i) BIOELISA®, Biokit and (ii) ELISA-Ortho®. Indirect immunofluorescence-IFI (Inmunofluor Chagas-Biocientífica®) was performed if any discordant result was found. Screening of St was performed in all patients presenting eosinophilia, screening of CD was performed in all patients from CD endemic areas that showed eosinophilia.

Results: Seventy-two out of 164 (44%) screened patients were diagnosed of St, 39 (54%) were male, mean age (38). Origin of patients was as follows, Bolivia (58), Democratic Republic of Congo (1) Ecuador (2), Equatorial Guinea (7), Honduras (2), India (1) Sierra Leone (1). All patients, except two, presented eosinophilia at the moment of diagnosis. Thirty-five of these 72 patients (49%) were found co-infected with CD. Co-infected patients were all Bolivian, 19 (54%) male, mean age (37). All, except one, showed eosinophilia. *T. cruzi* infection was confirmed in 71 patients by the two described ELISA tests, only in one patient discordant results were found between ELISA tests, and infection was confirmed by IFI.

Conclusions: We have found a high prevalence of co-infection of St and CD in patients from Latin-America attending to our clinic. Bolivian patients are the most important group, at the presence of eosinophilia, the screening for St and CD is strongly recommended. Taking into account the nature of these NTDs, their burden could be underestimated in non-endemic areas.

R2719 Short-term medical response in Haiti: epidemiology and model for delivery of care

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Objectives: This report describes an acute care medical mission clinic model and data collected on diseases seen during a trip organised by International Medical Relief (IMR) during the period from July 2–10, 2011 in Haiti.

Methods: The team consisted of 40 members including medical, dental and community education professionals in 7 locations within and around the capital, Port au Prince.

Results: Over an 8-day period, 1696 patients were seen, 641 male and 1042 female. Mean age was 24.6 years (range 30-days to 94 years). All patients sought medical attention and 254 patients also sought dental care. All patients and providers participated in the community education sessions. For women, number of pregnancies, live births and children currently was 3.38, 3.08 and 2.86 mean. The most common presenting complaint was fever, in 304 patients. Gastrointestinal illness was observed in 222 patients, which included gastrointestinal reflux, parasitosis, and acute diarrhoeal illness. One hundred and sixty-nine patients had respiratory illnesses, mostly upper respiratory illness, pharyngitis and otitis in children. Eye dryness occurred in 162 patients, and was related to dust and smoke in the air. Skin rashes including infections (wound infection, cellulitis, impetigo, ringworm and scabies) occurred in 153 patients. 151 patients complained of pain and malaise. Vaginal symptoms related to fungal, bacterial vaginosis and likely sexually transmitted illness was present in 118 patients. Post-traumatic stress related symptoms were common, often presenting as pain, vague abdominal complaints, anxiety and difficulty sleeping. Clinical presentations typical of tuberculosis, advanced AIDS, and severe cholera were uncommon. Mumps was suspected in one patient, there were no diagnosed cases of measles or rubella. Three patients were transferred to acute care hospitals, one for refractory seizures, a second for diarrhoea refractory to oral rehydration likely secondary to cholera and the third for a large intra oral, retropharyngeal abscess. 83% of patients said they had seen a provider in the past. 16% were previously diagnosed with a condition including malaria, TB or HIV. 45% had received prior immunizations; 58% of children had received polio immunization and 55% stated they slept with bed nets.

Conclusion: The intervention bundle described in this report of an acute care clinic staffed by short term medical and education workers provides a useful model for acute care in developing countries.

R2720 Evaluation of *Toxoplasma gondii* Liaison IgG and IgM assay

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Objectives: The serological diagnosis of toxoplasmosis is very complex. Diagnosis is especially difficult for pregnant women. In Slovenia we have established screening programme in 1995 to prevent congenital toxoplasmosis. We are testing pregnant women three times during the pregnancy. The purpose of this study was to evaluate the analytical performance of the Cobas Toxo IgG and IgM (Roche, USA) immunoassays and compare the results with those of the Liaison Toxo IgG and IgM (Dia-Sorin, Italy) assays.

Methods: We have done prospective study for *T. gondii*. IgG and IgM. In May and June, a total of 870 samples were collected from 870 pregnant women. Serums for Toxoplasma IgG and IgM testing were stored at 4°C until use. Each sample was tested on both machines in parallel. To confirm positive IgM cases we used ISAGA IgM assay (bioMerieux, France). To confirm acute toxoplasmosis we used testing of second serum that was taken 3–4 weeks after first serum.

Results: We detected two cases of acute toxoplasmosis. One was woman was infected early in first trimester. She had a rise in the values of IgG in the second serum sample. The second woman seroconverted in second trimester of pregnancy. The Cobas IgM values were all the time negative in the second case. The detection of IgG was not problematic in both assays. In seven cases we found false positive IgM with Cobas assay and four cases of false positive IgM with Liaison assay. In both of the cases of acute toxoplasmosis we could not detect IgM with Cobas assay.

Conclusion: In our opinion the Liaison assay is more reliable than Cobas assay. We discovered less false positive IgM with Liaison. Any false positive result on *T. gondii* testing presents great psychological burden for pregnant women. On the other hand we would miss acute infection, if we would use only Cobas assay.

Table 1: Results of discrepant cases.

Patient	COBAS IgG (IU/ml)	COBAS IgM (COI)	LIAISON IgG (IU/ml)	LIAISON IgM (AU/ml)	ISAGA
1	544,6	1,5	169	Neg	Pos 12
2	1106	1,14	70,7	Neg	Neg 3
3	20,6	2,03	19,9	Neg	Neg 3
4	1,23	Neg	Neg	Neg	Neg 3
5	1497	1,32	87,6	Neg	Pos 11
6	Neg	2,57	Neg	Neg	Neg 1
7	Neg	3,12	Neg	Neg	BV 6
8	4,8	Neg	Neg	Neg	Neg 3
9	513,4	1,09	93,6	BV	Pos 11
10	Neg	1,3	Neg	Neg	Neg 3
11	288,7	Neg	27,7	11,5	BV 8
12	Neg	Neg	Neg	9,7	Neg 1
13	230,7	1,23	73,7	Neg	Neg 3
14	Neg	2,7	Neg	Neg	Neg 3
15	Neg	Neg	Neg	8	Neg 2
16	Neg	Neg	Neg	8,3	Neg 0
17	41,3	Neg	Neg	BV	Neg 0
18 AT	195,2	Neg	51	11,1	Pos 11
19 AT	268,5	Neg	88	17,2	Pos 12

BV boarder value; Pos positive; Neg negative; AT acute toxoplasmosis.

R2721 Can PCR provide the clue to a diagnosis of swine cysticercosis?

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Introduction: Cysticercosis is a zoonotic disease due to Taenia solium, which involves pork as intermediate host. It is endemic in Madagascar, however very few data has been reported concerning porcine cysticercosis prevalence. Lack of ante-mortem diagnostic tools makes the prevalence evaluation difficult. Tongue palpation is very specific but of poor sensitivity. Serological tests detecting antigen or antibodies are sensitive for human cysticercosis diagnosis but are not yet considered as a gold standard in swine ante-mortem examination. PCR are wildly used to detect pathogens but poorly evaluated for the diagnosis of cysticercosis.
Methods: We compare the performance of PCR and ELISA assays on sixty seven pig serums: 22 from cysticercosis positive pigs (meat inspection) and 45 from cysticercosis negative animals (originating from a non-endemic country or grown in industrial and well isolated pigsties). Among the negative samples 19 were collected from pigs with trichinellosis and four from pigs with toxoplasmosis.

Table I. Evaluation of Antibody Elisa and 3 PCR for the diagnosis of swine cysticercosis in serum samples

	Elisa		B1B2-PCR		T3T4-PCR		T3T4-T1T2-PCR	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
Infected swine (N=22)	22 (100%)	0 (0%)	5 (23%)	17 (77%)	7 (32%)	15 (78%)	14 (64%)	8 (36%)
Non infected swine (N=22)	12 (27%)	33 (73%)	0 (0%)	45 (100%)	0 (0%)	45 (100%)	0 (0%)	45 (100%)

Table II. Sensitivity, specificity, NPV, and PPV of immunoenzymatic and PCR assays for the diagnosis of swine cysticercosis in serum samples

Evaluated assays		Sensitivity	Specificity	PPV	NPV
Ab-Elisa		100%	73%	65%	100%
PCR	B1B2	23%	100%	100%	73%
	T3T4	32%	100%	100%	75%
	T3T4-T1T2	64%	100%	100%	89%

Results: Results indicate that ELISA assay showed high sensitivity and good specificity while the PCR assays showed high specificity but a low sensitivity.

R2722 Post-malaria neurologic syndrome in falciparum malaria case

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Infection with *Plasmodium falciparum* is known to cause several neurological complications. The most deleterious presentation is cerebral malaria, carrying a mortality of 10–50% in treated patients. Patients can experience a neurological syndrome following successful treatment rarely. The syndrome has been defined as the acute onset of neurological or neuropsychiatric symptoms in patients recently recovered from *Plasmodium falciparum* malaria who have negative blood films at the time of onset.
We report a case of PMNS after successful treatment for *Plasmodium falciparum* malaria. He was travelled to Equatorial Guinea 2 weeks before admission and referred to a hospital with fever and fatigue. After referral to our hospital 6 days later with no diagnosis, he had taken to intensive care unit with cerebral malarial clinical presentation. Blood smears revealed *Plasmodium falciparum* parasites. He was put on quinine and tetracycline and improved in 2 days. Two weeks after discharge, he began to experience headache, mild hand tremor, psychosis and strange behavior. He readmitted to the hospital, and presented with irritability, visual hallucination. Blood smears showed no parasites. Blood biochemistry was normal. Neurologic examination and electroencelaphalography was normal. Cranial MRI was also normal except T2 hyperintensity in proximity of right trigeminal nerve. He improved with anti-psychotic medication (Olanzapin) and had no complaints in a week.
The first description of PMNS was a large case series from Vietnam and Thailand. Of 1176 patients with severe falciparum malaria, 21 (1.8%) developed PMNS. PMNS usually occurs in adults after recovery from severe falciparum malaria. Median onset was 4 days in the Vietnamese study and 15 days for the other cases. It is a postinfectious diffuse encephalopathy, characterized by >1 of the following: impaired consciousness, confusion, aphasia, seizure, tremor, psychosis, and in severe cases, ataxia. Patients often are febrile at presentation. PMNSis self-limited, lasting 2–14 days, and requires no specific treatment.

Resistance and mechanisms of action of antifungals

R2723 Antifungal susceptibility profile of bloodstream *Candida* isolates in an intensive care unit of a tertiary hospital

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Introduction: Incidence of nosocomial fungal infections have been increased in intensive care unit (ICU), especially in patients who underwent surgery. In recent years, azole prophylaxis, have led to change in the distribution of *Candida albicans* and non-albicans *Candida* species. Our objective is to evaluate the resistance profile of *Candida* spp. isolated from candidemic patiens followed-up in ICU of our hospital.
Materials and Methods: The patients are consulted by infectious diseases and clinical microbiology consultants daily in our 13 bed medical/surgical ICU. The patients with positive peripheral blood cultures for *Candida* species was evaluated from January 1st 2005 to December 31st, 2007. Stored *Candida* spp. at -70°C were subcultured onto Sabouraud dextrose agar and *Candida* spp. grown in cultures were evaluated by germ-tube test, according to the morphological characteristics of colonies in Sabouraud dextrose agar and CHROMagar, and has been identified by the commercial identification system API 32C. Antifungal susceptibility was performed by E-test method according to the manufacturer's instructions. Interpretations of minimal inhibitory concentration (MIC) levels for fluconazole, itraconazole, voriconazole, caspofungin

Table. Antifungal susceptibilty profile of *Candida* spp. isolated from blood culture in the ICU.

Species	MIC90 µg/mL	MIC50 µg/mL	Range µg/mL
C. albicans (13)			
Amphotericin B	0.5	0.38	0.19-0.5
Caspofungin	0.047	0.016	0.002-0.094
Anidulafungin	0.003	0.002	0.002-0.008
Fluconazole	0.5	0.19	0.004-0.75
Itraconazole	0.38	0.064	0.023-0.5
Voriconazole	0.023	0.008	0.008-0.47
Posaconazole	0.032	0.023	0.008-0.47
C. parapsilosis(6)			
Amphotericin B	0.25	0.19	0.10-0.38
Caspofungin	0.38	0.19	0.032-0.5
Anidulafungin	6	1	0.19-12
Fluconazole	3	0.25	0.094-3
Itraconazole	0.5	0.19	0.012-3
Voriconazole	0.094	0.012	0.008-0.32
Posaconazole	0.25	0.047	0.016-0.25
C. glabrata(6)			
Amphotericin B	0.38	0.19	0.006-0.5
Caspofungin	0.125	0.016	0.002-0.125
Anidulafungin	0.012	0.006	0.002-0.016
Fluconazole	2	6	1.5-12
Itraconazole	4	2	0.5-8
Voriconazole	0.19	0.19	0.002-0.25
Posaconazole	3	1.5	0.25-3

and anidulafungin were evaluated according to Clinical Laboratory Standards Institute. As there are no established breakpoints for amphotericin B, breakpoints proposed by Nguyen et al. were used.
Results: Thirty-two candidemia episodes were identified in 32 patients. Incidence of candidemia was 22 per 1000 admissions. The most frequent species were *C. albicans* (41%), followed by *C. parapsilosis* (19%), *C. glabrata* (19%), *C. tropicalis* (9%) and *C. dubliniensis* (9%). Overall susceptibility to amphotericin B, voriconazole and caspofungin were 100% among all *Candida* species. Although 100% of *C. albicans*, *C. parapsilosis*, *C. tropicalis* and *C. dubliniensis* were susceptible to anidulafungin, only 33% of C. parapsilosis were susceptible. Susceptibility to fluconazole were 100% among all *Candida* spp. except *C. glabrata* (83% susceptible). Posaconazole exhibited low MICs for all isolates, except three in *C. glabrata* (MIC >1 μg/mL). MIC50 and MIC90 levels of the *Candida* spp.are demonstrated in table.
Conclusion: In recent years, the distribution of *Candida* spp. has been shifted towards non-albicans *Candida* spp. But antifungal susceptibility results have demonstrated that fluconazole may be still used as a first choice in empirical therapy in our unit.

R2724 In vitro synergism of nine antifungal combinations against bloodstream *Fusarium solani* and *Fusarium oxysporum* isolates

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Objectives: Fusarial infections have both high mortality and morbidity rates among immunosupressed populations. Treatment is a major problem since two of the most prevalent species – *Fusarium solani* and *Fusarium oxysporum*- are usually resistant to antifungals. Therefore, the present study aimed to evaluate synergism of antifungals combinations against bloodstream *Fusarium solani* and *Fusarium oxysporum* isolates.
Methods: The clinical isolates were all from blood source. These were previously identified by the University of Texas Health Science Center at San Antonio University of Texas Health Science Center Fungus Testing Laboratory. Susceptibility was determined according to methods outlined in Clinical and Laboratory Standards Institute document M38-A2. Drug interactions were tested using the checkerboard microdilution method. Interaction was determined by calculating the fractional inhibitory concentration index (FICI) with standard definitions: synergy FICI ≤0.5, indifference FICI >0.5 and = 4, and antagonism FICI >4. The in vitro activities of Amphotericin B (AMB), Caspofungin (CAS), Poscanazole (POS), Voriconazole (VOR) and Terbinafine (TER) alone and in the following combinations AMB + CAS, TER + CAS, TER + AMB, POS + AMB, TER + POS, CAS + POS, AMB + VOR, CAS + VOR, TER + VOR were evaluated. Identification was based on morphology and *Paecilomyces variotii* ATCC 3257 was included as quality control strains for minimum inhibitory concentration (MIC) determination. The MIC50 and MIC90 were calculated using Microsoft Excel 14.0.
Results: We analyzed 14 *Fusarium solani* isolates and five *Fusarium oxysporum* isolates. Synergy was observed for the combination VOR+TER against 14/19 isolates (*F. solani* 9/14 and *F. oxysporum* 5/5). The MIC50 and MIC90 against TER alone (>4 μg/mL, >4 μg/mL) and VOR alone (4 μg/mL, >4 μg/mL) were higher than in combination (TER=2 μg/mL, 4 μg/mL and VOR = 1 μg/mL, 4 μg/mL). The following combinations had synergism against F solani isolates: AMB + CAS 2/14, TER + CAS 0/14 and TER + AMB 1/14. Synergy was noted fot the following combinations against F oxysporum: AMB + CAS 1/5, TER + CAS 1/5 TER + AMB 1/5, CAS + VOR 1/5 and TER + VOR 5/5.
Conclusion: The combination VOR + TER showed synergism against 73.7% of bloodstream fusarial isolates while the common used combination AMB + VOR was indifferent against all isolates. There were no antagonism.

Fungal infections

R2725 Epidemiology and outcome of invasive candidiasis in an intensive care unit of a central hospital in Lisbon

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Objective: To determine the incidence of invasive candidiasis, factors associated with risk of infection, and to identify the main infectious *Candida* species in the Intensive Care Unit (ICU) of the Hospital Egas Moniz.
Methods: A prospective observational study was conducted from May to December 2009 in ICU of Hospital Egas Moniz. Demographic information, risk factors, therapy and outcome of all patients in the ICU were reviewed. Statistical analyses were performed using Epi Info 2000 and SPSS 5.3, 18.0 Statistic PAWS. Mean ± SD was determined for quantitative data, and frequency was determined for categorical variables.
Results: During this period, 28 episodes of invasive candidiasis (IC) were reported in 26 patients, accounting for an overall incidence of 12.8% and for a candidemia incidence of 0.46%. In the five death cases associated with IC, five were due to *C. albicans*, from which two were mixed infections with *C. glabrata* or *C. parapsilosis*, accounting for 19.2% of mortality rate and a proportional mortality ratio of 11.1%. Patients with IC had mean age of 72.3 years, and patients hospitalized longer than 21 days accounted for 46% of the cases. In all age groups, IC occurred more frequently in males (73.1%). Hypertension (69.2%), chronic obstructive pulmonary disease (46.2%), heart failure (42.3%), diabetes mellitus (30.8%), central venous catheter (100%), and mechanical ventilation (88.5%) were the major underlying conditions. The use of broad-spectrum antibiotics, namely carbapenems (n = 22) and piperacilin+tazobactan (n = 12), was prevalent in patients with IC. In patients with previous quinolones use, a trend for the isolation of non-albicans species was noticed.
The most common pathogen was *C. albicans* (59.3%), followed by *C. glabrata* (15.5%), *C. krusei* (12.4%), *C. tropicalis* (9.7%) and *C. parapsilosis* (3.1%). 40% of IC was due to non-albicans species. Mixed infections due to two species were recorded in six cases (21.4%).
Conclusions: *Candida* species are an important cause of invasive infection in patients with co-morbidities and extremes of age are at highest risk. These results help to establish epidemiological measures for the control of IC. In addition, these findings reinforce the need for continued and active surveillance programs to address the changes in the species distribution which will help to develop effective, preventive and therapeutic strategies.

R2726 Detection of *Aspergillus* antigen galactomannan in diagnosis and follow-up of the *Aspergillus* infections

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Objectives: The invasive aspergillosis (IA) remains a common cause of morbidity and mortality in immunocompromised patients, although there is a lot of progress in diagnosis and therapeutic management. Aim of our study is to evaluate the detection of *Aspergillus* antigen galactomannan (GM) in diagnosis and following up in infected patients of our hospital.
Methods: During the period 2008–2010, 2499 clinical samples were examined for the detection of GM antigen. Samples (1–8/patient) were from 422 immunocompromised hospitalized patients. Patients were mostly neutrophenic with symptoms of infection. GM antigen was detected by Platelia *Aspergillus* EIA method (Biorad, Marnes-La-Coquette, France). Samples that had ratio (r) >0.5 were rated as positive. Statistical analysis was made using SPSS (paired samples t-test).
Results: The positive samples were 133/2499 (5.32%). These samples were from 71/422 (16.8%) patients. Apart from GM there were other clinical and laboratory findings that confirmed aspergillosis. Fifty-eight

patients were from the hematological clinic, eight from the intensive care unit, four from the pathological clinic and one from the surgical clinic. One hundred and eight from the 133 positive samples were blood samples, 12 were bronchoalveolar lavage (BAL) and 13 were cerebrospinal fluid (CSF). During the weekly detection of the GM antigen titer, r was 0.5–5.6 in blood samples and 0.8–6.7 in the rest of the samples. In infections of the central nervous system, levels of r were statistically higher ($p < 0.05$) in CSF (mean 4.76 ± 0.36) than in blood (mean 1.76 ± 0.64). A difference between levels of BAL and blood in the infection of the respiratory system was not statistically significant ($p > 0.05$). Levels of r correlated well with response to therapy.
Conclusions: Detection of GM antigen is a reliable marker for early diagnosis of IA and therapeutic management of high risk patients such as immunocompromised patients.

R2727 A fungi as a cause of hospital infection: the results of 2 years of active surveillance in a tertiary care hospital

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Background: It was aimed to investigate the frequency of fungal infections (FI), to determine typing of fungi that were isolated from patients and to evaluate risk factors associated with FI and mortality.
Methods: This study was carried out between Jan1, 2009 and Dec 31, 2010 as prospective and controlled in intensive care units (ICU), and the patients were observed with active surveillance. Colony morphology, and germ tube formation methods, and VITEK 2 Compact System kits were used for the identification of isolates.
Results: In this study, 2362 patients were followed for 16 135 patient days of hospitalization. During the study, 63 (27.5%) of 229 patients who developed nosocomial infection (NI) 77 episodes of FI were observed. Patients of 54% were male, 46% were women. Hospital stay (OR = 1.03, p = 0.007), hyperglycemia (OR = 17.93, p = 0.009), and accompanied by other infections (OR = 3.98, p = 0.001) were identified as independent risk factors for FI. *Candida* spp. were determined the only as cause of FI. 77 of 135 candida strains was isolated as causative pathogens. The distribution of isolates were 63.6% *C. albicans*, 14.2% *C. tropicalis*, 6.4% *C. glabrata*, 6.4% *C. parapsilosis*, 3.8% *C. kefyr*, 3.8% *C. dubliniensis* and 1.2% *C. zeylanoides*. High APACHE II scores (OR = 1.37; p = 0.002), and the use of central venous catheter (OR = 9.01; p = 0.04) were assigned as independent risk factors for mortality. The rates of mortality attiributable to candida infection and candidemia were 27%, and 18.3% respectively. The mortality rate related *C. albicans* and *C. tropicalis* was 12.2%.
Conclusions: As a result, FI rate is increasing in our hospital. FI and mortality can be prevented by controlling of some risk factors as well as length of hospital stay and hyperglycemia. Typing of fungi should be done routinely to treat FI successfully.

R2728 Species distribution and antifungal susceptibilities of yeast isolated from catheterised urine specimen

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Objectives: The aim of the present study was to evaluate the effect of urinary catheter on species distribution and susceptibilities of antifungals against clinical isolates of yeasts from catheter-associated urinary tract infection (CAUTI).
Methods: A total 281 yeast isolates from catheterized urine in a medical and surgical ward were collected. Species identification and antifungal susceptibulity test to amphotericin B, fluconazole, voriconazole and flucytosine were performed by VITEK 2 system (bioMérieux Inc., Hazelwood, MO, USA).
Results: The most frequent species was *Candida tropicalis* (48.8%), followed by *C. albicans* (24.6%), *C. glabrata* (15.7%) and *Trichosporon asahii* (5.0%). *C. tropicalis* and *T. asahii* were more frequently isolated in a surgical ward than medical ward ($P < 0.05$). Decreased susceptibilities to amphotericin B were observed in *C. albicans* and *T. asahii*. All isolates except *C. glabrata* and *C. krusei* were susceptible to fluconazole and voriconazole.
Conclusions: The results of this study suggest the possibility that urinary catheter may lead to influence on species distribution of yeast of CAUTI. There is an need for continuous surveillance of CAUTI by yeast for the control of CAUTI.

R2729 Fungal infections diagnosed in patients of haematological department

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The aim of this study was to test the utility of the detection of *Aspergillus* GM and fungal DNA in blood samples from patients with clinical signs of infection.
Methods: Within the period of 3 years 80 patients of the Department of Haematology who were treated with conventional chemotherapy due to leukaemia (40), lymphoma (7), multiple myeloma (8) or other malignancies: CLL (17) MDS (8) were examined for the presence of fungal biomarkers in the blood. The patients showed FUO or abnormalities in respiratory tract and/or central nervous system demonstrated in radiological examination. The laboratory examinations included testing of Aspergillus galactomannan (GM) by Platelia™Aspergillus (BioRad, France) and investigation of fungal DNA by previously described universal primers and *Aspergillus*- and *Candida*- specific TaqMan probes (White et al., CID 2006; Khlif et al. CMI 2009). DNA from EDTA-blood samples was extracted by mechanical cell disruption and commercial test QIAmp® DNA Mini kit (Qiagen. The presence of PCR inhibitors was tested using the TaqMan Exogenous IPC Reagents (Applied Biosystem).
Results: The multiple positive results of GM blood testing were obtained in two patients with ALL, of them one was also positive for Aspergillus DNA. Next three patients (two with ALL and one with MM were positive in Aspergillus TaqMan, despite of negative GM results, however clinical and radiological picture were in agreement with positive TaqMan sample.
Candida DNA was detected in the blood from seven patients, no one of them showed positive blood cultures, however in some patients pneumonia was diagnosed based on clinical and radiological findings. The patients were treated empirically with ketokonazol and voriconazol with good outcome. The blood culture positive for fungi were found in one patient with negative results of tested biomarkers. The causative organism was identified biochemically and by sequencing of ribosomal DNA as Trichosporon asahi. In the another patient (AML) the mucormycosis was detected by the culture of Absidia corymbifera from the respiratory tract. Applying the PCR with universal primers followed by sequencing of PCR product enabled to detect the DNA of this microorganism in patient's blood sample.
Conclusion: Molecular examination of blood samples together with molecular identification of cultured microorganisms seems to be an useful tool improving the diagnosis of IFI in haematological patients.

R2731 Candida blood infections in a 5-year period

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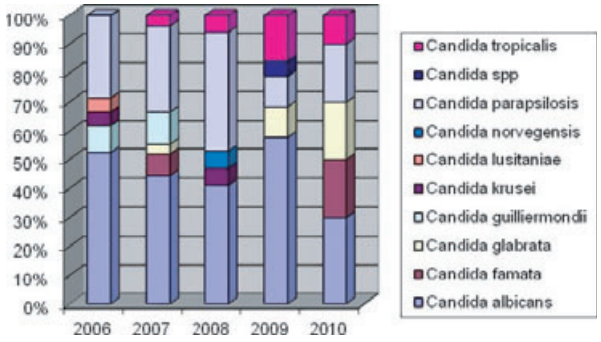
Objective: *Candida* species are a common cause of nosocomial bloodstream infections and are associated with a significant morbidity and mortality. The aim of this study is to determine the epidemiology of candidemia in our hospital over a 5 years period.
Methods: Retrospective study of candids isolates from blood samples of patients attended in the Hospital Universitario Miguel Servet from 2005 to 2010. Isolates were identified using chromogenic agar (CHROMagar®) and API 20C AUX (bioMèrieux)

Results: A total of 186 candidemia cases were identified among 28 480 patients with blood sample studied in this period. The candidemia cases in adults were isolated in 94 patients in adults in critical units care. 61.16% were male, age range 16–95 years old with a mean age of 63 years old. Isolation was most frequent in patients hospitalized in intensive care units (32.23%). The most frequent isolates are shown on table 1. Forty-four cases were isolated in children in critical care units under 14 years old (male 58.49%; age range: 1 day–13 years old). The most frequently isolated species were *C. albicans* (43.39%) and *C. parapsilosis* (28.30%) and *Candida guilliermondii* (13.20%). The pair urine-blood culture was obtained in 15 adult patients. In 10 (66.67%) cases, the urine and blood culture isolates belonged to same specie and the *Candida* isolates from urine and blood were different in five patients (33.33%).



Conclusion: *Candida albicans* remains the most common isolated specie.
The critical unit pediatric had experiment an important decrease in the candidemia in the last 3 years. Further studies are obliged to find out the predisponent factors of bacteremia.

R2732 Rare tropical fungi in Madagascar. When should we think of them?

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Emergent, unknown, misdiagnosed may qualify two type of fungal infection in Madagascar: sporotrichosis and chromoblastomycosis.
The aim for this topic is to summarize and illustrate the current localization of both diseases with updated data, the clinical patterns of the infection and the laboratory procedures to recover the pathogens; therepeutic options are also noted. Sporotrichosis is a subcutaneous disease due to Sporotriochosis schenkii, an environmental dimorphic agent that typically causes lymphnode enlargement following a lymphatic pathway, ulceration may occurs and can lead to secondary bacterial infections. The disease is currently found in the Malagasy highlands: with a fairly cold and dry weather and is directly related to the injure of wood splinters or plant thorns. Treatment is based on Potassium Iodine or Itraconazole. Pro and cons are listed.
Chromoblastomycosis occurs mainly in the dry and hot South-East of Madagascar where deamacious fungi harboured by cactuses and dry plants thorns are said to be responsible of this chronic subcutaneous disease displaying many kind of clinical lesions (displayed). pathology is the base of its diagnosis, fungi are hard to isolate thanks to the overgrowth of other environmental moulds. Its treatment relies mostly on terbinafine, even though a combination of antifungal agents is strongly advised.
Surgery may be used for limited lesions and relapses may occur due to a default of patient compliance or treatment inefficiency.

R2733 Invasive pulmonary aspergillosis and zygomycosis in an AIDS patient

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Introduction: Fungal Invasive filamentous lung infections are very rare conditions in AIDS but must be considered in patients with profound immune suppression.
Clinical Case: A 34-year-old female was admitted to our Unit in January 2010 with Listeria monocytogenes meningitis. She had a known history of AIDS and a plasmablastic lymphoma of the oral cavity diagnosed 1 month before admission and was on antiretrovirals and chemotherapy since then. She improved under antibiotic therapy but after the third week in hospital, she developed persistent cough, low grade fever, dyspneia and hypoxemia. The thoracic CT scan revealed interstitial infiltrates and cavitation on the upper right lobe. A bronchofibroscopy revealed a grayish lesion on the wall of the main right bronchus, macroscopically described as ''caseous granulomas''. Microbiological examination of BAL was negative. Antituberculous therapy was started without improvement. Histology of bronchial mucosa revealed extensive necrosis with fungal hyphae suggestive of Mucor. Another bronchoscopy was done and histology confirmed aspects of Mucor and CMV infection. The patient started liposomal amphotericin B and valganciclovir for pulmonary mucormycosis and CMV peumonia with clinical and radiological improvement. She completed 3 weeks on amphotericin B and switched to posaconazole due to hypokalaemia and to enable oral dosing. Despite a reduction of the cavitation and improvement of lung infiltrates, it was considered wiser to do a lobar lung resection that was performed on the 36th day of antifungal therapy. Histology revealed a cavitation containing grayish white material, with extensive necrosis and numerous hyphae compatible with Aspergillus and Mucor. Oral posaconazole was maintained for 2 months and she resumed treatment for lymphoma with local radiotherapy. She remains without evidence of lymphoma or fungal lung infection.
Discussion: The clinical presentation of fever and prolonged respiratory symptoms with pulmonary cavitation suggested tuberculosis. The endobronchial mucous material described as ''caseous granulomas'' was in fact endobronchial mucormycosis. Surgical approach was essential, in spite of clinical improvement with antifungal therapy. The concomitant finding of pulmonary aspergillosis and zygomycosis in the surgical specimen confirms the profound delicateness of patients with severely compromised immune systems.

AIDS and HIV infection

R2734 Prevalence of QTc prolongation among HIV-infected patients: a clinical prediction tool to determine high-risk patients

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Objectives: The objectives of this study were to (i) quantify prevalence of QTc prolongation and (ii) determine the predictors of QTc prolongation among HIV-infected patients.
Methods: A cross-sectional study was performed among HIV-infected patients receiving care at the Albany Medical Center between Jan 2007 and Jul 2011. A random sample of these patients was selected for screening. Inclusion criteria were: (i) age ≥18 years, (ii) documented HIV-infection, and (iii) availability of at least one echocardiogram (EKG) test in the patients' medical record. Trained reviewers extracted the following from the patients' medical records: demographics, co-morbid conditions associated with QTc-prolongation, EKG test results, and medication histories. Normal QT interval was defined as ≤430 milliseconds among men and ≤450 milliseconds among women. Results above these thresholds were considered abnormal. Logistic regression was utilized to determine the variables independently associated with QTc prolongation. Significant variables in the logistic regression analyses were used to determine the predicted probabilities of the outcome.
Results: There were 138 patients that met inclusion criteria. The mean (SD) QT interval was 420 (25.9) ms. Abnormal QT intervals were observed in 38 (27.5%) patients. The distribution of concomitant comorbidities differed significantly between patients with abnormal and normal QT intervals. The frequency of cardiac, hepatic and renal comorbidities were higher among patients with abnormal QT intervals compared to patients with normal QT intervals. Known medications with a high risk of causing QT prolongation were more prevalent among patients with an abnormal QT than patients with a normal QT interval. In multivariate analyses, the only variable to be independently associated with abnormal QT interval was the number of comorbidities (OR = 2.9, 95% confidence interval 1.81–4.66, $p < 0.001$). The observed and predicted probabilities of abnormal QT, stratified by number of comorbidities, are displayed in Table 1. Overall, there was good concordance between observed and predicted probabilities and the risk of abnormal QT interval increased monotonically.

Table 1: Observed and Predicted Probabilities of Abnormal QT Interval, Stratified by Number of Comorbidities

Number of Comorbid Conditions	Observed Probability	Predicted Probability
0 conditions (n=74)	16.2 %	13.4 %
1 conditions (n=39)	23.1 %	31.0 %
2 conditions (n=19)	57.9 %	56.6 %
3 conditions (n =2)	100 %	79.1 %
4 conditions (n=4)	100 %	91.7 %

Conclusion: This study included patients with an available EKG readings and demonstrated a high prevalence of HIV-infected patients with an abnormal QT interval. The number of comorbidities was the only variable independently associated with an abnormal QT interval.

R2735 Quantifying the incidence and severity of acute renal failure among HIV-infected adults receiving tenofovir-based antiretroviral therapy

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Objectives: (i) Quantify incidence of acute renal failure (ARF), (ii) Classify severity of ARF & (iii) Determine predictors of ARF.

Methods: A retrospective cohort study was performed among patients receiving tenofovir (TDF) at the Albany Medical Center between Jan 2007 and Jul 2011. Inclusion criteria were: (i) age≥18 years, (ii) HIV-infection, (iii) receipt of TDF for ≥1 month, and (iv) availability of laboratory results to classify outcome status. Trained reviewers extracted the following from the patients' medical records: demographics, co-morbid conditions associated with chronic kidney disease (CKD), serum creatinine (SCr), CD4 count, HIVRNA, and medication histories. Creatinine clearance (CLCR) was calculated using the Cockroft-Gault method. ARF was defined and classified using criteria proposed by the Acute Dialysis Quality Initiative Group as follows: Risk (SCr increase × 1.5 or CLCR decrease >25%), Injury (SCr increase × 2 or CLCR decrease >50%) and Failure (SCr increase × 3 or CLCR decrease >75%). Logistic regression was utilized to determine the variables independently associated with each ARF classification.
Results: There were 298 patients that met inclusion criteria. There were 173 (58.1%) males. The mean (SD) age at the start of TDF was 48.5 (9.0) years. The median (IQR) SCr and CLCR values were 0.9 (0.8–1.1) mg/dL and 95 (78–110) mL/minute, respectively. Concomitant nephrotoxins were used in 71 (23.8%) patients. The incidence of ARF was 22.8% during the study period. The number of patients that satisfied the Risk, Injury and Failure classification criteria were 68 (22.8%), 21 (7.0%) and 12 (4.0%), respectively. In bivariate analyses, variables associated with ARF-Risk were underlying CKD, concomitant abacavir use, and duration of TDF. Variable associated with ARF-Injury classification are displayed in Table 1. There were no variables significantly associated with the Failure classification. In multivariate (MV) analyses assessing ARF-Risk as the outcome of interest, underlying CKD was the only independent predictor variable (OR = 3.93, 95% CI: 1.44–10.73, $p = 0.008$) after adjusting for duration of TDF use. In MV analyses assessing ARF-Injury as the outcome, independent predictor were CKD (OR = 4.38, 95% CI: 1.31–14.62, $p = 0.02$) and hypertension (OR = 3.69, 95% CI: 1.34–10.13, $p = 0.01$).

Table 1: Variables Associated with Acute Renal Failure – Injury Classification (SCr increase x 2 or CLCR decrease >50%)

Covariate	Injury (n = 21)	No Injury (n = 277)	P-value
Race:			0.13
• White	7 (33.3)	132 (47.7)	
• Black	13 (61.9)	103 (37.2)	
• Hispanic	1 (4.8)	24 (8.7)	
• Other	0 (0)	18 (6.5)	
Males	13 (61.9)	160 (57.8)	0.71
Diabetes mellitus	3 (14.3)	28 (10.1)	0.47
Hypertension	15 (71.4)	97 (35.0)	0.001
Chronic Kidney Disease	5 (23.8)	12 (4.3)	<0.001
Concomitant Nephrotoxins	9 (42.9)	62 (22.4)	0.03
Median (IQR) TDF duration (months)	30 (23 – 34)	26 (15 – 36)	0.04
Concomitant Protease Inhibitors	17 (81.0)	156 (56.3)	0.04

All data presented as n (%), unless otherwise noted

Conclusion: The incidence of ARF varies as a function of severity. Underlying CKD and hypertension are important variables when predicting severity of ARF.

R2736 QTc interval prolongation in HIV-infected patients: a case-control study

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Objectives: QTc interval prolongation in HIV-infected patients has been associated with several drugs, autonomic dysfunction, and HCV coinfection. Since the duration of ventricular repolarization can change during the day, we assessed QTc interval by a 24-hour ECG recording.

Aim of the study was to evaluate the risk factors associated with QTc prolongation and the indices of cardiovascular autonomic control.
Methods: Twenty-seven consecutive HIV+ patients (group I) with known prolonged (>440 milliseconds) QTc interval as assessed by standard ECG, and 54 HIV+ patients with normal QTc interval at standard ECG (group II) matched 1:2 with group I individuals by gender and age (±5 years) underwent 24-hour Holter ECG. A case-control study was performed using as cases the patients of both group I and II with prolonged QTc interval as assessed by Holter ECG (mean QTc >440 milliseconds), and as controls (1:2) HIV+ subjects with normal QTc interval as assessed by Holter ECG, matched by gender and age. Autonomic nervous system function was evaluated by heart rate variability (HRV) analysis during 24-hour ECG recording.
Results: Eighteen out of the 27 group I patients (67%) had QTc interval prolongation confirmed by Holter ECG. Fourty of the 54 group II patients (74%) had a normal QTc interval at Holter ECG. Overall, 32 patients from both group I and II had a prolonged QTc interval (mean 475 ± 33 milliseconds) assessed by Holter ECG and were considered cases. They were matched by gender and age (±5 years) with 64 controls exhibiting a normal QTc interval assessed by Holter ECG. Duration of HIV disease was significantly longer among cases than among controls (10.9 ± 7.1 years vs. 7.8 ± 6.4 years; p = 0.04). Waist/hip ratio was higher among cases than among controls (0.93 ± 0.07 vs. 0.90 ± 0.07; p = 0.05). Antiretroviral drugs were not associated with QTc prolongation at a statistically significant level. HRV analysis showed the absence of physiologic decrease of low frequency (LF) in the night period in both cases and controls. The LF night in cases showed a statistically significant reduction when compared with controls (p = 0.007).
Conclusions: In our study group the QTc interval prolongation was associated with a longer duration of HIV infection and with a greater waist/hip ratio. HIV-infected patients with QTc interval prolongation and with a longer duration of HIV infection were more likely to have an impairment of parasympathetic and sympathetic cardiac component.

R2737 Assessment of factors influencing health-related quality of life in HIV-infected patients: the era of co-morbidities

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Objectives: To determine factors influencing health-related quality of life (HRQL) in a cohort of HIV-infected pts.
Methods: This was a single-center cross-sectional study in pts attending the outpatient clinic during a 3-month period in 2011. Exclusion criteria were: age <18 years, seropositivity diagnosis <3 mos, illiteracy, lack of print informed consent and acute disease. MOS-HIV questionnaire was used. Treatment adherence was assessed with SMAQ (Simplified Medication Adherence Questionnaire). Demographic, clinical and laboratory data were retrieved from the medical records. Co morbidities included dyslipidaemia, hypertension, diabetes mellitus, COPD, hepatitis B/C, psychiatric illness. Logistic regression models were used to identify HRQL determining factors.
Results: A total of 144 pts were enrolled (91% men, mean age 37.6 years, median time from seroconversion 5.5 years, actively on HAART 86.8%, CD4 > 500 61.8%, undetectable viral load 81.9%). Stage-distribution was: A-73.6%, B-15.3%, C-11.1%. Co morbidities were recorded in 32.6% (4.2% psychiatric illness). Adherence rate was 63.2%, significantly improved in non-experienced pts (p = 0.03) and negatively associated with the number of pills daily (p = 0.05). MOS-HIV domains did not differ by gender, stage, immunological status, treatment status, antiretroviral regimen type and past regimens. Co morbidities in total (and psychiatric illness in particular) were negatively associated with most MOS-HIV domains (excluding social functioning and health transition), as well as the Physical Health Summary score (PHS) and the Mental Health Summary score (MHS). PHS was also negatively associated with increasing age and unemployment. Treatment adherence was significantly associated with better scores in cognitive function, pain, quality of life and MHS.
Conclusions: In a patient cohort with stable HIV disease, the existence of co morbidities was a consistent and independent aggravating factor for most HRQL domains. Reinforcing treatment adherence and dealing effectively with coexisting chronic conditions (age or disease-related) may help in improving HRQL in HIV pts.

R2738 *Epstein-Barr virus*–associated smooth-muscle tumours in AIDS patients: the largest case series

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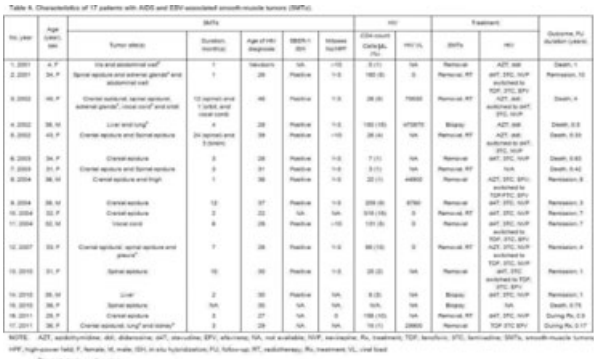
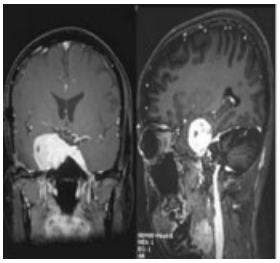
Objectives: Our previous report described the association of *Epstein-Barr virus* (EBV) and smooth-muscle tumors (SMTs) in AIDS patients. Most patients without immunological response had fatal outcomes despite complete tumor removal. The present study was thus aimed to determine the outcome of EBV-associated SMTs in higher number of AIDS patients receiving highly active antiretroviral therapy (HAART) with longer follow-up period.
Methods: Seventeen AIDS patients with SMTs were analyzed at King Chulalongkorn Memorial Hospital, Thailand from 2001 to 2011. All tissues were tested for SMTs and EBV by standard immunohistochemistry and in situ hybridization, and blood samples were assayed for EBV by real-time quantitative PCR.
Results: Of 17 study patients, there were 5 (29.4%) males and 12 females with the mean age of 34 + 9.2 years. Apart from two patients with SMTs as the first presentation, the median duration of diagnosis of HIV infection before SMTs diagnosis was 4 years with interquartile range (IQR) of 2–6.5 years. At the time of SMTs diagnosis, the median CD4 count was 26 cells/μL with IQR of 9.75–154.5 cells/μL and four patients had undetectable of HIV viral load. 8 (47.1%) patients had received HAART. There were eight and nine patients with single and multiple sites of tumor. The most common site was the cranial epidura (10 patients, 58.8%), followed by spinal epidura (7, 41.2%), lung, liver, vocal cord, abdominal wall, and adrenal gland (two patients each), pleura, kidney, thigh, orbit, and iris (one patient each). Of nine patients with multicentric SMTs, there were 3 (33.3%), 4 (44.5%), and 2 (22.2%) patients with central nervous system (CNS) only, CNS and extra-CNS, and extra-CNS only. All patients had evidence of EBV infection in the tumor, and two patients had detectable blood EBV viral load. Seven (41.2%), 7 (41.2%), and 3 (17.6%) patients received surgery, both surgery and radiotherapy, and neither surgery nor radiotherapy. The median follow-up duration was 1 year with IQR of 0.5–5.5 years. Overall mortality rate was 41.2%; all seven patients with detectable HIV viral load died. In contrast, all 10 patients with undetectable HIV viral load and immunologic improvement survived despite incomplete tumor removal in some patients.
Conclusion: The improvement of immune status by HAART, in analogy with treatment of EBV-associated posttranplantation lymphoproliferative disorder, will result in better outcome of EBV-associated SMTs in patients with AIDS.





Picture: MRI showing 2 enhancing epidural masses including one at right cavernous encasing carotid artery and another displacing cervical cord posteriorly.

R2739 **Changes in the metabolic status and the liver function tests in a cohort of newborns exposed in utero to antiretroviral agents**

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Objective: To describe the metabolic characteristics and results of the liver function tests of a cohort of neonates born to HIV positive mothers, who were exposed in utero to different scheme of antiretroviral agents (ARVs).
Methods: The study was conduced at the Instituto Nacional de Perinatología, Mexico, city, during the period of January 2008 to May 2011. This was a cohort study that evaluated the metabolic condition and results of renal and liver function tests (LFT) at born in infants exposed in utero to ARVs. A comparison was made of the results observed in the neonates born to HIV positive mothers, who received HIV treatment during their gestation, against the normal newborns data described at the literature. The infants were included in different comparison groups according to the following variables: (i) initiation of ARV treatment in the mother before 20 weeks of gestation, (ii) if the scheme included a protease inhibitor, (iii) if scheme included lopinavir-ritonavir, and (iv) if the scheme contained tenofovir-emtricitabine. Descriptive statistics were used, and the comparison between groups was made by Student's t test.
Results: The cohort consisted in 58 newborns. The average value of the components of LFTs, and serum glucose, cholesterol and triglycerides were statistically higher among newborns exposed to ARV that the normal newborn data described at the literature. The average value of serum urea and creatinine was similar to normal. No significant differences was observed in the laboratory results between cases of newborns whose mothers received ARV scheme that included a PI, lopinavir/ritonavir, tenofovir/emtricitabine or that the treatment was started before the 20 SDG, with the exception of an increase statistically significant in the serum creatinine of newborns exposed in utero to tenfovir/emtricitabine.
Conclusions: ARV treatment during pregnancy increases the values of LFTs, glucose, cholesterol and triglycerides in the newborn. Tenfovir/emtricitabine increase the newborn serum creatinine. It is important to monitor laboratory parameters in newborns exposed in utero to ARV.

Hepatitis

R2740 **The correlation between baseline biochemical and virological parameters with viral replication and liver fibrosis in patients with chronic hepatitis B infection**

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Objective: To evaluate the relation of biochemical liver function parameters with pathologically diagnosed liver fibrosis in treatment-naïve chronic hepatitis B (CHB) patients. Change in HBV-DNA, HBsAg and HBeAg during the 12 months (mo) of antiviral treatment were also evaluated.
Methods: This is a non-interventional, multicenter study was conducted at 34 centers in Turkey in 289 patients with CHB (67.7% males; mean (standard deviation; SD) age: 36.5 (10.7) years). After baseline data collection, 143 (92.3%), 138 (89.0%) and 148 (95.5%) patients were evaluated at the 3rd, 6th and 12th months (mo) of follow-up.
Results: HBeAg positivity was identified in 25.5% of the patients at the initiation, in 20.7% at the 3rd and 6th month and 19.0% at the 12th month. Reduction in baseline HBeAg titers was determined in 47.1 and in 62.5% of these patients at the 3rd and 6th month. Mean (SD) HBsAg titers were 277.2 (77.0) IU/mL at baseline 305.2 (201.7) IU/mL at 3 months and 313.0 (193.6) IU/mL) at the 6th month. Mean (SD) HBV-DNA (IU/mL) levels were significantly higher in HBe (+) patients compared to HBe (-) patients at baseline (7.7 (0.7) vs. 5.3 (5.1)), 3rd month (6.0 (2.2) vs. 2.9 (2.3)), 6th month (5.4 (4.6) vs. 2.4 (1.7)) and 12th month (4.1 (3.7) vs. 2.1 (1.7)) ($p < 0.001$ for each). Baseline mean (SD) AST level was 61.2 (46.8) U/L, ALT 97.3 (79.6) U/L, alpha-2 macroglobulin 265.5 (83.4) mg/dL, haptoglobulin 70.3 (39.6) mg/dL, apolipoprotein A1 145.8 (33.6) mg/dL, and Knodell score 7.6 (4.5) in the overall population. There was no difference between HBe (+) and HBe (-) patients in terms of baseline levels of AST (59.8 (39.1) vs. 61.9 (49.7) U/L), ALT (101.0 (57.5) vs. 96.4 (86.8) U/L) and total Knodell score (7.1 (4.5) vs. 7.8 (4.5)). Baseline HBsAg titer was negatively correlated with 3rd month ($r=-0.265$; $p < 0.001$) and 6th month titers ($r=-0.287$; $p < 0.001$) of HBV-DNA. No correlation between baseline AST and ALT levels and Knodell scores was found. Baseline HBeAg titer was positively correlated with baseline ($r = 0.488$; $p < 0.001$), 3rd month ($r = 0.393$; $p < 0.001$) and 6th month ($r = 0.579$; $p < 0.001$) HBV-DNA titers. Positive correlation of ALT ($r = 0.381$ and $r = 0.283$) and AST ($r = 0.334$ and $r = 0.267$) were determined with baseline and 3rd month HBV-DNA ($p < 0.001$ for each).
Conclusion: In conclusion, no direct relation between baseline biochemical liver function test results with Knodell scores showing in patients with CHB was detected. HBsAg and HBeAg titers were significantly related to HBV-DNA titers during the course of treatment.

R2741 **A case of hepatitis B virus reactivation with a mutant HBsAg virus in a renal dialysis unit: infection control and public health implications**

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Blood borne viruses (BBVs) are a recognised risk in renal dialysis units (RDU). *Hepatitis B virus* is the most infectious of the BBVs. Patients who are HBV uninfected are immunised against the virus prior to commencing on haemodialysis. We report a case of a successfully immunised patient whose low level HBV infection was missed who then went on to reactivate her infection with a virus bearing HBsAg mutants while undergoing dialysis.
Objectives: To report this unusual case of HBV in RDU and to propose ways to prevent similar cases occurring in other RDUs.
Methods: Case history: A 71-year old Bangladeshi lady was admitted with ESRF in February 2009 and commenced on dialysis in March 2009. Testing for HBV markers on March 2009 was negative for HBV surface antigen (HBsAg). She received three doses of immunisation between April to November 2009 and mounted a good antibody response. A routine sample taken in January 2010 showed a BsAb titre of 228 mIU/mL) but also a total anti-core (Anti-Core Ab) positive signal considered at the time to be of doubtful significance. The patient continued haemodialysis and samples taken on 3 monthly bases continued to be negative for HBsAg with ''protective'' HBsAb levels.

However, routine testing in July 2011 was positive for both HBsAg & Anti-Core Ab with undetectable levels of HBsAb. This remarkable change in the immune status of the patient (from immune to highly infectious) triggered an immediate investigation and the implementation of strict infection control measures.

Results: HBV DNA was detectable at 240 IU/mL in the January 2010 sample when low level anti-HBc was first detected. By the time routine testing first detected HBsAg, HBV DNA levels had increased to >108 IU/mL. The virus was shown to be genotype D with mutation analysis indicating the presence of multiple amino acid substitutions in the a determinant. Additional phenotyping analysis confirmed the deletion of HBsAg antigenicity. The patient to date remains HBsAg, HBeAg and HBV DNA positive. Comprehensive testing over a period of 2 months found no further cases.

Conclusion: This unusual case emphasised the need for more frequent testing of dialysis patients (4 weekly rather than 3 monthly). The implementation of strict infection control measures ensured no further transmission. The role of immunisation in selecting HBsAg mutants in this patient's virus remains speculative but individuals with positive anti-core Ab should be reviewed carefully before immunisation.

R2742 Seroprevalence of hepatitis E virus infection among HIV-infected men who have sex with men in Taiwan

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Objectives: Men who have sex with men (MSM) are at increased risk for infections with viruses that are transmitted through fecal-oral route, such as hepatitis A and hepatitis E virus (HEV). In this study, we aimed to investigate the seroprevalence of HEV infection among human immunodeficiency virus type 1 (HIV-1)-infected MSM who sought HIV care at a major university hospital in Taiwan.

Methods: Between 1 April 2011 and 30 September, 2011, blood samples were collected from HIV-infected MSM. A standardized computerized data record form was used to collect information on demographics, and clinical, virologic and immunologic characteristics of the subjects. Antibodies against HEV, HEV-specific IgG and IgM, were determined with the use of commercial kits by following the instructions of the manufacturer (Beijing Wantai Biological Pharmacy, Beijing, China).

Results: During the 6-month study period, 1260 HIV-infected MSM were enrolled, and 969 (76.1%) were receiving combination antiretroviral therapy. The median CD4 lymphocyte counts and plasma HIV RNA load for the study subjects were 478 cells per cubic millimeter (range, 0–2862) and 3.75 log10 copies/mL (range, 1.60–6.99), respectively. Overall, 83 subjects (6.6%) were seropositive for HEV-specific IgG and 5 (0.4%) tested positive for HEV-specific IgM. When the patients were stratified according to the age group, an increasing trend of seropositivity for HEV-specific IgG was observed, from 1.6% (5/307) in subjects aged between 20 and 29 years, to 25.5% (27/106) in those aged 50 years or greater. Compared with the subjects who were seronegative for HEV-specific IgG, subjects who were seropositive for HEV-specific IgG were older (median, 35 vs. 44 years), had a lower median CD4 count (483 vs. 421 cells per cubic millimeter), and lower plasma HIV RNA load (3.88 vs. 2.88 log10 copies/mL) in univariate analysis. In multiple logistic regression, age (per 1-year increase) and CD4 (per 1-cell per cubic millimeter increase) were independently associated with seropositivity for HEV-specific IgG, with odds ratio of 1.086 (95% confidence interval [CI], 1.063–1.111) and 0.999 (95% CI, 0.998–1.0), respectively.

Conclusions: We concluded that the HEV seroprevalence of HIV-infected MSM in Taiwan was estimated 6.6%, which increased with age.

R2743 Hepatitis B virus infection in Chinese population living in Barcelona, Spain

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Objective: To understand the main characteristics of hepatitis B virus infection in the Chinese population living in Baix Llobregat area (Barcelona).

Material and Methods: A retrospective study was performed among Chinese patients consulting in Primary Care centre from January 2008 to June 2011 and tested at the Clinical Laboratory of l'Hospitalet (Catalan Institute of Health). The Hepatitis B virus (HBV) infection was screened analysing the Hepatitis B surface antigen (HBsAg) and subsequently the hepatitis B e antigen (HBeAg) and HBV-DNA PCR was performed. We assayed HBsAg, HBeAg, anti-HBe and HB anti-core (anti-HBc) by chemiluminescent immunoassay (Vitros® Johnson&Johnson) and the Hepatitis B DNA viral load was quantified by Abbott Real Time HBV DNA Assay®. The immune tolerant phase was defined by HBeAg presence, HBV-DNA $>10^6$ UI/mL and normal ALT levels (<40 UI/mL), the immune reactive phase was determinated by HBeAg positive or negative with abnormal levels of ALT and the non replicative or low replicative phase by HBeAg negative and normal ALT levels.

Results: A total of 68 patients tested positive to HBsAg and were included in this study, the median age was 35 years and 42.6% (29) were women. HBeAg was positive in 30 (44.1%) patients and negative in 38 (55.9%). Of the total, eight (11.8%) were considered to be in the immune tolerance phase, 24 (35.3%) were defined as immune reactive patients and finally, 28 (41.2%) patients were included in non replicative or low replicative phase. Amongst the immune tolerance phase, six patients were women in childbearing age. Five (7.3%) patients from the total, were anti-HBc negative and presented DNA viral loads $>10^6$ UI/mL, reflecting extreme immune tolerance.

Conclusions: Chinese population living in Barcelona presents chronic HBV infection with similar characteristics to the country of origin. Some of them had an elevated replicative activity and therefore a high HBV-DNA viral load which is associated to high risk of sexual and perinatal transmission. Additionally, we found an atypical serological pattern with absence of anti-HBc and high levels of HBV-DNA in serum that might be associated to congenital infection.

R2744 Evaluation of the determination of the core antigen of the hepatitis C virus as a direct scoreboard for diagnosis of the infection

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Introduction: The diagnosis of infection by the virus of the hepatitis C (VHC) is based on the direct detection of antibodies (AB) against VHC (AcVHC) and on nucleic acid detection tests (NAT). A positive low or indeterminate result in the AcVHC's detection screening tests needs to be confirmed, which can be performed by means of immunoblot (BB) and / or viral replication tests. The AB's confirmation tests are poorly sensitive, costly in time and money and provide scarce information about the diagnostic value. The NAT tests are expensive and need more technology than the serologic tests. The majority of the studies done up to the moment have correlated the levels of Ag VHC with ARN-VHC's quantification, showing top levels of correlation to 0.75. The cost for determination of Ag VHC is four times lower than that of the ARN-VHC and two than that of BB.

Objective: To evaluate the determination of the AgVHC as a direct marker of infection in patients with AcVHC's results ≤3 times the cutoff of the test, which supposes about the 20% of the total of reactive samples analyzed for AcVHC screening.

Material and Methods: The VHC Ag quantification carried out in 33 samples of serum and plasma by means of chemiluminescent immunoassay using the ARCHITECTi2000 autoanalyzer (Abbott

Diagnostic), sent to the Laboratory for VHC infection screening. The selected samples had an AcVHC with S/CO's result ≤3. A confirmation test was performed on all the samples using the BB (Chiron RIBA HCV 3.0 Strip Immunoblot Assay) and the determination of VHC's viral load (CV-VHC) in the system COBAS Taqman® (Roche Diagnostic).

Results: Of the 33 samples analyzed with a positive serology, negative or indeterminate AcVHC, the result of the BB was negative in six of them, indeterminate in 21 and positive in 6. Whereas the result of the AgVHC was negative (<0.04 pg/mL) in 31 of the samples and in two positive (>0.13 pg/mL). All the samples with negative VHC Ag, the VHC viral load was <15 copias/mL. In two samples with values of positive AgVHC the viral load was positive.

		HCV BB			Total
		INDETERMINATE	NEGATIVE	REACTIVE	
HCV Ac	INDETERMINATE	17	3	0	20
	NEGATIVE	1	1	0	2
	REACTIVE	3	2	6	11
		21	6	6	33
		HCV Ag			Total
			NEGATIVE	REACTIVE	
HCV Ac	INDETERMINATE		19	1	20
	NEGATIVE		2	0	2
	REACTIVE		10	1	11
			31	2	33
		HCV CV			Total
			NEGATIVE	REACTIVE	
HCV Ag	NEGATIVE		31	0	31
	REACTIVE		0	2	2
			31	2	33

Conclusions: Ag's determination VHC might be considered a direct serological marker, easy to perform, with a short time of response and costs-safe, to evaluate the replication activity of the VHC and based in our results, it would allow us to classify the patients with AB levels of VHC with cutoff values ≤3 and negative VHC Antigen as not infectious.

R2745 Presence of the Toll-like receptor 8 A1G polymorphism affects the severity of liver histopathological hhanges in males with chronic HCV infection

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Background/Objectives: Toll-like Receptor 7 and 8 (TLR7 and TLR8) are key mediators of the innate immune response to RNA viral infections. The clinical impact of genetic TLR7/8 variation, however, is not well studied yet. We have previously shown that polymorphisms of the TLR7 gene influence the development of fibrosis and the therapy response in patients with chronic hepatitis C virus (HCV) infection. This study tests the hypothesis that the course of chronic hepatitis C is also affected by genetic variation of TLR8.

Methods: Nine hundred and eighty-six patients with chronic HCV infection and 809 HCV negative controls were genotyped for the TLR8A1G polymorphism. The association with the persistence of HCV infection and with the histological severity of chronic infection was analyzed. In addition, TLR8 specific stimulation of monocyte-derived dendritic cells (DCs) differing in their TLR8A1G genotype was performed for ex vivo functional analysis.

TLR8 A1G genotype frequencies in chronic HCV patients according to stage of fibrosis, grade of inflammation, and gender

	Gender	N	Number (%) of individuals in each TLR8 A1G			*P* value[1]	G present	*P* value[1]
			AA or A/-[2]	AG	GG or G/-[2]			
Stage of fibrosis	female	0-1 180	105 (58.3%)	61 (33.9%)	14 (7.8%)		75 (41.7%)	
		2-4 137	71 (51.8%)	54 (39.4%)	12 (8.8%)	0.512	66 (48.2%)	0.248
	male	0-1 157	121 (77.1%)	—	36 (22.9%)		36 (22.9%)	
		2-4 187	120 (64.2%)	—	67 (35.8%)	0.009	67 (35.8%)	0.009
Grade of inflammation	female	0-1 158	93 (58.9%)	51 (32.2%)	14 (8.9%)		65 (41.1%)	
		2-4 158	83 (52.5%)	63 (39.9%)	12 (7.6%)	0.371	75 (47.5%)	0.257
	male	0-1 154	120 (77.9%)	—	34 (22.1%)		34 (22.1%)	
		2-4 189	120 (63.5%)	—	69 (36.5%)	0.004	69 (36.5%)	0.004

[1] 2x2 or 3x2 χ^2 comparisons

[2] "-/-" denotes absence of the respective allele in hemizygous males

Results: The TLR8 A1G polymorphism was significantly related to higher stage liver fibrosis ($p = 0.009$) and inflammation ($p = 0.004$) in male but not female patients (table 1). Cytokine profiles of cells derived from hemizygous TLR8 mutation carriers equalled those observed of cells from wildtype hemizygotes.

Conclusion: This analysis suggests that the TLR8A1G polymorphism predisposes male patients to higher-grade hepatic inflammation and fibrosis. Since this polymorphism is also relevant for the course of HIV infection and pulmonary tuberculosis, further analysis of its functional impact is of major interest.

R2746 The ''protective'' role of high-dose intravenous immunoglobulin therapy in two patients with pemphigus vulgaris in clinical remission affected by hepatitis C virus-chronic liver disease

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Objectives: *Pemphigus vulgaris* (PV) is an autoimmune blistering disease rarely associated with hepatitis C virus (HCV). Among the most common side effects (asthenia, fatigue, anorexia, bone marrow suppression), the antiviral therapy include the unmasking or exacerbation of autoimmune disease. This report was performed to investigate the favorable outcome of two PV-patients in clinical remission with chronic liver disease associated with HCV treated with a combined protocol of pegylated interferon alfa-2a (PEG-IFN alfa-2a) plus ribavirin (RBV) and high-dose intravenous immunoglobulins (IVIg). Methods: Patients had a similar history of severe and recalcitrant mucocutaneous PV with excellent outcome to previous IVIg therapy at 2 g/kg/cycle. To be recruited, patients were both in complete clinical and partial immune-serological remission. One patient was genotype 1b-related and one patient 2a; both with HCV-RNA PCR positive. The routine blood tests and the thyroid function were determined before, during and post-treatment. The combined protocol with PEG-IFN alfa-2 plus RBV and IVIg was as follows: one dose of IVIg at 3gr/kg/cycle divided in three consecutive days 30 days before the first dose of PEG-IFN (1.5 mg/kg) plus RBV (400 mg twice a day) and performed one time a month for 12 months. The PEG-IFN and RBV were administered totally for 12 months, 1 time/week and daily respectively.

Results: Patients presented a clinical remission during the antiviral therapy with no bullous lesions on skin and mucosal sites and no serological PV antibodies. At the third month after the beginning of the antiviral therapy, patients were negative on HCV-RNA PCR and these data were substained until the end of the protocol. During the therapy we did not observe any major side effects, while asthenia and weight loss were reported for both patients.

Conclusion: Despite the immunomodulation of PEG-IFN, patients obtained a sustained clinical remission of PV without any flare during the follow-up period (18 months). We suggest a protective role of the IVIg therapy administered at 3 g/kg when a PV patient with an active chronic liver disease undergoes an antiviral therapy. We believe that the natural history of the two diseases is not been modified when they are present simultaneously in terms of clinical outcomes and prognosis. Further researches on a large number of patients are needed and the role of HCV virus in the autoimmune blistering diseases needs to be clarified.

R2747 Materno-foetal transmision of B viral hepatitis in Constanta

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Aim: To evaluate transmission of B hepatitis virus from mother to child in Constanta County during a period of 30 months (January 2009–June 2011).

Methods: Retrospective and prospective study on a group of 35 HbsAg positive pregnant women and their babies.

Results: Age of patients ranged from 1 to 38 years, average 25.07 years, eight women were HbeAg positive. Alaninamino-transferases (ALT) in 14 patients were within normal limits, the maximum value 3.5 × normal. HBV-DNA viremia was performed (method TaqMan) during pregnancy in 19 patients, with average 49000 IU/mL (limits: 28–23 500 000 IU/mL). 18 of the women had C-section and 20 didn't brestfeed. Five women were coinfected HBV and HIV. Fourteen children had specific prophylaxis with immunoglobulin anti hepatitis B in doses of 30–50 IU/bw, and vaccination with EngerixB was performed in all infants (schedule 0-2-6 months of age). Two infants were found HbsAg positive; they were born from mothers with high viral load (over 100 000 IU/mL), one from a HbeAg positive mother. All children borne from mothers with HIV and HBV coinfection present anti HBs after 1 year of age.
Conclusion: Rate of transmission for B virus was low (8.57%) in our group of patients. Careful monitoring of pregnant women limitted materno –fetal transmission of B virus infection. We do not register transmission of HBV or HIV in coinfected mothers to their children.

Virology non-HIV/non-hepatitis

R2748 Detection of *Norovirus* acute gastroenteritis in two hospitals in Sofia, Bulgaria

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Objectives: Noroviruses are considered as a global leading cause of non-bacterial gastroenteritis outbreaks, as well as being an important cause of sporadic cases. The aim of the prospective study was to evaluate the contribution of noroviruses to morbidity of sporadic acute gastroenteritis among children <10 years of age admitted for treatment in two infectious wards in Sofia, Bulgaria.
Methods: For 2-years period (2010–2011), a total of 280 stool samples were collected from children aged 45 days–10 years. All the patients were suffering from 2 to 10 episodes of vomiting and/or watery diarrhoea. The samples were screened for bacterial pathogens and rotavirus group A by standard bacterial methods and rotavirus antigen ELISA. Only rotavirus-negative samples were taken for norovirus detection through RT-PCR using primers toward norovirus polymerase region (JV12Y-JV13I, NI+E3). Amplicons of norovirus genogroup I and II capside region were obtained using primers G1SKF+R/G2SKF+R, and norovirus strain characterization was performed through sequence and phylogenetic analysis.
Results: Results revealed that all stool samples were negative for bacterial agents, and noroviruses were found in 48 (17.1%) of the samples tested. Thirty (62.4%) isolates were successfully amplified using G2SKF+R primer set and three (6.3%) using primers G1SKF+R toward noroviruses genogroup I, and 15 norovirus isolates (31.3%) could not amplified with the genotype-specific primers used. Sequence analysis of 23 norovirus isolates showed that norovirus GII.4 was the cause of most acute gastroenteritis cases, and norovirus GII.4/2006 and GII.4/2010 variants were the pre-dominant.
Conclusion: *Norovirus*-related gastroenteritis is responsible for a significant morbidity in Bulgaria. Implementation of routine norovirus testing among hospitalized patients of different age groups will help health authorities to estimate the real burden of norovirus gastroenteritis. In addition, it can complement the routine rotavirus surveillance system helping the epidemiology authorities to take preventive measures for control of norovirus spread.

R2749 Evaluation of 3151 cases applied to rabies vaccination centre, Istanbul

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Objectives: Rabies is aviral disease that produced almost uniformly fatal encephalitis in human and most other mammals. Every year, more than 15 million people worldwide receive a post-exposure preventive regimen to avert the disease. In this study, we aim to emphasize that rabies risk-contact cases are important public healthproblems for our region and importance of effective, accurate prophylaxies.
Methods: In this study; 3151 cases applied to rabies vaccination center of Umraniye Training and Research Hospital between January 2009 and July 2011 were evaluated.
Results: A total of 2242 (71%) cases were male, while 909 (29%) were female with an average age of 26.6 years. It was determined that 63.6% of the cases were bitten by dogs, 28% scratch by cats. The sites of injury were head-neck in 173 cases (%5.5), body in 189 cases (%6) cases, foot in 267 cases (8.5), arm in 315 cases (%10), leg in 773 cases (%24.5) and hand in 1434 cases (%45.5) cases. Three doses of protective vaccines were administered to 1470 (%46.7) of the cases and five doses to1516 (%48.1) of the cases. Additionally, 31 of the cases were treated with rabies immune globulin. Only wound care was performed in 91 cases. No rabies infections developed in any of the cases included into the vaccination program. Due to high mortality rate is almost %100 prophylactic measured are of utmost importance in rabies.
Conclusion: In conclusion, public education programmes and stray-animals control programmes are important to prevent rabies in our country and they might have an impact on unnecessary treatment costs.

Mycobacterial infections (including diagnosis)

R2750 Molecular detection of isoniazid and rifampicin-resistant *Mycobacterium tuberculosis*

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Background: Presently, tuberculosis (TB) reemergence and spread are of worldwide concern. The situation is aggravated by the increasing circulation of multidrug resistant (MDR) *Mycobacterium tuberculosis* strains that are defined as resistant to at least rifampicin (RIF) and isoniazid (INH), which comprise the back-bone of antitubercular chemotherapy.
Objectives: The aim of the present study was to test a simple and rapid PCR assay for detecting INH and RIF resistance in *M. tuberculosis* clinical isolates.
Methods: In this study, Twenty-five strains of *M. tuberculosis* were tested for their susceptibility to two antituberculous drugs (isoniazid and rifampicin) by proportion method and PCR. The strains were tested by PCR procedures for mutation detection. Multiplex PCR (MPCR) was used to detect mutation in codon 315 of katG gene denoting INH resistance. Amplification Refractory Mutation System (ARMS PCR) was used to detect mutation in codons 516, 531 and 526 of rpoB gene denoting RIF resistance.
Results: Susceptibility results for the 25 tested strains by proportion method, detected 7 (28%) strains resistant to INH. While for RIF 5 (20%) were found to be resistant. While out of the seven strains found to be INH resistant by the proportion method, of these, six strains were resistant by both the proportion method and by MPCR. Similarly, out of five strains found to be resistant to RIF by the proportion method, four were found to be resistant by ARMS. The sensitivity of the PCR compared to the proportion dilution method in testing susceptibility to INH was 85.71%, the specificity of the PCR was 100%. Reflecting a very good agreement of the PCR with the proportion method for INH susceptibility testing. The sensitivity of the PCR compared to the proportion dilution method in testing susceptibility to RIF was 80%, the specificity of the PCR was 100%. Reflecting a very good agreement of the PCR with the proportion method for RIF susceptibility testing.
Conculsion: The molecular method tested in the present study cannot completely substitute the proportion method for detecting INH and RIF sensitivity. Yet the described procedure may be used to detect a considerable proportion of INH and RIF resistant isolates, as it is rapid, easy to perform and interpret and its implementation would be useful for timely delivery of adequate antituberculous therapy.

R2751 Aetiological diagnostics of tuberculous spondylitis in HIV-positive and HIV-negative patients

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Purpose: Verification of tuberculous spondylitis (TS) diagnosis by bacteriological (B) and molecular-genetic methods (MG) in patients with HIV-infection and without it.
Methods: Purulence, granulations, caseous masses, bone and disk fragments were simultaneously analyzed using (MG) methods and (B) method by inoculation in Levenstein-Jensen (LJ) liquid culture medium (CM) (BACTEC MGIT 960) with determination of drug resistant (DR) of mycobacterium tuberculosis (MBT) using absolute concentration method. The material analyzed was carefully regrind and subjected to triple washing in order to eliminate erythrocytes to prevent growth inhibition in BACTEC system. Isolation of DNA and amplification of nucleotide sequence IS6110 – marker of MBT was carried out using test systems manufactured by ''DNA-Technologia'' (Russia) by real time PCR method (RT-PCR) using analyzer iCyclerQ, Bio-Rad (USA). Accelerated evaluation of MBT DR in surgical material was carried out using ''TB-BIOCHIP'' (TBch) system. This method helps identify IS6110 sequence in the sample and simultaneously identify mutations associated with DR in rpoB genes in rifampicin-(R)-resistant strains, katG, inhA and interregulatory area of ahpC-oxyR genes in isoniazid-(H)- resistant strains.
Results: First group included 26 HIV-infected patients. MBT was isolated by the way (B) in 15 cases of 26 (57.5%), (MG) method – 20 of 23 (86.0%). Nine MBT cultures had multidrug-resistant (MDR-TB), 1 – extensively drug resistant (XDR-TB) that in total has made 66.0%. Second group – 155 HIV-negative patients operated for TS. Among 155 samples – 51 were isolated by (B) and MBT was identified, that has made 32.9%, (MG) methods were used to receive DNA MBT – 92 positive tests (59.3%). Twenty-four cultures had MDR, 2 – XDR that in total has made 50.0%. DR to H was presented by gene katG mutations in codon 315 (50.0%). DR to R was determined by gene rpoB mutations in codons 531 (44%), 526, 516. Comparison of the results of DR determination by microbiological methods and using (TBch) technology has shown 100.0% conformity of the results.
Conclusion: Isolation of MBT in HIV-infected patients, operated for TS, using (B) methods (57.5%) is higher than in patients without HIV-infection (32.9%) (X2 = 4884; Er = 0.027). The result was confirmed by (MG) methods: first group – 86%, second group – 59.3% (X2 = 5411; Er = 0.02). There were no DR differences, but in both groups DR degree was high which is adverse prognostic factor.

R2752 Comparison of *Helicobacter pylori* diagnosis techniques: Moroccan prospective study

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Objective: The aim of our study is to compare rapid urease test (RUT), histology and PCR in *Helicobacter pylori* (*H. pylori*) diagnosis.
Methods: A prospective and randomized study has been conducted in the gastroenterology department of University Hospital Hassan II of Fez from May 2009 to July 2011. Consenting patients with abdominal pain or gastric discomfort and undergoing endoscopy has been enrolled in the study. For each patient, four biopsies (three antrum and one corpus) were collected and used for *H. pylori* detection by rapid urease test, PCR and histological examination (1antrum and one corpus). The comparison of different techniques was done using SPSS.
Results: During the 26 months of this study, 486 patients have been recruited. The mean age of the participants was 49 years (16–90 years). The prevalence of H. pylori varies according to the diagnosis technique with rates of 50%, 68.5% and 65% using RUT, Histology and PCR respectively. The concordance between pairwise techniques has been determined using Kappa coefficient. This coefficient show that the result obtained with RUT was more concordant with those obtained with PCR (0.362) than with histology (0.252). Then, PCR and histology gives the best rate of complementarities (87.4%). Among biopsies classified as positive by PCR, sensitivity of histology and RUT were 71.01% and 63.92% respectively.
Conclusion: PCR and Histology may be used as complementary techniques in H. pylori detection and therefore in better therapeutic management. Effectively, the PCR was more specific than histology however histological examination still essential to detect gastric lesions and to confirm gastric cancer diagnosis.

R2753 Optimisation of sputum microscopy with use of Naoh/ centrifugation method for the diagnosis of tuberculous and non-tuberculous mycobacterial pulmonary infections

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Objectives: Descriptive study of a collection of Acid-Fast Bacilli (AFB) smears optimized by the NaOH/centrifugation method, and isolation of Mycobacteria.
Methods: Direct examination and concentration of sputum or respiratory secretions with NaOH/centrifugation and direct visualization by Ziehl-Neelsen method in specimens sent to the mycobacterial laboratory from 2006 through 2010. Cultures were done with the use of MGIT 960. Isolates grown in liquid cultures were identified to the complex, and to the species levels with a modified IS6110/hsp65 multiplexPCR and PRA-PCR.
Results: During the study period 12 473 clinical respiratory specimens were examined. AFB smears performed in the non-concentrated materials yielded 487 positive tests. An additional 127 cases were found after concentration of the material. 5.25% of the samples showed a positive AFB smear. When compared to culture as the gold standard, the sensitivity for non-concentrated material was 33%, and 39% for the concentrated material. Positive predictive ratio was 74%, and 69% for the non-concentrated, and concentrated materials. 87% of Mycobacteria grown were identified as *M. tuberculosis*. When grown from positive-only AFBs made with concentrated materials, isolates had a higher chance of belonging to the Non-tuberculous mycobacteria group. Another 662 isolates were grown from materials that had simultaneously AFB negative smears made from non-concentrated and concentrated materials.
Discussion: AFB smear is a mainstay for the diagnosis of respiratory mycobaterial infections. Concentration of respiratory materials can add new cases of both MTC, and NTM infections. The low sensitivity of non-concentrated and concentrated AFB smears observed strongly indicates that mycobacterium culture plays an essential role in the diagnosis of tuberculosis. Semi-automated liquid cultivation techniques have improved the recovery of mycobacterium not detected by AFB

	AFB smear				
	Nconc+ /Conc+	Nconc- /Conc+	Nconc+ /Conc-	Nconc- /Conc-	Total
Positive cultures	344	80	14	662	1100
M.tb	308	62	NA	NA	370
M.szulgai	1	2	NA	NA	3
M.abscessus	5	3	NA	NA	8
M.avium	17	7	NA	NA	24
M.gordonae	1		NA	NA	1
M.kansasii	5	4	NA	NA	9
M.nonchromogenicum	1	1	NA	NA	2
M.terrae	2		NA	NA	2
M.fortuitum	1		NA	NA	1
M.intracellulare	3	1	NA	NA	4
Negative cultures	118	72	11	10450	10651
Total	462	152	25	11112	12175

smears in paucibacillary materials. Concentration of sputum or respiratory secretion, and universal mycobacterium cultures have to be mandatory in order to diminish the burden of tuberculosis among affected countries. There is a significant higher chance of growing non-tuberculous mycobacteria from only positive AFBs from concentrated respiratory secretions. Fast and simple identification techniques must be done in all mycobacterial laboratories to distinguish *M. tuberculosis* complex isolates from Non-tuberculous mycobacteria.

R2754 The genetic diversity of *Mycobacterium tuberculosis* complex in Azerbaijan by 24 MIRU-VNTR loci genotyping in association with susceptibility testing by conventional and molecular methods

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Objective: Tuberculosis (TB) is still a serious public health problem in Azerbaijan. According to the most recent World Health Organization (WHO) global TB drug resistance surveillance report, high multidrug-resistant (MDR) TB rate was recorded in Baku. The spread of the disease is also complicated by the emergence of hyper virulent strains from the Beijing/W lineage. We report an epidemiological surveillance study performed in the region of Sheki in Azerbaijan under a program supported by the Hellenic Ministry of Foreign Affairs.
Methods: Sixty-two *Mycobacterium tuberculosis* complex (MTB) clinical isolates were isolated from 182 patients during 7 months (9/3/10–17/10/10). Ziehl-Neelsen (ZN) staining was performed in all sputum samples, inoculation onto Löwenstein-Jensen slants (LJ, bioMérieux) and into Bactec/9000 MB culture vials (Becton Dickinson) was carried out. Susceptibility testing was performed by phenotypic and genotypic methods (MGIT, Becton Dickinson and GenoType MTBDRplus/GenoType MTBsl, Hain, respectively). Molecular typing was based on 24 loci including variable numbers of tandem repeats of mycobacterial interspersed repetitive units (MIRU-VNTR).
Results: Phenotypic susceptibility testing of 62 MTB showed that 14 were XDR, 24 MDR and among the remaining, 15 were sensitive to isoniazid, 23 to rifampicin, 22 to ethambutol and 12 to streptomycin. The molecular method showed that all XDRs were isoniazid/rifampicin/aminoglycosides/fluoroquinolones-resistant and 13/14 additionally ethambutol-resistant, while MDRs carried at least one mutation in inhA/katG and rpoB genes. Among the 15 phenotypicaly isoniazid-sensitive isolates, 10 carried the wild types and one mutation either in katG or in inhA genes, while 16 out of 23 rifampicin-susceptible isolates carried one mutation of the rpoB gene and two were identified as resistant. The 24 loci internationally-agreed MIRU-VNTR typing scheme classified the 62 isolates into four clusters: 52 strains belonged to Beijing family, four to LAM (Latin-American and Mediterranean), four to H37RV and two to Harlem lineages.
Conclusions: The MDR-MTB clinical isolates belonged to two lineages LAM and H37RV, while all the XDR-MTB clinical isolates to the Beijing family. Drug resistance among tuberculosis patients in the region of Sheki in Azerbaijan, could be associated with the spread of Beijing family strains.

R2755 A fine line between a major surgery and oral drug therapy: the diagnosis of sternal tuberculosis

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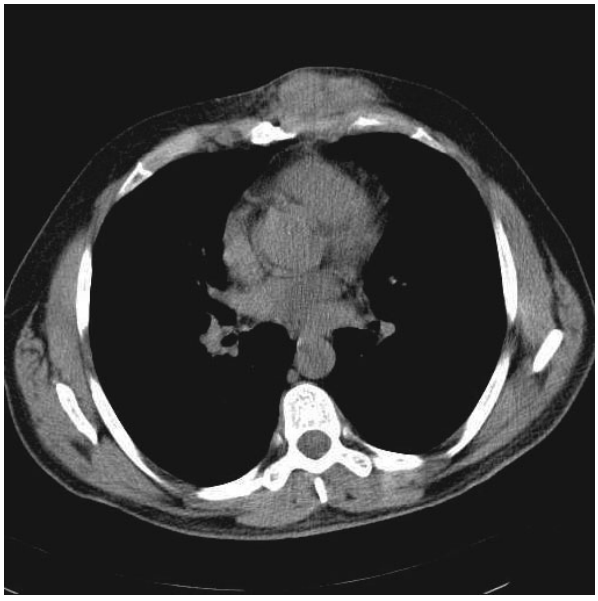
Case: A 53-year-old man presented with a 1-month history of chest pain and two distinct palpable masses on his lower sternal part of chest. He was a teacher and was living in a village. He denied cough, night sweating, loss of appetite, loss of weight, fever and any trauma. He had a history of pulmonary tuberculosis which occured 36 years ago. Previously when he had admitted with those complaints to a local hospital, ultrasound revealed 4×2 cm hypoechoic septated abscess formation localised near to the left of distal sternum and non-contrast enhanced computed tomography of thorax demonstrated a 6.7×4.2 cm lobulated soft tissue mass eroding the sternum (Figure 1). Ultrasound-guided fine needle aspiration yielded no microorganism and the cytology revealed polymorphonuclear leucocytes. When he was admitted to our hospital for an extensive surgery fort he removal of the mass, a pus drainage from the needle insertion area was seen on examination. The remaining systemic examination was normal. Anteroposterior chest radiograph showed no abnormalities with the lung parenchyma and bone structures. Sternal tuberculosis was suspected of first priority in differential diagnosis. Local debridement of infected tissue and punch biopsies from sternum was done under local anesthesia. Amoxicilin-clavulonate was given for 10 days because of the growth of methicillin-sensitive Staphylococcus epidermidis and he was discharged from hospital on the third day of the operation. Isoniazide, rifampin, ethambutol and pyrazinamide were started after the necrotising granulomatous inflammation was reported. Mycobacterium tuberculosis, that was not seen with acid-fast staining microscopically, was cultured from biopsy specimen on the 15th day with Bactec 460 TB system. By the end of 2 months of treatment, the masses were reduced in size and there was no drainage. The four-drug antitubercular treatment was switched to two-drug treatment. Isoniazid and rifampin was planned to be completed to 1 year.



Conclusion: Tuberculous osteomyelitis of sternum comprises <%1 tuberculous osteomyelitis. Of today, fewer than 40 cases of sternal tuberculosis were reported. Sternal tuberculosis, should be kept in mind in the differential diagnosis of mass involving the chest wall particularly in the endemic areas. This approach will prevent patients with sternal tuberculosis-but mimicking a tumoral lesion- undergoing a major surgery.

R2756 Early detection of genotypic resistance of *Mycobacterium tuberculosis* in direct specimen

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Objectives: The aim of this study was to determine the genotypic resistance pattern of *Mycobacterium tuberculosis* complex directly in

clinical samples to improve the response time of the laboratory and to optimize the treatment for tuberculosis patients.
Material and Methods: We studied 61 respiratory samples of different patients with tuberculosis diagnostic in Malaga province. All the samples were processed for cultivation and stains. Theses samples were selected for giving positive with Auramine-Rodamine. The *M. tuberculosis* complex strains were performed to study of sensitivity with the first-line anti-tuberculosis drugs. The clinical samples were worked following the manufacturer's instruction with the kit MTBDRplus (Hain LifeScience, Germany) for detection of genotypic resistance to Isoniazid and Rifampicim (detection of gen katG, inhA and rpoB).
Results: We obtained a total of 32 (52.46%) isoniazid-resistant strains, which have mutations in the katG gene in 13 (40.62%) and in the inhA in 6 (18.75%), which represents 59.37% of the clinical samples we find a mutation associated with resistance to isoniazid. Of all the strains, we have obtained a phenotypic resistance to rifampicin in 14 (22.95%), which detected 13 (92.86%) clinical samples with mutations in the rpoB gene. We have not found any resistance mutations with phenotypic sensitive strains.
Conclusions: The study of genotypic resistance to Isoniazid and Rifampicin in clinical samples has a good correlation with phenotypic sensitivity studies, especially to rifampicin. This facilitates clinical decision when managing a patient with tuberculosis, by decreasing the response time of 30–40 days to 24–48 hours. The conventional susceptibility testing cannot yet be replaced by genotypic study to interpret the behaviour of *Mycobacterium tuberculosis* complex against Isoniazid, because it does not detect all mutations involved in resistance.

R2757 Risk factors associated with surgical site infection caused by *Mycobacterium massiliense* in abdominal videolaparoscopies

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In Brazil, *Mycobacterium massiliense* infections after surgery have been described since 2004, mainly due to contamination of surgical materials. The purpose of this study was to identify risk factors associated with surgical infection after abdominal video surgery in a hospital of Rio de Janeiro and describe their clinical presentation. In a case-control study, controls were patients undergoing the same procedure in this hospital during the period. The classification and treatment were established by the Brazilian Health Department. Strains were tested by PRA-hsp65 and gen rpoB sequency. Forty nine (29%) patients: 13 proven, three probable and 33 possible were compared to 119 controls. Probable and possible were similar to proven (confirmed) cases. Confirmed cases had lower incubation time (14 and 27.5 days, $p = 0.001$) and greater local hyperthermia (77 and 25%, $p < 0.001$) than non confirmed ones (possible and probable). Pancreatitis ($p = 0.05$) and sterilization procedure ($p = 0.02$) were associated with this infection. At logistic regression, only sterilization was associated to infection (OR=2.31; 95% IC: 1.10–4.87; $p = 0.03$). All cases were submitted to surgical debridement followed by monotherapy or combination therapy (45% and 55%). Two patients died during treatment (4%). Success was observed at 10 (confirmed) and 12 months (not confirmed). About 90% of patients had an adverse reaction, digeus was the most frequent (71%), and 4% were severe. All the strains were identified as *M. massiliensis*. Conclusion:
Despite the low mortality, the incidence of this infection was high and it should be remind as a differential diagnosis of surgical infections.

Infection in the immunocompromised host and transplant recipients

R2758 Seroprevalence of hepatitis E among Iranian renal transplant recipients

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Background: Renal transplant recipients are known to be susceptible for viral infections with more severe clinical presentations compared to healthy persons. Hepatitis E is generally a self-limited disease which is caused by *Hepatitis E virus*. Recently, Hepatitis E becomes more important in organ transplant recipients, because of new findings regarding the chronicity potential in this patient group. This study was aimed to evaluate the seroprevalence of anti-HEV IgG among kidney transplant recipients of Urmia in the north-west region of Iran.
Methods: Ninety one patients were selected randomly among patients who underwent kidney transplantation in Urmia, Iran. Each patient was experimented for anti-HEV IgG using ELISA method (Diapro, Italy).
Results: Twenty eight subjects (30.8%) were seropositive for anti-HEV IgG. Seropositive cases are generally older than seronegative cases ($p = 0.009$). There was no correlation between HEV infection and the level of education ($p = 0.206$), the history of blood transfusion ($p = 0.164$), history of pre-transplantation hemodialysis ($p = 0.228$). There was not significant difference among the serum ALT level of anti-HEV seropositive and seronegative cases. Multinomial logistic regression indicated no significant relationship between HEV infection and increase in ALT levels, even when controlled for the treatment with azathioprine ($p = 0.79$, OR=1.12; 95% CI: 0.45–2.76).
Conclusion: The anti-HEV IgG has a high prevalence in Iranian kidney transplant recipients, and it is significantly higher in comparison with previous studies in general population or HD patients. This could be of great clinical importance considering the probable persistent HEV infection in the setting of graft recipients suggested in the literature.

R2759 Iron overload is a major risk factor for infectious complications in kidney transplant recipients

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Objectives: The impact of iron overload on the pathogenesis and outcome of infections has been documented in various patient populations but, to our knowledge, it has never been assessed in kidney transplant (KT) recipients.
Methods: In this observational cohort study we prospectively analyzed 159 patients (102 males; mean age: 53.6 ± 14.9 years) who underwent KT at our institution from November 2008 to August 2010. Serum iron markers (iron level, ferritin level, total iron-binding capacity [TIBC], and transferrin saturation) were measured within the first month after transplantation before any infectious event had occurred. Primary outcome was the occurrence of any episode of infection in the first 6 months post-transplant. Secondary outcome included all-cause mortality at the end of follow-up. Multivariate adjusted odds ratios (ORs) were calculated using those covariates that were found to be significant at $p < 0.10$ by univariate analysis. We compared Kaplan–Meier survival curves with the log rank test.
Results: During the first 6-month follow-up period, 95 (59.7%) recipients developed at least one episode of infection (bacterial in 44.7%, cytomegalovirus [CMV] in 23.9%, viral non-CMV in 8.2%, and fungal in 6.3%). Serum iron markers were assessed in samples taken at a median interval of 2 days after KT (interquartile range, 1–5 days) and a median interval of 24 days before any infectious event occurred (interquartile range, 12–64 days). Mean ferritin levels were significantly higher in those patients who developed any episode of infection (540.8 vs. 391.0 ng/mL; $p = 0.024$), as compared to the rest of the cohort. As an inverse marker of iron status, TIBC was lower in

the group with any infection (213.1 vs. 229.0 μg/dL; p = 0.048). After adjustment for other factors (including age, pre-transplant comorbidities, number of red blood cells transfused intraoperatively, and induction therapy), a ferritin level >600 ng/mL (above percentile 75) emerged as an independent risk factor for any infection (OR = 4.61; 95% confidence interval = 1.89–11.23; p = 0.001). Overall mortality at the end of follow-up (median 544 days) was 5.0% (infection-related mortality of 2.5%). Patients with ferritin levels >600 ng/mL exhibited a nearly significant trend towards a worse survival (p = 0.088).
Conclusion: Our results suggest that iron overload plays an important role on the risk of infectious complications after KT and may be considered as a potential marker of poor outcome.

R2760 Pulmonary infection caused by non-tuberculous mycobacteria in cancer patients: is treatment always necessary?

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Objectives: Cancer patients frequently undergo pulmonary imaging (usually chest CT) to monitor for recurrent or metastatic disease. Occasionally lesions seen on CT represent infection (predominantly fungal or mycobacterial). Many patients with Non-Tuberculous Mycobacteria (NTM) isolated from BAL are asymptomatic. Our objective was to determine whether antimicrobial therapy was necessary in such patients, and to assess treatment outcomes when it was deemed necessary.
Methods: Retrospective case review of 33 cancer patients with documented microbiologically NTM pulmonary infection.
Results: Twenty-two patients (67%) were women. The median age of patients was 69 years (range 35–82). Lymphoma, lung, and breast cancer were the most common underlying tumors. Nine patients were actively receiving chemotherapy when the infection was diagnosed. The NTM isolated were *M. avium*-complex in 26 patients (79%), *M. gordonae* in 2, and *M. abscessus*, *M. chelonae*, *M. fortuitum*, *M. kansasii* and *M. sculgai* in one patient each. Twenty one pts (64%) were asymptomatic. Sixteen of these (76%) chose not to, or were recommended not to, receive any anti-mycobacterial therapy. All have remained asymptomatic despite radiographic waxing and waning with a median follow-up of 48 months. Seventeen patients received standard therapy for the NTM isolated. Five (29%) discontinued therapy within 4–20 weeks due to serious side effects (intractable vomiting, rash, *C. difficile colitis*, tendinitis). Seven were able to complete 18 months of therapy. All have remained clinically stable after discontinuation of therapy (any length) despite persistent radiographic changes in most, and repeat positive cultures for NTM in five patients.
Conclusion: Therapy for NTM infections in asymptomatic cancer patients is not indicated. In patients who do need treatment, it can be quite toxic and may not lead to the eradication of the NTM in those who are able to tolerate a full course.

R2761 Bloodstream infection among bone marrow transplant outpatients: risk factors for hospitalisation and death

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Introduction: Bloodstream infection (BSI) is one of the most common medical complications in bone marrow transplanted patients. A few studies, however, evaluate the treatment of BSI among bone marrow transplated outpatients.
Objectives: Describe the bloodstream infection agents identified among bone marrow transplanted outpatients at HCFMUSP. Evaluate the proportion and risk factors associated with hospitalization and death of such patients.
Method: Retrospective analysis on patients' records of the Bone Marrow Transplant's clinics that presents positive blood culture gathered between January 2004 and December 2008. All the data was analyzed using the software Epi Info version 3.5.1, the significance level adopted was of 5%. Hospitalization and death in 30 days were the outcomes evaluated and which the risk factors associated were calculated and compared through the analysis bivariate and multivariate.
Results: A total of 743 patients were evaluated of which blood cultures were positive during the clinic follow up in 172 pts, the records of 146 were evaluated, with a total of 235 bloodstream infection episodes, being 207 (88%) monomicrobial episodes and 28 (12%) polimicrobial in both the occurrence of gram-negatives was predominant. The episodes were more frequent during the first 100 days after the transplant. The most important agents isolated were S. maltophilia (15%), SCN (12%), Acinectobacter spp (9%). The average age of the patients was 32 years old (range of 2–68 years), being 90 (61.6%) male and 87 (59.6%) allogeneic transplant. The hospitalization occurred in 26% of the episodes. Autologous transplant was found as protect for hospitalization on both analyses, bivariate and multivariate. Death in 30 days occurred only in 10% of the cases. BSI due to Gram-negative and presence of serious neutropenia were found as independent risk factor for death. The multivariate analysis found as protection factor for death in 30 days only MASCC score.
Conclusion: The gram-negatives were the most important agents isolated in our bone marrow transplat outpatients unit, highlighting the isolation of Stenotrophomonas maltophilia. The occurrence of hospitalization and death wasn't high. MASCC score could be a good predictor for death and the treatment of autologous outpatients with bloodstream infections seems to be safe.

R2763 Derivation and validation of a scoring system to identify patients with bacterial bloodstream infection and haematological malignancies at higher risk for mortality: He.M.A.B.I.S. (Hematological Malignancies Associated Bloodstream Infections Surveillance)

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Objectives: The aim of the present study, conducted in nine Italian large hospitals, was to develop and validate a reliable, easy-to-use clinical prediction rule that could be employed to identify patients with higher likelihood of mortality among those with hematological malignancies (HM) and bacterial bloodstream infections (BBSIs).
Methods: To identify risk factors for mortality in adult patients with HM and BSI, we conducted a cohort study in nine Italian haematological units. The derivation cohort consisted of adult patients with BBSI and HM admitted to the Catholic University Hospital (Rome), between January 2002 and December 2008. The outcome measured was mortality 30 days after BBSI onset. Survivors and nonsurvivors were compared to identify predictors of 30-day mortality. The validation cohort consisted of patients hospitalized with BBSI and HM, prospectively enrolled in eight other Italian hematological units between January 2009 and December 2010. Inclusion and exclusion criteria were identical to those used for the derivation cohort; patients included in the validation set were matched with those in the derivation set according to type and stage of HM (match ratio, 1:1).
Results: In the derivation set (247 episodes), the multivariate analysis (after excluding variables not evaluable at BBSI onset) yielded the following significant mortality-related risk factors: acute renal failure (Odds Ratio [OR] 6.44, 95% confidence interval [CI], 2.36–17.57); severe neutropenia (<100/mm3) (OR 4.38, 95% CI, 2.04–9.43); nosocomial infection (OR 3.73, 95% CI, 1.36–10.22); age ≥65 years (OR 3.42, 95% CI, 1.49–7.80), Charlson Comorbidity Index >= 4 (OR 3.01, 95% CI, 1.36–6.65). The equal-weight risk score model, which

signed one point to each risk factor, yielded good-excellent discrimination in both cohorts with areas under the receiver operating curve (AUROCs) of 0.83 vs. 0.93 (derivation vs. validation). The model was well calibrated in both derivation and validation sets (Hosmer-Lemshow p = 0.16 and 0.75, respectively).

Conclusion: The risk index accurately identifies patients with HMs and BBSIs at high risk for mortality; a better initial predictive approach may allow better therapeutic decisions for these patients, with an eventual impact on reducing mortality.

R2764 Epidemiology of *Candida* species in critically ill patients in ICUs in a multi-speciality hospital in India

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Aim: To study the epidemiology of *Candida* isolates in critically ill patients, its risk factors and their antifungal susceptibility pattern.

Methods: Retrospective study was conducted at a 650 bedded tertiary care centre: Medanta – The Medicity, Gurgaon, India between November 2009-October 2011. Data of 5456 samples from patients admitted in ICUs (from November 2009 – October 2011) was analyzed. Identification of the isolates and their antifungal susceptibility testing was done using VITEK 2 (bio Merieux).

Results: Out of 5456 clinical specimens 295 samples (5.4%) grew *Candida* Species. *Candida* was isolated from104 blood samples,115 from respiratory specimens (ET, sputum, BAL), 22 from body fluids (pleural, ascitic,) and 54 from urine.

Non albicans *Candida* (71.6%) was more frequent than *C. albicans* (29.4%). *C. tropicalis* (39.3%) was the most frequent isolate followed by *C. albicans* (29.4%) and *C. haemulonii* (8%). According to our results, Candida infections were nosocomially acquired in 83.7% of cases and patients frequently had severe co morbidities. Liver transplant, CVC, Neutropenia & Liver cirrhosis were associated risk factors. During the 2 years of the study, a fall in Candida associated BSIs was seen after implementation of CLABSI bundle. *C. tropicalis* was the most predominant species followed by *C. albicans*. Susceptibilty to routine antifungal agents varied according to the species .92% of isolates were susceptible to Voriconazole, 86.4% to Amphotericin B, 82.03% to Fluconazole, with variable sensitivity according to species. All *C. krusei* were resistant to fluconazole . 63% of C. hemulonii were resistant to Amphotericin B and ~50% were resistant to Fluconazole and Voriconazole.

Candidial infections were found to be more common in men (70.5%), those above 45 years of age (76.27%), those with hospital stay of more than 9 days and patients from gastroenterology ICU (24.4%).

Conclusion: Epidemiological studies including early identification, speciation and antifungal therapy are critically important in Candidial infections. Epidemiological data helps in risk assessment of cases and initiation of empirical treatment. Hence it is imperative to know the epidemiology of *Candida* spp. in one's own setup. New subsets of Non albicans Candida like *C. haemulonii* are emerging. Current antifungal agents have inadequate activity against current and emerging fungal pathogens. Use of newer generation antifungals like echinocandins improve outcomes in serious fungal infections.

R2765 Adult lung cancer complicated with invasive pulmonary aspergillosis

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Objectives: *Aspergillus* spp. can cause acute invasive disease in severely immunocompromised patients. However there are few cases reported of lung cancer complicated by invasive pulmonary aspergillosis (IPA) at the same site.

Methods: A retrospective review of all consecutive episodes of adult lung cancer complicated by IPA diagnosed in a hospital, from January 2009 to October 2011. A combination of socio-demographic characteristics, underlying diseases, clinical features, microbiological results, radiological findings, treatment and outcome were recorded.

Results: During the study period 856 patients with lung cancer were treated and 10 episodes (1.16%) of probable IPA were diagnosed. Eight episodes occurred in men and two in women, mean age 63 years. The most common underlying oncological disease was non-small cell lung cancer (eight patients). Clinical stages were III (3) or IV (7). Chronic lung disease was a co-existing illness in five persons, but none had previous *Aspergillus* colonization. Median time between cancer diagnosis and *Aspergillus* detection was 5 months. One episode occurred during neutropenia. Within 30 days prior to the diagnosis of IPA, five patients were receiving systemic steroids therapy and two chemotherapy. Two patients had received previous chest radiotherapy. The most common clinical presentations were fever (4), hemoptysis (4), dyspnoea (3), chest pain (3) cough (2) and distress syndrome (2). Aspergillus was detected in four sputum samples, three bronchoalveolar lavages, two tracheal aspirates and one percutaneous lung puncture. *A. fumigatus* (6) was the most frequently isolated, followed by *A. terreus* (3) and *A. flavus* (1). Nine patients had concomitant bacterial isolation. At time of *Aspergillus* detection, the most common radiological findings in thoracic computed tomography (TCT) were cavities (5), consolidation (3), nodular infiltrates (1) and air crescent signs (1). Since *Aspergillus* detection, TCT controls showed that all cavitary lesions expanded over time. All patients were treated with azoles (voriconazole or itraconazole) and one underwent surgical resection. Eight persons died after Aspergillus detection (average time 11 weeks) with two IPA related death.

Conclusions: The presence of lung cancer seems to be a factor triggering *Aspergillus* implantation, predisposing to IPA. Once localized in the damaged lung, the mould can grow and cause or expand cavities. In lung cancer patients *Aspergillus* detection entails a very poor prognosis.

R2766 An outbreak of *Burkholderia cepacia* bacteraemia in hospitalised haematological patients in northern Greece

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Objectives: *Burkholderia cepacia* (Bcc) is a common environmental bacterium that is resistant to disinfectants, and therefore is often encountered as a hospital-acquired pathogen. We describe a 3-month hospital outbreak of Bcc bacteremia among hospitalized hematological patients.

Methods: The study included nine hematological patients hospitalized between December 16, 2009 and March 13, 2010 who had blood culture positive for Bcc. Environmental samples were collected from benches, sinks, drains, bed rails, floors, refrigerators, bathroom surfaces, stethoscopes, distilled water, sterile saline and intravenous fluids. All positive blood culture isolates were identified to the species level and tested for antimicrobial susceptibilities by a commercial system Vitek 2 (AST-N093 bioMerieux, France).

Results: During the period of the outbreak 102 patients were hospitalized in the Hematology Department and 28.4% (29) of them suffered from acute myeloid leukemia (AML). Over the 3-months outbreak period, a total of nine patients with Bcc bacteremia were identified. Notably, all patients infected with Bcc suffered from AML and were neutropenic. Of them, 5 (55.5%) were men and the ages ranged from 43 to 75 years. Bcc was repeatedly isolated from the blood of the above nine patients. Four patients (4/9) had signs of pneumonia, but Bcc was never recovered from sputum and the rest of the index cases (5/9) had no other documented infection site but Bcc bacteremia. Three cases developed septic shock and died. All isolates were sensitive to ceftazidime and resistant to amikacin, gentamycin, tobramycin, imipeneme and colistin. The rates of susceptibility for the nine Bcc isolates to cefepime, ciprofloxacin, cotrimoxazole, piperacillin-

tazobactam and minocycline were 73%, 82%, 59%, 61% and 64% respectively. All environmental cultures did not grow Bcc. A point source was not identified and horizontal spread was suspected. Strict infection control measures terminated the outbreak.
Conclusions: *B. cepacia* is an opportunistic pathogen which can cause life-threatening disease to haematological malignancy patients and can produce a serious outbreak. Infection control measures, including contact isolation, are of outmost importance in containing the spread of Bcc among patients.

R2767 Surveillance of catheter-related bloodstream infections in a haemodialysis centre

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Objectives: To evaluate the frequency, risk factors, severity and the etiology of catheter-related bloodstream infections (BSI) in hemodialysis patients.
Methods: We reviewed the records of patients receiving maintenance hemodialysis three times per week in the period 2008–2011, the capacity of the dialysis center being 160–170 patients. Catheter-related BSIs were diagnosed if at least one positive blood culture, clinical manifestations of infection were present and positive semi quantitative or quantitative cultures from the segment of the removed catheter. The outcome measures were number of BSI per patient catheter- days, hospitalizations and deaths, comorbidity scores, and correlations with hospitalization and infection as the cause of deaths.
Results: Among the 165 hemodialysis patients currently cared, 47 (28%) have a tunneled catheter in place due to no other vascular access. Infection control interventions for the prevention of BSIs were folowed. The mean age was 64.7 ± 13.45 years, the dialysis duration 4.22 ± 3.71 years, the vascular-catheter in place 1.52 ± 0.84 years with 1.68 ± 1.86 infections/patient, catheter-related BSI rate was 3.16 per 1000 catheter-days. The infection death rate/year was between 22 and 25%. Most of the patients with BSIs were treated with systemic antibiotic alone in the dialysis center (4 weeks) and only critical patients or infective endocarditis were considered for hospitalization. The clinical picture was very similar in all patients and severe sepsis was rarely observed. The need of hospitalization was not significantly increased depending on the number of infections (≥2), OR 1.83 (95% CI 0.26–13.36), the duration of catheter in place for >1 year was significantly associated with increased number of infections, OR 5 (95% CI 1.16–23.8). The Comorbidity Charlson Index was significantly associated with hospital admissions and deaths (>3), OR 5.27 (95% CI 1.37–21.14). There was a predominance of staphylococci – 61% (meticillin resistant) and polymicrobial infections 26% (multidrug resistant). High procalcitonin levels were not clinical and statistical significantly associated with hospital admission or death OR 1.9 (95% CI 0.32–11.31).
Conclusion: The increased need for vascular access through vascular catheter was associated with excessively high number of BSIs. The risk of severe sepsis and deaths were mainly related to comorbidities than to the etiology or resistance pattern.

Community-acquired infections including CAP, sepsis, STD

R2768 The investigation of risk factors of patients with urinary tract infections due to *Escherichia coli*

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Objectives: To assess the relationship between community acquired urinary tract infection (CA-UTI) due to extended-spectrum beta-lactamase producing Escherichia coli (ESBL-*E. coli*) and demographic characteristics and risk factors.
Methods: Between 2009 and 2010, 100 outpatients diagnosed with *E. coli* urinary tract infection (UTI) were included in this study. Demographic characteristics, risk factors such as; hospitalization, antecedent UTI, urinary tract stone and anomalies, diabetes mellitus, pregnancy, invasive procedures were recorded in a form. Antimicrobial susceptibility test results were evaluated.
Results: Mean age of 100 patients was 47.4 ± 18.8. The rate of ESBL-*E. coli* among CA-UTI was 25%. There were no relationships between the number of people living at home, antecedent UTI, urinary tract stone, diabetes mellitus, pregnancy, renal insufficiency, invasive procedures in the last 3 months and ESBL-*E. coli* ($p > 0.05$). In contrast hospitalization ($p < 0.01$) and antibiotic use ($p < 0.009$) in the last 3 months were statistically significant with ESBL-*E. coli*. Rates (%) of resistance to gentamicin, levofloxacin and trimethoprim/sulphametoxazole were respectively 31, 43 and 40.
Conclusions: We show that ESBL-*E. coli* in patients with CA-UTI was high. Inappropriate antibiotic use, especially irrational use of levofloxacin in patients with CA-UTI and hospitalization in the last 3 months can be the factors predisposing ESBL production.

R2769 Body mass index as prognostic factor in community-acquired pneumonia

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Objective: Underweight and/or obesity have been associated with poor prognosis in many pathological processes including some infectious diseases. We aimed to evaluate the impact of body mass index (BMI) and waist circumference on mortality, other outcome parameters and etiology in patients admitted with community-acquired pneumonia.
Methods: A prospective observational study was conducted in two university hospitals in Spain between January 2008 and December 2010. In addition to epidemiological parameters, clinical and laboratory data, we collected weight, height, BMI and waist circumference in all patients with community-acquired pneumonia. According to BMI, subjects were stratified into five subgroups: underweight (BMI < 18.5), normal weight (BMI 18.5–24.9), overweight (BMI 25.0–29.9), obesity (BMI 30.0–39.9) and morbid obesity (BMI > 39.9). Association between anthropometric parameters and outcome (mortality during hospitalization, mortality post-discharge, morbid complications, admission to ICU, readmission, length of hospital stay, duration of fever and time to reach clinical stability) were evaluated in univariate and multivariate analyses.
Results: A total of 514 patients were enrolled in the study. Distribution of patients according to BMI subgroups were: 3% had underweight, 33% normal weight, 42% overweight, 20% obesity and 3% morbid obesity. Morbid obesity was associated with some poor prognostic indicators: mortality during admission (15% vs. 3%; $p = 0.014$), readmission at 30 days (18% vs. 4%, $p = 0.029$), duration of fever (5.8 vs. 2.4 days; $p = 0.010$) and time to reach clinical stability (8.3 vs. 3.5 days; $p = 0.004$). This relation was particularly evident among patients classified at admission as low-risk patients (PSI groups I/II/III) and among females. Multivariate analysis selected morbid obesity (OR:68.13; 95% IC:2.08-904-67), in addition to prior cerebrovascular diseases (OR:32.72; 95% IC:1.48–647.46), altered mental status (OR:18.94; 95% IC:2.49–95.60), tachypnea (OR:1.10; 95% IC:1.01–1.27) and low oxygen saturation levels (OR:0.91; 95% IC:0.86–0.96) as independent risk factors for in-hospital mortality. No associations were found between other BMI subgroups or waist circumference and outcome. Similarly, no associations were found between BMI subgroups and etiologies.
Conclusion: Our study suggests that among anthropometric parameters only morbid obesity could have a significant prognostic impact in patients with community-acquired pneumonia.

R2770 Effect of adjuvant therapy in pneumococcal meningitis: seizures and mortality

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Objectives: To evaluate the occurrence and prognostic relevance of seizures in pneumococcal meningitis (PM) and to compare clinical outcomes of pts treated with different strategies.
Methods: This is a cohort study of pts with community-acquired bacterial meningitis (CABM) 1977 to 2009 in a tertiary care hospital. Since 1987 all pts with PM were systematically treated with anti intracranial hypertension prophylaxis (Dexamethasone (DXM) for 48 h, a single dose of mannitol (MAN) and prophylactic phenytoin (PHT) in order to avoid seizures and other neurological complications. We considered three groups for analysis: Group 1 (G1) (1977–1986 without any prophylaxis); Group 2 (G2) (1987–2009 with DXM ± MAN prophylaxis and prophylactic PHT). Group 3 (G3) (1987–2009 with DXM ± MAN without prophylactic PHT due to contraindications). Forty-five points from G1 received DXM as treatment due to poor prognosis.
Results: Two hundred and sixty-three episodes of PM, mainly in adults, 110 W/153 M, mean age 53 years (7–93) have been treated. Seizures occurred in 75/263 pts (28%), 33 of them (12%) prehospital. Seizures in pts with CABM due to other etiologies was 62/729 (8.5%) $p < 0.05$. Time point of seizures was before arriving to the hospital in 33 pts (44%), first 24 hours in 19 (25%), 24–72 hours in 12 (16%), between 72 hours and 1 week in 7 (9%) and >1 week in 4 (5%). In PM overall mortality was 52 (20%). Mortality in pts presenting seizures was 29/75 (39%) vs. 23/188 (12%) in pts without seizures ($p < 0.05$). Mortality in pts with prehospital seizures was 3/33 (9%) while in pts with in-hospital seizures was 26/42 (62%) ($p < 0.05$). Among pts from G1 ($n = 110$), in-hospital seizures were present in 27/97 (28%), overall mortality was 40/110 (36%) and mortality among patients presenting seizures was 20/27 (74%). Among pts from G2 ($n = 132$), in-hospital seizures were present in 13/122 (10%) ($p < 0.05$ vs. G1), overall mortality was 18/132 (14%) ($p < 0.05$ vs. G1) and mortality among pts presenting seizures was 5/13 (38%) ($p < 0.05$ vs. G1). Among pts from G3 ($n = 21$), in-hospital seizures were present in 2/11 (18%) overall mortality was 2/21 (9%) and mortality among pts presenting seizures was 1/2 (50%).
Conclusion: Seizures in PM is related to a worse prognosis. Introduction of prophylaxis with DXM ± MAN and PHT has been effective. The exact role of PHT in avoiding seizures in PM is not clear besides the improvement due to DEX ± MAN, so a randomized clinical trial should be performed.

R2771 Clinical presentations of invasive pneumococcal disease and associated co-morbidities in adults in Spain

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Objectives: To analyse clinical presentations, associated comorbidities, and clinical outcome of invasive pneumococcal disease (IPD) in adults.
Methods: A prospective, active, hospital-based surveillance of all culture-confirmed IPDs in adults (≥18 years) was performed in seven Spanish hospitals (August 2010-June 2011). IPD was considered isolation of *S. pneumoniae* from normally sterile fluids. Clinical presentations were classified as complicated pneumonia -CP- (pneumonia with pleural effusion and/or empyema and/or multilobar presentation), non-complicated pneumonia (N-CP), meningitis (M), primary bacteremia (PB), sepsis (S), peritonitis (P) and others (O).
Results: One hundred and ninety-one cases (mean age 62.2 ± 17.8 years, 58.1% males) were included. Table shows by clinical presentation (with ≥8 cases) percentage of patients with comorbidities (present in at least 9% total patients), history of vaccination and outcome. The most frequent presentation was pneumonia (71.2%), with previous pneumonia as main comorbidity. Malignancies were mainly associated with PB and S (both accounting for 12.0% cases and showing the highest Charlson index) while chronic liver disease was the main comorbidity in patients with peritonitis (4.2% cases) and meningitis (9.9% cases).

	CP	N-CP	M	PB	S	P	Total
n	70	66	19	14	9	8	191
23v-vaccination (%)[a]	20.0	39.0	13.3	36.4	0.0	16.7	21.9
Charlson (X ± SD)	2.4 ± 2.4	2.5 ± 2.6	1.4 ± 2.8	4.0 ± 2.9	4.4 ± 3.1	3.3 ± 3.1	2.6± 2.6
Patients with comorbidities [n (%)]	65 (92.9)	63 (95.5)	15 (78.9)	14 (100)	9 (100)	7 (87.5)	178 (93)
Smokers	46.2	30.2	26.7	14.3	11.1	28.6	34.3
Previous pneumonia	26.2	25.4	6.7	21.4	33.3	14.3	23.6
Chronic liver disease	20.0	15.9	20.0	35.7	22.2	71.4	21.9
Malignancies	13.8	20.6	6.7	57.1	44.4	28.6	20.8
COPD	23.1	19.0	6.7	0.0	22.2	14.3	18
Diabetes mellitus	23.1	17.5	6.7	0	22.2	14.3	18
Chronic heart disease	20	17.5	6.7	7.1	22.2	0	15.7
HIV infection	18.5	12.7	13.3	14.3	0	0	14
Stroke	9.2	19.0	0	7.1	0	14.3	11.2
Chronic renal disease	9.2	6.3	13.3	14.3	11.1	0	9
LOS (X ± SD)	21.1± 24.7	10.9± 9.0	23.6± 12.7	12.9± 9.7	24.4 ± 23.2	16.6± 25.7	17.5± 19.2
ICU admission (%)	31.4	19.7	63.2	0	33.3	0	26.7
Mortality (%)	17.1	9.1	0.0	14.3	55.6	12.5	14

[a]Available in 153 patients

Conclusions: Previous pneumonia has been identified as one of the most frequent risk factor for IPD that represents a great burden of disease. Facing limitations of the 23-valent polysaccharide (21.9% total population had been vaccinated, reaching >36% patients presenting N-CP or PB), vaccination with conjugate vaccines could be a better strategy.

R2772 Seven-years retrospective epidemiological survey of uncomplicated community urinary tract infections in a tertiary hospital

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Objectives: To investigate the pathogens causing community-acquired acute uncomplicated urinary tract infections (UTIs) and their resistance during 7 years period (2004–2010) in a tertiary hospital of Athens, Greece.
Methods: Midstream urine samples were taken for culture and testing for the presence of leucocytes and /or bacteriuria from 30 106 community patients presenting with symptoms of acute UTI. The identification of isolated bacteria performed with established techniques and their antibiotic resistance by Kirby- Bauer method and MICs by VITEK-II system, E-test. Only patients with urine cultures yielding growth of pathogens ≥105 cfu/mL were included in the study.
Results: Recognized urinary tract pathogens were present in 9278 (31%) patients. The first group of isolated bacteria was Enterobacteriaceae (84%), followed by Gram positive cocci (9%) and Gram negative non fermentans (3.6%). The commonest isolated organisms were Escherichia coli (67%), *Klebsiella* spp. (6.7%), *Proteus* spp. (7%), *Enterococcus faecalis* (4.6%), Coagulase- negative staphylococci (CoNS) (2%), *Staphylococcus saprophyticus* (1.47%). Resistance of *E. coli* was most common to ampicillin (43%), co-trimoxazole (23%), followed by quinolones (6.8%) while to co-amoxiclav, cefuroxime, nitrofurantoin, gentamicin was <3%. Proteus mirabilis was resistant to ampicillin in 50% and co-trimoxazole in 29%, while Klebsiella spp was resistant to co-trimoxazole, quinolones more than 29% .In *P. aeruginosa* the resistance to carbapenemes, quinolones was 14% and 31% respectively. S. saprophyticus resistance to quinolones, co-trimoxazole was <3%. CoNS were resistant to quinolones (23%), co-trimoxazole (21%), methicillin (26%). The resistance of E. faecalis to quinolones was 27%.

Conclusions: The frequence of uncomplicated community urinary tract infections was 31% during 7 years period. Enterobacteriaceae was the most frequently isolated group organism (84%) with *E. coli* accounting for 67% of isolates. E. coli was resistant to ampicillin and co-trimoxazole more than 23%. So the most effective antibiotics for the empirical treatment of acute community-acquired urinary tract infections seems to be nitrofurantoin, co-amoxiclav, cefuroxime.

R2773 Elevated venous lactate levels predict mortality in community-onset norovirus enteritis – a retrospective cohort study

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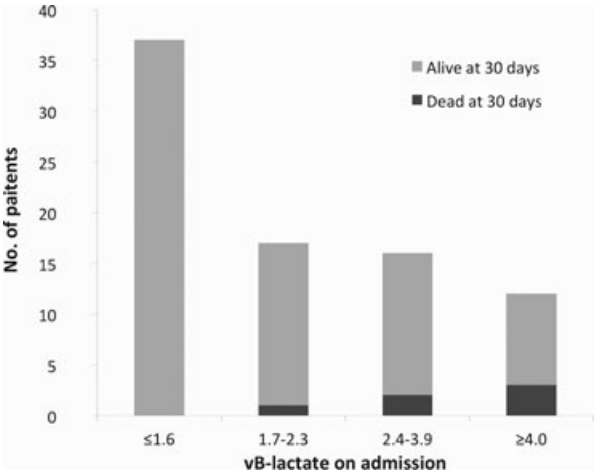
Objective: Serum lactate levels measured in the emergency department (ED) detect occult hypoperfusion and are associated with mortality. Since norovirus enteritis (NVE) causes fluid losses and has been associated with increased mortality, we hypothesised that lactate levels can be used to identify high-risk patients with norovirus (NoV) infection who require hospitalisation. The objective was to describe the association, in patients with community-onset NVE, between lactate levels on admission and 30-day mortality.
Methods: This was a retrospective cohort study set at the Sahlgrenska University Hospital, Gothenburg, Sweden during the NoV-season august 2008- june 2009. All hospitalised adult (>18 years) patients, admitted via the ED of the hospital, with a stool sample positive by PCR for NoV and community onset of gastroenteritis symptoms were included. Patients with concomitant serious bacterial infections, hypovolemic shock or surgical abdominal emergencies were excluded. Vital signs and venous lactate on arrival, co-morbid conditions, diagnosis at discharge and time of death were registered. The primary outcome was 30-day all-cause mortality. Chi-square test was used for comparisons of proportions, and Mann-Whitney U-test for continuous variables. One-step logistic regression was used for multivariate analysis.
Results: We included 82 patients with a median age of 77 years (IQR 53–86), of whom 47 (57%) were female and 49 (60%) had at least one major co-morbid condition. Lactate levels were above the upper limit of normal (ULN) 1.6 mM in 45 patients (55%). The overall 30-day mortality rate was 7.3% (6/82). Mortality was higher in patients with lactate >2.4 mM (>50% above the ULN) on admission (18% vs. 2%, $p < 0.05$). Higher lactate levels were associated with higher mortality (see figure). Patients who died had a higher median lactate compared to survivors, 4.5 (IQR 2.7–7.9) mM vs. 1.7 (IQR 1.2–2.6) mM, respectively ($p < 0.01$). After adjusting for other variables associated with mortality, the adjusted odds ratio for death within 30 days of a 1 mM increase in lactate was 3.4 (95% CI 1.05–11.1, $p = 0.041$).



Conclusions: Measurements of serum lactate may be a valuable tool in the clinical assessment of suspected NoV-infection.

R2774 Is bacterial vaginosis associated with the presence of *Candida albicans* and *Ureaplasma urealyticum* in reproductive-age women with vulvovaginitis?

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Objectives: At present, it is accepted that low acidity and a healthy appearing lactobacillary flora is typical for *Candida vulvovaginitis*, while bacterial vaginosis (BV), with decreased lactobacilli and increased vaginal pH levels, protects against *Candida* growth. In other words, it is suggested that the growth requirements of *Candida* and BV are completely opposite to each other. However, it has been reported that 6% of women with symptomatic *Candida vaginitis* had simultaneously BV and that a different proteolytic activity in the vaginas of women with BV can be involved in the pathogenesis of concomitant infection with *Candida*. Furthermore, women with BV were more likely to have a genetic polymorphism of the innate immune response. On the other hand, *Ureaplasma urealyticum* (UU) appears to be an important factor that enhances the virulence commonly associated with BV and was detected more often in women with BV than in those without BV. For the diagnosis of BV usually a Gram stain is used. Since genital mycoplasmas lack a cell wall and are thus resistant to Gram stain, their presence in studies that rely on Gram stain criteria can easily be missed. We aimed to assess the potential association of bacterial vaginosis with the presence of UU and *Candida* species in a group of symptomatic reproductive age women.
Methods: We examined 3648 vaginal and cervical specimens from an equal number of reproductive age women presenting to our hospital with signs and symptoms of vulvovaginitis during April 2009 to April 2011. Only 284 women with BV were tested simultaneously for mycoplasmas and this population was included in the present study. Gram stain preparations were examined from all specimens and Nugent criteria were applied for the diagnosis of BV. Vaginal and cervical cultures were performed under standard conditions. For the isolation of mycoplasmas the Mycoplasma IST2 (BioMèrieux, France) was used.
Results: Out of the 284 women with BV, 39 (13.7%) had vaginal candidiasis, too, while UU was isolated in 88 (31.0%) cases. Lastly, in 15 (5.3%) women BV was simultaneously present with UU and *Candida*.
Conclusions: BV was associated with UU in one third of the cases and in 5% with both UU and *Candida*. However, given the complexity of the vaginal flora and our relatively limited understanding of lower genital tract infections, further studies are needed to understand the role these pathogens play in causing disease.

R2775 Analysis of cases of infective endocarditis at Barts and the London NHS Trust over 1 year: clinical features, investigations and outcome

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Infective endocarditis (IE) is an important and challenging illness. Recent studies have reported a changing epidemiology related to increasing nosocomial IE with Staphylococcal aetiology. However, we are not aware of any recent published studies from the UK.
Objectives: To study the clinical features, microbial aetiology, management and outcome of all cases of IE admitted to Barts and the London NHS Trust (BLT) over 1 year. BLT receives direct inpatient admissions and is also a tertiary level referral centre for Cardiology and Cardiothoracic surgery.
Methods: All patients with clinical definite endocarditis (as per modified Duke's criteria) admitted from December 2009 to January 2011 were included. Demographic, clinical and laboratory data were collected prospectively.

Results: Thirty patients were studied. The age range was 19–85 years (73% were above 60 years). Sixteen patients (53%) had no comorbidities. Twenty four patients had native valve endocarditis and six had endocarditis of a prosthetic valve. Fever and respiratory symptoms were the commonest presentation and 10 (33%) patients were admitted within a week of symptom onset. Fourteen patients (47%) were admitted directly from the community (emergency admissions or GP referrals), and 16 (53%) were transferred from district hospitals for specialist care. Twenty six patients (86%) had community acquired IE and 3 (10%) had nosocomial IE. Blood cultures were positive in 24 (80%) patients. The most frequent organisms isolated were Viridians streptococci (7/24:29%), *Staphylococcus aureus* (6/24:25%) and Enterococcus faecalis (5/24:20%). Blood cultures were negative in 7 (29%) patients. Streptococci were identified in 6/10 (60%) patients presenting with acute IE. All patients underwent valve surgery. Twelve (40%) valve samples were culture positive, and 19 (63%) were PCR positive. A good outcome was observed in 29 (96%) patients, with a median follow up period of 3–6 months post discharge.
Conclusions: IE presenting to our centre occurred predominantly in elderly individuals with no obvious pre-existing valvular heart disease. Against expectations, Viridians streptococci were a significant cause of acute endocarditis. Earlier presentation, timely surgical intervention and predominance of Streptococcal aetiology might have contributed to the overall favourable outcome. Despite the limitation of an inherent selection bias, important conclusions about patients with IE presenting to a UK tertiary level service can be made.

R2776 Interventions for preventing recurrent urinary tract infection during pregnancy: a Cochrane review

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Objectives: Recurrent urinary tract infections (RUTI) are common in women who are pregnant and may cause serious adverse pregnancy outcomes for both mother and child including preterm birth and small for gestational age babies. Interventions used to prevent RUTI in women who are pregnant can be pharmacological (antibiotics) or non-pharmacological (cranberry products, acupuncture, probiotics and behavioural modifications). So far little is known about the best way to prevent RUTI in pregnant women.
Methods: We used standard Cochrane methodology (last search February 2011). The primary maternal outcomes were (recurrent) urinary tract infection before birth (variously defined) and preterm birth (<37 weeks). The primary infant outcomes were small-for-gestational age and total mortality.
Results: The review included one trial involving 200 women. This trial compared a daily dose of nitrofurantoin and close surveillance with close surveillance only. No significant differences were found for the described primary outcomes: recurrent pyelonephritis (risk ratio (RR) 0.89, 95% confidence interval (CI) 0.31–2.53), urinary tract infection before birth (RR 0.30 95% CI 0.06–1.38) and preterm birth (<37 weeks) (RR 1.18 95% CI 0.42–3.35). The incidence of asymptomatic bacteriuria (ASB) ($\geq 10^3$ colonies per milliliter) (secondary outcome), only reported in women with a clinic attendance rate of >90% (RR 0.55, 95% CI 0.34–0.89), was significantly reduced in women who received nitrofurantoin and close surveillance.
Conclusion: Daily dose of nitrofurantoin and close surveillance has not been shown to prevent RUTI compared with close surveillance alone, although a significant reduction of ASB was found in women with a clinic attendance rate of > 90% and who received nitrofurantoin and close surveillance. Furthermore, there is limited reporting of both primary and secondary outcomes for both women and infants. Due to lack of RCT, no conclusions can be drawn regarding the optimal intervention to prevent RUTI in women who are pregnant. RCT comparing different pharmacological and non-pharmacological interventions are necessary to investigate potentially effective interventions to prevent RUTI in women who are pregnant.

R2777 Severe community-acquired pneumonia

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Objective: This study was designed to identify etiological agents of severe community-acquired pneumonia (CAP) in patients who require admission to an intensive care unit (ICU).
Material and Methods: A multicenter, retrospective study of 12 hospitals in the Republic of Belarus was undertaken. Two hundred thirty-three patients with severe CAP were enrolled in the study during the period from March, 2009, to May, 2011. The diagnosis of CAP was based on the combination of chest radiograph findings, clinical signs and laboratory data (sputum specimens – 85.4%, bronchial aspirate – 12.9%, pleural fluid – 1.7%). We analyzed four age groups (15–29 years; 30–49 years; 50–69 years; more than 70 years). The first group included 45 patients with severe CAP (19.3%), the second group included 66 patients (28.3%), the third group included 78 patients (33.5%), and the fourth group included 31 patients (13.3%). Data was stored and analyzed using Microsoft Excel software (Microsoft, USA).
Results: During the study period, a total of 233 nonimmunosuppressed adult patients with severe CAP were admitted to ICUs, of whom 164 were men and 69 were women (mean patient age, 48 years; range, 15–91 years). Etiologic diagnoses were established in all cases. The most frequently identified pathogens were *Klebsiella pneumoniae* (61.8%), *Staphylococcus aureus* (34.8%), *Streptococcus pneumoniae* (2.6%) and *Haemophilus influenzae* (0.9%). All patients received antibiotic therapy.
Conclusion: The results of this study have stated a remarkable role of *Klebsiella pneumoniae* (61.8%) and *Staphylococcus aureus* (34.8%) in severe adult CAP.

R2778 Community-acquired pneumonia in an internal medicine service

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Objective: Community-acquired pneumonia (CAP) is a leading cause of death from infectious disease in western countries. The mortality is reported to be about 10%. The aim of this study was to analyze mortality in patients with CAP admitted to an Internal Medicine service and to study the value of the CURB-65 score as a prognostic tool.
Methods: The prospective cohort study was conducted at Hospital Sierrallana (Torrelavega, Spain) from March to October 2010. All patients admitted with CAP to the Internal Medicine Service were prospectively recorded. Clinical and epidemiological data and laboratory findings were examined. The CURB-65 score and the Charlson Cormobidity Index were calculated. Sensitivity, specificity, positive predictive value and negative predictive value were assessed. All data were analysed and processed with the SPSS version 15.0. Results are expressed as frequencies or as mean ± standard desviation.
Results: During the study period, there were 539 in-patients admitted to the Internal Medicine service, 39 (7%) with CAP (64% men and 36% women). The mean age was 78 ± 11 years and the mean duration of stay was 7 ± 6 days. One patient was admitted to Intensive Care Unit (ICU). According to CURB-65 criteria on admission, five patients presented low risk (13%); 15 intermediate risk (38%) and 19 high risk (49%), without significant sex-related differences. According to the Charlson Comobidity Index on admission, 13 patients (33%) presented high risk (44% men; $p < 0.01$). The most common chronic diseases causing comorbidity were pulmonary disease (31%) and dementia (28%). Overall, 30-day mortality was 20%. The mean age of patients who died was 82 ± 2 years, 50% were men. The CURB-65 score was significantly associated with the risk of death. The positive predictive value for a fatal outcome of high risk CURB-65 was 40%.
Conclusions: Seven percent of patients admitted to the Internal Medicine service presented CAP. Mortality was higher than in previous studies, which could be associated with the older age and

comorbidity of our patients. The CURB-65 score can also be used in these patients to assess pneumonia severity and the risk of death.

Lyme borreliosis, toxoplasmosis

R2779 Molecular identification of *Borrelia spirochetes* isolated from skin biopsies of patients characterised with an unusual dermal manifestation (prurigo pigmentosa) in Taiwan

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Objectives: To identify the genetic identity of *Borrelia spirochetes* isolated from skin biopsies of patients characterized with an unusual skin manifestation in Taiwan.
Methods: Serum samples and skin biopsy specimens were collected from patients in Taiwan. Serodiagnosis was performed by Western immunoblot assay and Borrelia spirochetes were isolated by culturing of skin biopsies. Total genomic DNA was extracted from individual culture by using DNeasy Tissue Kit (Qiagen). The genetic identity of Borrelia spirochetes were determined by analyzing the gene sequences amplified by a genospecies-specific polymerase chain reaction (PCR) assay based on the 5S-23S intergenic spacer amplicon gene of Borrelia spirochetes. Aligned sequences were analyzed by neighbor-joining (NJ) compared with maximum parsimony (MP) methods to estimate the phylogenetic relationships of these detected spirochetes.
Results: Serological evidence of *B. burgdorferi* infection was confirmed by elevated IgG and IgM antibodies against protein antigens of *B. burgdorferi*. A total of 10 patients were examined by Borrelia cultures and tested by PCR assay. Phylogenetic analysis reveals that all those detected spirochetes constitute two major separate clades distinguished from other Borrelia genospecies in both NJ and MP methods. Within these clades, two isolates of Borrelia spirochetes detected in skin cultures were closely related to the genospecies of *B. garinii* and one isolate were closely related to the genospecies of *B. afzelii*.
Conclusion: Our results describe the first identification of *B. garinii* and *B. afzelii* spirochetes isolated from skin biopsies of patients characterized with an unusual skin lesion of prurigo pigmentosa in Taiwan. Further investigations on Borrelia spirochetes detected in patients with various clinical manifestations would beneficial to the better understanding of genetic diversity of Borrelia spirochetes in Taiwan.

R2780 Detection of *Borrelia burgdorferi* sensu lato in urine specimens from patients with early and late Lyme borreliosis

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Objectives: Detection of *Borrelia burgdorferi* by polymerase chain reaction (PCR) in blood from patients with Lyme disease is often not enough effective to ensure reliable diagnosis. The aim of this study was to investigate appropriateness of urine and blood specimens for diagnosis of Lyme disease by PCR.
Methods: Urine and blood samples from 228 patients with symptoms attributable to Lyme disease were investigated by PCR. Of them, 81 patients were with typical erythema migrans and were not treated with antibiotics, 92 were with treated with antibiotics erythema migrans and 55 presented with symptoms of early disseminated and late Lyme borreliosis.
Results: *B. burgdorferi* was detected only in patients with untreated erythema migrans. The diagnosis was confirmed in 10 (12.3%) of 81 patients. The etiologic agent was detected in urine of 4/59 (6.8%) patients and in blood plasma of 6/81 (7.4%) patients.
Conclusion: Our results demonstrated that *B. burgdorferi* could be detected by PCR before specific IgM antibodies appearance and that PCR testing of urine samples may add to effectiveness of this diagnostic method in Lyme disease confirmation.

R2781 Lyme borreliosis and tick-borne encephalitis in Latvia: a comparative study of cases reported in 2007–2010

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Background: Tick-borne encephalitis (TBE) has been registered in Latvia since 1955, Lyme borreliosis (LB) – since 1986. The annual incidence rate during 2001–2010 ranged from 21.5 to 36.9 per 100 000 population for LB and from 7.5 to 22.0 – for TBE with the highest incidence rates in 2010. The objective of the study was to describe main epidemiological and some clinical characteristics of LB and TBE in order to improve public health advice.
Materials and Methods: Reports for notified individual cases of LB and TBE were analyzed for 2007–2010 with EpiInfo software. Retrospective epidemiological analysis covered incidence rates by territories, age and gender groups, seasonality, data on exposures, hospital admission rates and length of hospital admission, data on laboratory confirmed diagnosis, as well data on tick activity and pathogen prevalence.
Results: Altogether, 2664 cases of LB and 1177 cases of TBE were reported during the study period, including 75 co-infection cases with TBE and LB. The total number of LB cases 2.3 times exceeded the number of TBE cases, ranging from 3.7 in 2007 to 1.7 in 2010. The incidence rates of LB, TBE and co-infection varied by territories. Male/female ratio was 0.53 for LB and 1 – for TBE patients. Mean age of LB patients was 47.4 years (SD ± 18.2, range from 15 months to 88 years); mean age of TBE patients was 46.6 years (SD ± 18.2, range from 14 months to 87 years). Proportion of cases occurred outside of tick activity season was higher in LB cases. 67.3% (95% CI: 65.5–69.1%) of LB patients and 63.5% (95% CI: 60.7–66.3%) of TBE patients reported tick bite prior to disease onset. TBE patients were more likely to be hospitalized than LB patients (RR 28.9; 95% CI: 21.5–38.8, $p < 0.05$). On average, patients with single LB or TBE infection were hospitalized for accordingly 11.9 and 12.7 days. Comparing to LB cases, patients with LB/TBE co-infection were older, had higher hospitalization rate and stayed in hospital on average 3 days longer.
Conclusions: The results of the study revealed more or less similar trend in age distribution and hospitalization lengths; differences included unequal territorial distribution, higher hospitalization rate for TBE patients, and much higher proportion of LB women patients. Due to growing relevance of tick borne infections in Latvia it is necessary to sustain physicians and public awareness at high level for early diagnostic measures, as well conduct regular monitoring of disease careers and pathogens.

R2783 A rifampicin-containing antibiotic regimen is associated with better outcome in vascular graft infections

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Background: Vascular graft infection is a life-threatening complication after vascular surgery. Little is known about the optimal antibiotic management.
Methods: Ten-year retrospective analysis of all patients (pts) with vascular graft infection in a tertiary hospital between 2001 and 2011. Treatment failure was defined as death from graft infection and/or persistence of clinical or radiological signs compatible with vascular infection at 1 year after diagnosis. Cox proportional hazard models were used to estimate the hazard ratios (HR) of treatment failure at 1 year.
Results: Sixty-one points were included in this preliminary analysis. Characteristics: 52 males (85%), median age 65 years (IQR 57–70), cardiovascular comorbidity in 90% and diabetes in 16% of pts. Most pts (84%) received an aortic graft due to aneurysm, combined with valve replacement in 15 pts, and 16% received peripheral vascular graft due to

occlusive arterial disease. The median time to vascular graft infection was 37 days (IQR 9–267) after surgery. Wound secretion and fever were observed in 18% and 49% of pts, respectively. Coagulase-negative staphylococci and *Staphylococcus aureus* were the most frequent microorganisms (40%). Polymicrobial infections were found in 34% of pts. All pts were treated with antibiotics and 74% underwent surgical revision with graft replacement in 9 pts and extra-anatomical bypass in 8 pts. The median antibiotic therapy was 41 days (IQR 17–78). During the follow-up, 17 pts experienced treatment failure, 10 of those died from graft infection with a median time to death of 20 days (IQR 12–59) after diagnosis, i.e. 1-year success rate was 72%. In univariate analysis, risk factors of treatment failure at 1 year were older age (HR 2.0, 95% CI 1.2–3.3, p = 0.004, per 10 years increase), high C-reactive protein (HR 1.3, 95% CI 1.1–1.6, p = 0.013, per 50 mg/L increase), whereas infection with gram positive microorganisms (HR 0.2, 95% CI 0.1–0.6, p = 0.007) and a rifampicin-containing antibiotic regimen (HR 0.1, 95% CI 0.05–0.5, p = 0.009) were associated with better outcome. In multivariate analysis, after adjustment for age, vascular graft, C-reactive protein, microorganism and surgical treatment, an antibiotic regimen containing rifampicin was associated with lower risk of treatment failure (HR 0.1, 95% CI 0.1–0.8, p = 0.028).

Conclusions: Rifampicin-containing regimen significantly improved outcome in vascular graft infections in this study, as previously observed in orthopedic implant-associated infections.

R2784 Can bacterial infection be predicted in clinical trials on acute exacerbations of chronic obstructive pulmonary disease (AECOPD)? A retrospective analysis from the MAESTRAL patient cohort

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Objective: Acute exacerbations of chronic obstructive pulmonary disease (AECOPD) studies with improved design and modern methods have established that approximately 50% of exacerbations are caused by bacterial infection. In the MAESTRAL study, enrolling 1492 chronic bronchitis outpatients with moderate-to-severe COPD and an Anthonisen type 1 exacerbation, bacteria were isolated from sputum in 49% of the patients at enrolment. Using the MAESTRAL database, the objective of this analysis was to build up a scoring system that was easy for physicians to use, which could help predict bacterial infection in AECOPD patients.

Methods: This analysis first identified independent predictors associated with the presence of bacteria at baseline using a multivariate logistic regression method. These independent predictors were evaluated in terms of their frequency.

Results: The following factors were found to be independently associated with the presence of bacteria at enrolment: age ≥65 years/<65 years (51.6%/42.0%), history of cardiopulmonary disease (yes/no) (58.6%/47.7%), FEV1 predicted (≥30%/<30%) (50.6%/44.0%), sputum viscosity (very thick/not very thick) (55.6%/48.1%), colour of purulent/mucopurulent phlegm at AECB-SS (green or brown/other colours) (54.4%/47.0%), wheeze at enrolment (yes/no) (50.4%/43.0%), anti-cholinergics use (yes/no) (57.9%/47.7%). Although these were all statistically significant effects, the actual difference between categories did not exceed 10.9% and there was no category with an organism rate higher than 58.6%. When these factors were pooled and at least four of them were present, the probability of the presence of bacteria was 60.9% (Table). With five or more factors simultaneously occurring in patients, the rate was 68.2%, but only a few patients had all these factors. There were no patients with all seven factors present simultaneously.

Conclusion: This analysis suggests that using the combined independent factors listed above would have increased the proportion of bacterial infections in patients with an Anthonisen type 1 exacerbation, by only 10% in the MAESTRAL study. Applying additional risk factors to define a patient population would provide little benefit on increasing the proportion of patients with bacteria and would be too restrictive during enrolment.

Table. Cumulative percentages of presence or absence of bacteria

Number of factors	Presence of bacteria			
	No		Yes	
	Number of patients	Percentage	Number of patients	Percentage
0	7	77.8	2	22.2
1	65	69.1	29	30.9
2	201	56.1	157	43.9
3	281	52.6	253	47.4
4	111	39.1	173	60.9
5	21	31.8	45	68.2
6	6	57.1	3	42.9
7	0	n/a	0	n/a

R2785 A comparative randomised clinical trial against semi-synthetic penicillins and glycopeptides supports the use of daptomycin as first-line treatment of complicated skin and soft-tissue infections in the elderly

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Objectives: *Staphylococcus aureus* is the leading pathogen in skin and soft tissue infections (SSTIs). In comparison to anti-methicillin resistant *S. aureus* (MRSA) agents, including linezolid/tigecycline/glycopeptides, daptomycin has the most potent in-vitro activity against Gram-positives causing complicated SSTI, including methicillin susceptible *S. aureus* /MRSA. As therapy is initiated before availability of culture data, antibiotic coverage equally active against MSSA and MRSA is advantageous. Daptomycin's superior potency is a strong rationale for its use in empirical treatment, especially in the elderly. The objective of this study was to assess the effectiveness of daptomycin in the elderly.

Methods: Phase IIIb open label multicentre randomised study to assess safety/efficacy of i.v. daptomycin (4 or 6 mg/kg q24 hours) vs. pooled i.v. comparators (semi-synthetic penicillin 2 g q4 hours/6 hours or vancomycin 1 g q12 hours). Patients aged ≥65 years with cSSTIs with or without bacteraemia, treated for 14–28 days (stratified by baseline CrCl). The primary objective was to descriptively compare clinical success at the test of cure (TOC) 7–14 days after the end of treatment in the clinically evaluable (CE) set. Secondary objectives were microbiological outcome and safety.

Results: One hundred and twenty patients were randomised (81 to daptomycin; 39 to comparator) and 102 completed the study. Baseline characteristics were comparable in both groups. The most common infection was cellulitis; other common infections were ulcers and abscesses. Six patients had bacteraemia (daptomycin 4, comparator 2). Clinical success at TOC was numerically higher for daptomycin vs. comparator in the overall CE population (89% 65/73 vs. 83% 25/30, blinded) and in patients with S. aureus (90% 35/39 vs. 69% 9/13). In the full analysis set (nonevaluable response = failure) the clinical success was 82% for daptomycin and 72% for comparator. Rates of adverse events (AEs) and serious AEs were similar (respectively, 63% and 9% for daptomycin vs. 65% and 10% pooled comparator), rates of discontinuation due to significant AEs or SAEs were lower for daptomycin (4% vs. 10%).

Conclusion: Further studies are warranted to determine whether early use of anti-MRSA treatment in SSTI in settings with elevated MRSA rates may result in improved outcomes such as lower mortality, shorter length of hospital stay and lower total treatment costs. Results of the

present study support the use of daptomycin as first-line treatment of cSSTIs in elderly.

Paediatric infections

R2786 Childhood brucellosis-osteoarticular manifestations

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Objectives: Since 1980, brucellosis in Macedonia has persisted as an endemic and epidemic infectious disease of cattle and people too. The aim of this paper is to show the osteoarticular manifestations of human brucellosis in children.
Methods: This paper is a retrospective study of patients under 14 years of age, infected with brucellosis with predominant damages of the osteoarticular system. We have been working with a group of 49 children between 3 and 14 years of age. Nineteen patients were from Bitola, and 30 from its surrounding villages. 23 patients were female, and 26 male. The diagnosis was set according to the serum parameters (Rose-Bengal, Wright, Coombs, RVK Brucellae, ELISA), Rtg, scannographic, and KTM.
Results: Predominant symptoms were: fever (in 100% of the patients), sweating (85%), exhaustion (80%). Osteoarticular symptoms were evident in 64% of the patients: pain, limited mobility, almost immobility, with or without swollen joints. In 67%, the big joints of the lower limbs were infected, in 29% the joints of the upper limb, and in 4% the spinal and pelvic joints. Monoarticular form was present in 41%, and poliarticular in 59% of the children. All of the children had arthralgia, and complete immobility was present in 12%. The treatment lasted between 30 and 42 days; streptomycin and cotrimoxasol, rifampiciyn and cotrimoxasol were used, and also doxicycline was used in the treatment of children over 8 years of age. Relapse of the infection was registered in 6.3%, whereas sequel in a form of aseptic necrosis was registered in only one of the patients.
Conclusion: *Human brucellosis* is a grate health problem in the region of Bitola, *R. Macedonia*. It is important to organize more serious health education and very severe sanitary and veterinary control of the cattle, meat, milk and dairy products in order to eradicate the disease.

R2787 Urinary tract infection in a paediatric hospital in southern Iran

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Objectives: Urinary tract infections (UTI) are the most common infections in children and their complications result in significant morbidity and mortality and consume resources. The aim of this study was to find out the incidence and distribution of urinary tract infections in childhood in south of Iran and analysis their clinical presentation.
Methods: In this cross sectional study, during a 6-month period, 125 children who admitted to the pediatric hospital for treatment were prospectively investigated. Demographic characteristics, clinical and paraclinical findings, organism type, their sensitivity and drug resistance based on antibiogram were recorded in a pre-designed checklist. UTI was defined by >10 organisms/mL on laboratory culture of urine.
Results: Of 125 children, UTI rate in female children was significantly higher than that in male children (55% vs. 45%, $p < 0.05$). The greatest proportion of the patients was in 1–12 months age group (33%, $n = 41$) and then in 5–9 years age group (26%, $n = 32$). The most common symptom was fever, which was found in 79% of the cases. Some of non-specific symptoms were found in 75% of the children, with the highest rate in <2 years old children. Specific symptoms were mostly in older children. There was a significant relationship between age and the symptoms ($p < 0.05$). Most common causative organism for both genders and in all age groups was *E-coli* (74%). *Klebsiella* was common organism in both genders, but more prevalent in under 2 years old children. There was no significant relationship between organism type and gender, but it was significant in different age groups. The greatest susceptibility of E-coli and Klebsiella was to ampicillin, septrin and gentamicin and *Proteus* showed susceptibility to septrin. Kidney sonography of 42 children showed abnormalities such grade four reflux, urinary stone and hydronephrosis in seven children.
Conclusion: The main objectives in childhood urinary tract infections are rapid recovery from complaints and prevention of infection-related complications. Predominant organisms causing the infection were *E-coli*, *Klebsiella* and *Proteus*, whereas they show susceptibility to ampicillin, septrin and gentamicin. Consequently, further studies should be conducted to establish a guideline for management of these infections and to recognize innovative practical approaches in order to control the infections.

R2788 Two year study of neonatal intensive care unit blood cultures in a Greek maternity hospital

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Objectives: The purpose of this study was the assessment of blood culture isolates and their antibiotic sensitivities in the neonatal intensive care clinic of the IASO Maternity hospital.
Material and Methods: The study concerns the blood cultures of two ntensive care unit (ICUs) out of four of the IASO Maternity Hospital. Blood cultures have been performed by the Bactec system. Antibiotic sensitivity was accomplished by the Kirby Bauer method on Mueller Hinton agar.
Results: Out of 8224, four hundred ninety one (491) blood cultures were positive. The pathogenic microorganisms isolated were as follows: 75.4% *Staphylococcus epidermidis*, 5.3% *Escherichia coli*, 4.5% *Enterococcus*, 3.6% Streptococcus group B, 3% *Enterobacter cloacae*, 1.4% *Serratia marcescens*, 0.4% *Klebsiella pneumoniae*, 0.4% *Pseudomonas aeruginosa* and 6% *Candida* spp. (*Candida albicans* and *Candida krusei*). Penicillin G, exhibited the lowest percentage of sensitivity among both Gram positive and Gram negative species. Imipenem was the most efficient antibiotic with the highest percentage of sensitivity among gram negative. Most of the isolates (100% gram + and 90% gram-) showed a b-lactamase activity which was reversed in the presence of clavulanic acid. Resistance to second generation cephalosporins was highly prevalent for Gram positive, but 90% of Gram negative isolates were sensitive. Resistance to third generation cephalosporins was prevalent in 50% of gram positive strains, and about to 10% of gram negative with the exception of one strain of Serratia marscecens which was highly resistant to most of the b-lactam antibiotics. Gram negative strains showed 100% and gram positive strains showed 80% sensitivity respectively to ciprofloxacin. Resistance to vancomycin was shown in only 1% and to teicoplanin to 7% respectively of the S. epidermidis isolates. Among the aminoglycosides, netilmicin showed the highest percentage of sensitivity both for gram positive and gram negative strains.
Conclusions: We conclude that the most common microorganism isolated in blood cultures of our neonate ICU is Staph. epidermidis and that the most resistant phenotype was shown in only one Serratia marcescens isolate. About 95% of *Candida* spp. were sensitive to the azoles. Until now the management of ICU neonates with positive blood cultures was efficient and without treatment failure. We however are vigilant and we accomplish a monthly protocol for the survey of microbial carriage and antibiotic sensitivity in the ICU.

Immunology, host defences, immunotherapy

R2789 Effects of usage of immunomodulators of different pharmacological groups on eradication and immune indexes in *Helicobacter pylori* induced peptic ulcer disease

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Objectives: **1** To investigate influence of immunomodulators Licopid® (synthetic bacterial preparation) and Derinat® (nucleic acid preparation) in combination with antihelicobacter ''quadro-scheme'' therapy (QST) on efficiency of eradication in patients with peptic ulcer disease (PUD) caused by H. Pylori (HP).
2 To reveal interrelation of changes of intensity of specific (levels of Immunoglobulins and population T-lymphocytes) and non-specific immunity (lysozyme activity in saliva and gastric juice).
Methods: **1** This research is carried out on 75 patients within the age range of 20–55 years with PUD of stomach caused by HP.
2 Depending on types of received therapy, three groups of patients have been allocated:-
(i) Group A (control group) consisting of 25 patients receiving ''quadro-scheme'' therapy (QST) consisting of bismuth colloidal subcitrate, omeprazole, amoxicillin and clarithromycin.
(ii) Group B of 25 patients receiving QST with Licopid®
(iii) Group C of 25 patients receiving QST with Derinat®
3 Before and 6 weeks after treatment, the histomorphological tests of biopsy material taken endoscopically from stomach, blood immune test and nephelometric test of saliva and gastric juice from patients are done.
Results: Six weeks after treatment, cytological tests revealed that in Group A, parameter of eradication (percentage of absence of H. Pylori on biopsy samples) is essentially higher in Group B and C ($p < 0.01$) compared to Group A. Presence of coccal (resistant) forms are noted in Group A but are absent in Group B and C. Blood immune test revealed that levels of CD-3, -4 and -8 T-lymphocytes and Immunoglobulin A, M and G are increased in Group B and C ($p < 0.01$ compared to initial level). In contrast, their levels are decreased in control Group A. The maximal authentic increase of lyzosymal activity in saliva and gastric juice is observed in Group B ($p < 0.01$ compared to initial level) whereas in Group C its activity reliably increases ($p < 0.05$). In Group A, the lowest increase in lyzosymal activity of saliva and gastric juice is obtained ($p > 0.05$).
Conclusion: Usage of immunomodulators in combination with QST leads to increased eradication of HP and improvement of immune indexes, more pronounced at application Licopid®. In this connection, combination of immunomodulators with QST can be recommended for effective eradication of *H. Pylori* in patients with secondary immunedeficiency and prevention of serious complications of PUD (malignancy, bleeding, perforation, etc).

R2790 *Varicella zoster* antibodies among patients with chronic renal failure candidate for renal transplantation in a referral teaching hospital, Tehran, Iran

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Objectives: This study was designed to evaluate the immune status of patients with chronic renal failure (CRF) candidate for renal transplantation in a referral teaching hospital, Tehran, Iran, and to compare the history of chickenpox infection with the presence of varicella antibodies in this population.
Methods: In this cross-sectional prevalence study serologic testing for varicella zoster was performed for 187 patients with CRF between January and May 2010. A checklist was completed including demographic data and the history of varicella. Individuals with acute varicella infection, those under immunosuppressive therapy, or those having blood transfusion during the year 2009, were excluded from the study. Blood samples were collected from each individual and the separated serum was stored at -20° centigrade prior to testing. EIA for varicella-specific IgG was performed using commercial virus-specific IgG EIA kits (varicella IgG EIA well, RADIM, Italy; sensitivity 100%, specificity 88%). Statistical analysis was performed using the spss 15 software. The p value of <0.05 was considered statistically significant. We compared the history of varicella infection in patients with the results of testing for antibody against varicella zoster antigen to assess the reliability of the history of varicella in this population. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated.
Results: One hundred and eighty seven patients evaluated in this study, 49.2% were female and 50.8% were male. Mean age was 57.51 ± 16.17 years. One hundred and thirteen (60.42%) patients didn't have any history of varicella disease. One hundred and eighty three patients (97.86%) had positive antibody titers. Mean age of seronegative patients was significantly lower than seropositive patients (29.50 ± 10.41 vs. 58.13 ± 15.74, p value<0.001). Logistic regression reveals correlation between age and antibody titer ($B = 0.132$, p value<0.001). Sensitivity, specificity, PPV, and NPV of the history of varicella for the seropositivity were 39%, 50%, 97%, 1.7% respectively.
Conclusion: Most of kidney transplantation candidate in our study were seropositive and patients age was correlated to seropositivity, nevertheless the vzv antibody titer evaluating seams necessary in these patients. Negative history for varicalla zoster infection is not reliable to consider patients as seronegative.

R2791 Immune response to *Cryptosporidium parvum* in immunocompetent mice

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Objectives: *Cryptosporidium parvum* is an intracellular parasite causing enteritis which can become life-threatening in immunocompromised host. Immunoregulatory T cells (Tregs) play a central role in the regulatory network of the host. Specific therapeutic or preventive interventions are not yet available so understanding the immune response to the parasite is required. In this study, we propose to characterize cell composition in peripheral blood and spleen through infection and reinfection with *C. parvum*.
Methods: Specific-pathogen-free BALB/C mice were inoculated with 1×10^6 oocysts of *C. parvum* at days 0 and 22. Fecal and blood samples, spleens and small intestines were collected for analysis on specific days. Immune cells were characterized, by flow cytometry, in peripheral blood and spleen samples.
Results: The presence of gDNA of *C. parvum* in feces was confirmed after a nested PCR of the gp60. Small intestine sections from infected mice were screened for intracellular stages of the parasite, which could be observed in the villi of the intestine, although no evidence of pathology has been observed. After infection, mice presented higher values of neutrophils, eosinophils, NK cells and CD4 + CD25high T cells (days 0–21) in peripheral blood. After reinoculation, this upward tendency was maintained in the following days in infected mice, for all the four populations. At day 35, the values observed in infected mice were close to the control group values, except for CD4 + CD25high, which maintained higher values in infected mice. Statistical analysis using the nonparametric Mann–Whitney test showed a greater variability after reinfection stage. Despite the small sample size, the greater variability observed after reinfection stage might be age-related, which is particularly relevant in conditions of disease, and probably due to the different maturation of the immune system.
Conclusion: Our data presents preliminary information which seems to be important for the knowledgment of the specific cell-mediated response to the infection by *C. parvum* in immunocompetent mice. These data it is expected to contribute for a better understanding of the role of innate and Treg cells in the initial clearance process of this parasite. Complementary studies are needed to clarify the role of innate immune response in the context of C. parvum infection, and to corroborate the results presented in this work.

R2792 Luminex® xMAP: high-throughput technology for quantification of cytokines and immunoglobulins in *Cryptosporidium parvum* infection

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Objectives: *Cryptosporidium parvum* is the enteric parasite most frequently associated with diarrhea outbreaks. Infection with this organism triggers a complex array of innate and cell mediated immune response. To date, there are no studies applying the Luminex technology to determine profiles of cytokines and immunoglobulins in the context of an infection by *C. parvum*. In this study, we intent to analyze these immune mediators in serum of mice inoculated with C. parvum oocysts, using a Luminex® xMAP technology.
Methods: Specific-pathogen-free BALB/C mice were inoculated with 1×10^6 oocysts of C. parvum at day 0 and re-challenged at day 22. Peripheral blood was aseptically collected from mice euthanized, on specific days, and serum was obtained after centrifugation. Two kits were used to assess and quantify mouse cytokines and immunoglobulins in serum, respectively: the Bio-Plex Pro™ Mouse Cytokine Standard Group I 23-Plex and the Milliplex® MAP Mouse Immunoglobulin Isotyping, using the xMAP technology. Both assays were performed according to the manufacturer's instructions.
Results: Infection was confirmed by the presence of *C. parvum* DNA in feces, collected from each group of mice, by a nested PCR of the 60-kDa glycoprotein. The preliminary analysis showed a significant increase of IgM during the primary infection and a decrease after reinfection as expected. A significant rise of IgA and IgG2a was observed after reinoculation. The analysis of the produced cytokines suggests a preferential Th1 over the Th2 response, with the consequent production of IFN-gamma, TNF-alfa and IL-12 over IL-5 and IL-10.
Conclusion: This work aims to understand how the immune system responds to an infection by C. parvum. In this context, the greatest advantage of using Luminex® xMAP technology is allowing the quantification of the small amounts of immunoglobulins and cytokines produced during this infection in an animal model, from a single small volume sample.

R2793 Impaired early intravaginal cell recruitment in response to *Chlamydia muridarum* in mice pretreated with progesterone

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Objectives: Chlamydia is a widely spread chronic urogenital infection that essentially affects reproduction health worldwide. There is well acknowledged evidence that Chlamydia infection induces a range of immunopathological reactions, while the components of early immune response that might lead to protection from re-infection require further elucidation. It is also well established that progesterone treatment can boost the host susceptibility to Chlamydia and increases its acute infectivity and shedding. However, the effects of progesterone treatment on long lasting immunity to the pathogen and control of re-infection has not been assessed. In this study we are analyzing the early local responses to intravaginal *C. muridarum* primary infection in mice treated with covinan (progesterone analogue) and their responses to the secondary infectious challenge.
Methods: Mice were infected intravaginally with 106 IFU of Chlamydia and numbers and phenotypes of cells from vaginal washes were analyzed 24 hours post infection (PI) by flow cytometry.
Results: We have found that Chlamydia intravaginal infection induced very robust cell recruitment in mice untreated with progesterone 24 hours post infection (10-fold increase compared to uninfected controls and 30-fold increase compared to covinan-treated mice). Interestingly, uninfected but covinan-treated mice had increased numbers of recruited cells compared to intact control. Phenotype study of cells from vaginal washes revealed the increase in percentage of Gr-1+ cells (neutrophils) and CD11c+CD86+ (activated dendritic cells) in infected but covinan-untreated compared to intact controls. Covinan-pretreated infected mice had decreased levels Gr-1+ cells and completely none of CD11c+ cells, while covinan-pretreated uninfected control had apparent CD11c+CD86+ cell population (none of these cells were found in intact control) We also observed an increase in CD8 + T cells in both infected groups (slightly higher in covinan-untreated mice) compared to uninfected controls. Cytokine profiles and responses to secondary infectious challenge of covinan pretreated mice are currently under the study.
Conclusion: Our preliminary results demonstrate that early introvaginal recruitment of neutrophils and dendritic cells seems to be impaired in Chlamydia infected progesterone pretreated mice, while T cells are still recruited. Whether this affects the ability to control re-infection in our model needs further investigation.

R2794 Tumour necrosis factor -308 G/A and 238 G/A polymorphism: has it any role in the course of infective endocarditis?

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Objectives: The role of single nucleotide polymorphisms (SNPs) of the TNF gene in infective endocarditis (IE) remains unknown. The impact of SNPs at the promoter regions -308 (G/A) and -238 (G/A) of the Tumour necrosis factor (TNF) gene in the physical course of IE was studied.
Methods: Sampling was done from 82 patients with definite IE and from 108 healthy Greek individuals. Genomic DNA was extracted from whole blood by the Puregene kit. The TNF 308G/A and 238 G/A SNPs were detected by polymerase chain reaction, incubation with the enzyme NcoI and electrophoresis of the restriction fragments. Clinical data were recorded.
Results: For the 308 SNP, 67 patients were carriers of both wild-type G alleles; 13 patients were heterozygotes for the SNP A allele; and two patients was homozygous for both SNP A alleles. Respective frequencies within the 108 controls were 92, 14 and two patients (pNS) For the 238 SNP, 68 patients were carriers of both wild-type G alleles; and 14 patients were heterozygotes for the SNP A allele. Respective frequencies within the 108 healthy controls were 104 and 4 (p: 0.003 vs. patients). Double SNP phenotype (-308 and -238) was associated with death (OR: 7.0, 95% CI 1.0–48, $p = 0.05$) in a multivariate logistic regression model adjusted for diabetes mellitus, female gender and persistent bacteremia.
Conclusions: These results denote for the first time a role of SNPs of genes related with the immune function in the physical course of IE.

R2795 IgG antibody response to *Plasmodium falciparum* schizont extract

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Background: Schizont extract can be obtained from malaria culture using each plasmodium falciparum isolate and also can be used as a fast and easy method to evaluate the immune response against malaria. The current study aimed to evaluate the age dependent immune response against crude shizont extract.
Materials and Methods: Age-dependent immunity was analysed testing the sera of 179 individuals, randomly selected from the Keneba Serum Collection using crude schizont extract. Cultivation of the erythrocyte stages of *P. falciparum* was done according to the technique of Trager and Jensen. Crude schizont extract was obtained using centrifugation methods. Schizonts were concentrated from culture either by using the plasmagel flotation technique or the Percoll gradient technique according to modification of methods described by Kramer and Lambros & Vanderberg. Total IgG and IgG subclasses was measured using ELISA. Data were analyzed using Spearman correlation method, ANOVA and Post hoc analyses methods.

Results: About 78% of the individuals within all age groups had IgG responses to schizont extract with the mean OD values above the cut-off. 66% of individuals whose IgG responses to the schizont extract were below the cut-off belonged to the 0–5 year ages and only 2.5% belonged to the age group 51+. About all individuals in age group 16–30 and 91% of those in age group 51+ showed IgG responses higher than background to schizont extract. The correlation between age and OD values to schizont extract was positively significant ($r = 0.296$ and $p < 0.01$).
Conclusion: Most sera strongly recognized the crude schizont extract in an age dependent manner while these responses were associated with an increase in haemoglobin levels and a decrease in parasitaemia, suggesting that IgG response to crude schizont extract may be a useful mean for further studies of protective immunity against plasmodium falciparum.

Vaccines

R2796 Efficacy of hepatitis B vaccination in Iranian thalassaemic patients

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Objectives: Thalassemia is still a major health problem in south of Iran and thalassemic patients are at risk of transmission infection diseases such as hepatitis B. The aim of the present study was to examine the efficacy of vaccination against hepatitis B in thalassemic patient in a thalassemia centre in south of Iran.
Methods: A random sample of 406 thalassemic patients including major thalassemia, sickle thalassemia, intermediate thalassemia who had regular blood transfusion and had received a full course of vaccine against hepatitis B were investigated. Antibody levels after vaccination were measured by an enzyme immunoassay method. Levels >10 mIU/mL were considered to show a positive response.
Results: Of 406 patients, 69.70% of the cases were with major thalassemia, 19.46% intermediate thalassemia and 10.84% sickle thalassemia. One hundred and ninety-six cases (48.28%) were male with mean age of 13.3 years (SD=7.3) and 210 cases (51.72%) were female with mean age of 12.27 years (SD = 6.8). 61 cases had booster dose and 23 cases had three more doses in addition to routine vaccination. HBsAb in 79.2% of the cases with routine vaccination was positive. It was positive in 86.89% of the cases with booster dose and in 86.96% of the cases with three extra doses. Overall, 78 of the 406 individuals in the study (19.21%) showed no antibody at all or had levels <10 mIU/mL. The response rate was significantly different in those who received routine vaccine and those who received a combination of routine vaccine and booster dose or three extra doses. There seemed to be no demographic characteristics that significantly distinguish responders from the non-responders. Moreover, thalassemia type, frequency of blood transfusion per month and duration between the vaccination and serology did not have significant effect on antibody response.
Conclusions: Despite this fact in the literature that hepatitis B vaccination is effective in raising antibody level in general population, this study almost confirm that it cannot be effective in thalassemic patients. It seems that for these patients routine antibody screening must be called every 4–5 years and accordingly advice booster dose or extra three doses.

R2797 Antibody response to hepatitis B vaccination in health care workers

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Objectives: Hepatitis B is an important cause of acute and chronic hepatitis, cirrhosis and hepatocellular carcinoma. This disease is 10 times more prevalent among health care workers. At present, vaccination is the most effective and cost saving means of prevention of hepatitis B virus infection. The main aim of this study was determining hepatitis B surface antibody (HBsAb) level following vaccination and examining the relationship between HBsAb level with age, sex, body mass index, Anti-HBV titre, time elapsed since last dose of vaccination and vaccine combination.
Methods: This cross sectional study was performed among 165 health care workers in a main general hospital in south of Iran, who had a full course of vaccination against hepatitis B virus. Data on Anti-HBV titer, combined vaccination history, body mass index and time elapsed since last dose of vaccination were collected retrospectively by reviewing their medical records. Antibody levels after vaccination were measured by an enzyme-linked immunoadsorbent assay (ELISA) method and the results expressed in mIU/mL. levels <10 mIU/mL were considered negative, 10–100 mIU/mL relative responses and more than 100 mIU/mL full responses.
Results: According to the results, 92% (152 individuals) of the health workers showed a complete immune response, 8% (13 individuals) had partial immunity. The response rate did not differ appreciably whichever combination of vaccine was given. There was no significant relationship between Anti-HBV titer, age, sex, body mass index and duration between the vaccinations with antibody level.
Conclusions: The results confirm that hepatitis B vaccination is effective in raising antibody level in health care worker. Given the relatively high cost in both time and labour that vaccination programmes entail, routine antibody screening after vaccination must be called into question except perhaps for those who are at highest risk, such as those who work in renal or drug addiction units.

R2798 Vaccination rates among adult cancer patients at a comprehensive cancer centre

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Adult patients with cancer are at a high risk for pneumonia from both influenza and pneumococcal infections. The risk increases in patients that are still smoking. Mortality rates from pneumonia can be seen as high as 33%. The CDC recommends all individuals at high risk including immunocompromised patients to receive both vaccines.
Objective: Our primary objective was to determine our influenza and pneumococcal vaccination rates in adult solid tumor cancer patients diagnosed with pneumonia in the Emergency Center (EC) at a comprehensive cancer center.
Methods: A retrospective chart review was completed over a 5 month period in 2010.
Results: A total of 157 solid tumor cancer patients [median age: 61 years (range 18–91), 87 males (55%)] were included. Influenza vaccines were screened in 123 patients (78%). This is an improvement from 65% in 2008. Nurses screened 118 patients while physicians screened 22. There were 42 patients that received the vaccine prior to their EC visit. Seventy-four patients qualified to receive vaccines upon EC discharge. None of these patients received vaccines or a recommendation if they were admitted. Four patients received vaccination prior to discharge from their primary team. Pneumococcal vaccines were screened in 112 (71%) patients out of the 157. This number is slightly up from 67% in 2008. Nurses screened more often than physicians, 107 vs. 21 patients. Thirty-three patients received pneumococcal vaccines prior to their EC visit. Again, no recommendations were made for vaccination and none were given prior to EC discharge. Fifty-seven (36%) patients were 65 years old or older. Of these patients, 40 patients were screened. Sixteen (28%) patients received vaccines prior to their EC visit. No recommendations or vaccines were given in the EC. Of the 157 patients that were diagnosed with pneumonia only 37% were screened for smoking. Twenty-one of the patients were considered active smokers. Four of the active smokers were screened and one recommended to be seen by the Tobacco Smoking Cessation Program.
Conclusion: More education is needed to all healthcare providers associated with cancer patients about the importance of influenza and

pneumococcal vaccines. A new nurse driven program is needed to ensure that our patients receive the appropriate vaccines prior to discharge from the clinic, EC and/or hospital.

R2799 Astragalus adscendens extract is a novel adjuvant increasing the immune responses in mice compared to Quil A and Allum

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Objectives: Astragalus adscendens is a perennial plant of family astragalus that its polysaccharide extract is usually used as a popular sweetie in Iran for years called GAS ANGABIN. Several studies demonstrated that Astragalus membraneous extracts could be safely used as an adjuvant with low or non-haemolytic effect. As there is no study assessing such potential ability in A. Adscendens the current study was designed to evaluate the hemolytic and adjuvant activities of this plant in mice.
Materials and Methods: Four groups of ICR mice were subcutaneously immunized with OVA 100 μg alone or OVA and Astragalus extract (ASE as a new adjuvant), QuilA and Allum on Day 1 and 15. Two weeks later (Day 28), concanavalin A (Con A)-, lipopolysaccharide (LPS)- and OVA-stimulated splenocyte proliferation and OVA-specific antibodies in serum were measured. Haemolytic activities of ASE was evaluated using 0.5% rabbit red blood together with its adjuvant potentials on the cellular and humoral immune responses at both 100 and 200 μg/mL doses.
Results: ASE showed no haemolytic effect, at the concentration of 100 and 200 μg/mL. ASE significantly enhanced the Con A-, LPS-, and OVA-induced splenocyte proliferation in the OVA-immunized mice at both doses of 100 and 200 μg. The IgG total and IgG sub-class responses in the serum of mice were significantly higher using ASE as adjuvant compared with QuilA, Allum and control group.
Conclusion: This study demonstrated that ASE has a considerable adjuvant activity with non-haemolytic effect at both 100 and 200 μg/mL doses superior to Allum and QuilA.

Internet and electronic resources

R2800 Management of infective endocarditis: an innovative multidisciplinary pathway to manage a complex disease – clinical experience from a Lancashire cardiac centre

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Infective endocarditis (IE) is a complex disease with poor prognosis and high mortality. Cardiologists, cardiac surgeons and the microbiologists from Lancashire Cardiac Centre collaborated and reviewed the practical limitations of identification and referral of IE and the available guidelines to set up an IE care-pathway (Endocarditis Pathway-EP). We present data and clinical experience from the use of EP over a 12-month pilot.
Methods: Analysis of database (55 patients). Patients with suspected IE are e-logged (intranet based registration of patients on EP) and paper copy is added to the notes and completed during patients' hospital stay.
Results: Mean age: Fifty-eight years; 67% Males; Mean weight 72 kg; 95% Caucasian. Forty patients had an initial diagnosis by echocardiogram of IE (transthoracic: 38; transesophageal: 14); 62% (34/55) with left-sided IE; 4% (2/55) right-sided and 7%(4/55) both sided IE; indeterminate 20% (11/55). Valve involvement: aortic 18; mitral 9; aortic+mitral 7; mitral+tricuspid 4; tricuspid 2. Echocardiography was not recorded for three patients. Thirty-nine patients had positive blood cultures. IE specific isolates: Meticillin-sensitive Staphylococcus aureus (MSSA), 13; *Streptococcus* spp., 11; *Enterococcus* spp., 6; HACEK group, 3; S. epidermidis, 3; meticillin-resistant *S. aureus*, 2; MSSA + Strep, 1. Two patients were also culture-positive for *Candida albicans*. Thirty-three patients underwent surgical procedures. Antibiotics: gentamicin in combination 35%; rifampicin in combination 32%; flucloxacillin 35%; daptomycin 27%; benzyl-penicillin 23%; vancomycin 15%. Six patients required continuous veno-venous haemodialysis (CVVHD). Outcome was cured or improved in 42 patients (76%). Mortality was 23% (13/55), of which 10 patients died from complications or conditions other than IE.
Conclusions: The EP was piloted at the Lancashire Cardiac Centre with plans to extend it for consultation of the regional cardiac network with an aim to standardise care throughout the region. The multidisciplinary input from cardiologist, cardiac surgeon and microbiologist prevents delay and optimises management. Results and clinical experience from this pilot have been used to inform revised user-friendly version of the EP to be available on the new trust electronic patient record system. Details to be presented.

R2801 Colony Morphology Ontology (CMO): a standard nomenclature for the classification of human pathogenic bacteria colony morphology

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Phenotypic and genetic variations are the most important mechanisms adopted by microorganisms to survive adverse stress conditions. Bacteria persistence is a noteworthy downside in clinical settings (even after disinfection and antibiotic procedures) and studies have pointed out that colony morphology variations are a key factor motivating pathogenicity, virulence and resistance of infection-causing microorganisms.
Ongoing studies on colony morphology switching aim to apply colony observation mechanisms to medical diagnose and clinical decision making. Colony observation approaches share a common principle, but they lack standard laboratorial guidelines and consensual criteria to identify the morphological characteristics of the colonies. Clearly, the comparison of colony morphologies is only possible under similar experimental conditions and nomenclature standardisation is recommended to avoid ambiguity or misinterpretation. Particularly, visually observed phenotypes, which cannot be directly measured quantitatively, are often in the form of free text descriptions plagued by semantic ambiguity (i.e. a single concept has different meanings, though sometimes the differences can be very subtle), heterogeneity (i.e. the same visually observed phenotype is described with different concepts), and low granularity (i.e. the level of detail used in concept definition). Standardisation efforts are thus needed to ensure the understanding of the annotations and large-scale phenotype comparison in real-time clinical decision making.
Till date, no ontology covers for the annotation of colony morphologies. This work aims to fill in this gap, proposing the Colony Morphology Ontology (CMO). CMO considers the following top annotation classes: microorganisms (e.g. identification, source), culture conditions (e.g. culture type, growth mode), plating conditions (e.g. media, incubation time), stress conditions (e.g. physical, chemical stressors), susceptibility profiles (e.g. antibiotics, disinfectants) and morphological characterisation (e.g. shape, size, colour). The ability to search for and compare similar phenotypic appearances within and across species is believed to have vast potential in biofilm-associated infection research. Therefore, CMO is made publicly available at http://193.137.90.5/morphocol/. In the future, we envision the development of a framework suitable for the modelling and analysis of the computable representation of biofilm phenotypic signatures.

R2802 **BiofOmics: a database on high-throughput biofilm susceptibility and resistance tests**

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Biofilms are structured communities of bacteria that are able to survive virtually everywhere in Nature because of their ability to adhere to a surface and embed in a protecting, self-produced matrix of extracellular polymeric substances. Due to their persistence and resistance to antimicrobials, including clinically relevant antibiotics, biofilm-associated infections are at the basis of a range of problems in clinical settings. Much research has been devoted to the understanding of biofilm-growing bacteria susceptibility and resistance in the last decades.

Similarly to what has happen in other research domains, biofilm research has benefited from technological evolution. The development of high-throughput biofilm-forming devices (e.g. the 96-well microtiter plate and the calgary device) has enabled the simultaneous testing of large sets of conditions. The implementation of automated spectrophotometry and microscopy systems allow addressing different features of biofilms in a large scale. Furthermore, the so-called ''omics'' platforms are enabling the disclosure of the transcriptome proteome and metabolome of biofilms.

As biofilm research grows to be a data-intensive discipline, the need for suitable bioinformatics approaches becomes compelling. The large amount of results being generated claims for proper means of data organization, normalisation and storage. Also, in order to convert the large sets of data into valuable and interchangeable knowledge, researchers are in need of analytical tools, capable of dealing with the ever growing size of the data as well as supporting advanced statistics and, eventually, data mining. However, our analysis of current procedures in biofilm research shows that no real effort has been made to introduce bioinformatics into the community.

The purpose of this work is thus to describe a novel approach to the large-scale, standardized and systematic documentation of published evaluations on biofilm-growing bacteria susceptibility and resistance. Specifically, we establish the basic requirements in terms of data normalisation and describe a systematic and computer-amenable approach to data organisation. The approach has been validated using our laboratories' experiments and it is already in practice, supporting the operation of the BiofOmics database (publicly accessible at http://biofomics.org). This new resource provides the means for large-scale study comparison and interchange, promoting inter-laboratory collaborations.