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ratio and CAS at 0.015 mg/l was noted against PL cells ($82.2 \pm 6.5\%$ vs $43.6 \pm 3\%$, $p < 0.001$ and $82.2 \pm 6.5\%$ vs $62.8 \pm 11\%$, $p < 0.05$), no significant collaboration between MNCs and CAS was observed against BF at any MNC/target ratio and any CAS concentration used.

Conclusions: *C. albicans* BF are more resistant than PL cells of CA to MNCs, to CAS and to the combination of MNCs with CAS. While MNCs and CAS exhibit an additive effect against PL, no significant collaboration between MNCs and CAS exists against BF. The mechanism(s) behind resistance of CA BF to host defences need to be determined.

P627 Effect of disinfectants and caspofungin against planktonic and sessile cells of *Candida* spp.

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Objectives: The aim of this study was to evaluate the activity of 3 different disinfectants chlorhexidine digluconate, Akacid plus[®] and hydrogen peroxide compared to the antifungal caspofungin against planktonic and sessile cells of *Candida* spp.

Methods: As test strains 40 clinical isolates of *Candida* spp. including *C. albicans*, *C. krusei* and *C. tropicalis* were used. The activity of active substances was determined against planktonic cells according to CLSI guidelines for antifungals using broth microdilution method. For antifungal susceptibility testing of sessile cells, isolates were incubated overnight in yeast peptone dextrose resuspended in RPMI 1640 to a cellular density equivalent to 1.0×10^6 CFU/mL. Cells were grown for 48 h in 96-well-microtiter plates, and then treated with 100 μ l of Akacid plus[®], chlorhexidine and hydrogen peroxide at a final concentration of 0.25, 0.5, 1, 2 and 4% compared to caspofungin at a concentration of 64, 128, 256 and 512 mg/l for 48°C at 35°C. The cells were fixed and stained with crystal violet. The mean optical density was used for quantification using a routine microtiter-plate-reader at 490 nm. Additionally, fungal growth following antimicrobial treatment was examined.

Results: MICs of Akacid plus[®] and caspofungin against planktonic cells of *Candida* spp. were comparable and reached MIC values of 0.03–8 mg/l, whereas MICs of chlorhexidine and hydrogen peroxide ranged from 16 to 32 mg/l and from 128 to 256 mg/l. Low concentrations of caspofungin at 64 mg/l caused a 62% reduction of the sessile cells of *Candida* spp. Treatment with 0.25% chlorhexidine and Akacid plus[®] led to reduction of the sessile cells in 59 and 74%, whereas hydrogen peroxide showed no effect. No viable cells of *Candida* spp. were detected in biofilms treated with 0.25% Akacid plus[®] and chlorhexidine or 0.1% caspofungin.

Conclusion: Caspofungin and cationic antimicrobials showed high activity against sessile and planktonic cells of *Candida* spp., whereas hydrogen peroxide was found to be ineffective.

P628 Response of *Candida albicans* biofilms to bacterial factors

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Introduction: Candidoses affecting immunocompromised patients are likely to be associated with certain commensal bacteria attracted by the inflammatory microenvironment. Although the precise outcome of such a cohabitation is not known, a plausible scenario would credit bacteria for influencing the survival of *Candida* spp. To gain more insights into this aspect of fungal infection, we are looking at the response of *Candida* biofilms to colonisation by bacteria normally present in the human microflora.

Methods: *Candida albicans* isolated from an immunocompromised patient were grown in collagen gel to produce multiple biofilms. Conditioned media from *Pseudomonas aeruginosa* (CMPa), *Staphylococcus aureus* (CMSa) and *Lactobacillus casei* (CMLc) were filtrated and freeze-dried as stock solutions. After 5 hrs in collagen gel, nascent *Candida* biofilms were treated once with the several conditioned media for approximately 10 hrs. Relevant morphological parameters were detected and cell survival/gel was determined by the CFU test.

Results: The value of CFU in the control group was 1×10^7 cells/mL or above. Conditioned media affected differently the survival of *C. albicans*. Specifically, 90% of *Candida* cells died out following treatment with CMPa. CMLc was somewhat less effective in that approximately 50% of the cells survived the treatment. However, CMSa did not damage *Candida* cells, that displayed a value of CFU comparable to that of the control group. CMPa implemented filamentation of *Candida* biofilms, resulting in what it appeared a selective inhibition of yeast forms. CMLc and CMSa did not interfere with this process.

Conclusions: The present results indicate that *P. aeruginosa* releases factors capable of inhibiting *Candida* biofilms in collagen gel. While the content of CMPa was not analysed, most reports agree that aryl homoserine lactones, quorum sensing (QS) factors regulating the homeostasis of *P. aeruginosa* biofilms, are mainly responsible for the biological activity of this Gram-negative bacteria. This suggests that certain bacterial QS factors could help to keep under control fungal biofilms, an observation worthy of further investigation. The finding that *Lactobacillus casei*, a probiotic, can inhibit the growth of *Candida albicans* has clinical relevance, in that the association of probiotics with existing antimycotic drugs may provide a most effective procedure to cure candidoses. (Supported by Pfizer Italia).

P629 In vitro activity of essential oils and their major components against *Candida albicans* yeasts growing planktonically and as biofilms

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Objectives: Candidiasis can be associated with the formation of biofilms on bioprosthetic surfaces and the intrinsic resistance of *C. albicans* biofilms to the most commonly used antifungal agents has been demonstrated. In this study, we report on the antifungal activity of 13 terpenes and essential oils on *C. albicans* growing planktonically or as biofilms.

Methods: The strain ATCC 3153 of *C. albicans* was used. Nine terpenes (carvacrol, citral, eucalyptol, eugenol, farnesol, geraniol, linalool, menthol and thymol) and 4 essential oils (tea tree, palmarosa, oregano and rosemary) were tested. The anti-biofilm activity of the tested compounds was evaluated using an in vitro model of *C. albicans* biofilm associated with polystyrene surfaces and the metabolic activity of yeasts within the biofilm was assessed with XTT method.

Results: The majority of the tested compounds showed a significant antifungal activity (MIC < 0.4 mg/mL). Two essential oils exhibited an “intermediate” antifungal activity – tea tree (MIC = 2.25 mg/mL) and rosemary (MIC = 1.10 mg/mL) – and two terpenes (farnesol and eucalyptol) were not efficient against planktonic *C. albicans* (MIC > 74 mg/mL).

Citral, eugenol, palmarosa and rosemary induced a significant inhibition of the metabolic activity of the yeasts included in the *C. albicans* biofilm ($p < 0.001$) when added at a concentration < 2.25 mg/mL during the early step of the fungal biofilm growth. The concentration needed for carvacrol, geraniol, linalool, oregano and thymol to achieve a significant reduction of the biofilm development was < 5 mg/mL. The higher efficient concentrations were obtained for farnesol and menthol and corresponded to 35.5 mg/mL and 17.8 mg/mL respectively.

Conclusion: This study demonstrated the efficiency of almost all the tested terpenes and essential oils to inhibit the biofilm growth which could therefore represent good candidates in the prevention of biofilms associated with implanted medical devices.

P630 *Aspergillus fumigatus* biofilms are refractory to antifungal challenge

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Background: *Aspergillus fumigatus* may cause infections in immunocompromised patients including patients with cystic fibrosis. *A. fumigatus* conidia are readily inhaled and are not cleared by the innate immune system. Respiratory infections are typified by the presence of

dense filamentous networks of hyphae in the pulmonary cavity and airways. These fungal mucus plugs are inherently difficult to treat, and have characteristics which resemble biofilms.

Objectives: To develop an in vitro *A. fumigatus* biofilm model to assess the ability of antifungal agents to inhibit and/or kill these structures.

Methods: Spores were collected from AF293 and standardised at various densities in RPMI medium and grown in 96-well polystyrene plates. Biofilm growth kinetics were then observed over 48 h (1, 2, 4, 6, 24 and 48 h) microscopically and by both metabolic (XTT) and a biomass (crystal violet) assays. Developing biofilms were visualised using confocal laser scanning microscopy (CLSM) and electron scanning microscopy (SEM). The specific effects of voriconazole (0.125–256 µg/mL) was also examined to measure its effectiveness pre- and post challenge.

Results: Optimal spore concentration for confluent biofilms after 24 h was determined to be 1×10^6 spores/mL. The initial biofilm growth kinetics involved an adherence stage (0–4 h); development of a monolayer of cells (4–8 h); and formation of a three dimensional biofilm structure after 24 h. Voriconazole and caspofungin were ineffective against mature biofilms. Amphotericin B was effective between 0.5 – 1.0 µg/mL, however 90% killing was never achieved. When voriconazole was added at the initial stages of adhesion, a dose dependant effects was observed at therapeutic concentrations.

Conclusions: We have developed a robust reproducible in vitro *A. fumigatus* biofilm model was developed. Early exposure of spores to voriconazole prevented filamentation and biofilm formation. Overall voriconazole potentially offers excellent prophylactic properties against invasive aspergillosis.

Viral infections in the immunocompromised host

P631 Community respiratory virus infections in patients with haematological malignancies

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Objectives: Viral infections are the important cause of morbidity and mortality in immunocompromised patients (pts) with haematological malignancies. With regard to these agents the focus has been on herpes viruses, particularly human Cytomegalovirus (CMV). The studying of last decade showed that community respiratory viruses also play the important role in serious respiratory illnesses in pts with haemoblastosis. The current study was designed to determine the frequency and clinical features of respiratory viral infections in pts with different forms leukaemia.

Methods: Our study included 91 pts with different forms haemoblastosis. The patients were studied during the episodes of respiratory illnesses. Material – blood and nasal swabs were studied by means of polymerase chain reaction (PCR) with primers to the batteries of viral genomes: Adenoviruses, Respiratory syncytial virus (RSV), Influenza type A and B, Parainfluenza type 3 and Coronaviruses.

Results: The signal of Adenoviruses was detected in nasal swabs in 12 (13.1%) cases of respiratory illnesses, RSV in five (5.5%) cases, Influenza A in five (5.5%) cases, Influenza B in two (2.2%), Parainfluenza type 3 in eight (8/8%) and Coronaviruses in 13 (14.3%) cases. In blood the signal of Adenoviruses was detected in 5.4% cases. It is interesting that in all these cases the signal in nasal swab was not founded. In one case Influenza B virus was founded simultaneously in blood and nasal swabs. The main clinical symptoms of respiratory illnesses were the chill (87.5%), fever (87.5%), mostly higher than 38°C, lymphocytosis – 43.7%. In the 50% patients respiratory illnesses were complicated by pneumonia. We did not find any clinical peculiarities of the community respiratory illnesses of different viral etiology.

Conclusions: The community respiratory viral infections are serious illnesses in pts with haemoblastosis. These infections must be controlled as well as CMV and other herpes infections. PCR is adequate method of monitoring viral infection in this group of patients.

P632 Viral infection is responsible for acute renal dysfunction and chronic allograft lesions in paediatric renal-transplant recipients: a prospective study

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Objectives: Follow-up evaluation of paediatric renal-transplant recipients in order to assess the contribution of viral infection to acute and chronic nephropathy and allograft rejection.

Methods: The presence of viral DNA (i.e., EBV, CMV, HHV6, HHV7, HHV8, VZV, BKV, JCV, SV40 and parvovirus B19) and viral load was prospectively investigated by quantitative real-time PCR methods in the peripheral blood, urine, and in allograft biopsies obtained from a series of 77 consecutive children (31F, 46 M, R/D mean age: $11.9 \pm 7.6/11.9 \pm 5.3$ years) undergoing kidney transplantation in the period 2000–2004. Immunosuppressive therapy included basiliximab, steroids, FK506 or cyclosporinA ± mycophenolate mofetil. Follow-up allograft biopsies were performed at the time of transplantation and at 6, 12, and 24 months post-transplantation; diagnostic biopsies were performed in 11 patients because of acute renal dysfunction. Virological findings were compared with histological analysis according to Banff 97 criteria.

Results: At the time of transplantation, the allografts were positive for parvovirus B19 in 33% of cases, HHV6 23%, BKV 5%, SV40 3%. The cumulative incidence of chronic lesions was 29%, 52%, and 83% at 6, 12, and 24 months post-transplantation, whereas the cumulative incidence of viral DNA detection in biopsies was 63%, 69% and 71%, respectively (coinfections in 25%, 22%, 24%, respectively; the most frequent: EBV, HHV6, BKV, B19). The prevalence of viral genomes was higher in biopsies showing acute (Banff III, IV) or chronic (Banff V) lesions than in normal histology cases, but viral infection or histological damage did not correlate with renal function tests. Moreover, children who developed chronic lesions generally had early and persistent kidney infection (especially from BKV, B19, EBV). Viral-genomes were isolated in 7/11 biopsies performed for acute renal dysfunction: 2 tubulo-interstitial nephropathy (BKV), 3 thrombotic microangiopathy and 1 acute vascular rejection (parvovirus B19), and 2 acute rejection (EBV). A correlation between virological findings in biopsies and viral DNA detection in blood and urine was observed, although the rate of positive tests was higher in biopsies. Evaluation of test predictivity is ongoing in a long-term follow-up study.

Conclusions: Viruses not only are responsible for acute renal dysfunction in kidney transplanted children, but also contribute to the development of chronic allograft lesions due to persistent infection.

P633 Whole blood real-time PCR for cytomegalovirus DNA quantification: analysis of PCR data and pp65 antigen test in a cohort of solid-organ transplant recipients

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Cytomegalovirus (CMV) is a major opportunistic agent in solid organ transplantation (SOT). Pre-emptive therapy and a strict infection monitoring with highly sensitive methods have significantly decreased CMV morbidity and mortality. Recently introduced real-time PCR tests for routine CMV DNA quantification require correlation studies with pp65-antigen test as the gold standard. Moreover, there is no consensus as the most appropriate blood compartment (i.e. whole blood, leukocytes, plasma) for PCR test.

Aims: 1) to study the correlation between pp65-antigen test and CMV DNA as quantified by real-time PCR in whole blood (WB) in a cohort of SOT recipients and 2) the identify a CMV DNA cut-off level for pre-emptive anti-CMV therapy.

Methods: WB samples (n=397) from 41 asymptotically infected patients (18/41 undergoing pre-emptive therapy with ganciclovir) were monitored the first year after SOT by pp65 antigen test and real-time PCR for the UL123 gene, IE1 exon 4 (Nanogen, I). Extraction was