mycoses
Diagnosis, Therapy and Prophylaxis of Fungal Diseases

Abstracts

Poster Presentations

P001

Management of aflatoxine producing fungi in peanut (*Arachis hypogeae*) varieties in central tigray through soil solarization and planting time

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Objectives Peanut is invaded before harvest mainly by *Aspergillus flavus* and *A. parasitics*, which are potential aflatoxin producers. However, no research efforts have been directed to develop suitable management options against aflatoxigenic fungi in Northern Ethiopia. Therefore, this study was undertaken to determine the effect of soil solarization on *A. flavus* inocculum in the soil and to evaluate the effect of soil solarization and planting time on peanut seed invasion by Aspergillus spp. and yield of peanut varieties.
Methods Soil samples were taken from 5 and 10 cm soil depths in three rounds and analyzed for aflatoxigenic population. Soil solarization reduced fungal inoculum and increased groundnut yields. Individual and total cfu g^{-1} of soil was determined before, after solarization and at harvest. Four *Aspergillus species* namely, *A. flavus, A. parasiticus, A. niger* and *A. terreus* were plated on Rose Bengal Agar media (selective medium for Aspergillus spp.) and morphologically identified. To determine the natural seed infection by Aspergillus spp. and other fungi, undamaged pods from the middle two rows were carefully hand-shelled and 100-seed of each plot were surface sterilized by soaking in 5% aqueous solution of sodium hypochlorite (NaOCl) for 3 minutes and immediately rinsed with sterile distilled water, and plated on Czapek-Dox agar medium and incubated at room temperature. After 7 days the growing colonies of fungi were visually recorded. Finally, fungi identification was carried out based on macro- morphological (reverse and surface coloration of colonies, presence of pigment, and colony texture) and micro- morphological characteristics (conidia size, conidial head, shape of vesicle). Standard text (Klich, 2002) was used in the identification process.
Results Their densities were significantly ($P < 0.05$) reduced at after solarization. In the solarized plots, *A. flavus* and *A. parasiticus* were found reduced by 53.8 and 45% cfu g^{-1} at Ramma and 36.4 and 44% cfu g^{-1} at 5 and 10 cm soil depths at Mayweyni, respectively, after soil solarization in the solarized plots than the nonsolarized plots. At harvest, *Fusarium* spp., *A. flavus* and *A. terreus* were detected. Pod yields were found increased by 265.6 and 182.22 kg ha^{-1} on solarized plots at Mayweyni and Ramma, respectively. Increase in yield related parameters (14.8% increase in number of seed per plant and 7.4% increase in number of pods per plant) were found from early planting dates as compared to later planting time at Mayweyni.
Conclusions Generally, yields varied across locations; mean pod yield in Mayweyni was 360.9 kg ha^{-1}higher than the yield in Ramma. Three *Aspergillus* species namely, *A. flavus*, *A. niger*, and *A. parasiticus* were isolated from seed samples plated on Czapek-Dox Agar medium. Early planting of the varieties showed the lowest level of seed infection by *A. flavus* (22.8%). Four Aspergillus species namely, *A. flavus, A. parasiticus, A. niger* and *A. terreus* were isolated before soil solarization and after solarization. Both the total propagule density and individual fungi cfu g^{-1} were counted and *A. niger* was found as the most dominant.

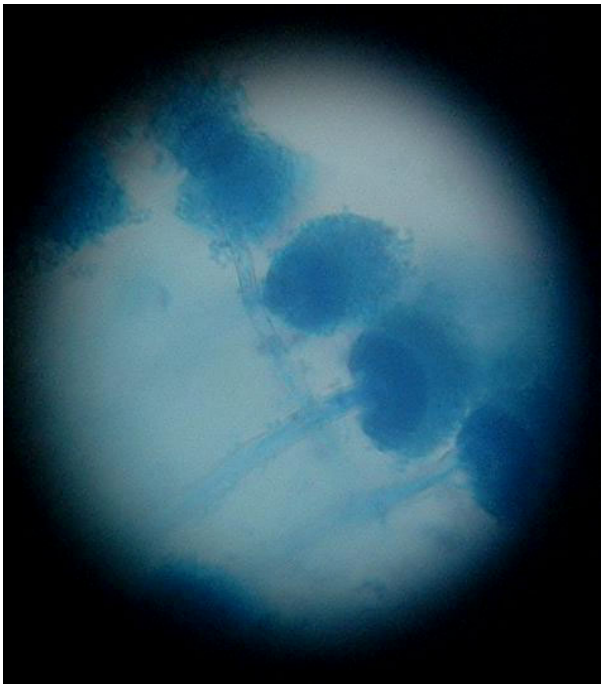

Figure 1 Structure of *Aspergillus* spp.

P002

An amino acid substitution (G470R) in lanosterol 14-alpha demethylase (Erg11p) is critical for fluconazole resistance in a case of recurrent cryptococcosis

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Objective *Cryptococcus neoformans* is one of the most frequent cause of meningitis in AIDS patients from Asia and Subsaharian-Africa. After initial treatment with AmphotericinB, with or without flucytosine, lifelong maintenance with fluconazole is recommended to prevent recurrence. Although persistence of the original infecting organism is assumed to occur in cryptococcal meningitis, it is possible that antifungal therapy eradicates the initial infection and that recurrences are due to infection with a new strain. Moreover, the use of fluconazole as maintenance therapy has increased the number of azole resistant *C. neoformans* strains. However, the underlying azole resistance mechanisms have not been widely described. In this study we have characterized six *C. neoformans* strains from a patient with recurrent cryptococcosis to elucidate possible mechanisms involved in the development of fluconazole resistance.
Methods Six strains of *C. neoformans* were isolated from an AIDS patient with recurrent cryptococcosis. The first episode of cryptococcosis was detected in February 1994. During the following two years, five more episodes of cryptococcosis were proven by cultures from cerebrospinal fluid and blood samples. The six isolates were identified by agglutination test and the amplification and sequencing of the ITS and IGS-1 regions from the rDNA. Mating-type analysis and genotyping studies by RAPD analysis were also performed. Susceptibility testing was performed by E-test methodology. The entire sterol 14-α demethylase gene (*ERG11*) was amplified and sequenced. Since the *ERG11* sequences from serotype A and D are quite different, a 700 bp PCR fragment of the distal *ERG11* (nucleotide 1,563 to 2,274), from DNA of each of the six strains, was cloned and the sequences from several clones within each DNA were analyzed.

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Results All the isolates were recovered from cerebrospinal fluid excepting for the last one which was recovered from blood culture. Five out of six strains were identified as aAαD hybrids (CNG1, CNG3, CNG4, CNG5 and CNG6) and the second one (CNG2) as αA. A clonal origin was demonstrated for all of the aAαD strains except for the isolate recovered from blood (CNG6). Nucleotide sequence analysis of the *ERG11* gene detected in all aAαD strains a point mutation responsible for the amino acid substitution (G470R) in the encoded protein. For all the strains showing the same genotype, the mutation was heterozygous in strains CNG1 and CNG3 and homozygous in strains CNG4 and CNG5. The G470R amino acid substitution in homozygosis is responsible for an increase in the fluconazole MICs (from 16 μg/ml to 256 μg/ml). The strain recovered from blood (CNG6) shown to be more susceptible to fluconazole (MIC = 8 μg/ml) although it was also heterozygous for the point mutation.
Conclusions (i) Recurrent episodes of cryptococcosis could be due to both, the same strain or the infection with a new strain; (ii) the G470R amino acid substitution in the Erg11p could be involved in the development of fluconazole resistance in some of the strains aAαD recovered from the case patient; (iii) more studies to prove the relevance of this mutation in the *C. neoformans* azole resistance in the clinical setting are needed.

P003

Antifungal activity of echinocandins against invasive isolates of *Candida* spp.

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Objectives The echinocandins anidulafungin (ANF), caspofungin (CSF), micafungin (MCF) represent a new antifungal group with potent activity against *Candida* species. As non-competitive inhibitors of beta-1,3-glucan synthase they have distinct mechanisms of action and target the fungal cell wall and thus present an alternative for the tratment of candidiasis. A commercially prepared dried colorimetric microdilution panel (Sensititre Yeast One, TREK Diagnostic Systems, Cleveland, OH, USA) is able to determine the susceptibility of echinocandins and demonstrated excellent corelation with the reference method M27-A3 from the Clinical Laboratory Standards Institute (Pfaller 2012). The aim of this study is to evaluate the *in vitro* activity of ANF, CSF and MCF against clinically significant *Candida* strains by Yeast One colorimetric microdilution method.
Methods A total of 77 *Candida* strains were isolated from bloodcultures (n = 34), cultures of urine samples of symptomatic patients (n = 33), bronchoalveolar lavage (n = 1) and sputum from pediatric patients with cystic fibrosis (n = 4), abscess (n = 3) and eusophageal biopsy (n = 2) samples. Isolated strains were checked of purity and were identified by classical morphological tests including germ tube formation in human serum at 37°C for 3 h, blastoconidia, pseudohyphae, true hyphae and chlamydoconidia formation on corn meal agar-Tween 80, integrating with the results of chromogenic agar (CHROMagar, HiMedia, India) and of API 20C AUX kit (Bio Mérieux, France). Identification kit procedure and colorimetric microdilution tests were performed according to the manufacturer's instructions. Quality control was ensured by testing CLSI-recommended strains *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258. Recently described species-specific clinical breakpoint (CBP) values were used to categorize the minimum inhibitory concentrations (MICs) of echinocandins as susceptible (S), intermediate (I) and resistant (R) against the strains of *Candida*.
Results Among 77 *Candida* isolates, 39 were *C. albicans*, 2 were *C. glabrata*, 28 were *C. parapsilosis*, six were *C. tropicalis* and one of each was *C. kefyr and C. krusei*. Regarding to the MIC values obtained, resistance to ANF was observed in one *C. albicans* and two *C. tropicalis* strains; resistance to CSF was observed in one *C. albicans* an done *C. parapsilosis* strains; and resistance to MCF was observed in one *C. albicans* and one *C. tropicalis* strains. Activity of CSF was found as I against one *C. albicans* and two *C. tropicalis* strains, and of MCF was found as I against two *C. parapsilosis* strains. Resistance to all three echinocandins were observed only in one *C. albicans* strain which showed multidrug resistance including to azoles and amphotericin B. The remaining strains were *in vitro* susceptible to all echinocandins tested.
Conclusion Based on newly established breakpoints for species-specific interpretive criteria, the results of this study affirmed that echinocandins exhibit excellent activity against the *Candida* species most frequently involved in human infections.

P004

Aspergillus terreus and amphotericin B resistance: correlation of *in vivo* and *in vitro* – susceptible versus resistant strains in a hematogenic mouse-model

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Objective Amphotericin B (AMB) is one of the most commonly used antimycotics to treat fungal infections in humans. It is active against most pathogenic fungi and used to be the antas in therapy for critically ill patients with invasive mould infections (IMI) and invasive aspergillosis (IA). IMI and IA have become increasingly common among immunocompromised or immunosuppressed patients including solid-organ or haematopoietic stem-cell transplant recipients and individuals who are on immunosuppressive drug regimens. The majority of IA is caused by *Aspergillus fumigatus* followed by *Aspergillus terreus* (*A. terreus*) and *Aspergillus flavus* (*A. flavus*). In particular, *A. terreus*, a widespread soil saprophyte and producer of several secondary metabolites, is a common cause of infection at the UniversityHospital of Innsbruck (UHI), Austria. In vivo and in vitro data indicate that AMB resistance and emergence of resistance during AMB therapy is rare but almost all *A. terreus* isolates are intrinsically resistant to AMB, and a high mortality rate is associated with this particular mould. It is unclear whether low AMB-MICs (< 0.20 μg/ml) reflect clinical response and thus correlate with the *in vivo* situation; no data are available on strains with AMB-MIC < 0.20 μg/ml. For this study we compared AMB resistant *A. terreus* strains (ATR; AMB-MIC ≥ 4 μg/ml) and a rare *A. terreus* variant showing enhanced susceptibility (ATS; AMB-MIC ≤ 0.20 μg/ml) in response to AMB in vivo. ATS and ATR were susceptible to other antifungal agents (e. g. azoles).
Methods Inbred BALB/c mice were intravenously (i.v.) injected with 200 mg of cyclophosphamide/kg of body weight on day 3 and every 5th day to produce prolonged immunosuppression. All the mice were inoculated with ATR or ATS on day 0 by intravenous injection of 1×10^6 conidia (100 μl of a 10^7-conidia/ml stock solution in 0.9% NaCl) into the tail vein. Mice were intraperitoneally treated with 5 mg/kg Amphotericin B deoxycholate and 4 mg/kg Voriconazole (VORI). Organ fungal load (cfu) was checked via fungal plate count.
Results ATS infection *per se* killed 50% of the mice by day 3 and was significantly more lethal than ATR ($P < 0.05$). At day 18, all mice infected with the ATS strain died, whereas 17% of the animals infected with ATR who received no treatment survived. AMB treatment improved survival in ATS infection up to 75%, while this was not the case with ATR infection ($P < 0.05$). Survival outcome of ATS infected mice with AMB treatment was comparable with VORI therapy. VORI therapy in ATR infected mice significantly enhanced survival compared to AMB. Survival was independent of fungal organ load and showed significant differences between the AMB and VORI groups.
Conclusion Our in vivo murine model of disseminated aspergillosis showed that ATS was highly virulent, indicating that loss of fungal fitness is not associated with the appearance of AMB susceptibility. AMB therapy enhanced survival outcome of ATS infected mice. AMB and VORI treatment rescued ATR infected mice, yet independent of the fungal organ load.

P005

Feces of *Nymphicus hollandicus* (cockatiels): emergence and dissemination of azoles resistant yeast?

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Objectives To evaluate the occurrence and distribution of yeasts in feces of cockatiels and to know their antifungal susceptibility profile.
Methods In the period from November 2011 to April 2012, feces samples from39 cockatiels (captive birds), were cultured for fungi. All the mycological analyses occurred at the Microbiology Laboratory from Medical Schoolof São José do Rio Preto (FAMERP). Biochemical and commercial tests, including ID 32C (BioMerieux- France) were performed to define the yeast species. The evaluation of the antifungal activities of Fluconazole, Itraconazole, Ketoconazole and Amphotericin B was done according to disk diffusion M44-A2 and microdilution M27-A2 methods (CLSI).
Results Yeasts occurred in 83.33% (N = 30) which were recovered from 36 samples. The most common species were *Trichosporon asahii* (8), *Candida famata* (7), *Candida albicans* (6), *Candida guilliermondii* (6) and *Candida tropicalis* (6), *Kodamaea ohmeri* (4). In addition to this prevalent organism, *Candida membranifaciens*, *Cryptococcus albidus*, *Rhodotorula mucilaginosa*, *Sporobolomyces salmonicolor*, *Trichosporon mucoides* (3), and *Candida lusitanea, Cryptococcus laurentii, Cryptococcus neoformans* and *Rhodotorula glutinis* (1) presented low frequency. Considering species-specific groups, the profile of resistance and dose-dependent susceptibility for Fluconazole and Itraconazole was commonly observed. For the first one, dose-dependent susceptibility and resistance occurred among 5 species (12,5% to 100%) and to Itraconazole, 11 (14% to 100%), respectively. All isolates were susceptible to amphotericin B and Ketoconazol.
Conclusion Organisms isolated and their significance as potential pathogens in birds wereprior discussed.This study, alert about the need of veterinary supervision for preventive measures in cockatiel breeding, emphasizing the zoonotic potentialof resistant pathogenic yeasts. Considering that *Nymphicus hollandicus* is the second most popular pet parrot in the world, new surveillance parameters for domestic birds must be create including microbial monitoring of bird dropping.

P006

Antifungal activity of Iranian honeybee with different botanical sources against *Candida*, *Aspergillus* species and *Trichophyton rubrum*

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Objectives Due to limitation for choice suitable antifungals, search for new antifungals is necessary and stimulates research on new chemotherapeutic agents in natural products. The current study was carried out to assess the antifungal activity of different Iranian honey from different botanical origin against some important fungi, as well as to determine activity against azoles-resistant *Candida* species.
Methods Seven types of honey samples include *Thymus* vulgaris, *Alfalfa*, *Citrus*, *Zizyphus*,*Astragalus*, *Chamaemelum nobile* and multi flora *honey* were collected. *Candida* yeast strains included *Candida albicans, C. dubliniensis, C. parapsilosis*, *C. tropicalis*, *C. glabrata*, *C. Krusei, C. kefyr*, *Aspergillus niger*, *Aspergillus fumigates*, *Aspergillus flavus*, and *Trichophyton rubrum* was used in this study. Clinical *Candida* strains identified and were confirmed by PCR-RFLP. MICs microdilution broth method was used for antifungal activity of honeys based on the CLSI, M27-A2. Stocks and dilutions of honeys and fluconazole (for *Candida* species) were prepared in sterile distilled water. Final drug concentrations in the microdilution plates ranged from 64–0.125 μg/ml for fluconazole and from 80–10% (v/v) for all of the honeys. The microdilution plates were prepared by using the RPMI 1640 broth medium with MOPS & glutamine and without sodium bicarbonate. Yeast suspensions were prepared after vortexing and adjusting to a 0.5 Mc Farland standard transmittance at a wavelength of 530 nm. The final inoculum yielded was of $0.5 \times 10^3 - 2.5 \times 10^3$ cells/ml. MICs were visually determined at 24 h of incubation at 35°C, and were observed for the presence or absence of growth.
Results All of the honey samples had antifungal activity against the tested microorganisms, ranged from 15–60% (v/v).The results revealed variations in the antifungal activity of the different honey samples. *Alfalfa* & *Thymus* honeys were significantly more effective and *Zizyphus* & *Chamaemelum* honeys exhibited lowest antifungal activity. The differences were considerable, the order of activity in terms of overall activity against all; *Alfalfa* > *Thymus* > *Astragalus* > *multifloral* > *Citrus*> *Chamaemelum* > *Zizyphus. Trichophyton rubrum* was the most and *C. krusei* was the least susceptible of the fungal species. Significant differences were determined between susceptibilities of *T. rubrum* and *Candida* species to *Multiflora, Thymus, Alfalfa, Citrus, Zizyphus* and *Chamaemelum* honeys and not for *Astragalus*. No statistically significant differences of susceptibility to honey samples, between fluconazole resistance and susceptible *Candida* species were determined.
Conclusion Honey could be applied in the therapy of fungal infections includes oral, vaginal and cutaneous candidiasis, dermatomycosis and dermatophytosis. On the other hand, the fungal infections have emerged as a major cause of disease and mortality in immunocompromised patients. Accordingly, emergence of fungi resistant to antifungal agents, side effects and limited spectrum has created a need for antifungal agents. With using particular originated honeys, researchers and practitioners could select the honey with defined origin source for different types of fungal infection, superficial mycoses, and resistant *Candida* species and with a high probability of successful results.

P007

P-27530: In vitro evaluation of antifungal activity against a broad spectrum of yeast and filamentous fungi: investigation of the evolution of resistance in *Trichophyton rubrum*

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Objectives (1) To evaluate the antifungal activity of P-27530 against a broad range of yeast and filamentous fungi, by minimum inhibitory concentration (MIC) assay. **(2)** To evaluate the natural resistance frequency and the evolution to resistance of P-27530 in *T. rubrum*, compared to amorolfine, terbinafine, itraconazole and ciclopirox.
Methods (1) Test isolates included 5 clinical isolates each (3 susceptible and 2 resistant strains where possible) of yeasts (*Candida albicans*, *C. glabrata*, *C. parapsislosis*, *C. krusei*, *C. lusitaniae*, *C. tropicalis* and *Cryptococcus neoformans*), moulds (*Fusarium solani*, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. terreus*, *Pseudallescheria boydii*, *Scopulariopsis brevicaulis*, *Scedosporium* spp., *S. prolificans*, *Cunninghamella bertholletiae*, *Rizopus oryzae, Mucor plumbeus, M. fragilis*) and dermatophytes (*Trichophyton rubrum, T. mentagrophytes, Epidermophyton floccosum, Microsporum canis*). MIC testing was performed according to the CLSI M27-A3 and M38-A2 standards for the susceptibility testing of yeasts and filamentous fungi, respectively. **(2)** The frequency of spontaneous resistance of 3 *T. rubrum* strains was evaluated by counting the number of colonies obtained following incubation of agar plates inoculated with a total of about 10^9 CFU on agar plates containing

drug concentrations higher than the minimal fungicidal concentration; the evolution of resistance was evaluated in 2 *T. rubrum* strains serially propagated for 10 transfers on agar plates containing subinhibitory drug concentrations. After the 5th and the 10th transfer, colonies were transferred on plates containing inhibitory drug concentrations and assessed for growth following incubation.

Results (1) The *in vitro* susceptibility of the tested strains to P-27530 was as follows: Yeasts P-27530 MIC range of 0.06–1 μg/ml was recorded, following 24 hours incubation, for all yeasts but several *C. glabrata* isolates which were read at 48 hours (MIC range of 0.06–2 μg/ml). P-27530 performed similarly against both *Candida* susceptible strains and strains resistant to caspofungin, fluconazole, miconazole, and voriconazole. Slightly reduced activity was observed against *Cryptococcus neoformans* with a MIC range of 2–4 μg/ml. Moulds P-27530 performed similarly against the susceptible strains and the strains with elevated MICs to amphotericin B, caspofungin, itraconazole, and miconazole with a MIC range of 0.06–2 μg/ml. Dermatophytes P-27530 demonstrated similar activity against the isolates (range of 0.25–2 μg/ml) and performed similarly (within one dilution) against both susceptible and resistant (terbinafine and fluconazole) *T. rubrum* and *T. mentagrophytes* strains. **(2)** no spontaneous resistant mutant to P-27530 and CPX was isolated; resistance frequency was 10^{-7} for itraconazole and 10^{-9} for terbinafine and amorolfine. All mutants collected confirmed the loss of susceptibility. P-27530 and CPX did not induce any resistance; frequency was 10^{-5} for itraconazole and 10^{-7} for terbinafine and amorolfine. In both experiments itraconazole resistant mutants showed increased resistance also to amorolfine and terbinafine.

Conclusions P-27530 shows a good antifungal activity *in vitro* against a broad spectrum of yeasts, moulds and dermatophytes, without evidencing any cross-resistance potential. Moreover, the lack of resistant mutant isolation in the *in vitro* assays suggests a low propensity of *T. rubrum* to develop resistance against P-27530.

P008

Antifungal susceptibility of bloodstream *Malassezia furfur* isolates by CLSI protocol in various media

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Objectives The microdilution antifungal method (CLSI BMD, M27-A3) was used for testing the antifungal susceptibility of *Malassezia* species. Optimal broth media that allow sufficient growth of *Malassezia* yeasts producing reliable and reproducible Minimum Inhibitory Concentration (MIC) using the CLSI BMD protocol are established only for *Malassezia pachydermatis* (Cafarchia et al., Vet Microbiol 2012) but not for *Malassezia furfur*. In this study, the susceptibility of *M. furfur* to posaconazole (POS), voriconazole (VOR), itraconazole (ITZ), fluconazole (FLZ) and amphotericin B (AMB) was evaluated by the CLSI BMD method using Christensen's urea broth (CUB), RPMI 1640 containing lipid supplementation (mRPMI) and Sabouraud dextrose broth with 1% tween 80 (SDB).

Methods A total of 24 *M. furfur* strains were employed. The strains were isolated from bloodstream infection (BSI) of 6 neonatal and 2 pediatric patients, three of which received systemic antifungal prophylaxis with fluconazole (3 mg/Kg/72 h). After the onset of fungemia, all patients were treated with intravenous AMB (5 mg/Kg/day) and recovered. *M. furfur* isolates were classified as susceptible (S), susceptible dose dependent (SDD), or resistant (R) using the breakpoints established for *Candida* spp. (i.e., ITZ:S ≤ 0.125 mg/L, SDD 0.25–0.5 mg/L, R ≥ 1 mg/L; FLZ: S ≤ 8 mg/L, SDD16–32 mg/L, R ≥ 64 mg/L; VOR: S ≤ 1 mg/L, SDD 2 mg/L, R ≥ 4 mg/L; POS: S ≤ 1 mg/L, SDD 2 mg/L, R ≥ 4 mg/L; AMB: S ≤ 1 mg/L) (Rex et al., Clin Infect Dis 1997). The interpretive categories for each medium were compared and results falling within the same category were considered in categorical agreement (CA) (Iatta et al., J Chemother 2011).

Results ITZ and POS displayed lower MIC values than those registered for VOR, FLZ and AMB regardless of the media employed. A large number of FLZ-resistant *Malassezia* strains were observed using SDB (58.3%) and mRPMI (45.8%). All *M. furfur* from the patients receiving prophylactic treatment with FLZ resulted FLZ resistant only when tested in SDB. All *M. furfur* isolates were AMB resistant regardless of the media with the lowest mMIC (mean values) registered in CUB (12.5 mg/L) and SDB (13.3 mg/L). The highest CA for all the drugs, except for POS, was registered comparing the results obtained using RPMI and SDB (from 66.7% to 100%).

Conclusion Based on the findings herein detected, RPMI and SDB gave comparable MIC results using CLSI BMD protocol, even if SDB is the only media able to detect FLZ resistant isolates in patients receiving FLZ prophylaxis and the lowest MIC values for AMB. Future studies are needed to confirm this hypothesis.

P009

Analysis of the main resistance mechanism to echinocandins in a large collection of clinical *Candida* isolates from Spain

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The use of echinocandins as a first-line therapeutic agent for the treatment of candidemia has increased. Consequently, it is of great importance to know the susceptibility patterns of Candida to these agents and determine which are the mechanisms that confer a lower susceptibility to them.

Objectives The main objective of this study was to analyze the hot spot regions of the genes fks1 and fks2, in order to determine if the principal underlying resistance mechanism described for the echinocandins is present in a collection of *Candida* isolates which are not susceptible to caspofungin (CFG), micafungin (MFG) and/or anidulafungin (AFG).

Methods The susceptibility of clinical Candida isolates to CFG, MFG and AFG were determined according to CLSI document M27-A3 or using the commercial kit Sensititre® YeastOne.

The molecular resistance mechanism to echinocandins was determined in 35 *Candida* spp. isolates. For this, in all *Candida* species the HS1 and HS2 regions of the *fks*1 gene were sequenced whereas the regions HS1 and HS2 of the *fks*2 gene were only analysed in *C. glabrata* and *C. parapsilosis*. The reference strains: *C. parapsilosis* (ATCC® 22019) and *C. krusei* (ATCC® 6258), were included as controls.

Results 35 *Candida* spp. isolates not susceptible to at least one of the three echinocandins were studied molecularly. Including: 12 *C. glabrata*, 7 *C. krusei*, 6 *C. parapsilosis*, 5 *C. tropicalis* and 5 *C. albicans*.

Seven isolates (20%) showed a mutation within the HS1 or HS2 regions in *fks1* or *fks2* genes (six of the 12 *C. glabrata* isolates and one of the 7 *C. krusei*):

– Two *C. glabrata* demonstrated the same mutation **S663P** in *fks2*. One isolate (M1105) was R to the 3 echinocandins (MIC CFG: 8 μg/mL, MIC MFG: 4 μg/mL and MIC AFG: 2 μg/mL), whereas the other (CG-68) was only R to MFG (MIC: 1 μg/mL) and AFG (MIC: 2 μg/mL).

– The *C. glabrata* 1176LL showed the equivalent mutation **S629P** in *fks1* and was R to CFG (MIC: 0.5 μg/mL) and I to AFG (MIC: 0.25 μg/mL).

– Two isolates of *C. glabrata* (CG-65 and CG-74) presented a mutation in the same position in *fks***2**, but with a different amino acid substitution, **F659S** and **F659C**, respectively. These two strains were R to CFG (MIC: 1 μg/mL) and I to AFG (MIC: 0.25 μg/mL).

– The isolate 912LL (*C. glabrata*) showed a heterozygous mutation **D666E/D** in *fks2* and was only R to CFG (MIC: 0.5 μg/mL).

The *C. krusei* isolate CY-118 was R to CAS (MIC: 4 μg/mL) and MFG (MIC: 1 μg/mL), I to AFG (MIC: 0.5 μg/mL), and showed the mutation **R1368G** in *fks1*.

Conclusions

- Our non-susceptible *Candida* isolates analysed presented a very low percentage of mutations in the *fks* genes.
- The mutation S663P in *fks2* gene detected in *C. glabrata* isolates confers the highest MICs for the 3 echinocandins, as has been previously described.
- In *C. glabrata* the lowest MICs for the three echinocandins was observed in a heterozygous mutation in the *fks1* gene.

P010

In vitro antifungal susceptibility testing of the clinical *Fusarium* strains in the Belgian fungal BCCM/IHEM-collection suggests alternative treatments for different types of *Fusarium* infection

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Objectives The objective of this study was to assess *in vitro* antifungal susceptibility profiles of the most important human infection-associated *Fusarium* species.

Validated *F. solani*, *F. lichenicola*, *F. oxysporum*, *F. verticillioides*, *F. musae*, *F. proliferatum*, *F. dimerum* and *F. delphinoides* strains of clinical origin, from the BCCM/IHEM-collection, were investigated.

Correlations between antifungal susceptibility and infection type were tested.

It was evaluated whether amphotericin B (AMB), the current drug of choice for fusariosis therapy, remains the best treatment for all *Fusarium* infections or whether other less toxic antifungals also exhibit sufficient efficacy.

Methods The BCCM/IHEM-collection contains 320 *Fusarium* strains, half of which are clinical isolates belonging to one of the 5 most important infection-associated *Fusarium* species complexes.

Presumptive identification of these strains was obtained by morphology and MALDI-TOF mass-spectrometry, and validated by gene sequencing using ITS, BT, EF1α and LSU.

In vitro antifungal susceptibility testing was performed according to EUCAST-E.DEF9.1 methodology. Twofold serial drug dilutions were prepared in 96-well plates, with concentrations ranging from 64 to 0,032 $\mu g.ml^{-1}$ for fluconazole (FLC) and 5-fluorocytosine (5FC), and from 16 to 0,008 $\mu g.ml^{-1}$ for voriconazole (VOR), itraconazole (ITR), ketoconazole (KTZ), terbinafine (TER), AMB and AMB+VOR.

Minimal 100% inhibitory concentrations (MIC-100) were determined and principal coordinate analysis (PCoA) was done.

Results The PCoA of MIC-100 gives a summary of the susceptibilities of each *Fusarium* strain against all tested antifungals and identified 5 clusters of strains (Figure 1).

Cluster 1 comprises the multiresistant strains of *F. solani* and *F. oxysporum*, with only susceptibilities for AMB and AMB+VOR (Figure 2).

In cluster 2, the *F. solani*, *F. lichenicola* and *F. oxysporum* strains show additionally limited VOR-susceptibility.

The 3rd cluster contains the remaining *F. solani* strain, which has relatively high VOR-susceptibility and limited KTZ-susceptibility.

Cluster 4 contains *F. proliferatum*, *F. oxysporum*, *F. dimerum* and *F. delphinoides* strains. The *F. proliferatum* and *F. oxysporum* strains display relatively high MIC-100 values for AMB, AMB+VOR, VOR and TER as opposed to the *F. dimerum* and *F. delphinoides* strains.

In cluster 5, additionally, KTZ-susceptibility was observed for the present *F. proliferatum*, *F. verticillioides* and *F. musae* strains. The MIC-100 values for KTZ were lowest for the *F. proliferatum* strains.

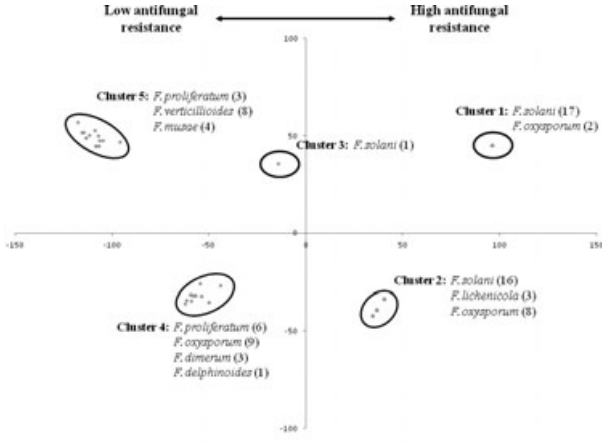

Figure 1 Principal coordinate analysis (PCoA) of the minimal 100% inhibitory concentrations (MIC-100).

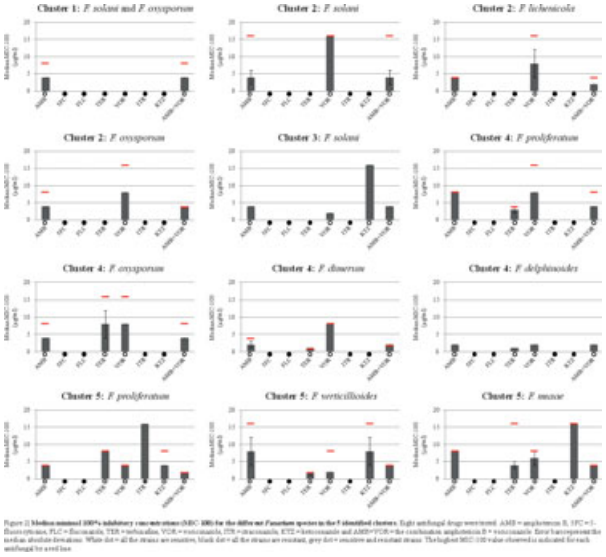

Figure 2 Median minimal 100% inhibitory concentrations (MIC-100) for the different Fusarium species in the 5 identified clusters.

The combination AMB+VOR did not statistically increase susceptibility as opposed to AMB or VOR alone.

Conclusion The results of these *in vitro* antifungal susceptibility tests suggest that AMB is not always the best option for fusariosis treatment.

Better efficacy was noticed with TER against *F. dimerum* and *F. delphinoides*, and VOR or TER against *F. verticillioides*.

Similar efficacy was obtained with VOR against *F. delphinoides* and *F. musae*, TER against *F. proliferatum* and KTZ against *F. verticillioides*.

AMB remains the best treatment against *F. solani*, *F. lichenicola* and *F. oxysporum* due to occurrence of multiresistant strains.

In vivo tests are needed to confirm our conclusions, but nevertheless this study emphasizes the utility of diagnosis and identification at species level in *Fusarium* infection to improve patient treatment by avoiding AMB therapy, causing adverse side-effects.

P011

Should echinocandin doses be increased against *Candida* species? An *in vitro* and *in vivo* study of caspofungin against *Candida albicans*, *C. krusei* and *C. inconspicua*

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Objectives The aim of our study was to compare the activity of caspofungin in RPMI-1640 and in 50% serum against three *Candida* species and in vitro and in a murine model as well.

Methods Caspofungin MICs and killing activity were determined against three *C. albicans*, three *C. krusei* and three *C. inconspicua* isolates in RPMI-1640 and 50% serum (Table 1). The killing kinetics of caspofungin from 1 to 32 mg/L in both media (RPMI-1640 and 50% serum) was analyzed. One-way ANOVA with Tukey post-testing and *T* test (with Welch's correction where appropriate) were used to compare killing kinetics in different concentrations and different media, respectively.

BALB/c female neutropenic mice were infected intravenously. The infectious dose of *C. albicans*, *C. krusei* and *C. inconspicua* were 5×10^4, 4×10^6 and 2×10^7 CFU/mouse, respectively. Five-day intraperitoneal treatment with 1, 2, 3, 5 and 15 mg/kg CAS was started after 24 hours postinfection. Kidney burden was analyzed using the Kruskal–Wallis test with Dunn's post-test for multiple comparisons.

Results MICs and time-kill results are shown in Table 1 The relationship between the killing rate and CAS concentrations was linear in both media for the three *Candida* species (Fig. 1). In case of *C. albicans*, the highest killing rate was noticed at 1 mg/L (the mean *K* value was −0.81 CFU/ml/h) in RPMI-1640, but significantly decreased at 16 and 32 mg/L (the mean *K* values were −0.34 and −0.41 CFU/ml/h, respectively) when compared to the *K* value at 1 mg/L ($P < 0.01$ and 0.05, respectively) (paradoxical growth). In 50% serum, *K* values were comparable ($P > 0.05$) (Fig. 1). At the same caspofungin concentrations in RPMI-1640 and 50% serum, the killing rates were similar ($P > 0.05$).

Table 1 MICs and killing activities of caspofungin

isolates	MIC (mg/L)		effect in time-kill studies in (mg/L)	
	RPMI	50 % serum	RPMI	50 % serum
C. albicans ATCC 10231	0.03	0.5	≥0.03 static	≥0.5 static
C. albicans 183	0.03	0.25	≥0.03 static	≥0.25 static
C. albicans 5265	0.015	0.25	≥0.5 cidal^{PG}	≥1 cidal
C. albicans 34350	0.015	0.25	≥0.25 cidal^{PG}	≥1 cidal
C. krusei ATCC 6258	0.25	2	≥1 cidal	≥8 cidal
C. krusei 5029	0.25	2	≥2 cidal	≥8 cidal
C. krusei 4363	0.25	2	≥8 cidal	≥8 cidal
C. krusei 27393	0.25	2	≥1 cidal	≥16 cidal
C. inconspicua 12060	0.125	0.5	≥0.5 cidal	≥1 cidal
C. inconspicua 22027	0.125	0.5	≥0.5 cidal	≥0.5 cidal
C. inconspicua 20114	0.06	0.25	≥0.5 cidal	≥1 cidal

PG: paradoxical growth

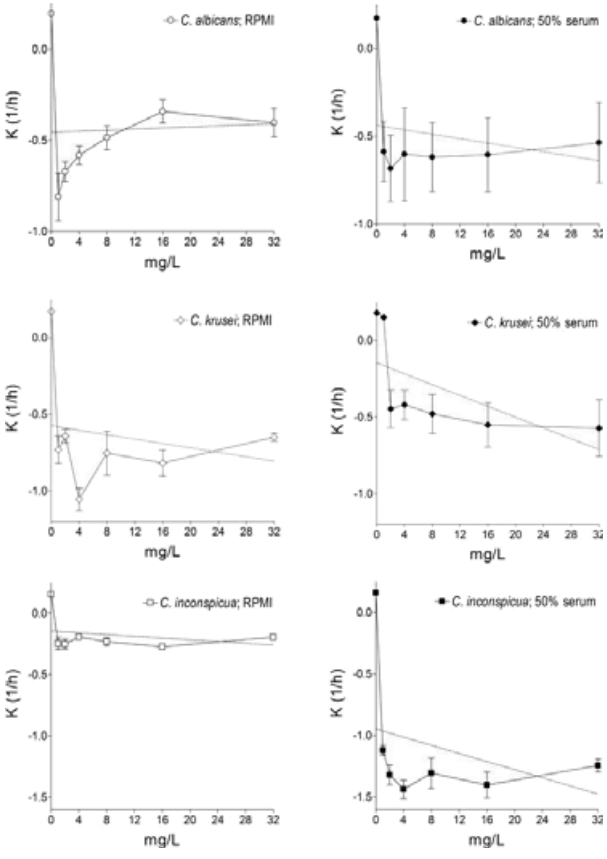

Figure 1 Relationship between caspofungin concentration and killing activity.

In case of *C. krusei* highest killing rate was noticed at 4 mg/L caspofungin concentration in RPMI-1640, which was significantly better than at 2 and 32 mg/L ($P < 0.05$). In 50% serum the mean *K* values (ranges from −0.42 to −0.57 CFU/ml/h) at effective caspofungin concentrations (4–32 mg/L) did not differ significantly (Fig. 1). The killing rates at 1, 2 and 4 mg/L of CAS concentrations were significantly better in RPMI-1640 than in 50% serum ($P < 0.01$–0.005).

Adding 50% serum increased caspofungin killing activity against *C. inconspicua* significantly at any concentrations tested between 1–32 mg/L (ranges −1.12 to −1.44 CFU/ml/h; $P < 0.0003$ for all tested concentrations).

All treatment arms significantly decreased the fungal tissue burden in the kidneys against *C. albicans* and *C. inconspicua* ($P < 0.05$–0.001). Against *C. krusei*, doses 3, 5 and 15 mg/kg were effective ($P < 0.05$–0.01). All effective doses were comparable in efficiency for all three species ($P > 0.05$).

Conclusion Fifty percent serum decreased caspofungin killing activity against *C. albicans* and *C. krusei* but increased it against *C. inconspicua*. In 50% serum caspofungin, killing activity of all concentrations above the MIC was comparable (concentration independent killing) suggesting that the efficacy of dose escalation is questionable. These in vitro results were also supported by the murine model.

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P012

Distribution of invasive isolates of *Candida* spp. and *in vitro* susceptibility to antifungal agents: multicenter study in Russia

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Objectives The aim of this study was to present the distribution of invasive isolates of *Candida* spp. and range of susceptibility to echinocandins and azoles in hematological (Hem) and ICU patients (pts).
Methods Susceptibility testing of 346 Candida spp. isolated from blood and other sterile specimens to antifungal agent was performed by the CLSI the broth microdilution method (CLSI 2012). Isolates were collected from 9 Hem centers (2003–2012) and 11 ICU centers (2009–2012).
Results A total of 346 *Candida* spp. (307 from blood and 39 from other sterile specimens; 167 from Hem pts and 179 from ICU pts) were included. Species distribution was as follows: 40% (138) *C. albicans*, 25,4% (88) *C. parapsilosis*, 6,6% (23) *C. krusei*, 5,7% (20) *C. glabrata*, 5,7% (20) *C. tropicalis*, 5,2% (18) *C. guilliermondii*, 4,3% (15) *C. pelliculosa*, 3,5% (12) *C. lusitaniae*, 3,5% (12) other. In candidemia the rate of *C. albicans* was 29% in Hem and 43% in ICU pts; *C. parapsilosis* ranked second in Hem (25%) and ICU (31%), than *C. krusei* (11%) was in Hem and *C. glabrata* (6,5%) in ICU. *C. albicans* was the predominant species (75%) from the other sterile specimens. MIC_{90} values for *Candida* spp. isolated from Hem and ICU pts are provided in the table 1. Overall, we detected decreased susceptibility to fluconazole for 30 *Candida* spp. (11 *C. albicans*, 17 *C. parapsilosis*, 1 *C. tropicalis*, 1 *C. lusitaniae*) and to voriconazole for 26 isolates (14 *C. albicans*, 12 *C. parapsilosis*). Including intrinsically resistant fungi this rate was to fluconazole for 73 (21%) *Candida* spp. and to voriconazole for 46 (13%) isolates. Thus, 17 (18,4%) of 88 isolates of *C. parapsilosis* demonstrated resistance to fluconazole especially in ICU patients (n = 13, 27%). All *C. parapsilosis* (n = 48) from ICU were echinocandin susceptible and only 2 isolates of *C. parapsilosis* from Hem pts were echinocandin resistant. Acquired resistance to anidulafungin was for 7 isolates (2 *C. parapsilosis*, 5 *C. guilliermondii*) and to caspofungin for 16 *Candida* spp. (2 *C. parapsilosis*, 4 *C. krusei*, 8 *C. guilliermondii*, 1 *C. glabrata*, 1 *C. lusitaniae*).

MIC90 values for Candida spp. isolated from Hem and ICU pts are provided in the table 1.

Table 1 MIC90 values (mg/L) for Candida isolated from Hem and ICU patients.

Candida spp.	n/n*	MIC90 of antifungal agents against *Candida* spp isolated from Hem pts. MIC 90 of antifungal agents against *Candida* spp isolated from ICU pts.			
		Anidulafungin	Caspofungin	Fluconazole	Voriconazole
C. albicans	51/87	0.032/0.032	0.064/0.064	4/2	0.5/0.125
C. parapsilosis	40/48	0.25/0.25	0.5/0.5	1/32	0.25/0.25
C. krusei	17/6	0.032/0.032	8/16	32/64	0.25/0.25
C. tropicalis	11/9	0.016/0.125	0.064/0.25	0.5/4	0.032/0.064
C. glabrata	8/12	0.032/0.032	0.25/0.016	64/32	2/2
C. guilliermondii	16/2	8/0.064	16/0.064	2/2	0.25/0.032
C. pelliculosa	12/3	0.032/0.008	0.064/0.064	2/4	0.125/0.25
C. lusitaniae	7/5	0.5/0.032	16/0.5	32/1	0.25/0.016
C. famata	3/2	0.25/0.5	0.25/0.25	0.5/1	0.25/0.064
C.kefyr	2/2	0.032/0.016	0.125/0.064	0.5/1	0.064/0.032
Other	0/3	n.a./0.125	n.a./0.016	n.a./1	n.a./0.5

*n/n – Candida spp. isolated from Hem pts/ Candida spp. isolated from ICU pts.

Conclusion *C. albicans* was the predominant *Candida* spp. especially in ICU pts. High resistance was demonstrated to fluconazole for *C. parapsilosis* isolated from ICU pts. There was not significant change in MIC_{90} values of anidulafungin, caspofungin and voriconazole against *Candida* spp. isolated from Hem and ICU pts except for *C. guilliermondii*. MIC_{90} values of fluconazole were different for common *Candida* spp. isolated from Hem and ICU pts.

P013

In vitro activities of current and new antifungal agents against *Candida* blood isolates according to new CLSI epidemiological cut-off values and clinical breakpoints

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Objective To evaluate the antifungal activity of the triazoles (fluconazole, posaconazole, voriconazole), echinocandins (anidulafungin, caspofungin and micafungin) and a polyene (amphotericin B) against *Candida* blood isolates according to new Clinical and Laboratory Standards Institute (CLSI) epidemiological cut-off values and clinical breakpoints.
Methods In vitro activities of amphotericin B (Sigma), fluconazole (Sigma), caspofungin (MSD), posaconazole (MSD), anidulafungin (Pfizer), voriconazole (Pfizer) and micafungin (Astellas), against 100 blood *Candida* isolates were assessed by the CLSI M27-A3 method. Isolates included 40 *Candida albicans*, 20 *Candida glabrata*, 20 *Candida parapsilosis*, 10 *Candida dubliniensis* and 10 *Candida krusei*. A 24 h prominent inhibition endpoint for determination of MIC was selected and susceptibility categorization was made according to the new CLSI fluconazole, voriconazole and echinocandins breakpoints for *Candida albicans*, *Candida glabrata* and *Candida parapsilosis*. CLSI epidemiological cut-off values and clinical breakpoints were determined as described by Pfaller MA and Diekema DJ (J Clin Microbiol 2012; 50: 2846–56). Reference strains, *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258 were included as controls.
Results Amphotericin B, anidulafungin and voriconazole were the most active against all *Candida* species (geometric means -GM- 0.953 μg/ml, 0.085 μg/ml and 0.041 μg/ml, respectively). All *Candida glabrata* isolates were susceptible dose-dependent to fluconazole, and *Candida krusei* isolates were resistant. Echinocandins showed lower activity against *Candida glabrata* isolates than amphotericin B or voriconazole. Ten isolates were resistant to caspofungin (GM 0.307 μg/ml), and four and fourteen isolates showed intermediate susceptibility to anidulafungin and micafungin (GM 0.050 μg/ml and 0.088 μg/ml, respectively). Moreover, five out of 10 isolates of *Candida krusei* were found to be resistant to caspofungin and micafungin. According to epidemiological cut-off values for caspofungin, 20 out of 40 isolates of *Candida albicans* and most isolates of *Candida glabrata* and *Candida krusei* (18 and 9 isolates, respectively) were classified as non-wild type isolates. When epidemiological cut-off values were applied, something similar was observed for micafungin against *Candida glabrata* and *Candida krusei* (17 and 8 isolates, respectively). For posaconazole, only epidemiological cut-off values were considered, so, nine isolates of *Candida albicans* were categorized as non-wild type isolates because clinical breakpoints have not been yet defined.
Conclusion Anidulafungin and voriconazole displayed excellent antifungal activities against most frequently blood isolated species of *Candida*.
Funding This study was financed in part by projects PI11/00203 (FIS, Gobierno de España), and GIC12 210-IT-696-13 and S-PR12UN002 (Gobierno Vasco-Eusko Jaurlaritza).

P014

Influence of hypoxia on antifungal susceptibility, sterol pattern and biomarker release of *Aspergillus* spp.

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Objectives Invasive aspergillosis (IA) is a major life-threatening disease in immunocompromised patients, with mortality rates from 40% up to 90% in high-risk populations. The most common species causing aspergillosis is *Aspergillus (A.) fumigatus*, accounting for approximately 90% of infections. Depending on regional distinctions, *A. flavus* and *A. terreus* are frequently reported. During infection, fungal pathogens must adapt to microenvironmental stresses, including hypoxia as well as high CO_2 levels. Such oxystress conditions are usually not taken into account in current *in vitro* models of infection, the assessment of antifungal sensitivities or the release of biomarkers used for diagnosis.

Methods Using Etest strips,the *in vitro* activity of amphotericin B (amB), different azoles and echinocandins in hypoxic conditions (1% O_2, 5% CO_2) to their activity in normoxic conditions against aspergilli. For evaluation of biomarker release, the amount of β-1,3 glucan (BG) and galactomannan (GM) in Aspergillus supernatants was determined by commercially available detection kits (Platelia/Fungitell).

Changes in the sterol pattern or the amount of ergosterol was evaluated by GC-MS.

Results On surface cultures, we found a reduction of the minimal inhibitory concentration (MIC) for amB for all aspergilli in hypoxic conditions. Similarly, a significant reduction in the MIC for all tested azoles was demonstrated for *A. terreus* isolates, while for *A. fumigatus* isolates differences were less pronounced. For echinocandins, little or no change in the MEC (minimal effective concentration) was detected between hypoxic and normoxic conditions for all *aspergilli.* Most interestingly, *A. terreus* strains, that are resistant to amB in normoxia, exhibited sensitivity to amB in hypoxic conditions, defining a breakpoint of > 2 μg/ml. Notably, for none of the strains tested, MIC/MEC values increased in hypoxia. Our results so far indicate, that there is no significant difference in the amount of ergosterol whether mycelia is grown in hypoxia or normoxia.

The detection of circulating fungal antigens in serumfor *Aspergillus* galactomannanor *β*-D-glucan has become an accepted diagnostic strategy. However, sensitivity and specificity vary extremely and the reasons are only partially clear; therefore, we are currently checking whether hypoxia influences the physiological kinetics of GM and β-glucan release.

Conclusion ECOFFs in hypoxia differ from those in normoxia for antifungal drugs targeting ergosterol or its biosynthesis. Supplementation of test media with blood or ergosterol abrogated the reduction of the MIC, but there is no direct link between ergosterol content and increased susceptibility to amB and azoles, but further analysis of sterol intermediates needs to be done in more detail.

P015

2005–2012 prevalence of triazole-resistant *Aspergillus fumigatus* isolates in a cohort of patients with cystic fibrosis from Haute-Normandie, France

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Objectives The aim of this work was to evaluate retrospectively the prevalence of azole-resistance in *Aspergillus fumigatus* from a large cohort of patients with cystic fibrosis..

Methods *Aspergillus fumigatus* isolates obtained from 334 sputa and bronchial aspirations of 97 cystic fibrosis (CF) patients (mean age = 22 years, range 4–58) attending the Rouen University Hospital from December 2005 to June 2012 were cryopreserved. After thawing, 326 isolates (93 patients) were grown and plated on Sabouraud's agar medium containing 4 mg/ml itraconazole (ITZ). Minimal Inhibitory Concentrations (MICs) to itraconazole were determined according to the EUCAST standardized methodology. Species identification of *A. fumigatus* isolates was obtained by sequencing of the b-tubulin gene. Sequencing analysis of the *CYP51A* gene and its promoter region was performed.

Results 6/326 (1.84%) isolates identified as *Aspergillus fumigatus* species obtained from 6/93 (6.4%) patients were resistant to itraconazole (MICs >2 mg/ml) (Table). Three out of 6 isolates (3 patients) obtained since 2011 displayed the TR_{34}/L98H mutation that suggest acquisition through environmental exposure of de novo azole-resistant isolate in these patients. Mutations at residue 248 have been previously described although their involvment in azole resistance remains to be established. Strikingly, a non-*CYP51* mediated azole resistance mechanism was evidenced for two patients (Pt1 and 5) as one isolate had the wild-type sequence whereas the remaining exhibited mutations previously reported in azole-susceptible isolates (M172V, E427K).

Table Characteristics of the six itraconazole-resistant *Aspergillus fumigatus* isolates obtained for patients with cystic fibrosis

Conclusion In the present study, we report a 6.4% prevalence of itraconazole-resistant *Aspergillus fumigatus* mostly driven by TR_{34}/L98H In CF patients from the Haute-Normandie. This large cattle breeding area also showed some agricultural treatments based on azole fungicides as prothioconazole, fluzilazole, epoxiconazole and tebuconazole. Finally these results underline the need for routine azole susceptibility testing. Additional studies are ongoing to determine the molecular mechanisms leading to azole resistance in some of these isolates.

P016

Identification and in vitro response to antifungal drugs of clinical isolates of *Pithomyces* spp.

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Pithomyces is an anamorphic genus of filamentous fungi with species commonly found colonizing dead leaves or stems of many different plants. *Pithomyces chartarum*, the most cosmopolitan species of the genus, has been reported to cause facial eczema in some animals, including sheep, cattle, goats and deer, a cutaneous response due to a liver damage caused by a mycotoxin (sporisdesmin) produced by this fungi. Recently, this species has been also reported as agent of onychomycosis, and some studies have associated the presence of *Pithomyces* spores in the air around asthma patients. However, an accurate identification of the species associated to different clinical process has never been done. The aim of this study is to identify a set of clinical isolates of *Pithomyces* at the species level, on the basis of a morphological and molecular characterization, to evaluate the spectrum of *Pithomyces* species from clinical sources and to determine the in vitro activity of different antifungal agents against the species identified. We studied 42 isolates from different clinical specimens, provided by the Fungus Testing Laboratory of the University of Texas Health Science Center (USA) and isolated between 2003 and 2012. Identification was done by standard mycological procedures and DNA sequencing of ITS and D1/D2 regions of rDNA. The predomiant species were *P. chartarum* (n = 14) and *P. atro-olivaceous* (n = 14), followed by *Pithomyces* sp I (n = 12), and *Pithomyces graminicola* (n = 2). Although seven of this isolates did not sporulate, they were identified on the basis of the combined sequence analysis. The most common anatomic sites of isolation were superficial tissue (50%),

respiratory tract (21.4%), nasal region (19%), and deep tissue (4.7%), and unknown (4.7%).

With the exception of one isolate of *P. atro-olivaceous*, which showed high MICs to amphotericin B and voriconazole, the rest of isolates showed low MICs to the 8 antifungal drugs tested.

In conclusion the most prevalent species were *P. chartarum* and *P. atro-olivaceous*. We found no correlation between the species identified and the infection source. The majority of the species identified were susceptible to the drugs tested.

P017

Comparison of minimal inhibitory concentration elevation in *Candida dubliniensis* and *Candida albicans* after fluconazole exposure *in vitro*

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Objective *Candida dubliniensis* was the closest and phynotypically related to *Candida albicans*. While fluconazole has been commonly used for treatment of candidiasis and prophylaxis treatment in immunocompromised patients, the stable fluconazole resistance *C. dubliniensis* has been reported to be able to induce *in vitro* after the drug exposure. To prove whether *C. dubliniensis* or *C. albicans* can be easier induced or develop drug resistance.

Methods Forty-one isolates of *C. dubliniensis* and forty-five isolates of *C. albicans* were used. *C. albicans* ATCC 10231 and *C. dubliniensis* CBS 7987 were reference strains and *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019 were used as control strains in antifungal drug susceptibility test. The fluconazole susceptibility of both *Candida* species before and after fluconazole exposure *in vitro* was investigated by broth microdilution method. The fluconazole exposure was carried out with each of 20 isolates of *C. albicans* and *C. dubliniensis* as described by Moran and coworkers in 1997. Colonies of fluconazole-sensitive isolates of *C. albicans* and *C. dubliniensis* were inoculated on yeast peptone dextrose (YPD) agar containing 0.5 μg/ml of fluconazole. After 48 hrs of incubation at 37°C, each colony was transferred to new fresh medium, containing 0.5 μg/ml of fluconazole, and incubated for 48 hrs. Then each colony was subcultured twice on YPD agar containing 1, 5, 10, 25, and 50 μg/ml of fluconazole and incubated for 48 hrs. Colonies which were able to grow on medium containing 50 μg/ml of fluconazole were considered to be putative fluconazole-resistant derivatives, and these colonies were subcultured twice on fluconazole-free medium, and subsequently subjected to fluconazole susceptibility testing for Flu MICs. The resistant isolate or the isolate which displayed the highest elevated-MIC was repeatedly subcultured 10 times on fluconazole- free medium. In each subculture, Flu MIC was determined to evaluate the stable fluconazole-resistant phenotype. In addition, the high Flu MIC derivatives of both species were 10 times exposed to extreme concentration of fluconazole (64 μg/ml) and Flu MICs were again determined.

Results All 41 isolates of *C. dubliniensis* isolates were susceptible to fluconazole (MICs ranging from 0.125 to 2 μg/ml) whereas 44 isolates of *C. albicans* were susceptible to fluconazole (MICs ranging from 0.125 to 1 μg/ml) and only 1 isolate showed susceptible – dose dependent phenotype (MIC 16 μg/ml). After *in vitro* exposure to increasing doses of fluconazole, 45% of *C. dubliniensis* exhibited increasing of fluconazole MICs and the stable fluconazole MIC elevation could be induced while 70% of *C. albicans* showed the increasing of fluconazole MICs but not stable.

Conclusion *C. dubliniensis* developed stable fluconazole MIC elevation whereas *C. albicans* exhibited transient fluconazole resistance. These suggest that for long term drug exposure, *C. dubliniensis* tends to persist and gradually resist to drug than *C. albicans*.

P018

Azole resistance in *Aspergillus fumigatus* isolates from lung transplant recipients with cystic fibrosis: preliminary results

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Objectives Azole resistance in *Aspergillus fumigatus* is an emerging problem. The mechanism of resistance is mostly related to mutations in the *CYP51A* gene encoding 14-alpha-demethylase, the target of azoles drugs.

Azole resistance can be probably acquired through two distinct ways that are long term azole treatment or inhalation of *de novo* azole resistant isolates from our environment. Importantly, both scenario can be distinguished as their underlying mechanisms differ, the latter involving not only mutations in the *CYP51A* gene but also a tandem repeat in its promoter region.

The aim of the study was to screen for azole resistance a collection of *A. fumigatus* isolates recovered from cystic fibrosis lung-transplant recipients (CF-LTx) over a 12-year period (2001-2012) at a single center. The molecular mechanisms leading to azole resistance were also investigated.

Methods The screening was performed by subculturing each isolate on RPMI agar plates supplemented with itraconazole (4 mg/L) and voriconazole (1 mg/L). *In vitro* resistance was confirmed by the EUCAST reference method. EUCAST clinical breakpoints were used to categorize the isolates. For each azole resistant isolate, the whole *CYP51A* gene and its promoter were sequenced and compared with that of a wild type isolate (GenBank acc. number AF338659).

Results We present here preliminary results for three patients (Table) with *A. fumigatus* azole resistant isolates.

Most of the isolates displayed azole cross-resistance. TR_{34}/L98H was the most prevalent mutation being recovered from 5 out of the 8 isolates (in patients 1 and 2). At least for patient 1, our data support the acquisition of the azole resistant isolate through environmental exposure as this patient had not been given azole therapy before recovering the first azole-resistant isolate. Other amino acid substitutions (codons 54 and 216) have been previously reported in patients receiving long term therapy suggesting that both mutations are probably the result of selective pressure in the patient itself.

Conclusions This preliminary study confirmed the occurrence of azole resistance (with distinct molecular mechanisms) in *A. fumigatus* isolates recovered from cystic fibrosis lung transplant patients. These

Table 1

Patient #	Isolate ID	Sample	Date of isolation	MIC (μg/mL)			Promoter / *CYP51A* sequence	Previous azole exposure
				ITZ	VRZ	PSZ		
Pt1	1895	Bronchial fluid	20/11/2003	>8	2	0.5	TR_{34}/L98H	None
	2372	BAL	06/10/2004	>8	2	1	TR_{34}/L98H	None
	2438	BAL	03/12/2004	>8	2	1	TR_{34}/L98H	ITZ
	2659	Bronchial fluid	10/05/2005	>8	2	1	TR_{34}/L98H	None
Pt2	3974	Sputum	30/05/2009	>8	0.125	>8	P216L	ITZ
	4083	Sputum	28/09/2009	>8	4	1	TR_{34}/L98H	VRZ
Pt3	4017	Bronchial fluid	21/07/2009	>8	0.25	>8	G54W	ITZ, VRZ
	4020	Sputum	21/07/2009	>8	0.25	>8	G54W	ITZ, VRZ

MIC: Minimum Inhibitory Concentration; ITZ: Itraconazole; VRZ: Voriconazole

findings highlight again the need for systematic antifungal susceptibility testing of *Aspergillus* spp. recovered in this patient population.

P019

In vitro susceptibility of *Cryptococcus neoformans* VNI and *Cryptococcus gattii* VGII to antifungal drugs from HIV positive and HIV-negative patients in midwest of Brazil

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Objetive Cryptococcosis is a life-threatening, systemic mycosis affecting humans and animals. In Brazil, cryptococcosis caused by *C. neoformans* occurs in all regions; however, *C. gattii* behaves as a primary pathogen infecting native immunocompetent hosts. In vitro susceptibilities and molecular types of 27 isolates of *Cryptococcus* spp. were analyzed. The isolates were HIV (positive and negative) patients from the university hospitals of Cuiabá, midwest of Brazil.
Methods The species were determined through morphological and physiological tests and genotypes were determined by RFLP-URA5. Antifungal susceptibility of clinical isolates to amphotericin B, fluconazole and voriconazole were determined by the method ETEST (AB Biodisk).
Results Following the species and genotypes were Identified: *Cryptococcus neoformans* VNI (17/27, 62.97%), *Cryptococcus gattii* VGII (10/27 37.04%). The isolates of *C. neoformas* VNI were predominant in HIV-positives patients and *Cryptococcus gattii* VGII were predominant in HIV-negative patients. The ranges of the minimal inhibitory concentrations (MIC) for antifungals were: fluconazole (2 to >256 μg/ml), voriconazole (0.016–0.094 μg/ml) and amphotericin B (0.094–0.75 μg/ml). *C. neoformans* VNI isolates from acquired immune deficiency syndrome (AIDS) and non-AIDS patients were less susceptible to fluconazole than other antifungals.
Conclusion It is very important to note that VGII is a genotype predominant affecting individuals HIV-negative in Cuiabá-midwest Brazil, our results provide new information on the molecular epidemiology of *C. neoformans* and *C. gattii* in Brazilian areas. The continued surveillance of antifungal susceptibility of clinical strains of *C. neoformans* and *C. gattii* is desirable to monitor the emergence of any resistant strains in order to ensure more successful therapy of cryptococcosis.

P020

Susceptibility to fluconazole of *Cryptococcus neoformans* isolates from patients in Saint Petersburg, Russia

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Cryptococcosis is a life-threatening infection which is encountered mostly in immunocompromised patients. Fluconazole is widely used in antifungal therapy of patients with cryptococcosis.
The aim of the study To determine susceptibility to fluconazole of *Cryptococcus neoformans* isolates obtained from patients in Saint Petersburg,Russia during 2004–2013 yrs.
Methods Susceptibility testing was performed by disk-diffusion method according to CLSI M44A Document.
Results During 10 years of observation 118 *C. neoformans* clinical isolates were tested. Annual rate of isolation was as follows: 2004 y. – 4, 2005 y. – 2, 2006 y. – 7, 2007 y. – 12, 2008 y. – 15, 2009 y. – 21, 2010 y. – 9, 2011 y. – 23, 2012 y. – 20, 2013 y. (from January to April) – 5.

C. neoformans isolates were obtained predominantly from HIV-infected patients – 88%, oncohematological patients – 7%, renal transplantation – 2%, patients with unknown risk factors – 3%.

Most of strains were isolated from cerebrospinal fluid (91,5%), other were isolates from blood, bronchial washings, sputum, vertebra, brain tissue.

Distribution of *C. neoformans* clinical isolates according to susceptibility to fluconazole: susceptible (S) – 75%, susceptible-dose-dependent (SDD) – 14%, resistant (R) – 11%.
Conclusion Frequency of cryptococcosis in Saint Petersburg, Russia is increasing since 2006 year. HIV-infection is a predominant risk factor for cryptococcosis. Eleven per cent of *C. neoformans* isolates obtained from patients during 2004–2013 yrs. were resistant to fluconazole.

P021

Effect of 50% human serum on the killing activity of micafungin against *C. dubliniensis, C. lusitaniae, C. guilliermondii* and *C. kefyr* using time-kill methodology

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Objectives Though five *Candida* species account for ≥95% of all candidemia, the less common other species may cause problem in the treatment of invasive candidiasis due to high probability of resistance development to the commonly used agents fluconazole (*C. dubliniensis*) or amphotericin B (*C. lusitaniae*). Echinocandins became a useful alternative for the treatment of invasive candidasis, especially among neutropenic patients. Echinocandins are highly protein-bound (from 96.5% to 99.8%), which decreases the drug concentration available in the serum and tissues. Therefore, aim of our study was to compare micafungin *in vitro* activity in RPMI-1640 and 50% serum against four rarely isolated *Candida* species.
Methods Four *C. dubliniensis*, four *C. lusitaniae*, four *C. guilliermondii* and four *C. kefyr* clinical isolates were used in the study. MICs were determined using the CLSI standard broth microdilution method. Micafungin (Astellas) final concentration ranged between 0.015–8 and 0.06–32 mg/L in RPMI-1640 and in RPMI-1640 plus 50% human serum (from a human male, type AB, Sigma, Budapest, Hungary), respectively. MICs were read after 24 h using the partial inhibition criterion (CLSI, 2008).

In killing studies micafungin activity was measured in both test media (in RPMI-1640 with and without 50% human serum). The highest micafungin concentration used in the study was 32 mg/L. Samples (100 ml) were removed at 0, 4, 8, 12, 24 and 48 hours, serially diluted tenfold, plated (4 × 30 ml) onto Sabouraud dextrose agar and incubated at 35°C for 48 hours. Fungicidal or fungistatic activity was defined as a 99.9% or less reduction in viable cell count compared to the starting inoculum, respectively.
Results Micafungin MIC ranges in RPMI-1640 for *C. dubliniensis, C. guilliermondii, C. lusitaniae* and *C. kefyr* were 0.06–0.12, 1–2, 0.12–0.25 and 0.06–0.12 mg/L, respectively. In killing studies micafungin was fungistatic at ≥1× MIC against all tested isolates, while was fungicidal at ≥0.25 and ≥4 mg/L against all *C. kefyr* and 2 out of 4 *C. lusitaniae* isolates, respectively.

In 50% serum MIC ranges for *C. dubliniensis, C. guilliermondii, C. lusitaniae* and *C. kefyr* were 0.5–1, 16–32, 16 and 8–16 mg/L, respectively. Micafungin was fungistatic at ≥1 and ≥16 mg/L concentrations against *C. dubliniensis* and *C. lusitaniae*, respectively. *C. kefyr* isolates were killed by 16–32 mg/L micafungin concentrations after 12–24 h. Killing curves for *C. guilliermondii* at any concentrations were similar to controls (no effect).
Conclusion Serum decreased micafungin activity for all tested species. Micafungin showed good activity against *C. dubliniensis* even in serum, against *C. kefyr* and *C. lusitaniae* addition of serum dramatically decreased activity, while MICs and killing pattern against *C.*

guilliermondii were similar to those seen in case of *C. parapsilosis* for which the activity of echinocandins is debated. Although serum-based susceptibility testing has not yet been standardized, our results strongly suggest that micafungin and probably other echinocandins should be used with caution not only against the 'psilosis' group but against other rare *Candida* species as well.

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P022

Species distribution and antifungal resistance profiles among *Candida* spp. isolated from bloodstream infections in a Belgian university hospital

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Objective The incidence of candidemia has significantly increased worldwide, representing an important complication in hospitalized patients. The aim of this study was to describe the *Candida* species distribution and the antifungal susceptibility for documented episodes of candidemia at Erasme Hospital in the last 3 years.

Methods A retrospective study was conducted between January 2010 and December 2012 at Erasme Hospital. All hospitalized patients with at least one *Candida* positive blood culture during this period were enrolled. Clinical data were collected by medical chart review. Different episodes of *Candida* bloodstream infections in the same patient were counted. Blood cultures were performed using the Automated BACTEC FX (BD) system. Yeasts were identified using phenotypic methods including chromogenic media, VITEK 2 (BioMerieux) and Maldi-Tof mass spectrometry. In vitro susceptibilities were determined by Sensititre YeastOne. CLSI M27A3 and the recent CLSI species-specific clinical interpretative breakpoints (2012) were used.

Results Fifty-seven candidemia episodes from 53 patients were analysed: 22 in 2010, 22 in 2011 and 13 in 2012. Isolates included *Candida albicans* 21 (37%), *C. glabrata* 21 (37%), *C. tropicalis* 6 (10%), *C. parapsilosis* 4 (7%), *C. krusei* 3 (5%), and *C. guillermondii* and *C. lusitaneae* 1 each (2%). The proportion of *C. albicans* and non-albicans was similar during the three years. Antifungal susceptibility results (Table 1) showed that all isolates were susceptible to amphotericin B and caspofungin. *C. guillermondii* and *C. lusitaneae* were susceptible to all tested drugs. *C. albicans*, *C. glabrata* and *C. tropicalis* were non-susceptible to azoles in 10 to 17% of cases. Twenty-one patients (40%) were admitted or developed candidemia in intensive care department at the moment of *Candida* isolation in blood culture. The more frequent underlying diseases were solid tumor (28%), solid organ transplantation (13%) and cardiovascular disease (11%). Sixteen (30%) patients died during the month after *Candida* bloodstream isolation.

Table 1: Antifungal susceptibility percentages and MIC 50 - MIC 90 for different species of *Candida*

Species (n of isolates)	*C. albicans* (n=21)	*C. glabrata* (n=21)	*C. tropicalis* (n=6)	*C. parapsilosis* (n=4)	*C. krusei* (n=3)
Amphotericin B MIC50-MIC90	100 0.5-0.5	100 0.25-1	100 0.5-0.5	100 0.25-0.5	100 0.5-0.5
Caspofungin MIC50-MIC90	100 0.06-0.25	100 <0.06-0.125	100 0.125-0.25	100 0.5-0.5	100 0.25-0.5
Flucytosine (MIC50-MIC90)	100 <1-<1	100 <0.06-<0.06	100 0.06-0.25	100 0.06-0.125	0 8-32
Itraconazole (MIC50-MIC90)	85 0.125-16	85 0.25-16	83 0.125->8	100 0.125-0.125	100 0.25-0.25
Fluconazole (MIC50-MIC90)	85 0.5-256	85 8-256	83 1-256	100 0.5-1	0 32-64
Voriconazole (MIC50-MIC90)	85 <1-8	90 0.125-4	83 0.03->8	100 <1-<1	100 0.25-0.25

Conclusion Our results showed that *C. albicans* and *C. glabrata* are the most common species causing candidemia. Amphotericin B and caspofungin were the most active antifungal in all *Candida* spp. The frequent cross-resistance to azole (up to 17%) of both albicans and non-albicans strains support the necessity to a rapid identification and a continuous surveillance monitoring including antifungal susceptibility testing.

P023

Prospective evaluation of azole resistance in *Aspergillus fumigatus* clinical isolates in France

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Objectives Azole resistance in *Aspergillus fumigatus* is emerging in Europe, particularly in the Netherlands and UK, but has also been reported in several other European countries such as Austria, Belgium, Denmark, France, Germany, Italy, and Sweden. In France, although azole-resistance in clinical isolates has been detected more than 10 years ago, the prevalence remains largely unknown.

The objective of the present study was to evaluate the prevalence of azole resistance in *A. fumigatus* clinical isolates prospectively recovered over a 1-year period (2012) at two centers in the same city, near Paris.

Methods All isolates of *A. fumigatus* recovered over a period of 1 year (from January 2012 to December 2012) in clinical samples of patients from two centers (Hôpital Henri Mondor (HMN) and centre hospitalier intercommunal Créteil (CHIC)) were prospectively identified and stored for subsequent analysis.

Isolates were revived from storage and in vitro susceptibility to azoles was screened by subculturing each isolate on RPMI agar plates supplemented with itraconazole (4 mg/L) and voriconazole (1 mg/L). *In vitro* resistance was confirmed by the EUCAST reference method. EUCAST clinical breakpoints were used to categorize the isolates. For patients with resistant isolates, clinical data including underlying disease and previous exposure to azole drugs were retrieved.

Results HMN and CHIC are tertiary hospitals with 805 and 509 beds, respectively. Both immunocompromized patients (hematological malignancies, solid organ transplantations (liver, kidney and heart)), and patients with chronic respiratory diseases including cystic fibrosis are hospitalized in these centers.

During the study period, 1737 respiratory samples (sputum, bronchial fluid, BAL) were received at the laboratory for mycological cultures. From these samples, 165 isolates of *A. fumigatus* were recovered from 130 patients.

Three isolates highly resistant to azoles were recovered from 3 patients (Table). Evaluation of the mechanism of resistance involved in these isolates is in progress.

Patient 1 was treated for an aspergilloma (excavated lesion in the left apex) by voriconazole and surgery with a favorable outcome.

Table 1

Patient #	Age (years)	Underlying disease	Type of infection	Sample	Date of isolation	MIC (µg/ml)			Previous azole treatment
						ITZ	VRZ	PSZ	
1	58	Lung cancer	Aspergilloma	Bronchial fluid	06/12/2012	>8	4	1	VCZ
2	62	COPD	Colonization	Bronchial fluid	11/04/2012	>8	4	1	no
3	82	COPD	ABPA	sputum	11/10/2012	>8	4	1	no

COPD: Chronic Obstructive Pulmonary Disease; ABPA: Allergic Broncho-Pulmonary Aspergillosis; MIC: Minimum Inhibitory Concentration; ITZ: Itraconazole; VRZ: Voriconazole

Patient 2 was colonized by *A. fumigatus* in a context of bacterial pulmonary nodules treated by antibiotics. Patient 3 was successfully treated by a combination of antibiotics and corticosteroids for ABPA exacerbation.

Conclusions The present study demonstrated the occurrence of azole resistance among unselected *A. fumigatus* clinical isolates, with an overall prevalence of 1.8%. A larger multicenter study is warranted to better characterize the prevalence of azole resistance in France.

P024

Prospective surveillance of azole resistance in *Aspergillus fumigatus* isolated from patients with cystic fibrosis at Nantes University Hospital, France

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Objectives Azole resistance in *Aspergillus fumigatus* is increasingly reported in several European countries. One mechanism, relying on the TR_{34}/L98H mutation has probably emerged as a consequence of the widespread use of triazole fungicides in our environment. Recently, we reported an 8% prevalence of azole resistance in *A. fumigatus* during a retrospective study in patients with cystic fibrosis (CF)[1]. We found four patients with *A. fumigatus* azole-resistant isolates and demonstrated that resistance was mainly driven by TR_{34}/L98H. Since January 2012, we conducted a prospective survey of azole resistance in CF patients followed up at our center. Here are presented the preliminary results.

Methods All respiratory samples from CF patients received at our laboratory since January 2012 were included prospectively. Routine mycological identification of the mould isolates was based on macroscopic and microscopic morphological characteristics. *Aspergillus* section *Fumigati* isolates were screened for azole resistance by subculture on agar plates containing 4 mg/L itraconazole (ITZ). Each isolate that grew on ITZ-containing agar plates was identified at the species level by β-tubulin gene sequencing and analysis of the full coding region of the *AfCYP51A* gene and its promoter were performed. Minimal inhibitory concentrations of ITZ were determined according to the EUCAST standardized methodology. Data regarding azole exposure were collected.

Results Preliminary analysis allowed us to identify 5 *A. fumigatus* ITZ-resistant isolates (5 patients, table below). All these patients have been previously exposed to azole antifungals. Various alterations in the *AfCYP51*A gene were evidenced. TR_{34}/L98H isolates were found in two patients. Mutations G54E and F219I, both previously linked to azole resistance, were found in two patients (1 and 3). Interestingly, one ITZ-resistant isolate only harbored *AfCYP51A* mutations that have been reported in susceptible isolates suggesting non-Cyp51A-mediated azole resistance.

Conclusions This prospective study of azole resistance in CF patients allowed us to identify 5 additional patients displaying *A. fumigatus* azole-resistant isolates. Azole resistance in CF patients relies on both, *de novo* acquisition of resistant isolates from the environment as a consequence of triazole fungicides use and *in vivo* selection through long-term azole therapy. Finally, these preliminary findings confirm the relatively high prevalence of azole resistance in CF patients at our center and reinforce the need for regular susceptibility testing in this patient population.

Table 1

Patient	Sex/Age	Isolate	Sample	Date of isolation	Promoter/AfCyp51A alteration(s)	Azole exposure
1	F/22	70102550	Sputum	02/01/2012	G54E	Yes
2	M/28	70103434	Sputum	12/01/2012	TR_{34}/L98H	Yes
3	M/24	70105843	Sputum	09/02/2012	F219I	Yes
4	M/28	70108773	Sputum	14/03/2012	TR_{34}/L98H	Yes
5	M/33	70119422	Sputum	13/07/2012	F46Y, M172V, N248T, D255E, E427K	Yes

[1]Morio F, Aubin GG, Danner-Boucher I, Haloun A, Sacchetto E, Garcia-Hermoso D, Bretagne S, Miegeville M, Le Pape P. High prevalence of triazole resistance in *Aspergillus fumigatus*, especially mediated by TR/L98H, in a French cohort of patients with cystic fibrosis. J Antimicrob Chemother. 2012 Aug;67(8):1870–3.

P025

Genotyping and antifungal susceptibility of *Candida glabrata* isolated from patients with candiduria

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Objectives *Candida glabrata* is the second fungal most common of nosocomial infection. In critically ill patients treated in intensive care units, this specie is the main agents of candidemia and candiduria and often associated with high mortality rates, similar to *C. albicans*. The urinary tract infection (UTI) risk by yeast to evolve into blood infection has been increasingly valued. This species should receive special attention due to its high frequency as well as its low susceptibility to antifungal agents, mainly azoles. Nevertheless, little has been published on *C. glabrata* isolates from UTI. The aim of this study was to analyze *in vitro* the antifungal susceptibility and genotypic variability of *C. glabrata* obtained from nosocomial UTI.

Methods To analyze *in vitro* the antifungal susceptibility and genotypic variability 20 isolates of *C. glabrata*. Minimum inhibitory concentration was determined for amphotericin B, ketoconazole, itraconazole, fluconazole and voriconazole according to the document M27-A3. Yeast genotyping was done by three markers of the microsatellite region (RPM2, ERG3, and MTI) and primers P4 and M2 by RAPD (Random Analysis of Polymorphic DNA).

Results Four, six and seven different alleles were found for the loci RPM2, ERG3, and MTI, respectively. The combined discriminatory power of these markers was 0.92. RAPD showed four and eight different profiles, with *SAB* 0.9 ± 0.09 and 0.64 ± 1.00 for primers M2 and P4, respectively. All isolates were susceptible to amphotericin B, however, more than 50% were susceptible-dose dependent or resistant to azoles (Fig 1). The predominant genotype gathered isolates with this resistance profile.

Conclusions Our results suggest that some *C. glabrata* subpopulations are more predominant than others, and that genetic profile it can be associated with the susceptibility profile to antifungals.

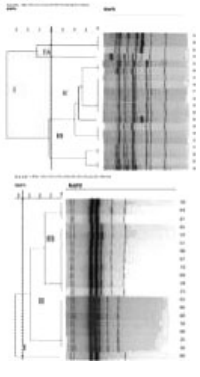
Figure 1. In vitro susceptibility and genotypes generated by microsatellite analyses of *C. glabrata* from urinary tract infection isolates

Fig. (A)

Samples	Genotype	Antifungal susceptibility profile			
		Fluconazole	Voriconazole	Itraconazole	Ketoconazole
59	A	S	S	S	S
81	B	S	DDS	DDS	S
60	C	S	S	S	S
19	D	S	DDS	DDS	S
67	E	S	S	S	S
66		S	S	S	S
58	F	S	S	S	S
68	G	DDS	DDS	R	DDS
23		DDS	S	DDS	DDS
69	H	DDS	S	DDS	S
25		S	S	R	DDS
56		DDS	DDS	R	DDS
24	I	DDS	DDS	DDS	R
37		DDS	DDS	R	R
65		R	DDS	DDS	DDS
80		DDS	DDS	DDS	DDS
82		DDS	DDS	DDS	DDS
61	J	DDS	S	DDS	S
67		S	DDS	DDS	S
64	K	DDS	S	S	DDS

S: Susceptible; DDS: dose-dependent susceptible; R: Resistant

Fig. (B)



P026

In vivo and *in vitro* resistance mechanisms to voriconazole in clinical *Candida krusei* strains

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Objectives We assessed the mechanisms of resistance to voriconazole (VOR) acquired *in vivo* and *in vitro* by clinical *C. krusei* strains.

Methods Five *C. krusei* successive isolates were recovered from the urine of a kidney transplant patient who received a 3-week VOR treatment: isolate 628 L (before therapy), isolates 633L and 640L (9th and 16th day of VOR therapy), 657L and 671L (10th and 20th days after VOR discontinuation). We genotyped the isolates using both microsatellite analysis and intergenic repeat-PCR (CKRS-1). In addition, we chose four independent clinical strains of *C. krusei* that were VOR-susceptible and isolated from VOR-free patients (P1, P21, P24, P34). They were grown in brain-heart infusion (BHI) containing 0.001 mg/l of VOR, with daily subcultures in fresh BHI-VOR for 30 days, in order to obtain VOR-resistant derivatives. VOR MICs, in the presence or not of 100 mg/l of the efflux pump inhibitor FK506, were assessed according to CLSI M27-A3 protocol. The expression of *ABC1* and *ABC2* genes (coding for ATP dependent efflux pumps) and *ERG11* gene (coding for lanosterol-14α-demethylase target enzyme) was assessed by RT-qPCR using *ACT1* gene (coding for actin) as the reference. VOR-resistant isolates with an expression of the target genes higher than 2-times that of its susceptible parent was considered as an overexpressing isolate.The expression of genes in the pre-treatment isolate was used as reference and set at 1.0.

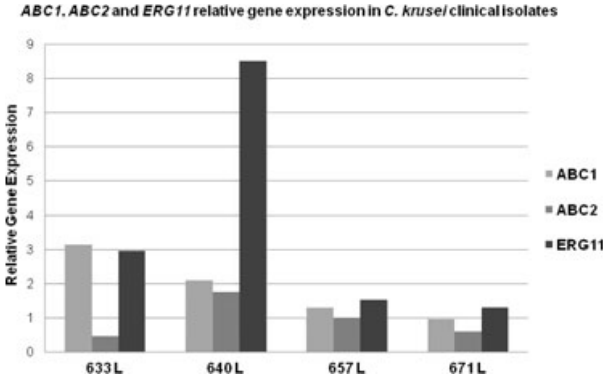

Figure 1

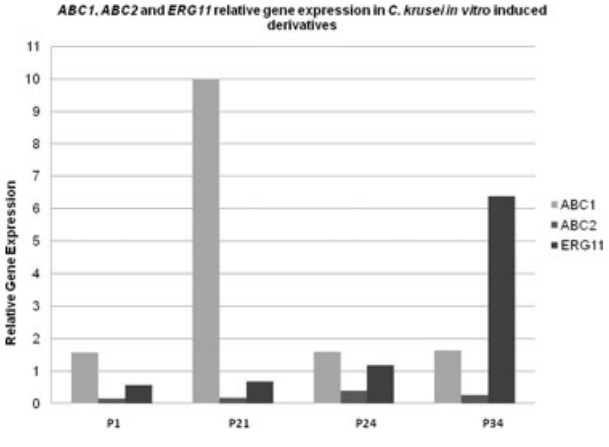

Figure 2

Results All the strains isolated from the kidney transplant patient were clonal. VOR MIC increased during therapy from 0.25 (isolate 628L) to 4.0 mg/l (isolates 633L and 640L) and decreased after VOR discontinuation (isolates 657L and 671L, VOR MICs 0.25 mg/l). The efflux inhibitor FK506 restored the susceptibility of the VOR-resistant isolates (from 4.0 to 0.125 mg/l). *In vitro* exposure of VOR-free *C. krusei* clinical strains to low VOR concentrations generated VOR-resistant derivatives with MICs ranging from 4 to 16 mg/l. Here again, the efflux inhibitor FK506 restored the susceptibility to VOR. *ABC1*, *ABC2* and *ERG11* expression is detailed in Figures 1 and 2.

The expression of *ABC1* was higher than that of *ABC2* in both *in vivo* and *in vitro* VOR-resistant isolates. The expression of *ABC1* and *ERG11* was increased in *in vivo* VOR-resistant isolates (633 L, 640 L) and went back to basal level in post-therapy isolates (657 L, 671 L). Surprisingly, the four *in vitro* VOR-resistant isolates showed different gene expression profiles: two (P21, P34) overexpressed exclusively *ABC1* and *ERG11*, respectively, while the two others overexpressed none of the tested resistance genes.

Conclusions The VOR-resistant isolates of *C. krusei* overexpressed *ABC1* and/or *ERG11*. The restoration of the susceptibility to VOR by FK506 confirms the role of the efflux in the resistance to VOR. Our results highlighted the diversity of resistance mechanisms to azole in *C. krusei.* Interestingly, two VOR-resistant isolates did not overexpress *ABC1*, *ABC2* or *ERG11* suggesting yet unknown resistance mechanisms.

P027

Comparison of the 'susceptible-intermediate-resistant' categorization of fungal clinical isolates in four mycology departments using previous breakpoints and newly revised 24 h clinical breakpoints

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Context Early and optimal antifungal therapy is an essential element for a successful outcome of invasive fungal infections. The emergence of resistance to antifungal agents including the latest triazoles and echinocandins enhances necessity of a rapid and reliable determination of in vitro MIC. New CLSI clinical breakpoints (CBPs) have been proposed for an interpretation of MICs after 24 hours (Pfaller, JCM 2012).

Objectives

– Compare the interpretation of MICs of fluconazole, voriconazole and caspofungin against 18 clinical isolates in four hospital centres, using previous CLSI breakpoints (BPs) and newly revised CLSI CBPs.

– Determine if the new 24H CLSI CBPs take into account a late apparition of resistance (after 48 h incubation), particularly for azoles.

Methods 160 MICs of 18 clinical isolates were determined in four labs of Mycology from French University hospitals (Poitiers, Reims, Rennes and Toulouse).

Strains: 5 Candida species (6 *C. glabrata*, 4 *C. krusei*, 4 *C. parapsilosis*, 2 *C. albicans* and 2 *C. tropicalis*)

Antifungal agents: fluconazole (except for *C. krusei*), voriconazole (except for *C. glabrata*), caspofungin were tested.

Antifungal susceptibility profile of the strains: 8 of 18 strains (44%) were chosen because presenting a decrease susceptibility for at least one antifungal molecule.

In vitro antifungal susceptibility study: MICs were determined using the E-test method® on RPMI agar medium.

Reading: at 24 h and 48 h.

Interpretation of MICS values: the categorization using new 24 h-CBPs was compared to the categorization obtained at 24 h and 48 h using previous BPs. The respective categorical agreements 24 h-CA and 48 h-CA were evaluated.

Results The 24 h-CA and 48 h-CA were 81%, 76,2%, 88,6%, 78% and 88,1%, 85,7%, 94,3%, 87,8% respectively for Poitiers, Reims, Rennes and Toulouse centres. Comparing interpretation using new 24 h-CBPs and previous 48 h-BPs, we obtained a different categorization for 18 of the 160 MIC (11,25%). Two late resistances at 48 h were not detected using the new 24 h-CBPs (SDD) for 2 pairs of *C. glabrata*/fluconazole (classed SDD). Three categorizations S (n = 1) or SDD (n = 2) at 48 h were ranged R using the new 24 h-CBPs, respectively *C. krusei*/caspofungin, *C. parapsilosis*/voriconazole and *C. parapsilosis*/fluconazole. The remaining 13 cases (8 strains) were categorized "S"using the previous BPs and "S"with the new 24hCBPs.

Conclusion The MIC determination at 24 h with application of the newly revised CBPs provide a good CA with categorization at 24 h and 48 h using previous BPs. The CA was better with 48 h-values in all centres, underlining that, in most cases, these new 24 h-CBPs take into account late apparition of an elevated MIC.

P028

Uncovering yeast recovery pathway to liposomal amphotericin B-induced stress

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Objectives Liposomal amphotericin B (L-AmB) is antifungal that targets to ergosterol, the principal sterol in the fungal cytoplasmic membrane. L-AmB exhibits broad fungicidal activity at very low concentrations. Nonetheless, in spite of the observed high in vitro susceptibility, in vivo response to L-AmB is often reduced. Pharmacokinetic studies indicate that serum levels decline rapidly during the first 2 h after the administration. In order to understand what happens to yeasts exposure to L-AmB serum concentrations, a kinetic study was performed in *Candida* spp., *Saccharomyces cerevisiae* and *Debaryomyces hansenii* by flow cytometry.

Methods Two *Candida* spp. clinical strains, one *S. cerevisiae* and one *D. hansenii*, all susceptible to liposomal amphotericin B were studied. Cells were incubated for 90 min with L-AmB 3 μg/ml, then submitted to three treatment conditions: 3, 0.3 and 0 μg/ml of L-AmB and incubated at 35°C, 180 rpm. After 90 min, 3 h, 6 h and 24 h of incubation cells were stained with $DiBAC_4$, (a membrane potential fluorescent probe) for 30 min in the dark, and analyzed in a FACSCalibur cytometer at FL1 (530 nm). Time-kill experiments were also performed to validate flow cytometry results.

Results The results showed that yeast cells are totally depolarized following exposure to L-AmB 3 μg/ml for 24 hours. Conversely, when exposed to the same concentration only for 90 min followed by 0 or 0.3 μg/ml of L-AmB in the remaining hours, the population re-polarizes following 3 hours of incubation only. Time-kill assay performed under the same conditions showed that cells exposed to 3 μg/ml of L-AmB (90 min, 3 h, 6 h or 24 h) are unable to replicate. However, in the presence of lower L-AmB concentrations, cells recovered the replication capacity. This phenomenon was observed in *Candida* spp., *S. cerevisiae* and *D. hansenii*.

Conclusions The expected L-AmB fungicidal effect is only observed when the concentration is high for long periods of time. The phenomenon of cell recovery at lower concentrations seems to be common on the studied yeasts, a conserved pathway should be present and can be related to a poor outcome.

This work was supported by Gilead Sciences.

P029

In vitro susceptibility testing of *Candida albicans* by using Micronaut-Am method

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Objectives Microbiologists are looking for standardized AFST (antifungal susceptibility testing) to determine the minimal inhibitory concentration (MIC) without *trailing effect* and to monitor *Candida* resistance. The knowledge concerning antifungal resistance rate among *Candida* sp. is still lacking, thus a susceptibility testing of superficial yeast is of great importance to improve it and finally to better manage the treatment of opportunistic infections. The aim of the study was to determine the susceptibility of *Candida albicans* isolates using AFST systems.

Methods 170 *Candida albicans* strains were tested against six antifungals using MICRONAUT-AM system and doubt results were verified by E-test and reference procedure.

Results A total of *Candida albicans* isolates were susceptible to ketoconazole, fluconazole, voriconazole and 5- flucytosine according to CLSI while 4 (2%) were considered intermediate to fluconazole (MIC = 4 μg ml^{-1}) by EUCAST. Thirteen isolates (8%) were resistant to itraconazole (CLSI). Up to 48 isolates (28%) exhibited MIC of amphotericin B – 2 μg ml^{-1} but the MIC values were significantly reduced by E-test (0.094–0.38 μg ml^{-1}). *Trailing endpoints* for azoles were clearly inhibited.

Conclusion MICRONAUT-AM system seems to be a useful tool for *in vitro* yeast susceptibility testing to azoles while E-test is still the most reliable to determine MIC of amphotericin.

P030

Molecular identification and antifungal susceptibility of clinical *Scedosporium/Pseudallescheria* isolates from a Spanish teaching hospital

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Objectives The taxonomy of the *Scedosporium/Pseudallescheria* species complex has been extensively modified in recent years. However, there is still limited data about the rate at which the different species of this fungal group are recovered from patients. In this study we identified to the species level a collection of *Scedosporium/Pseudallescheria* isolates using molecular techniques. Additionally, for all available isolates we also determined their antifungal susceptibility.

Methods We studied 91 clinical isolates collected from 43 patients attended at our institution from 1996 to 2012, and morphologically identified as *Scedosporium* spp. Molecular identification of isolates was performed by partial sequencing the beta-tubulin encoding gene. Susceptibility to amphotericin B (AMB), isavuconazole (ISA), itraconazole (ITZ), posaconazole (POS), voriconazole (VCZ), anidulafungin (AND), caspofungin (CAS) and micafungin (MYC) was evaluated by the CLSI broth microdilution method.

Results The following species (isolates/cases) were identified: *Pseudallescheria boydii* (PB, 37.4%/37.2%), *Scedosporium apiospermum* (SA, 24.2%/32.6%), *S. prolificans* (SP, 37.4%/27.9%) and *P. ellipsoidea* (PE, 1%/2.3%). The results of antifungal susceptibility testing are shown in the table.

Table 1

		PB	SP	SA	PE
AMB	range	4-16	16-16	2-16	2
	MIC_{90}	16	16	16	NA
ISA	range	0.25-2	8-16	1-4	0.5
	MIC_{90}	2	16	4	NA
ITZ	range	1-2	16-16	1-4	1
	MIC_{90}	2	16	4	NA
POS	range	0.5-1	16-16	0.5-1	0.5
	MIC_{90}	1	16	1	NA
VCZ	range	0.125-0.25	4-16	0.125-0.5	0.25
	MIC_{90}	0.25	16	0.5	NA
AND	range	2-8	8-16	4-8	8
	MIC_{90}	8	16	8	NA
CAS	range	4-32	16-32	16-32	32
	MIC_{90}	32	32	32	NA
MYC	range	128-128	128-128	128-128	128
	MIC_{90}	128	128	128	NA

NA: not applicable (a single isolate was available)

Conclusion Molecular-based methods are of great help in discriminating between morphologically similar species of *Scedosporium/Pseudallescheria* which can nevertheless show important differences in antifungal susceptibility and, consequently, in therapy.

S. Álvarez-Pérez is recipient of a 'Sara Borrell' contract (Instituto de Salud Carlos III, CD12/00419).

P031

Emergence of multi triazole-resistant *Aspergillus fumigatus* carrying the TR46/Y121F/T289A mutations in cyp51A gene from Indian environment

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Objective Aspergillosis due to multi-triazole resistant (MTR) *Aspergillus fumigatus* strains occur both in azole treated as well as in azole naïve patients. The commonly reported mechanism of MTR, TR34/L98H has increasingly been reported from Dutch patients and their environment as well as in other European countries and more recently from China, India and Iran. Molecular studies from Europe and India suggest that clinical azole resistant isolates originated from environmental or fungicide driven route of resistance development. Recently, a new resistance mechanism TR46/Y121F/T289A in *cyp*51A gene responsible for voriconazole resistance in *A. fumigatus* has been reported in clinical and environmental isolates from the Netherlands. This study investigated occurrence of TR46/Y121F/T289A in *A. fumigatus* strains from the environment in India. We also studied cross resistance of environmental TR46/Y121F/T289A *A. fumigatus* isolates to commonly used azole fungicides in India.

Methods A total of 102 environmental soil samples from the agricultural fields of Yamuna bank, Delhi (n = 50) and Varanasi, Uttar Pradesh (n = 52), were investigated during October 2012 to April 2013. The samples were inoculated on Sabouraud dextrose agar (SDA) plates and *A. fumigatus* colonies were subcultured on SDA plates supplemented with 4 μg/mL itraconazole and 1 μg/mL voriconazole. Identification of ITC+ and VRC+ *A. fumigatus* strains was confirmed by ITS, β- tubulin and calmodulin gene. All the ITC+ and VRC+ *A. fumigatus* were subjected to a mixed-format real-time PCR assay for detection of mutations and genotyped with microsatellite analysis. *In* vitro activity for all the standard azole antifungals and the 8 azole fungicides (bromuconazole, cyproconazole, difenoconazole epoxiconazole, metconazole, penconazole, tebuconazole and triadimefon) most commonly used in India were investigated using CLSI M38-A2 broth microdilution.

Results Of 102 soil samples, 48(47%) showed *A. fumigatus*. Of these, 126 colonies of *A. fumigatus* were isolated on SDA. A total of 10 isolates grew on 1 μg/mL voriconazole (6 from Varanasi, U.P) and 4 μg/mL itraconazole (4 from Delhi) SDA. Six VRC+ *A. fumigatus* originated from 2 soil samples of Varanasi. Of these, 5 (83.3%) were from a potato field and 1 (16.6%) was from *Trigonella foenum-graecum* (common name Fenugreek) field. Four ITC+ *A. fumigatus* isolates originated from Yamuna bank, Delhi. Overall, 5.8% of the samples harbored voriconazole and 6.8% had itraconazole resistant *A. fumigatus*. The geometric mean MICs of 6 VRC+ *A. fumigatus* isolates showed reduced susceptibility of voriconazole (10.07 μg/mL) and isavuconazole (8 μg/mL). These isolates exhibited low itraconazole MICs (1.78 μg/mL) but showed cross- resistance to all the fungicides tested. All six VRC+ *A. fumigatus* isolates exhibited the TR_{46}/Y121F/T289A mutation and had the same genotype as Dutch isolates. In addition 4 ITC+ *A. fumigatus* were of genotype TR34/L98H.

Conclusion This is the first environmental survey outside Europe revealing emergence of the new resistance mechanism TR46/Y121F/T289A in *A. fumigatus*. Microsatellite typing of these environmental isolates showed them to be very similar to Dutch clinical isolates. The widespread application of triazole fungicides and its persistence in the environment could have contributed to spread of azole resistant *A. fumigatus.*

P040

Suprascapular chromoblastomycosis: a case report from Portugal

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Chromoblastomycosis is a fungal infection of the skin and subcutaneous tissues, caused by various species of dematiaceous fungi. The disease is predominant in tropical and subtropical climates, but has been reported worldwide.

We report a caucasian 76 year-old man, living in a rural area, woodcutter, observed in the outpatient consultation for unilateral and asymmetrical erythematous and vegetating confluent plaques covered with scales, located on the left scapular region, with indolent evolution of several years.

The clinical diagnosis hypothesis were: tuberculosis, mycobacteriosis, cutaneous lymphoma or squamous cell carcinoma.

Skin scales and punch biopsies were performed for mycological and histopathological tests. The direct examination done with potassium hydroxide (KOH) showed muriform brown cells with thick walls, a double membrane and a central septum. Cultures were done in several media with and without antibiotics and incubated at 24º and 37º C for 3 weeks, and allowed the isolation of velvety and black colonies. Slide culture on diluted Sabouraud led us to identify those colonies as *Phialophora verrucosa.* The histopathology showed a granuloma with marked hyperplasia, as well epithelioid, giant and muriform cells.

Taking into account the size and location of the lesions and patient's age a combined surgical and cryosurgery treatment was done with marked improvement. There is no clinical or laboratory evidence of the infection in the last evaluation.

Chromoblastomycosis is caused by traumatic implantation of fungal elements into the skin, from fungi in soil, plants wood and plants, which accounts for its increased frequency among farmers and rural workers.

On a more careful anamnesis, we found that the patient, carried baskets of firewood on the back. This fact justifies the location of the lesions.

P041

Identification of clinical relevant fungi by rDNA sequencing and multilocus PCR coupled with electrospray ionization mass spectrometry

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Objectives We evaluated the performance of two molecular methods, i.e, rDNA sequencing, and multilocus PCR coupled with electrospray ionization mass spectrometry (PCR/ESI-MS), for the identification of a series of clinically important yeasts and filamentous fungi including 13 reference strains and 99 clinical/environmental isolates.

Methods For all fungal strains, the following three regions of rDNA were amplified and sequenced in our study: the 5' end of the large subunit rRNA gene (D1/D2 region), the internal transcribed spacer 1 (ITS1), and the internal transcribed spacer 2 (ITS2). For the identification of both yeasts and filamentous fungi, as revealed from our results, combined sequencing of D1/D2, ITS1, and ITS2 regions had the best performance (species level identification rate, 96.9% and 84.8%, respectively).

Results When tested separately, better identification performance was achieved by D1/D2 for yeasts (93.8% species level identification), while the ITS1/ITS2 combination were better for identification of filamentous fungi (84.8% species level identification). For PCR/ESI-MS,the broad fungal assay kit was used, and results were evaluated against microbial identifications (Micro IDs), which was acquired by combining results of phenotypic method and rDNA sequencing. With regard to the 65 yeasts that fell within the range of identifiable species according to the instructions of the broad fungal assay kit, 93.8% (61/65) achieved species level identification, and 100% (61/61) of which were concordant to microbial identification on species/species complex level. For 37 strains filamentous fungi that were covered by the instructions of the broad fungal assay kit, 94.6% (35/37) achieved either species (75.7%, 28/37) or genus (18.9%, 7/37) level identification. Among the 35 filamentous fungi identified by PCR/ESI-MS, the species/species complex and genus level concordance rates were 80% (28/35) and 97.1% (34/35), respectively.

Conclusions The rDNA sequencing and pairwise alignment method provides the most objective identification information, and better results are acquired by combination of ITS1, ITS2 and D1/D2 sequencing. The high identification accuracy of PCR/ESI-MS analysis, along with its ability to characterize mixtures of organisms, short turnaround time, and low DNA concentration requirement, makes it a promising method in both fungi identification and detection.

P042

Detection of (1,3)-β-D-glucans *in situ* in a *Candida albicans* brain granuloma

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(1, 3)-β-D-glucan (BDG), a major fungal cell wall component, is a useful biomarker of invasive fungal infections (IFI), particularly candidosis, aspergillosis and pneumocystosis. The European Organization for the Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) included this test for the diagnosis of IFI using serum samples. Nowadays, BDG is a helpful non-invasive biomarker to screen patients at high risk of IFI. Some studies have reported BDG detection in other samples: bronchoalveolar lavage, cerebrospinal and peritoneal fluid. To our knowledge, no studies have dealt directly with BDG testing in tissues.

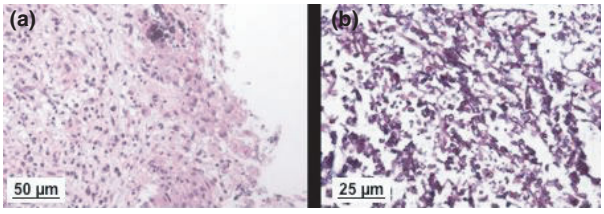

Figure 1 (a) Haematoxylin-eosin-safran staining, magnification x200. (b) Periodic acid Schiff staining, magnification x400.

We recently diagnosed a *Candida albicans* brain granuloma in an immunocompetent 2-year- old child. The medical history included prematurity, with ventricular bleeding and hydrocephaly complications, requiring a ventriculoperitoneal shunt. A left frontal mass was observed on an annual brain CT-scan and was confirmed by MRI. The child was asymptomatic. A stereotaxic biopsy of the lesion was then performed and stained with toluidine blue O. Direct microscopic examination of the specimen was negative. Cultures of biopsy and cerebrospinal fluid (CSF) were negative. However, examination of histopathological sections after Haematoxylin-eosin-safran and Periodic acid Schiff staining revealed fungal elements, including pseudomycelia and blastoconidia with granuloma (Figure 1a and 1b). BDG (Fungitell; Associates of Cape Cod, Inc) and *Candida* mannan (Mn) antigen (Platelia™; Bio-Rad Laboratories, Marnes-la-Coquette, France) tests on serum and CSF samples were negative. Brain biopsy (volume: 3 mm^3) was homogenized in a Potter grinder, suspended in 2 ml glucan-free water in an aseptic environment (reagent grade water) and centrifuged at 2500 g. Tests for BDG and Mn carried out on the homogenate supernatant were positive for both markers at a dilution of 1:8 (BDG: >500 pg/mL, Mn: >500 pg/mL), suggesting a *Candida* infection. PCR sequencing of brain biopsy was performed, targeting the transcribed intergenic spacers ITS1 and ITS2. This revealed a *C. albicans* specific sequence, with 100% identity with that of the *C. albicans* ATCC 18804 type strain (sequence deposited in GeneBank, accession number: KC585058). First-line therapy including liposomal amphotericin B and flucytosine was then initiated.

This case highlights the usefulness of BDG and Mn testing of biopsy material, when cultures remain negative. This non-invasive, rapid test may be useful for the diagnosis. In this case, the BDG test supported the histopathological observations and was very useful for patient management.

This case also illustrates *Candida* granuloma formation in an immunocompetent host, as it has been shown in an *in vitro* model that *Candida* spp., surrounded by a cellular response (macrophages, cytotoxic and T-helper cells), can persist in granulomatous structures. This defence mechanism is probably involved in the exclusion of fungal cells and mycelia from tissues. Altogether, our observations demonstrate that BDG detection is a useful alternative to culture-based methods for the diagnosis of fungal tissue invasion. In contrast to serum and CSF samples which remained negative, high levels of BDG were detected in ground biopsy material and granuloma lysis. This approach deserves to be extended to other culture-negative tissue biopsies where fungal elements are observed by direct examination.

P043

Rapid identification and susceptibility testing of yeasts from positive blood cultures by combination of direct MALDI-TOF mass spectrometry and direct inoculation of Vitek-2

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Objectives Since *Candida* septicemia is associated with high mortality rates and increasing resistance rates have to be considered, choice of appropriate antifungal therapy is essential for patient outcome. Slow growth of yeasts hampers timely species identification (ID) and antifungal susceptibility testing (AFST). Therefore, an approach for combined direct ID and direct AFST without prior time-consuming sub-culturing of yeasts from positive BCs is urgently needed.
Methods MALDI-TOF mass spectrometry (MS) using Sepsityper kit (Bruker Daltonik GmbH) was performed for direct ID from 15 blood culture bottles positive for yeasts as indicated by Gram stain. After lysis of blood cells, wash steps and ethanol/formic acid (E/FA) protein extraction, yeast cell pellet was used for MALDI-TOF MS. The same yeast cell pellet (except for the extraction steps) was used for suspension preparation and direct inoculation of Vitek-2 (bioMérieux, Marcy l'Etoile, France) for AFST (Vitek-2 Card AST-YS06). For comparison, MALDI-TOF MS ID and Vitek-2 AFST were performed from yeast subculture on Kimmig agar after 24 h incubation at 36°C. Viable cell count was performed on positive BC bottles, on suspension prepared from cell pellet and on standardized suspension (1.8–2.2 McFarland turbidity) from 24 h sub-cultures.
Results Applying expanded thresholds for species ID (score $\geq$ 1.7 with 3 identical consecutive propositions), 40.0% of BCs were identified by direct MALDI-TOF MS while MALDI-TOF MS from 24 h cultures provided valid result in 86.7% and 80.0% of BCs with and without E/FA extraction, respectively. AFST results could be generated for 85.7% of cultures directly inoculated into Vitek-2 (75.0% with complete AFST profiles) and for 100% (complete AFST profiles in all cases) of standardized suspensions from 24 h cultures. Thus, AFST comparison was possible for 49 isolate-antifungal combinations. Essential agreement (minimum inhibitory concentration difference $\leq$1 double dilution step) was 89.8%. Very major errors (false-susceptibility), major errors (false-resistance) and minor errors (false categorization involving intermediate result) amounted to 50% (3 false results for each fluconazole and voriconazole), 0% and 0% providing 87.8% categorical agreement. Mean viable count was 9.1×10^7 cfu/ml and 1.0×10^7 cfu/ml for BCs with successful direct ID (score >1.7) and failed direct ID, respectively. For suspension from cell pellet and for standardized suspension from 24 h cultures, mean viable count was 0.6×10^7 cfu/ml and 1.0×10^7 cfu/ml, respectively. Mean sample proceeding time required for Sepsityper kit was 18 min for one sample (51 min in total for direct MALDI-TOF MS including E/FA extraction and drying time). This method saved in average 23.7 h compared to routine ID by MALDI-TOF MS performed with 24 h cultures. For AFST, the result from direct inoculation of cell pellet suspension into Vitek-2 was available 18.2 h earlier than the result from standard method.
Conclusion Direct MALDI-TOF MS using Sepsityper kit with yeast positive BCs combined with direct Vitek-2 inoculation from Sepsityper pellet accelerates ID and AFST for a part of samples, however, testing from subculture remains indispensable to validate the direct finding.

P044

Bronchoalveolar lavage lateral-flow device test for diagnosing invasive pulmonary aspergillosis: a multicenter study

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Background Timely diagnosis is a key factor in successful treatment of invasive pulmonary aspergillosis (IPA). Limitations of galactomannan (GM) testing are varying turnaround time and availability. These limitations may be overcome by the Lateral- Flow Device (LFD) test, a single sample point-of-care test that is based on the detection of an Aspergillus extracellular glycoprotein antigen by monoclonal antibody JF5. This study evaluates the LFD test by using bronchoalveolar lavage (BAL) samples.
Methods A total of 173 BAL samples from 167 patients that were tested routinely for GM between between 2011 and 2013 at the Austrian University Hospitals of Graz (98 samples) and Innsbruck (75 samples) were included. 53 had probable/proven, 52 possible, and 68 no IPA. Diagnostic accuracy of LFD for probable/proven IPA was evaluated. For IPA grading fungal cultures as well as BAL GM (cut-off 1.0) were used.
Results Sensitivity and specificity of LFD test for probable/proven IPA were 75.5% and 84.2%, respectively (Graz: 95% and 84%; Innsbruck: 63% and 84%). PPV was 68%, NPV 89%. BAL GM levels were available in 108 samples (98 from Graz, 10 from Innsbruck) and significantly lower in patients with negative than in those with positive LFD result ($P < 0.0001$). LFD resulted negative in 13 patients with probable IPA. BAL GM was tested in two of those only and revealed levels of 1.23 and 1.35. LFD resulted positive in a total of 15 patients with possible and 4 without IPA (weakly to moderately positive LFD result in 17/19 cases). In 7 of these cases corresponding GM values were between 0.6 and 0.7.LFD resulted negative in proven/probable cases of infection due to Mucorales, Scedosporium spp., Fusarium spp., and Cryptococcus neoformans.
Conclusions The LFD test of BAL specimens is performed easily and provides accurate and rapidly available results. Therefore, this new point-of-care test may be a very promising diagnostic approach for detecting IPA in BAL specimens.

P045

Comparision of galactomannan and beta-D-glucan serum assays for diagnosis of invasive fungal infections in hematologic malignancy patients

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Background Galactomannan (GM) testing is an important tool for diagnosis of invasive fungal infections (IFI). In contrast, the role of beta-D-Glucan (BDG) assay is not fully evaluated yet and sensitivity and specificity varies between trials and centres. We compared performance of serum GM and BDG for IFI diagnosis in patients with hematologic malignancies.
Methods This prospective study was performed at the Division of Haematology, Medical University Graz for 6 months in 2012. Patients at risk for/with clinical suspicion of IFIs were included.

Twice weekly BDG and GM were evaluated and results compared to clinical+radiological findings, BAL-GM, culture and histology results.
Results GM and BDG were measured in 719 serum samples obtained from 98 patients (31/98 with alloHSCT). 8/98 cases had possible, 24 probable IFI and 3 proven candidemia according to modified EORTC criteria; 63 had no IFI. In 11 probable cases mycological evidence was brought by BDG-testing only. These cases would have been categorized as possible IFIs in absence of BDG-testing. Sensitivity of BDG for probable/proven IFI was 68.3% when single values were compared to GM values. Sensitivity per patient with probable/proven IFI was 89%. Specificity was 87.5% for BDG and 97.6% for GM. In 13/27 probable/proven IFIs BDG-assay became positive before fulfillment of clinical/radiological criteria (median 5 days, IQR 1,5–14.5 days).
Conclusions BDG-testing resulted in a significant increase (11.2 to 24.4%) of probable IFIs. Further, positive BDG-assay predicted IFIs in about 50% of cases; drawback, however was the lower specificity when compared to GM testing. Combination of BDG and GM-testing may be a sensitive method for ruling out IFIs among patients with hematologic malignancies.

P046

Rapid identification of five clinically relevant *Candida* species using multiplex PCR and high-resolution melting analysis

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Objectives Systemic candidiasis remains an increasing source of morbidity and mortality in immunecompromised patients. Even though *Candida albicans* is the most common and clinically relevant pathogen of the genus, the emergence of other species, such as *C. glabrata, C. parapsilosis* and *C. Kefyr,* resistant to antifungal agents, makes the accurate identification of *Candida*at species level crucial for the determination of an appropriate antifungal therapy.

The High-Resolution DNA Melting Analysis (HRMA) represents the most recent advance in the diagnosis of fungal infections.
Methods We describe the development and evaluation of single-tube multiplex PCR methods and HRMA analysis for the detection of medical relevant *Candida spp*. (*C. albicans*, *C. glabrata, C. kefyr, C. parapsilosis* and *C. guilliermondii*) and *Saccharomyces cerevisiae* using primers selected within the *C. albicans* gene encoding a 65 KDa mannoprotein (MP65). This technique consists of a 2 min rapid closed-tube assay using a saturating dye of double-stranded DNA (LCGreen): melting curves from different amplicons can be differentiated on the basis of shape, by heating the tubes during data acquisition at 79–100°C using HRM software. We applied HRMA to our collection of fungi using only a primers pair (ALL1–ALL2), previously described.
Results We tested the reproducibility of this assay in triplicate samples using, for each reference species, 1–500 ng of chromosomal DNA. We obtained optimal reproducibility with 10–50 ng chromosomal DNA per PCR reaction tube. We tested the sensitivity in blinded experiments, on DNAs extracted from simulated serum samples spiked in triplicate with 10^4 cells/ml of different strains of *Candida* and *S. cerevisiae*. Also in this case we successfully differentiated the *Candida* species and *S. cerevisiae*
Conclusions HRMA is an effective method to easily and rapidly identify different *Candida* spp. The risk of contamination is far lower than in a multi-step procedure. It is less expensive and more readily adaptable to *Candida* detection in clinical laboratories.

Recently, we started a collaboration in a multicenter study, being the primary objective of the project the evaluation of the combined use of HRMA, – a method developed by us – and BG antigen, in the diagnosis of invasive candidiasis in critically ill patients hospitalized in the ICU (Intensive Care Unit) for a therapeutic strategy.

The study enrols patients admitted to the ICU with a >0.5 colonization index or with suspected sepsis, patients undergoing surgery for perforation of the digestive tract and immunocompromised patients in general. The proven and possible cases of candidiasis will be defined upon the criteria of the European Organization of Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) that have been amended in view of the application in non-neutropenic patients. For each patient and for a period of two weeks, a monitoring will be provided twice a week performing the research of fungal DNA by means of HRMA, and of BG antigen, on the same sample of serum.

P047

PCR-based detection of *Acremonium strictum*

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Objectives Fungi of *Acremonium* genus are ubiquitous in the human environment. They are considered as common contaminants of laboratory and food samples. Human infections caused by *Acremonium* (one of the prevalent species is *A. strictum*) are not very frequent, but in opposite to the other filamentous fungi they are related to individuals with proper immunity. The most common manifestation of *Acremonium* infection is mycetoma developing after trauma (*Medicine (Baltimore)*, 1991, 70(6): 398–409). Ulceration and nodular infiltrations (*hialohyphomycosis*) as well as onychomycosis could appear. There were also reported cases of keratitis, endophthalmitis, endocarditis, meningitis and osteomyelitis caused by *Acremonium (Medicine (Baltimore)*, 1991, 70(6): 398–409) (*J Am Acad Dermatol*, 1999, 41: 938–44) (*Cutis*, 2011, 88(6): 293–5, 299). In addition, *Acremonium* are capable of developing opportunistic infections e.g. in patients after bone marrow transplantation (*Medicine (Baltimore)*, 1993, 72, 78–89) or prematurely born children (*J Med Microbiol*, 2003, 52(9), 835–837).

Traditional methods applied for detection and identification of fungi are generally based on examination of morphological, serological an biochemical features. Fungi belonging to *Acremonium* spp. must be distinguished especially from *Ascocoryne*, *Fusarium*, *Lecythophora*, *Phialemonium*, *Phialoacremonium*, *Sporothrix* and *Verticillium* genera. Identification of fungi is often laborious and time-consuming, so more and more often molecular methods, based on the examination of nucleic acids, are applied. Molecular diagnostics methods have many advantages: high sensitivity, repeatability, specificity and discrimination potential, as well as the short time of the test. Moreover, procedures and results interpretation are quite easy to perform and they are not related to the researcher's experience, so there is a possibility to identify even rare species.

The aim of the study was to develop *Acremonium strictum* detection kit based on PCR.
Methods The study included DNA isolated from 208 fungal specimens (moulds, dermatophytes, *Candida*) using Brillowska-Dąbrowska method (*J Clin Microbiol,* 2007, 45(4): 1200–1204).

Primers Acr1for247 and Acr1rev247, specific for *A. strictum*, were designed on the basis of the fungal rDNA nucleotide sequences comparison. Then PCR conditions were optimized: 94°C – 180 s; 35 cycles: 94°C – 30 s, 59°C – 30 s, 72°C – 30 s; 72°C – 600 s. Finally, PCR results were visualized by agarose gel electrophoresis.
Results Characteristic products (247 bp) were obtained only when DNA of *A. strictum* was present in a sample.
Conclusion This study shows the utility of Acr1for247 and Acr1rev247 primers in detection of *A. strictum*. Proposed PCR protocol of *A. strictum* detection and identification is much shorter than the traditional method of *A. strictum* identification. Moreover, the test performance is easy and not related to the researcher's experience that makes it possible to identify this rare human pathogen even in non-reference laboratories.

P048

Development and validation of a quantitative real-time PCR assay for the early diagnosis of coccidioidomycosis

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Objectives Coccidioidomycosis is endemic in the southwestern United States and Latin America. Although primary disease is self-limited, coccidioidomycosis also presents with disseminated disease, especially in immunocompromised patients. Early diagnosis of coccidioidomycosis is difficult, as most clinical signs are non-specific, and cultures often negative. Moreover, serology may be also negative, especially early after infection or not indicative of acute infection. Thus, the development of molecular methods for early diagnosis is needed. The aim of this study was the development of a Real-Time PCR assay for the early diagnosis of coccidioidomycosis, and validation of the assay in a murine model of disseminated infection.
Methods A Real-Time PCR (RT-PCR) was developed for the detection of *Coccidioides immitis* and *C. posadasii* DNA. Specific primers and a molecular beacon probe were designed to amplify 267 bp from the ITS2 region of the rDNA. An internal control, based on a jellyfish-derived sequence, was also included in each assay. Standard curves were constructed with PCR results from 5 repetitions of 10-fold dilutions of genomic DNA ranging from 1 ng to 1 fg/µl from *C. immitis* (CNM-CM 7056) and *C. posadasii* (CNM-CM 2912), belonging to the mould collection of the Spanish National Center for Microbiology, and *C. posadasii* strain Silveira. The specificity of the assay was assessed using DNA from 10 unrelated clinical *Coccidioides* spp. strains and DNA from 17 other fungal species, and also mouse and human DNA. A murine model of disseminated coccidioidomycosis was used for RT-PCR validation. Female 5-week-old CD-1 mice were infected iv with 250 arthroconidia of *C. posadasii* strain Silveira. Fungal burdens were determined by CFU and RT-PCR in spleen, lung, and liver on days 5, 10 and 14 days postinfection. Uninfected mice served as controls. CFU and CE (conidia equivalent) values were correlated.
Results The RT-PCR assay showed high reproducibility ($r > 0.99$) and specificity (100%); the lower detection limit was 1 fg of genomic DNA per microliter of sample. Fungal burdens after 5, 10, and 14 days of infection were higher by RT-PCR than by CFU enumeration for all tissues analyzed; RT-PCR was negative for blood on those days. No PCR inhibition was detected. Spearman correlations of RT-PCR and CFUs results showed a significant correlation for lungs ($p < 0.05$) but not for liver or spleen ($p > 0.05$). Comparative linear regression analysis of RT-PCR and CFUs showed similar slopes ($p > 0.05$) for temporal burden of infection in all organs, indicating RT-PCR, while more sensitive, is equivalent to CFU for disease progression.
Conclusions We have demonstrated the utility of a fast, sensitive, and specific RT-PCR for the detection of coccidioidomycosis in clinical samples from mice. RT-PCR appeared to better reflect the progression of infection. Further studies are warranted.

P049

A novel tool for *Aspergillus fumigatus*-free DNA detection in serum targeting a single copy gene

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Objectives Although molecular approaches are promising for early diagnosis of Invasive Aspergillosis (IA), lack of standardization has hampered inclusion of fungal nucleic acid detection into the EORTC/MSG criteria. The European Aspergillosis PCR Initiative (EAPCRI) has pointed out sample preparation and false positivity among the main limiting factors towards standardization. Circulating fungal genomic DNA (gDNA) detection in serum specimens has been widely suggested for early diagnosis of IA. Serum use overcomes long and complex sample preparation procedures and allows the same specimen to be used for both galactomannan (GM) and PCR assays. The present work highlights an effort towards standardizing fungal free gDNA detection in serum specimens. A. fumigatus is the first targeted species as it inflicts the majority of reported IA cases. Our study illustrates analytical performance data obtained using an integrated semi-automated procedure allowing detection of A. fumigatus gDNA from spiked serum using a single copy gene as a molecular target.
Methods Environmental and clinical A. fumigatus isolates were used for ribosomal protein locus sequencing. Strains Af293 and CBS144.89 were used to obtain gDNA for amplification and spiking. Sample preparation was based on fungal gDNA extraction, from 1 mL spiked healthy donor serum samples, using the easy-MAG® system. A single copy ribosomal protein gene was used as detection target in a Taqman Real Time PCR setting. Cross-reactivity testing was carried out against several mould, yeast and bacteria species gDNA. Purified human gDNA effect on A. fumigatus gDNA detection performance was checked.
Results The ribosomal protein locus sequence was determined in several A. fumigatus strains for PCR design purposes (avoid regions presenting intra-species polymorphisms). Real Time PCR design screening and reaction optimization were carried out using as template A. fumigatus gDNA. The bioMérieux easyMAG® system enabled optimizing a semi-automated sample preparation approach, including sample pretreatment/lysis and fungal gDNA extraction/purification, from spiked healthy donor serum. The whole optimized procedure (sample preparation and amplification-detection) was subjected to analytical performance evaluation, showing: (i) An apparent Limit of Detection (LoD) at 10 genomes/mL of serum (60/60 samples detected). This LoD value remained unchanged by varying parameters, such as serum batch, spiking approach, apparent extraction or PCR efficiency, the use of one or two PCR replicates per serum sample. (ii) Serum spiking with 5 genome equivalents/mL can lead to detection (20/30 tested samples). (iii) Cross-reactivity only to Neosartorya fischeri gDNA. (iv) 0% false positivity (140 negative controls tested). (v) No PCR inhibition after serially diluting spiked serum sample eluates nor by human gDNA.
Conclusion We have built an integrated, semi-automated, robust procedure for A. fumigatus free gDNA detection in spiked serum, using the easyMAG® system and a single-copy-gene based Real Time PCR approach, which fills the main required sensitivity and specificity standards set by EAPRCI (designated threshold of 10 genomes/mL serum, no false positives). Cross-reactivity with N. fischeri is not considered a major issue as the corresponding IA reported cases are rare. Focus is now on clinical validity evaluation of this tool, i.e., building a clinical study model and clinical sample testing from retrospective collections.

P050

Prospective clinical evaluation of yeast traffic light assay PNA FISH assay

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Objectives To analyse the performance and clinical consequences of PNA FISH in identification of Candida spp. directly from positive blood culture bottles in a prospective clinical study.
Methods Peptide nucleic acid probe fluorescence in situ hybridization (PNA-FISH) was performed directly from blood culture bottles positive for yeast growth documented by gram staining. The Yeast Traffic Light probes for C. albicans/C. parapsilosis, C. tropialis, and C. glabrata/C. krusei were used in the study. Results were compared to identification by conventional methods including the VITEK 2 system.

Results A total of 101 patients with positive blood cultures were tested prospectively. Only one blood culture per patient was included in the study. Using conventional methods 57 C.albicans, 24 C.glabrata, 4 C.tropicalis,5 C.parapsilosis, 5 C. dublinensis and one each of C.krusei, C. norvegensis, C. lusitaniae, C. pelliculosa, Rhodotorula and Saccharomyces cerevisiae were detected. The PNA FISH Candida assay showed 100% sensitivity and specificity for the Candida species included in Yeast Traffic Light kit. For two C.albicans tested in the beginning of the study, initial assay results were negative. However, repeated staining of these samples showed that both were positive for the C.albicans/C.parapsilosis probes indicating that lack of experience in sample preparation was the underlying factor for the negative result. Six Candida species (C. norvegensis, C. dublinensis, C. lusitaniae, C. pelliculosa, Rhodotorula and Saccharomyces cerevisiae) not included in the Yeast Traffic Light probes were negative as expected. PNA-FISH was 24–48 h faster in identification of Candida spp. compared to conventional methods. The possible clinical effect of rapid PNA-FISH result on antifungal management was also investigated. The current antifungal treatment on each patient at the time of identification of Candida spp. with PNA-FISH assay was recorded at laboratory information system. The present results have shown that the antifungal treatment could be optimized in 74 patients who had no antifungal treatment. Interestingly, 5 patients, four with C. glabrata and 1 with C. parapsilosis received ineffective antifungal treatment at the time of identification by PNA-FISH. These five patients could receive effective treatment 24–48 h earlier with the help of PNA-FISH data.
Conclusion The present study shows that the Yeast Traffic Light PNA FISH assay can provide rapid and reliable identification of five clinically relevant Candida species from positive blood cultures. The significantly faster identification of Candida spp. by PNA-FISH can be used as guidance for early, effective antifungal therapy.

P051

Early diagnosis of histoplasmosis by nested PCR

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Objectives To study the efficacy of nested PCR to detect *Histoplasma capsulatum* in various clinical specimens (sputum, lymph node, bone marrow and paraffin embedded tissues) in comparison with routine methods.
Methods Genomic DNA was directly extracted from 649 clinical specimens (including 479 concentrated sputum, 82 lymph node, 12 bone marrow and 76 paraffin embedded tissues). *H. capsulatum* was detected by nested PCR using Msp primers. The Msp primers (Msp1F/Msp2R and Msp2F/Msp3R) generated products of approximately 318 and 269 bp for the first and second round PCR, respectively. All positive bands were purified for sequencing analysis. PCR results were compared with routine fungus cultures for lymph node and bone marrow, PAS staining for concentrated sputum and GMS staining for paraffin embedded tissues. Sensitivity and specificity of the primers were also determined.
Results 1Twelve of 649 (1.85%) specimens were positive by nested PCR. These specimens included five concentrated sputum, five paraffin embedded tissues and two lymph node specimens. Only one lymph node specimen was positive for *H. capsulatum* by the routine culture method. Five of twelve paraffin embedded tissues positive for GMS staining for *H. capsulatum* were positive by nested PCR. The negative results of the rest of the paraffin embedded tissues may be explained by either DNA degradation caused by formalin fixation or those yeasts seen by GMS staining were not *H. capsulatum*. Smears of five concentrated sputum specimens were positive by nested PCR and by PAS staining showing small budding yeasts. Sequencing analysis of PCR products showed 97-99% identity to the nucleotide sequences of the gene encoding M antigen of *Ajellomyces capsulatus*. Determination of the sensitivity of the nested PCR method revealed that the minimal yeast cells and DNA of *H. capsulatum* per reaction resulting in a positive PCR were 10 cells and 100 fg, respectively. The nested PCR method showed high specificity of the Msp primers for *H. capsulatum* when tested with various species of bacteria and fungi.
Conclusion A comparison between PAS staining and nested PCR for concentrated sputum specimens showed significantly excellent correlation for the detection of *H. capsulatum*. The correlative classification of culture and nested PCR methods for lymph node and bone marrow specimens was significantly good. However, the correlative classification of GMS staining and nested PCR method for paraffin embedded tissue specimens was significantly fair. These findings suggest that the nested PCR method using Msp primers be a sensitive, specific and rapid method for early diagnosis of histoplasmosis in clinical specimens especially of sputum, lymph node and bone marrow tissues.

P052

Quantitative galactomannan detection in comparison to real-time PCR method in diagnosing invasive aspergillosis

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This work was supported by the Polish State Committee for Scientific Research (grant no. N N401 042738)
Objectives Invasive aspergilosis (IA) is an acute infection with a mortality rate of almost 70%. An etiological factor in more then 90% of cases is *Aspergillus fumigatus*. The most common clinical manifestation is lung aspergilosis – 75% of all IA cases, *rhinosinusitis* (infection of the nasal mucosa and nasal sinuses) – 5–10%, disseminated multiorgan – 25%, IA with an affected central nervous system makes out 10–40% of all cases. Traditional methods: as a "golden standard"in the diagnostic of invasive aspergilosis until now, remains the culture of a strain from the sample clinical material and identification of fungi by histopathology. As supportive tests can be used: detection of galactomannan antigen and fungal DNA in bronchioalveorial fliud or in serum. The aim of the study was to compare two metods: immunoenzymatic detection of galactomannan antigen (Platelia-Aspergillus Ag, BioRad, Hercules, USA) and molecular method Real-Time PCR for the detection of Aspergillus spp. genomic DNA (MycAssay™ Aspergillus, Myconostica, Manchaster, UK).
Methods Serum samples were collected through routine serological diagnostic procedures from patients hospitalized in the Institute of Transplantation Medicine. Patients were selected retrospectively on the base of availability of serum samples (at least two samples from one patient), clinical sympthoms of IA and the results of culture method. The tests were performed using serum samples of 2 groups of patients: patients with clinical symptoms of invasive aspergillosis and patients with positive reasult of galactomannan antigen test. Both methods were conducted according to manufacturer protocols.
Results In group of patients with positive galactomannan antigen test only 50% were positive in Real-Time PCR method. Serum samples collected from patients with clinical symptoms of invasive

aspergillosis were all negative for galactomannan antigen. However, in MycAssay test all collected serum samples of one patients were positive. In the rest of the serum samples from other patients the obtained results were both positive and negative. None of the specimens from this group gave the unequivocal confirmation of the infection.

Conclusions None of the compared method might be used as a single indicator of Invasive Aspergillosis.

The result of MycAssay were frequently unequivocal and difficult for interpretation.

The immunoassay test in comparison to MycAssay provided the divergent results.

P053

Evaluation the performance of the matrix-assisted laser desorption ionization-time of flight mass spectrometry for the identification of *Candida* isolates recovered from blood cultures

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Objectives The objective of this study was to evaluate the performance of MALDI TOF-MS method for the identification of *Candida* species isolated from blood cultures and to compare these results with those of conventional identification methods.

Methods A total of 216 stocked clinical *Candida* isolates recovered from blood cultures and 9 reference strains were included in this study. *Candida* isolates were previously identified with conventional methods by using routine mycological techniques and API ID 32C, if necessary. These isolates were analyzed by Bruker Daltonics MALDI TOF mass spectrometry. The data analysis was performed by MALDI Biotyper software version 3.1.

Results The MALDI-TOF MS spectral scores for the reference strains and clinical *Candida* isolates are summarized in Table 1 and Table 2, respectively. The eight of nine reference strains including 6 species had the spectral scores ≥1.70, but one (no reliable identification for *C. glabrata*). Of 216 clinical isolates, the 100 (46%) strains were correctly identified with scores of ≥2.0 and 194 (90%) with scores of ≥1.70. When the score threshold was lowered to ≥1.8, the species level identification increased to 78% and 85% for the reference strains and clinical *Candida* isolates, respectively (Table 2). All test scores of <1.70 (22 clinical isolates and one reference strain) provided 100% correct genus and species agreement, although these scores are accepted as "not reliable identification" according to the testing criteria.

Table 1. Scores obtained for the identification of reference *Candida* strains.

Reference strains	MALDI-TOF	
	Identification	Scores
C. albicans ATCC 10231	*C. albicans*	1,897
C. albicans ATCC 24433	*C. albicans*	1,832
C. albicans ATCC 90028	*C. albicans*	1,871
C. dubliniensis CBS 7987	*C. dubliniensis*	1,753
C. glabrata ATCC 90030	No ID (*C. glabrata*)	1,633
C. krusei ATCC 6258	*C. krusei*	2,075
C. parapsilosis ATCC 22019	*C. parapsilosis*	2,027
C. parapsilosis ATCC 90018	*C. parapsilosis*	2,033
C. tropicalis NRRL Y-12968	*C. tropicalis*	1,963

Table 2. Scores obtained for the identification of clinical *Candida* isolates.

Species (no. of isolates tested)	No. of isolates with spectral score of:				% Correct to genus by definition* (spectral score ≥1.7)
	<1.7	1.7-1.799	1.8-1.999	≥2.0	
C. albicans (48)	3	5	39	1	94
C. parapsilosis (90)	5	2	17	66	94
C. glabrata (26)	10	3	13	-	62
C. tropicalis (26)	2	1	6	17	92
C. krusei (14)	1	-	3	10	93
C. kefyr (5)	-	-	1	4	100
C. lusitaniae (4)	-	-	2	2	100
C. dubliniensis (2)	-	-	2	-	100
C. norvegensis (1)	1	-	-	-	0
Total (216)	22	11	83	100	90

*MALDI Biotyper 3.1 User Manual

Conclusion Overall, a total of 216 clinical *Candida* isolates were correctly identified by 90% and 85% when ≥1.70 and ≥1.80 thresholds were applied, respectively. For species identification, we concluded that the reduction in identifying threshold may be needed for the optimal utility.

P054

1,3-Beta-D-glucan for the diagnosis of invasive pulmonary aspergillosis in patients with chronic obstructive pulmonary disease

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Background Patients with chronic obstructive pulmonary disease is at risk for invasive pulmonary aspergillosis (IPA) particularly under corticosteroid therapy. Here, we evaluated the role of 1,3-beta-D-glucan (Fungitell, Associates of Cape Cod) detection for the diagnosis of IPA in COPD patients with IPA.

Methods The records of mycology laboratory was searched to identify the COPD patients with Aspergillus isolation from lower respiratory tract (LRT) samples, retrospectively The inclusion criteria were 1- the isolation of Aspergillus from LRT culture 2- COPD diagnosis established according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria 3- COPD patients who had a history of steroid use in the last 3 weeks before admission 4- the isolation is not considered as colonization 5- At least one 1,3-beta-D-glucan (BDG) measurement from serum before antifungal therapy. Patients were classified as having proven IPA or probable IPA on the basis of the criteria established by the European Organization for Research and Treatment of Cancer and Mycoses Study Group (independent of the BDG results). Galactomannan antigenemia (GM) (Platelia Aspergillus ELISA; Bio-Rad Laboratories) and BDG (Fungitell kit; Associates of Cape Cod, East Falmouth, MA, USA) tests were carried out in accordance with the manufacturers' specifications. The cut-off for BDG positivity was set at 80 pg/ml and for GM was set at 0.5.

Results We were able to identify four COPD patients with IPA (Two patients with proven IPA, two patients with probable IPA)) who had a BDG measurement. *Aspergillus fumigatus* was isolated from the sputum of two patients and transbronchial biopsy of one patient and sputum culture yielded *Aspergillus* niger from one patient. While BDG was positive in 3 out 4 patients, GM was positive in one patient (Table 1).

Conclusions Detection of BDG in serum can be helpful for the diagnosis of IPA in patients with COPD. However, prospective clinical studies are required to understand the exact role of BDG measurement in this patient group.

Table 1 Clinical and Demographic characteristics of the patients with IPA.

	Age/ Gender	Diagnostic Type	Thorax Computed Tomography	Culture	*Aspergillus* spp	Galactomannan index	1,3-Beta D-Glucan (pg/mL)
1	70/ Male	Probable	Infiltration, cavity, nodule	Sputum	*A. fumigatus*	0.22	> 523
2	60/ Male	Proven *	Consolidation, nodule	Sputum	*A. fumigatus*	0.54	59
3	61/ Male	Probable	Infiltration, nodule	Sputum	*A. niger*	0.17	90
4	78/ Male	Proven *	Infiltration	Transbronchial biopsy	*A. fumigatus*	0.23	> 523

P055

Microbiological and immunological study in immunocompetent patient compromised by cryptococcosis

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Introduction The fungal disease of great medical importance due to mortality and morbidity it causes, cryptococcosis, has a strong association between the incidence and the presence of HIV, lymphoproliferative disorders or immunosuppressive therapies.
Objective Clinical study of immunocompetent patient with cryptococcosis and association with immunological studies of their leukocytes as well as the microbiological aspects of the strain in question.
Case report Man aged 68 healthy, practicing of the racing, he developed intermittent headaches in July 2010. This picture was becoming more intense and frequent until in February 2011 was associated with vomiting, nausea, dizziness and temporal headache. Was diagnosed by cerebrospinal fluid and cranium Ct with cryptococcal meningitis. In this period was diagnosed with esophageal candidiasis by endoscopy and was initially treated with amphotericin B and 5 fluorocytosine (5 FTC), after amphotericin and fluconazole and when things began to improve only with liposomal amphotericin, fluconazole and 5 FCT. After discharge was introduced Fluconazole for another 6 months. The patient had no sequelae.
Laboratory methodology We performed biochemical and phenotypic study as well as virulence factors study and genotyping of the strain in question Also immunophenotyping of leukocytes test were performed.
Results (Clinical, immunological and microbiological) The patient in question trained race in a eucalyptus grove where there were too many pigeons, in his history of benign prostatic surgery in 1994, his father who died of prostate cancer and a mother who died of breast cancer. Immunophenotyping showed CD3, CD4, CD8, CD19 and CD3+16+56 within the ranges set, but it was proved cancer of the bone marrow in which 1% of their lymphocyte antigen showed coexpression of CD19 B and lymphoid antigens of the NK (9% of NK lymphocytes were of the CD3-CD19-CD56+ and 20% of the lymphocytes were of the type CD19+ CD56+ CD57+, not being shown restraint light chain populations of the lymphoid B), it is concluded that there was the presence of population lymphoid anomalous type (CD56+. CD19+). In the microbiological study was identified positive assimilation for inositol and glucose, negative for lactose, assimilation of nitrogen sources to Peptone and negative for KNO_3; fermentation of C sources (glucose) negative, growth in Sabouraud medium at 37°C, CBG and urease positive, micro and macroscopic analysis consistent with the findings for the genus *Cryptococcus* spp., study of the felonoxidase positive, phospholipase and proteinase with strongly positive expression, genotyping showing to be VGII type (*C. gattii*).
Conclusions Cryptococcal meningitis is a disease that usually occurs in immunocompromised patients. Our patient throughout the diagnosis was apparently healthy, but during the investigation was diagnosed with a population of lymphoid cells aberrant. These cells could have caused a secondary immunodeficiency that could be the basic cause of meningitis by *C. gatti*. The yeast in question is the most aggressive form of this genus and suggest that whenever we encounter a patient with cryptococcal meningitis was investigated any primary or secondary immunodeficiency, particularly neoplasms hidden like this.

P056

Comparison of different laboratory methods in the diagnosis of onychomycosis

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Introduction Onychomycosis, fungal infection of the nails, is the most common cause of onychodistrophy (50%). Dermatophytes are the predominant aetiological agents of onychomycoses, followed by yeasts and non-dermatopyte molds. Reliable diagnostic method for detection and identification of species/genus of fungi is necessary for proper and rational use of antifungal drugs because duration of treatment is long and may have some serious side effects.
Objectives The objective of this study was to evaluate the most frequent causative agents of onychomycosis and to perform comparative testing of individual diagnostic method or combination of laboratory methods that are applied to confirm diagnosis of onychomycosis.
Methods Study included 65 patients with suspected onychomycosis, examined by specialists of dermatology, submited to Medical mycology reference laboratory at Institute of microbiology and immunology, Faculty of medicine, University of Belgrade, Serbia from November 2012. until February 2013. Nail samples were collected by clipping or scraping depending on clinical type of onychomycosis. Each sample was divided into two portions: one for direct microscopy and rest for fungal culture. Direct microscopic preparations were made with 15% potassium hydroxide solution (KOH) and fluorescent dye Blankophor. Second part of specimen was planted into three mycological media: Sabouraud's dextrose agar, diluted Sabouraud's dextrose agar, Dermatophyte test medium and incubated for three weeks using standard mycological technique. To analyze the data, a criterion standard for the diagnosis of onychomycosis was defined as is generally accepted in clinical practice: clinical morphologic findings suggestive of onychomycosis plus at least one positive test result. For each laboratory test, we determined sensitivity and negative predictive value. To determine additional characteristics of tests like specificity, positive predictive value and clinical significance of applied tests, Blankophor was selected as 'gold standard' for statistical analysis.
Results The most common clinical form of onychomycosis in our patients was distal and lateral subungual onychomycosis found in 68.6% patients. A total of 70 nail samples were taken of which 46 (60.5%) were positive. From 46 positive nail samples fungi were isolated in 41 samples, while in remaining 5 samples the presence of fungi was observed only by direct microscopy. Dermatophytes were isolated in 33/41 (80.5%) followed by *Candida* spp. (9.8%), *Aspergillus* sp. (4.9%), *Alternaria* sp. (2.4%) and *Fusarium* sp (2.4%). Most common dermatophyte was *Trichophyton rubrum* (76%). Best sensitivity and negative predictive value of test combination showed Blankophor in combination with Dermatophyte test medium (95.6% and 92.3% respectively) followed by combination of Blankophor with diluted Sabouraud's dextrose agar (93.5% and 88.9% respectively). Compared to Blankophor as the 'gold standard', KOH gave the best specificity (90.6%), and the best combination for positive predictive value was KOH with diluted Sabouraud's dextrose agar (85.4%).
Conclusion Blankophor as an individual test is the most sensitive method for diagnosis of onychomycosis. Combination of Blankophor with Dermatophyte test medium or diluted Sabouraud's dextrose agar provides good sensitivity and allows identification of fungi. Recommendation is to combine multiple tests and including new methods (PCR) in diagnosis of onychomycosis.

P057

Identification of clinically important filamentous fungi by Maldi Biotyper Fungi Library in a clinical microbiology laboratory

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Objectives The objective of this work was to evaluate whether Fungi Library for Maldi Biotyper (Bruker Daltonics) would be useful for diagnostics of clinically important filamentous fungi in a clinical microbiology laboratory. Maldi-TOF is already widely used for identification of yeasts isolated from clinical samples but its use for identification of filamentous fungi hasn't been as widely studied. Yet it might provide a fast and cost-effective identification method that is also less subjective and less dependent on the expertise of the analyst.

Methods 35 mould strains and 34 dermatophyte strains were analysed in this study. Both reference strains and clinical strains were included. The strains were cultivated in sabouraud broth tubes (Sabouraud Liquid Broth, Modified, Becton Dickinson) for 16–48 h at 30°C. After incubation the tubes were left on a workbench in order to allow the fungal biomass to sediment to the bottom of the tube. The pellet was then washed twice using ultra-pure water. The standard extraction method for Maldi Biotyper using ethanol and formic acid was then carried out. 1 µl of resulting supernatant was pipetted onto a target plate on two consecutive spots. Each spot was overlaid with 1 µl of matrix solution and allowed to air dry prior to MALDI-TOF MS. The strains were identified using Fungi Library for Maldi Biotyper (Bruker Daltonics). The work was carried out at the Clinical Microbiology Laboratory of Turku University Hospital, Finland.

Results The results are summarised in table 1 and figure 1. Altogether, 83% (29/35) of the mould strains were correctly identified to the species level with score values ≥2.0 and 11% (4/35) with score values 1.7–1.999. For 6% (2/35) of the mould strains no reliable identification could be achieved. None of the mould strains was misidentified. 71% (24/34) of the dermatophyte strains were correctly identified to the species level with score values ≥2.0 and 9% (3/34) with score values 1.7–1.999. For 12% (4/34) of dermatophyte strains no reliable identification could be obtained and 9% (3/34) of them gave incorrect identification results.

Conclusions The Fungi Library gave excellent identification results for moulds with no misidentifications. Two strains (Mucor hiemalis and Fonsecaea monospora) were not identified because they were not included in Fungi Library. As expected, the identification results of dermatophytes were not as consistent since incorrect identifications were common. Some misidentifications were due to the fact that the correct species was not represented in the database. Microsporum audouini misidentified as Microsporum canis was not included in the database; neither was the unidentified Trichophyton terrestre. However, Microsporum gypseum and Trichophyton violaceum strains were also misidentified as M. canis and T. rubrum, respectively, despite the fact that both of these species were represented in the database. This is probably due to close genetic relation of these species which is reflected in their protein composition.

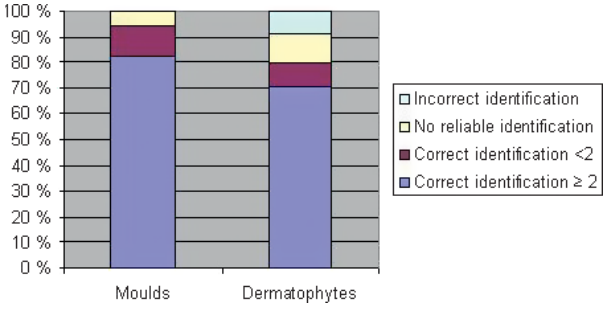

Figure 1 Overview of identification results for moulds and dermatophytes.

Table 1 Identification results by fungal genera.

Moulds		
Reference id	Species/strains tested	Maldi id
Aspergillus strains	9/18	All correct identifications ≥ 2.0
Fusarium strains	3/3	*F. solani* ≥ 2.0, *F. oxysporum* ≥ 2.0, *F. dimerum* 1.850
Paecilomyces strains	2/2	All correct identifications ≥ 2.0
Rhizopus species	2/2	*R. oryzae* ≥ 2.0, *R. microsporus* 1.969
Rhizomucor pusillus	1/1	*R. pusillus* ≥ 2.0
Mucor hiemalis	1/1	No reliable id (not in Fungi Library)
Lichtheimia corymbifera	1/1	*L. corymbifera* ≥ 2.0
Chrysosporium keratinophilum	1/1	*C. keratinophilum* ≥ 2.0
Fonsecaea monospora	1/1	No reliable id (not in Fungi Library)
Scopulariopsis brevicaulis	1/1	*S. brevicaulis* ≥ 2.0
Pseudallescheria boydii	1/1	*P. boydii* ≥ 2.0
Scedosporium prolificans	1/1	*S. prolificans* ≥ 2.0
Penicillium chrysogenum	1/1	*P. chrysogenum* 1.894
Chaetomium globosum	1/1	*C. globosum* ≥ 2.0
Sporothrix schenckii	1/1	*S. schenckii* 1.739
Dermatophytes		
Reference id	Species/strains tested	Maldi id
Trichophyton rubrum	12/12	All *T. rubrum* ≥ 2.0
Trichophyton interdigitale/ mentagrophytes	3/3	All *T. interdigitale/ mentagrophytes*, 2 strains ≥ 2.0, 1 strain 1.78
Trichophyton tonsurans	3/3	All *T. tonsurans* ≥ 2.0
Trichophyton mentagrophytes var. erinacei	1/1	*T. mentagrophytes var. erinacei* ≥ 2.0
Trichophyton terrestre	1/1	No reliable id (not in Fungi Library)
Trichophyton violaceum	1/1	Misidentified as *T. rubrum*
Epidermophyton floccosum	3/3	1/3 *E. floccosum* 1.7, 2/3 no reliable id
Microsporum canis	2/2	All *M. canis* ≥ 2.0
Microsporum audouini	1/1	Misidentified as *M. canis* (not in Fungi library)
Microsporum fulvum	1/1	*M. fulvum* 1.966
Microsporum gypseum	1/1	Misidentified as *M. canis*
Microsporum persicolor	1/1	*M. persicolor* ≥ 2.0

P058

Diagnosis of pulmonary aspergilloma by serological *Aspergillus* antibody detection: comparison of a new commercial immunoblot-based test with detection by immunoprecipitation

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Diagnosis of pulmonary aspergilloma is based on chest radiographic images (fungus ball) and specific antibody detection by serologic procedures. Concerning *Aspergillus* serology the reference method remains detection by immunoprecipitation although this method lacks standardization.

Objective To compare the commercially available IMB kit " Aspergillus WB IgG"(LBDBio Diagnostics, Lyon, France) with the immunoprecipitation method used in our laboratory. A panel of sera from patients with proven aspergilloma (lung biopsy showing mycelium and culture of *Aspergillus fumigatus*) was tested using the two methods in parallel.

Methods We used a total of patient 32 sera. Nine were collected at our hospital and the 23 sera were provided by 4 other hospitals. IMB was used in accordance with manufacturer's instruction. A serum was considered as positive if a minimum of 2 bands were detected among the 4 specific *Aspergillus* antigen bands (16, 18–20, 22, 30 kD). IPD used commercial somatic and metabolic antigens (Bio-Rad, Marne-La-Coquette, France) and a commercial *Aspergillus* positive control serum (bioRad,Marne-La-Coquette,France). A serum was considered as positive if one somatic or metabolic antigen was precipitated.

Results Of these 32 sera 19 were positive with IPD (detection from one to nine antigens). The sensitivity was 59%. Thirty-three sera were positive with IMB (detection from 2 to 4 bands). The two bands the most often observed were P16 and P18-20. The sensitivity was 97%. IMB showed significantly higher sensitivity than IPD. Specificity of IMB previously performed on blood donors (n = 213) was evaluated to 96%. IMB offers important advantages over IPD: small amounts of serum are required (15 µl versus 60 µl) and in contrast to IPD the criteria for interpreting positivity is simpler. The antigen-positive bands are much easier to detect than precipitated antigens.

Conclusion IMB appears a better method to diagnose pulmonary aspergilloma.

P059

Development of a MIQE-compliant *Aspergillus terreus* specific qPCR assay

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Objectives A reliable method for the early and accurate identification of *Aspergillus terreus* is becoming more important as it increasingly emerges as a causative agent of Invasive Aspergillosis. Moreover, most members of this species have decreased susceptibility to the antifungal drug Amphotericin B *in vitro* and *in vivo* (1). As a consequence, the ability to distinguish *A. terreus* from other species of *Aspergillus* is important to the clinician for therapeutic decision-making and prognosis. Although in theory real-time PCR (qPCR) is an effective method for the sensitive and accurate identification of this pathogen, the practical usefulness of qPCR in fungal diagnostics remains uncertain. Lack of specificity, poor amplification efficiencies and absence of standardised protocols have resulted in assays that are unreliable, not sufficiently optimised or validated and not fit for purpose as routine diagnostic tests. The MIQE guidelines aim to provide a blueprint for best practice in assay design and reporting and are widely viewed as an important contribution to translating qPCR-based assays from a research into a practical diagnostic setting (2). We followed these guidelines to generate a robust, sensitive and rapid assay that is specific for *Aspergillus terreus*.

Methods The first step was the *in silico* analysis of primers and amplicon, with stringent design criteria which resulted in two assays taken forward for several empirical optimisation tests where the reaction conditions and the assay performance were evaluated. The qPCR assays were then validated using DNA extracted from fungal cultures to assess their specificity. The two assays were comparable in their performance, but the probe assessment was carried out for only one of them, given its higher specificity. No amplification was seen in the negative controls - human DNA and non-template control wells – in any plates.

Results We obtained an *A.terreus*-specific assay that has an efficiency of 98% which translates into a limit of detection of 0.6 copies of *A.terreus* genome and that can be completed in less than 90 minutes. Furthermore, the results of the specificity analysis run show that there was no amplification of any non-*Aspergillus* species or any other *Aspergillus* species such as *A. fumigatus*, *A.niger*, *A.nidulans* or *A.flavus*. Amplification only occurs with *A. terreus*, at a Cq of 27 for the 2 pg/µl dilution, which corresponds to 60 copies of *A. terreus* genome/µl.

Conclusion This highly sensitive and specific *A.terreus*-specific assay is suitable for clinical application and the rapid identification of this particular Amphotericin B-resistant *Aspergillus* species.

1) Steinbach et al., Infections due to Aspergillus terreus: A multicenter retrospective analysis of 83 cases. Clinical Infectious Disease,2004; 39(2): 192–198

2) Bustin et al., The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments, 2009, Clinical Chemistry, 55:4, 611–622

P060

Diagnostic utility of a microarray based on 11 proteins of *Candida albicans* for the diagnosis of invasive candidiasis

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Objective The purpose of this study was to set up a protein microarray test based on 11 proteins of *C. albicans* for the detection of IgG antibodies in sera from patients with invasive candidiasis (IC) and determine its diagnostic utility.

Methods and patients The microarray was set up with 10 *C. albicans* recombinant proteins: Eno1, Als3-N, Hwp1-N, Eno1-RM, Bgl2, Grp, Pgk1, Fba, Pdc, Adh1, and an enolase purified from a dithiothreitol-extract of cell walls from *C. albicans* mycelial phase (CW-Eno). Antigens, IgG standard curve, and controls were printed onto glass slides using computer controlled high speed robotics. The arrays were processed with sera from IC and control patients and then fluorescence labeled secondary antibodies were added. The signal captured by each spot was detected by a laser scan reader and quantified. When possible, the cut-off values were calculated as mean mass of antibody detected in the control group sera plus 2 times the standard deviation. Twenty three sera from 15 patients with proven IC due to *C. albicans* and 33 sera from 28 patients at risk for IC but without clinical or microbiological data confirming a fungal infection (control group) were studied.

Table 1 Clinical performance determined for each antigen.

Antigen	Sensitivity (%)	Specificity (%)	Positive predictive value(%)	Negative Predictive value(%)
Eno1	33.33	100	100	73.68
Als3-N	20	100	100	70
Hwp1-N	20	96.43	75.00	69.23
CW-Eno	80	96.43	92.31	90.00
Eno1-RM	6.67	100	100	66.67
Bgl-2	20	100	100	70.00
Grp	33.33	96.43	100	72.97
Pgk1	46.67	92.86	77.88	76.47
Fba	6.67	96.43	50	65.85
Pdc	13.33	100	100	68.29
Adh1	26.67	100	100	71.79

Table 2 Diagnostic utility of the whole microarray and antigen clusters.

Antigen	Sens(%)	Spec(%)	PPV (%)	NPV(%)
All antigens	100	85.71	78.95	100
CW-Eno, Pgk1, Eno1 and Grp	100	89.29	83.33	100
CW-Eno and Pgk1	93.33	89.29	82.35	96.15

Results The performance of the microarray assay for each antigen is shown in Table 1 The best results were obtained with CW-Eno, showing a sensitivity and a specificity of 80 and 96.43%, respectively. Pgk1, Grp and Eno1 exhibited lower sensitivity values (33–47%), but their specificity reached values equal or greater than 93% Also, for all the other antigens, the specificity was very high with values above 96%. Furthermore, we clustered those antigens which separately had returned the best sensitivity. As shown in Table 2, the clustering raised the sensitivity up to 100%, while the specificity ranged between 85 and to 89% .

Conclusions The detection of serum IgG antibodies against proteins of *C. albicans* by a protein microarray exhibited moderate diagnostic utility values when assessed independently, being CW-Eno the main exception. However, when considering the microarray results either as a whole or as clusters of selected proteins (CW-Eno, Pgk1, Eno1 and Grp; or CW-eno and Pgk1), the system proved to be an efficient diagnostic tool for IC.

Further studies with a larger number of patients are needed to confirm the results of the present study.

P061

Performances of three real-time PCR methods for the diagnosis of *Pneumocystis jirovecii* pneumonia

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Pneumocystis jirovecii (PJ) is responsible for severe pneumonia (PJP) in immunocompromised patients, especially in HIV infected patients. The diagnosis of PJP is based on clinical and radiological signs, associated to PJ detection in pulmonary samples. The implementation of laboratory tests requires bronchoalveolar lavage (BAL) considered as reference sample. Currently, molecular methods have showed high sensitivity, particularly in low fungal loads and allow the use of less invasive samples such as sputum.

Objectives The main objective of this study was to evaluate the concordance rate of three methods of real-time PCR for the detection of *Pneumocystis jirovecii* DNA using BAL, sputum or bronchial aspirates.

Methods 107 pulmonary samples from patients with suspected PJP were tested, corresponding to 62 BAL, 11 bronchial aspirates, 33 sputum, and one pulmonary biopsy.

Direct examination (DE) was systematically performed for BAL by using May-Grünwald-Giemsa and Gomori-Grocott staining. DNA samples were automatically extracted (Biorobot® EZ1, Qiagen®) with the EZ1 DNA Tissue kit (Qiagen®). Three real-time PCR were performed: (i) 'in house' PCR according to Fillaux *et al.* (2008) protocol and two commercial kits were tested (ii) 'Real-time PCR *Pneumocystis jirovecii*' (Bio-Evolution®) and (iii) 'AmpliSens® *Pneumocystis jirovecii (carinii)*-FRT PCR kit'. Commercial kits were used according to the manufacturer's recommendations. The PCR instruments used were LightCycler® 480 (Roche®) for commercial kits and LightCycler® 2.0 (Roche®) for "in house"PCR. The study was approved by the ethics committee of Nîmes University hospital.

Results The number of positive samples by at least one of the three PCR methods was 65/107. The results were similar with the three methods for 87 samples (45 positive and 42 negative). 14 BAL, four sputum and two bronchial aspirates showed discordant results: "in house"PCR and AmpliSens PCR detected PJ while Bio-Evolution PCR remained negative. For BAL, these discrepancies corresponded to high cycle threshold (Ct) (>28) obtained with "in house"PCR. Table 1 presents the results obtained and concordance between the three molecular methods. Analysis of the results of BAL showed a similar distribution of the Ct obtained with "in house"PCR and AmpliSens PCR (Figure 1). As regards direct examination performed on 62 BAL, only 38.2% were positive among PCR positive.

Conclusion Among the three real-time PCR, the results are similar with "in house"PCR and AmpliSens PCR. Twenty samples remained negative with Bio-Evolution PCR while the other methods were positive with Ct consistently > 28. According to interpreting results for BAL proposed by Fillaux *et al.* (2008) (Ct <22: proved PPJ, 22 < 28: possible PPJ and Ct >28: improbable PPJ), these patients were considered only colonized but not suffering from PPJ. However, BAL interpreting thresholds must be adapted to each method and correlated with clinical data. Moreover, thresholds remain to be determined on the sputum and bronchial aspirates, whatever the method used.

Figure 1. Distribution of Ct results for BAL with the 3 molecular methods

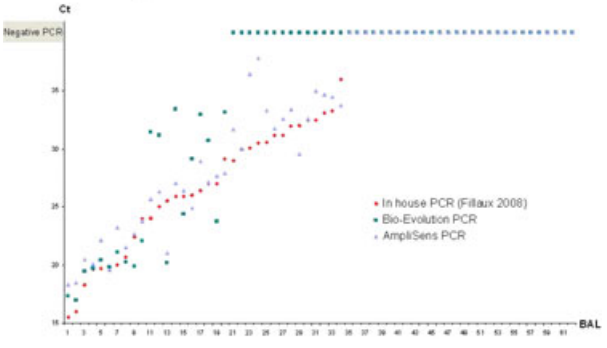

Table 1. Results and concordance of the 3 molecular methods.

		Bio-Evolution PCR			AmpliSens PCR		
		positive	negative	total	positive	negative	total
« in house » PCR *Fillaux et al. 2008*	positive	45	20	65	65	0	65
	negative	0	42	42	0	42	42
	total	45	62	107	64	42	107
AmpliSens PCR	positive	45	20	65			
	negative	0	42	42			
	total	45	62	107			

P062

Evaluation of two blood culture media for the detection of fungi

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Objectives The aim of this study was to evaluate the sensitivity of two blood culture media developed for Bactec 9240 system (Becton Dickinson, USA), namely BACTEC Plus Aerobic/F [Aerobic], designed for aerobic microorganisms, including yeasts, and BACTEC Mycosis [Mycosis] designed exclusively for fungi.
Methods The study was performed on six ATCC *Candida* strains and sixty clinical blood or cerebrospinal fluid isolates belonging to 12 fungal species. The blood culture bottles were first inoculated aseptically with 5 mL of sterile sheep's blood and subsequently with fungi (10–20 cells per bottle; each strain in duplicate or triplicate). The samples were incubated in BACTEC 9240 compartment until positivity, then control subcultures on solid Saboraud and Columbia media were performed.
Results All tested strains grow on Mycosis as well as Aerobic bottles and the time to detection obtained for Mycosis was shorter for 33/60 clinical isolates as well as for *C. glabrata* ATCC 90030 (difference 36 h). The highest and statistically significant differences ($p < 0.05$) in the time to positivity was found for *Candida glabrata* (8/8 isolates) and *Cryptococcus neoformans* (8/8), when Mycosis proceeded Aerobic in 20–48 h (mean 34.5 h) and 0.7–64 h (mean 22 h), respectively. Mycoses was earlier also for isolate of *Exophiala jensemani* (16 h) and some isolates of *C. albicans* (8/14), *C. kefyr* (2/2), *C. guilliermondii* (1/2), and *C. parapsilosis* (4/12).

On the contrary, Aerobic cultures of *C. krusei* (6/6; $p < 0.05$) and 21 other isolates were earlier positive, than Mycosis. This differences were rather low (up to 2 h), except of *C. spherica* (1/1) and *Blastoschizomyces capitatus* (2/3), for which reached 16 and 3 hrs.
Conclusion The study proved that both tested media serve right condition for the culture of fungi and that thy varied significantly in the detection time of some species. This result could suggest that simultaneously use of Aerobic as well as Mycosis media may improve time of diagnosis in many patients, especially those infected with *Candida glabrata* or *Cryptococcus neoformans.* The evaluation of the media should be continued on blood samples from patients with invasive mycoses.

P063

Performance in EQA for fungal identification – a 10-year experience in United Kingdom National External Quality Assessment Service

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Objectives To review participant performance of culture and identification of fungi distributed as part of the UK NEQAS Mycology EQA scheme between 2002 and 2012.
Introduction The United Kingdom National External Quality Assessment Service (UK NEQAS) for Microbiology Mycology EQA scheme provides participants with the opportunity to assess their performance in culture and identification for a variety of clinically significant. fungi.

The objective of an EQA scheme is educational allowing participants to gain experience with genera and species of fungi commonly encountered and fungi rarely isolated in their laboratory. EQA provides the benefit to learn from any failures. Achieving correct results may demonstrate that appropriate characteristic tests are being employed.

Methods A total of 120 fungal isolates were dispatched as panels of four simulated specimens, distributed three times per year. Specimens encompassed 50 species of filamentous fungi and 15 species of yeasts for culture and identification over the 10 year period. Isolates included the most common dermatophytes, non-dermatophytes, opportunist fungi and yeasts, and emerging pathogens that have been previously isolated in clinical specimens. Reported results from participants were analysed for concordance with intended results.
Results Data sets (ranging from 413 participants in 2002 to 444 in 2012) of results entered by participants were analysed. Results showed a range of outcomes from good performance for fungal identification, shown to be very much dependant on the Genus and species of fungus, to significant mis-identifications being reported.

Significant improvement in correctly identifying an isolate, is illustrated by the distribution of the anthropophilic dermatophyte, *Epidermophyton floccosum*. Participant performance showed only 50% concordance in 2008, increasing to 86% in 2010 and 92% when distributed in 2012.

Results showing significant mis-identification or insufficient identification, (which could ultimately result in inappropriate target treatment of the infection) was illustrated with a *Scedosporium prolificans* distributed in 2005. Results returned determined 50% of participants reported to Genus level and 34% of participants reported an incorrect identity of *S.*(*auranticum*) *apiospermum*.

A basidiomycete, Malassezia pachydermatis a recognised cause of catheter associated sepsis in individuals on total parenteral nutrition and is particularly seen in neonatal units was distributed in 2002 and 2010. Identifying correctly to species level was shown with 64% and 75% concordance respectively. Common incorrect identity reported, were species of *Candida*, 28% in 2002 and 15% in 2010.
Conclusions Overall, participants of this scheme have demonstrated marked improvement in identification of the fungi distributed in the simulated specimens.

Analysis of participants results has highlighted identification of some fungi remains challenging.

Advent of molecular techniques such as PCR and pyrosequencing and more recently the introduction of mass spectrometry (MALDI-Tof) to identify fungal organisms, may have a positive influence on the final identification for the future. However, at present laboratory staff need to maintain competence in identifying fungi, to support clinical management of patients. EQA is an important tool in providing evidence of competence.

P064

Veronaea botryosa: molecular identification with amplified fragment length polymorphism and *in vitro* antifungal susceptibility

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Inter- and intraspecific genomic variability of 18 isolates of *Veronaea botryosa* originating from clinical and environmental sources were studied using amplified fragment length polymorphism (AFLP). The species was originally described from the environment, but several severe cases of disseminated infection in apparently healthy individuals have been reported worldwide. All tested strains of *V. botryosa,* identified on the basis of sequencing and phenotypic and physiological criteria prior to our study, were confirmed by AFLP analysis, yielding a clear separation of *V. botryosa* as a rather homogeneous group from related species. *In vitro* antifungal susceptibility testing resulted in MIC_{90}s across all strains in increasing order posaconazole (0.25 μg/ml), itraconazole (1 μg/ml), voriconazole (4 μg/ml), terbinafine (4 μg/ml), caspofungin (8 μg/ml), anidulafungin (8 μg/ml), isavuconazole (16 μg/ml), amphotericin B (16 μg/ml), and

fluconazole (32 μg/ml). Overall, the isolates showed a uniform pattern of low MICs of itraconazole and posaconazole, but high MICs for remaining agents. The echinocandins (caspofungin and anidulafungin) had no activity against *V. botryosa*. There was no statistically significant difference between susceptibilities of environmental (n = 11) and clinical (n = 7) isolates of *V. botryosa* (P > 0.05).

P065

Prospective evaluation of PNA-FISH yeast Traffic Light® and Panfungal® for direct identification and detection of fungi in blood cultures

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Objectives Prospective evaluation of Yeast Traffic Light® (YTL) and Panfungal® (PF) (AdvanDx, Denmark), in 70 blood cultures flagged positive at our Centre (June-December 2012).
Methods Yeast Traffic Light (YTL) and panfungal® (PF) PNA-FISH was tested on specimens from 50 BacT/Alert and 20 BACTEC blood culture vials from intensive care patients (n = 34) and haematology/oncology patients (n = 36). Positive vials were screened by gram-stained smears and fungi-positive ones were concomitantly tested by conventional microbiological methods, for routine diagnosis, and by the YTL and PF probes according to the manufacturers' instructions. YTL and PF results were compared with those of conventional cultures. *Candida albicans, C. parapsilosis* (s.l) and *C. glabrata* (s.l) isolate characterization was confirmed by sequencing of ITS and D1/D2 regions in order to identify *C. dubliniensis* and potential cryptic species within the *C. parapsilosis* and *C. glabrata* complexes. Sequencing identified clinical isolates *C. orthopsilosis, C. metapsilosis, C. bracarensis* and *C. nivariensis* stored at UOA/HCPF 929 Collection (http://www.wfcc.info/ccinfo/collection/by_id/929) were inoculated in blood culture vials,each at 0.5 McFarland standard suspensions, incubated until flagged positive and tested by PNA-FISH YTL to confirm detection of cryptic species within the respective species complexes. Filamentous fungi were identified by morphotyping, ITS, and coding gene sequencing, *Aspergillus* (β-tubulin) and *Fusarium* (TEF). Fungi-negative blood cultures were also confirmed by real time PCR.
Results In total, 30/70 blood cultures were fungi positive (20/30 yeasts and 9/30 filamentous fungi and 1/30 basidiomycetous yeast), 38/70 were positive for bacteria, and 2/70 were negative. Of the yeast positive blood cultures 17/20 were from ICU patients and 3/20 from haematology oncology patients. YTL directly identified 8/20 *C.albicans*/*C.parapsilosis*, 4/20 *C. tropicalis*, and 8/20 *C. glabrata*/*C. krusei*. Sequencing confirmed detection/identification of yeasts as follows: *C. albicans* (n = 5), *C. dubliniensis* (n = 1), *C. parapsilosis* (n = 1), *C. metapsilosis* (n = 1); *C. tropicalis* (n = 4); *C. glabrata* (s.s) (n = 6) and *C. krusei* (n = 2). The overall agreement of YTL and sequencing-identified clinical isolates, as well as agreement of the detected cryptic species that were spiked in blood culture vials was 100%. PF detectedfilamentous fungi in 9/10 vials, all from haematology/oncology patients. Polyphasic identification of isolates confirmed isolation of 4/10 *Aspergillus fumigatus*, 2/10 *A. terreus*, 1/10 *A. flavus*, 2/10 *Fusarium solani*, 1/10 *Malassezia furfur*.
Conclusion The assay was found highly sensitive and specific for *C.albicans* /*C.parapsilosis*, *C. tropicalis*and*C. glabrata*/*C. krusei* (100%). Detection of cryptic species within the *C. parapsilosis* and *C. glabrata* complexes and *C. dubliniensis*, adds a clinical advantage. PF® directly detected common and rare fungal pathogens in immunocompromised patients displaying high sensitivity, however with no specificity. No mixed yeast or filamentous fungi infections were identified in our patient cohort. As expected, the sensitivity of the YTL® assay was identical in BacT/Alert and BACTEC blood culture systems. Though this study is limited regarding the number of patient blood cultures tested, use of the assay supports rapid and reliable yeast identification leading to targeted therapeutic intervention and improved outcomes. Routine use of the assay may contribute in selection of cost-effective antifungal treatment regimens and reduction of hospitalization time.

P066

Molecular identification of *Magnusiomyces capitatus* from paraffinized tissue samples

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Objectives *Magnusiomyces capitatus* (syn. *Geotrichum capitatum*, *Blastoschizomyces capitatus*, *Dipodascus capitatus*) is a filamentous fungus ubiquitous in soil, water, air plants and dairy products. It also colonizes the skin and bronchial and intestinal tract of healthy people producing serious opportunistic infections in patients with haematological malignancies, especially acute leukaemia. Deep infections are difficult to differentiate from *Candida* infections. The aim of this study was to design a PCR assay for rapid and specific detection of *M. capitatus* directly from fixed and paraffinized tissue samples.
Methods For the PCR assay two primers, Bcap-F and Bcap-R, were designed for hybridizing to specific sequences of *M. capitatus* 18S and ITS1 regions (according to NCBI data base). Tissue samples were first deparaffinized and then transferred to MagNA Pure Compact (Roche) for automated DNA isolation. PCR was run in parallel with DNA extracted from fungal cells grown in culture: 3 reference strains of *G. capitatum* (IHEM 5665, 5666 and 6803), 5 clinical isolates identified as *G. capitatum*, and other fungal species that usually cause invasive fungal infections such as *Candida* spp., *Aspergillus fumigatus* and *Saccharomyces cerevisiae*.
Results PCR performed with the designed primers, Bcap-F and Bcap-R, and DNA extracted from paraffinized tissue rendered a specific PCR product with a similar size (approx. 90 bp) to those obtained from DNA of the five *G. capitatum* isolates and the 3 *G. capitatum* reference strains (Fig. 1). No detectable amplification occurred for the other species of fungus tested. The amplicon from the infected tissue was cloned into the pCR2.1 vector (Invitrogen) and the highest matching results corresponded to the 18S and ITS regions of strains registered as *M. capitatus, D. capitatus* or *B. capitatus* in the NCBI database.
Conclusion PCR performed with the designed primers, Bcap-F and Bcap-R, amplifies a 74-nucleotide sequence that correlates with the *M. capitatus* sequences described in public data bases. It is equally

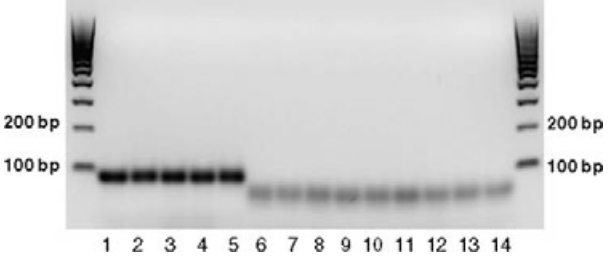

Figure 1 Products of PCR amplification using primers Bcap-F and Bcap-R. Target DNA was extracted from deparaffinized tissue of a patient, or from G. ca.

useful for DNA isolated from cells in culture than for fixed and paraffin included tissue sections. The remaining fungal species tested in the present work, *Candida* spp., *Aspergillus fumigatus* and *Saccharomyces cerevisiae* showed no detectable amplification. The early diagnosis of *M. capitatus* would permit to re-orientate the preemptive or empirical antifungal treatments that usually are not effective against this fungal species.

P067

Aspergillus-lateral flow device as a point-of-care test for the diagnosis of invasive fungal infections in patients with leukemia or receiving allogenic stem cell transplantation

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Background *Aspergillus*-lateral flow device (LFD) is of interest for the diagnosis of invasive aspergillosis due to easy applying methodlogy. Recent studies reported a considerable performance for *Aspergillus*-LFD in serum underwent a pre-treatment procedure. The aim of this study is to investigate the performance of *Aspergillus*-LFD as a point-of-care test for the diagnosis of invasive fungal infections (IFI).
Patients and methods The study was conducted at Erciyes University Haematology Clinics and stem cell transplantation centre, Kayseri, Turkey. Patients who had undergone chemotherapy for acute myelogenous leukaemia (AML), acute lymphocytic leukaemia (ALL), or allogenic stem cell transplantation (ASCT) with an expected neutropenia period longer than 7 days were included in the study. During the study period, a total of 10 mL serum was sampled twice a week from the patients. 5 mL was submitted to the laboratory for galactomannnan (GM) antigenemia test as part of routine care and the other 5 mL was stored for further *Aspergillus*-LFD (provided by Dr Thornton, University of Exeter, UK) and 1,3-beta-D-glucan (BDG) testing. GM (Platelia Aspergillus ELISA; Bio-Rad Laboratories) and BDG (Fungitell; Associates of Cape Cod, East Falmouth, MA, USA) were performed based on the manufacturers'instructions. For LFD testing, serum samples were diluted with tissue culture medium (1:1) and 100 µl serum was applied to the LFD kit. Results were read after 15 and interpreted as negative, weak positive, moderate positive or strong positive. Screening tests were started at the day of an absolute neutrophil count of $< 500/mm^3$ until recovery of neutropenia or diagnosis of IFI. IFI were catogorized based on definitions of the European Organization for Research and Treatment of Cancer and Mycoses Study Group.
Results A total of 75 neutropenic episodes in 64 patients were prospectively followed between February 2012 and January 2013. Median age of the patients were 41 years (range, 18–69 years), and 34 of them were female. The underlying hematological disease was AML in 38 patients, ALL in 24 patients, biphenotypic leukemia in 1 patient, and aplastic anemia in 1 patient. 7 out of 24 patients with ALL, 4 out of 38 patients with AML, and 1 patient with aplastic anemia underwent ASCT. Neutropenia was complicated with fever in 71 out of 75 episodes. IFI were detected in 15 patients (Probable IFI in 13, and fungemia in 2). *Aspergillus*-LFD was strongly positive in the serum of one patient with probable IFI and mederately positive (despite two days of amphotericin B therapy) in the bronchoalveolar lavage of an other patient with possible IFI whose all biomarkers in serum were negative. *Aspergillus*-LFD was positive in serum of two patients (one strongly positive, the other weakly positive) without any radiological evidence of IFI. Fever resolved after empirical caspofungin therapy in an AML patient with strongly positive *Aspergillus*-LFD result with out any radiological finding.
Conclusion *Aspergillus*-LFD can be helpful for the rapid diagnosis of IFI as a point-of-care test while waiting the results of other laboratory tests. However, pre-treatment of serum samples seems to be required for optimum performance of this novel kit.

P068

A direct spot to identify yeast

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Objective Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI tof MS) has considerably changed the way of working in many bacteriology labs. MALDI tof MS is a smart, powerful and cost effective tool which gives fast results with a simple protocol. The database from Bruker has been built by using an extraction. However, to speed up the methodology, the identification of bacteria was tested using a simple spot with the subsequent addition of matrix. That method has been validated and is used by different laboratories. Nevertheless, a simple protocol is still needed to be validated to allow the identification of yeast. This study demonstrates the possibility to use a direct spot to identify *Candida* spp. by shifting the cut off of the score result from 2 to 1.9.
Methods First, 150 yeasts strains isolated in Human specimen during a period of 3 months were collected. Among those yeast identified were 78 *C. albicans*, 2 *C. dubliniensis*, 32 *C. glabrata*, 2 *C. guilliermondi*, 4 *C. kefir*, 8 *C. krusei*, 2 *C. lusitaniae*, 1 *C. nivariensis*, 9 *C. parapsilosis*, 7 *C. tropicalis*, 1 *Cryptococcus neoformans*, 3 *Saccharomyces cerevisiae*, 1 *Pichia fermentans*. The identification was based on three different protocols: (1) the phenotypic method, (2) the identification with MALDI tof (Bruker) with adjunction of formic acid 70% and in parallel (3) the identification of a direct spot. Thereafter, the discrepancy of any of those three results was analyzed with the extraction method provided by Bruker and a sequencing of the rRNA 28s.
Results 55.1% of 78 *C. albicans* showed a score above of 2 by adding the formic acid, 21.8%, 12.8% had a score of 1.9–2 and 1.7–1.8 respectively and 10.3% were misidentified (<1.7). The identification of *C. albicans* using a direct spot showed a score above 2 in 38.5% of the cases, 42.3% were between 1.9–2, 14.1% between 1.7–1.8 and 5.1% were under 1.7.

75% of 32 *C. glabrata* showed a score above 2 by adding the formic acid, the same percentage of 9.4% was seen in scores between 1.9–2 and 1.7–1.8. The score below 1.7 represented 6.2% of the cases. The direct spot identification showed that 53.1% were above 2, 21.9% and 12.5% were between a score of 1.9–2 and 1.7–1.8 respectively and 12.5% were under 1.7 (not reliable). For the most common yeast encountered in routine clinical microbiology, by using a cut-off score of 1.9 for species level ID, no misleading results were obtained.
Conclusion Many articles (REF) have been published on the excellent identification of yeast by MALDI-TOF MS by using extraction protocols. Our group studied the possibility to "jump over"the extraction step, with direct spotting followed by extraction of matrix. We could show that, using a cut-off value of 1.9, MALDI-TOF MS without extraction is a safe way of identifying common yeasts encountered in routine clinical microbiology specially *C. albicans* and *C. glabrata*.

Table 1

Phenotypic ID	MALDI TOF ID	Extraction method	score (%) >2	score (%) 1.9-2.0	score (%) >1.9	score (%) 1.7-1.8	score (%) <1.7	Sequencing	total of ID
C.albicans	C.albicans	Formic acid	55.13	21.79	76.92	12.82	10.26		78
		Direct	38.46	42.31		14.10	5.13		
C.glabrata	C.glabrata	Formic acid	75.00	9.38	84.38	9.38	6.25		32
		Direct	53.13	21.88	75.00	12.50	12.50		
C.Krusei	C.Krusei	Formic acid	71.43	14.29	85.71	14.29	0		7
		Direct	71.43	14.29	85.71	14.29	0		
C.tropicalis	C.tropicalis	Formic acid	71.43	14.29	85.71	0	14.29		7
		Direct	71.43	0	71.43	14.29	14.29		
C.albicans	C.dubliniensis	Formic acid	0	0	0	100	0		2
		Direct	0	0	0	0	100		
		Extraction	100	0	0	0	0		
		Sequencing						2	
Candida spp	C. kefyr	Formic acid	100	0	0	0	0		4
		Direct	100	0	0	0	0		
Candida spp	C. nivarniensi	Formic acid	0	0	0	100	0		1
		Direct	0	0	0	100	0		
		Extraction	100	0	0	0	0		
		Sequencing						1	
Candida spp	C. guillermondi	Formic acid	100	0	0	0	0		2
		Direct	100	0	0	0	0		

P069

Evaluation of an in-house panfungal real-time PCR assay for detection of fungal infections

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Objectives The rise in the incidence of fungal infections and the expanding spectrum of fungal pathogens make early and broad detection as well as accurate identification of fungal pathogens essential. While species- or genus-specific PCR assays assume a certain infection, panfungal real-time PCR enables the unspecific detection and quantification of any fungal DNA present in a clinical sample. In a panfungal PCR assay, universal primers should target sequences specific for all fungal species to guarantee the detection of all fungal taxa. For species identification, PCR amplicons have subsequently to be sequenced and phylogenetically analysed. Thus, in addition to improving turn-around time of microbiological identification, panfungal PCR can provide results which in some cases lead to potentially surprising diagnoses, as enabling diagnosis of also uncommon and rare fungal infections.

Methods In this study a new panfungal HybProbe real-time PCR assay was designed and analytically as well as clinically evaluated. The panfungal real-time PCR assay targets the complete fungal ITS2 region using the LightCycler instrument (Roche®). Due to the broad-range feature of the primers and probe set, the assay allows for the detection of any fungal pathogen. Fungal DNA can be detected by a positive amplification curve at 640 nm in the LightCycler instrument (Roche®). Species are identified by subsequent phylogenetic sequence analysis (BLAST).

After having developed the PCR assay 401 clinical samples derived from patients with clinically suspected invasive or superficial fungal infection were investigated. Results were compared to conventional methods (culture, KOH and histology) and clinical signs of infection.

Results The samples consisted of 90 BALs and 6 bronchial secretions, 52 tissue samples, 66 samples of various sterile fluids, four paraffin tissue sections, 60 EDTA blood samples and 133 dermatological samples (90 nail samples and 43 skin scrapings). Out of these 401 samples 206 showed concordant positive or negative results. 20 samples showed positive results only by the panfungal PCR thus allowing for diagnosis which would have been missed if only culture would have been used. Especially, the use of PCR in blood and tissue samples showed better results than culture. However, there were cases when PCR detected airborne contamination (e.g. *Cladosporium* sp.) or colonization (e.g. *Candida* spp. in respiratory samples) In sum, fungal pathogens were properly identified by the panfungal assay.

Conclusion Results showed that the new assay improved the early diagnosis of fungal infections. The molecular approach helped to identify the species of culture negative but histologically positive samples. The assay was able to reduce time to detection and identification from two weeks down to two days. It was further able to successfully detect rare emerging pathogens, particularly in specimens from invasive infections. Its evident benefits make it a valuable tool especially where accurate and fast detection is necessary, such as the emergency setting, or where culture does not provide a clear or no result. As fungi due to colonization or airborne contamination can be detected as well as the infecting agent, results have to be interpreted in context with conventional methods and clinical data for reliable diagnosis.

P070

Identification of *Candida* and *Aspergillus* species using a new multiplex PCR-based methodology

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Fungal pathogens are the major eukaryotic agents of serious infection in Europe, between them, *Candida* spp. and *Aspergillus* spp. represent the most common and clinically important etiological agents. Due to their different susceptibility to antifungal drugs, the rapid and correct identification of these infecting species is crucial. Several methods have been developed for species identification, but they still present several limitations, such as low specificity and sensibility. In order to overcome these limitations, the analysis of microsatellite sequences represents an excellent alternative for the differentiation and characterization of clinically important species.

In this way, the main purpose of this study is the development of a multiplex PCR strategy for the identification of the major *Candida* spp. and *Aspergillus* spp. species. In order to accomplish this, markers for *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. krusei*, *A. fumigatus*, *A. flavus*, *A. niger* and *A. terreus* were selected for loci specific amplification and were combined into a multiplex strategy. This new methodology was tested in 120 strains from 15 different species for specificity, sensibility and reproducibility.

All the selected loci were tested in single and multiplex conditions and they have demonstrated 100% of specificity and sensibility. This multiplex system developed showed to be a fast, accurate and reproducible method, allowing the accurate identification of all 9 fungal species. The methodology developed is easy to perform and can be implemented at relatively low cost for routine identification in microbiology laboratories.

P071

The comparison of matrix-assisted laser desorption ionization-time of flight mass spectrometry with API 20 AUX test for the identification of *Candida* spp.

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Objectives We evaluated the differences in the identification of *Candida* spp. by MALDI-TOF MS and API 20 AUX methods.

Methods A total of 365 *Candida* spp. isolated from blood and sterile sites (January 2005 - April 2013) were evaluated. *Candida* spp. identification was performed by MALDI-TOF MS and API 20 AUX (bioMerieux, France). Mass spectra were compared with the Bruker database (4613 reference spectra, 2012).

Results MALDI TOF spectra were successfully obtained from 360 /365 (99%) isolates and identified to species, (spectral score ≥2.0) and genus (score ≥1.70)-level, respectively.

MALDI TOF and API had correct best match for the following species: 90% *C. albicans* (126/140), 89% *C. parapsilosis* (83/93), 100% *C. glabrata* (23/23), 100% *C. guilliermondii* (18/18), 100% *C.pelliculosa* (15/15), 87% *C. tropicalis* (20/23), 50% *C. lusitaniae* (6/12), 67% *C. kefyr* (4/6).

Four strains of *C. pelliculosa* had score below 2.

All species that were identified by API as *C. famata* were identified by MALDI TOF as *C. abicans* (n = 1), *C.tropicalis* (n = 1), *C.orthopsilosis* (n = 1), *C. lypolitica* (n = 2) with score range 2,080-2,374.

Five isolates (1.2%) were «not identified» (score of<1.7) by MALDI TOF.

Conclusions MALDI-TOF MS and API gave correct best match in 88% (318/360) of *Candida* isolates. Both methods appear to be reliable for *Candida* spp. identification.

Additional methods must be considered for the identification of *C. parapsilosis, C. lusitaniae, C. tropicalis* and *C. famata.*

P072

Rapid screening of bronchoalveolar lavage samples from immunocompromised patients for *Mucormycetes* using PCR followed by high resolution melt analysis

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Background Rapid diagnosis of invasive mucormycosis and early initiation of effective therapy are crucial for patient outcome. There are no serological tests available for detection of *Mucormycetes* and therefore a use method of molecular biology is appreciated. We used previously published seminested PCR targeting 18S-rDNA. Contrary to original protocol, species identification by sequencing was replaced by high resolution melt (HRM) analysis. Positive results were confirmed and fungal load determined by species-specific real-time PCR.
Methods DNA was isolated from 2 ml of BAL fluid using commercial kit using bead-beating method. Between 1/2009 and 12/2012 we analyzed 99 BAL samples from 86 patients using PCR HRM. Ninety BAL samples were negative, 2 samples from patients with proven mucormycosis were positive, 7 were weakly positive (2× possible invasive fungal disease, 4× other infection, 1× non-infectious process). All HRM positive samples were retested by real-time PCR specific to species identified – 4 were positive and 5 negative.
Results This method is suitable to detect of *Rhizopus* sp., *Rhizomucor pusillus*, *Lichtheimia corymbifera* and *Mucor* sp. in samples from immunocompromised patients at risk of invasive invasive fungal disease. Each species provides unique melting curve enabling easy identification. Results might be obtained within 5 hours (including DNA isolation). If quantification of fungal load is desired real-time PCR takes another 2.5 hours. Sensitivity and specificity of PCR HRM alone were 100% and 93%. Confirmation of the positive result by real-time PCR increased the specificity up to 96%. Despite using non-sterile clinical material (BAL) frequency of false-positive results was very low (<5%). We did not register any false negative results and negative result of the test is therefore able to rule out the pulmonary mucormycosis.
Conclusions PCR HRM a great tool for screening of BAL samples for the four most common *Mucormycete* species. It represents same day diagnostic method, cheap and fast alternative to sequencing that is usually necessary to identify the causative species. Confirmation of the results by quantification of fungal load may enable to distinguish colonization of the airways from active infection.

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P073

Can panfungal PCR detect and identify fungal species in culture negative samples from patients suspected of invasive fungal infection?

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Background Apart from cases of invasive aspergillosis and invasive mucormycosis, are also increasingly common infections caused by the so called "rare fungi". If culture of clinical material is negative, represents PCR and subsequent sequencing, the only possibility to determine the etiological agent. For this application seems to be ideal panfungal PCR, in which are the primers directed to conserved regions fungal genome. Identification of species is done by sequencing. Panfungal PCR is very suitable for fast and accurate identification of cultures of morphologically similar species of yeasts and filamentous fungi. The great advantage of PCR over culture is little chance of influencing the outcome as a result of the initiation of empirical antifungal therapy, because PCR is able to detect DNA of non-viable organisms. On the other hand, we face the difficulty of interpretation of positive detection of fungal pathogens in non-sterile clinical samples (e.g. bronchoalveolar lavage, sputum, samples from autopsy).
Material and Methods In our study, we tested various clinical samples using two independent panfungal assays (one-round PCR and nested PCR) bordering the ITS rDNA gene. Fungal species was determined by sequencing of PCR products. Since we often got mixed sequences by direct sequencing of PCR products, we decided to clone PCR products first. After transformation were colonies screened by panfungal primers, analyzed on an agarose gel and PCR products of different length were selected for sequencing. Overall, between 2009–2012 we tested samples from 108 patients (79 BAL, 19 tissues). Indication for the examination of clinical samples using panfungal PCR was mostly positive result of other diagnostic methods (histology, microscopy, galactomannan or 1,3-beta-D-glucan) and negative culture.
Results In our cohort of patients, we were able to identify several rare pathogens as causative agents of invasive infections – e.g. *Aspergillus terreus*, *Rhizomucor pusillus*, *Cladophialophora bantiana*, *Neosartorya pseudofisheri*, *Fusarium proliferatum*, *Schizophyllum commune*. Most of them were later confirmed by culture. The best results are achieved when we were testing tissue samples (either fresh or fixed) with positive histology. Interpretation of results of testing of BAL samples was difficult due to frequent positivity of several fungal species including those that are mostly considered to be contaminants.
Conclusions Panfungal PCR proved to be an excellent tool for precise identification of rare fungi in fungal cultures. We were also able determine the causative species in histologically positive tissues. Due to difficult interpretation of the positive results has panfungal PCR only limited use for testing of non-sterile clinical material (BAL, sputum).

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P074

Isolation of *Candida famata* from an equine aborted fetus: a case report

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Objectives Equine abortion of infectious origin has been reported to be primarily due to bacterial causes (Brown *et al.*, 1979; Williamson *et al.*, 1984). It is also established that the use of intravenous or urinary catheters, endotracheal tubes and multiple antibiotics treatments increase the risk of contracting systemic mycoses in horses (Reilly, 1994). In the horse, candidiasis is often associated with forms of arthritis (Madison *et al.*, 1995). Specifically, *Candida famata* infection has been found in association with arthritis resulting from trauma during training (Riley *et al.*, 1992). In this case report, we describe the isolation of *C. famata* from an equine aborted fetus.
Methods An equine fetus aborted in the 9th month of pregnancy was subjected to necropsy and tissue specimens were collected for bacteriological, mycological and virological exams. For mycological cultures, liver, gastric content, kidney, lung, and mother placenta samples were seeded on Sabouraud Dextrose Agar (SDA) and

incubated at37°C for 48–72 hours. For the identification of isolated colonies, standard methods (macro- and microscopical observation, Gram staining, germ tube, urea hydrolysis, API ID32C® and Vitek® systems) were compared to the Matrix-Assisted Laser Desorption Ionization-Time Of Flight (MALDI-TOF) mass spectrometry-based method.
Results Bacteriological and virological tests were negative. On SDA, smooth and glabrous white- to cream-colored colonies, grew in 48 hours from all specimens. Microscopic examination showed numerous Gram+ ovoid cells (2.0–3.5 × 3.5–5.0 μm), without pseudohyphae. Germ tube and urea hydrolysis were negative, whereas growth at 37°C was positive. API and Vitek® systems identified the yeast as *C. famata*. In contrast, MALDI-TOF mass spectrometry identified it as *C. guilliermondii* (score 1.861).
Conclusions *C. famata* is a common environmental yeast, however it is seldom found in human and animal clinical specimens. In our case report, in the absence of other pathogens in the fetus, it is reasonable to speculate that *C. famata* could be responsible for abortion, in agreement with the fact that the yeast was present in all organs examined. In accordance with previous reports (Ball *et al.*, 1987, Riley *et al.*, 1992), it is conceivable that colonization of the maternal body by the yeast was facilitated as a result of prolonged antibiotic therapy, manipulations during fertilization, or previous trauma of the mare. Contamination of the stallion sperm cannot be excluded. As previously reported for fungal strains of human origin (Buchan *et al.*, 2013), we found that MALDI-TOF mass spectrometry and standard methods may eventually produce conflicting results in the identification of yeasts of animal origin. This underlines the need of further adjustments of this yeast identification method, such as improved extraction procedures, modified scoring thresholds and expanded fungal reference libraries.

P075

Additionnal MALT extract agar plate as isolation medium improves recovery and identification of filamentous fungi

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Objectives Despite the development of several biomakers in the last 20 years such as galactomannan, glucan, and DNA detection, mycological culture remains the cornerstone for the diagnosis of invasive fungal disease (IFD). Culture is also of utmost importance for antifungal susceptibility, fungal identification at the species level in the era of the emergence of new species, and the delineation of cryptic species with intrinsic resistance to various antifungal drugs (1). To improve fungal culture that is known to have variable sensitivity (2), we tested the interest of MALT extract agar plate in addition to the Sabouraud slanted tube for filamentous fungi isolation in our routine laboratory.
Methods From January to April 2013, respiratory and deep cutaneous specimens were prospectively analyzed. Each specimen was cultured on one chromagar BBL plate (BBL, BD) at 35°C for 4 days, one Sabouraud Dextrose Agar slant with gentamycin chloramphenicol (SDA, Biorad) at 30°C for 3 weeks, and one Malt extract agar plate with gentamycin chloramphenicol (MEA, Merck) at 30°C for 2 weeks. The delay of recovery, the delay of identification, and the number of fungal species per sample were recorded.
Results From 162 specimens with positive mold recovery (136 patients), 150 (92.6%) were low respiratory samples (sputum, bronchial aspiration, broncho-alveolar lavage fluids (BALF)), 5 (3.1%) were high respiratory samples (nasopharyngeal aspirate, sinus aspiration) and 7 (4.3%) were deep cutaneous biopsies (burn skin). Seventy-seven (47.5%) were positive for yeasts and 203 mold isolates were recovered (40 on BBL, 144 on MEA and 95 on SDA). In 47 (29%) specimens with identical species recovered in MEA and SDA, the delay of microscopical identification was significantly improved in MEA compared to SDA ($p = 0.01$) with a benefit of one day, whereas the delay of recovery was not ($p = 0.27$). Mold recovery was significantly improved using MEA ($n = 74$) compared with SDA ($n = 33$) in yeast-positive specimens, ($p < 0.0001$) and not in yeast-positive specimens ($p = 0.14$). Using BBL, mold recovery was significantly less efficient compared to MEA and SDA ($p < 0.0001$). *Aspergillus* and *Penicillium* recovery on MEA was significantly higher on MEA ($n = 73$ and 57) compared to SDA ($n = 65$ and 15) ($p = 0.0002$). In addition, mixed mold recovery was observed in 14 specimens using MAE compared to 6 using SDA.
Conclusion To add MAE agar plate to Chromagar BBL and Sabouraud dextrose agar tube increases fungal recovery and speed up identification. Improving the recovery of molds in BAL fluids is important for the interpretation of galactomannan detection in BALF since all septate filamentous fungi can produce galactomannan.This inexpensive medium can impact on diagnosis and management of IFD in increasing the number of molds detected.

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2. Arendrup et al. Bone Marrow transplant. 2012

P076

Serum and urine galactomannan testing for screening in patients with hematological malignancies

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Background Serum galactomannan (GM) has been established as an important method for diagnosing invasive aspergillosis (IA). In this study we evaluated performance of GM test in urine specimens and compared results with those obtained in serum.
Methods The study was performed from July 2012–March 2013 in adult patients with underlying hematological malignancies hospitalized at the Department of Haematology at the Medical University of Graz in Austria and was approved by the local ethics committee. Serum and urine samples were collected and tested twice weekly (always on the same day). For serum samples an optical density index (ODI) ≥0.5 was considered positive.
Results Each 242 serum and urine samples were collected in 75 patients (33 of those had undergone recent allogeneic stem cell transplantation). 21/242 (8,7%) serum samples from 13 patients resulted GM positive. Sensitivity, specificity, PPV and NPV for different cut-offs for urine samples (compared to serum results) were as follows: 0.15 ODI: 33,3%, 90,5%, 25%, 93,5%; 0.1 ODI: 47,6%, 86%, 24,4%, 94,5%. A cutoff of 0.1 ODI was chosen for urine samples. 11/21 positive serum GM samples gave negative results in urine; 4 of those were derived from a patient with increasing positive serum GM levels in whom urine samples became positive with a 2 week delay. In 2 probable IA cases initially positive urine samples became negative under appropriate antifungal therapy while decreasing serum samples remained positive. 3 serum samples were considered false positives, 2 urine sample false negatives. Urine resulted positive in 31/221 negative serum GM samples. 26 of those were considered false positives. Spearman-Rho correlation analysis revealed a significant positive correlation between serum and urine samples ($p < 0.001$; $\rho = 0.252$).
Conclusions A significant positive correlation was found between urine and serum GM results. Further studies are needed to evaluate the potential role of urine GM testing in IA diagnosis

P077

New *Aspergillus* antigen detection LFD kit for the IPA diagnostic: What results with positive and negative galactomannan ELISA sera?

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Objectives Diagnosis of probable invasive pulmonary aspergillosis (IPA) according to EORTC criteria relies, in part, on the detection of

Aspergillus galactomannan (GM) in patient's sera. GM detection is achieved by using the Platelia™ *Aspergillus* ELISA BioRad, but the test is constrained by false positive and false negative results. Alternative tests that detect surrogate markers of infection include the newly developed *Aspergillus* lateral-flow device (LFD) that is currently in the process of commercialization. Our objective was to evaluate the prototype LFD as a diagnostic test for IPA and to compare it retrospectively to the GM ELISA for detection of probable disease in different patient groups.
Methods Probable IPA was defined according to EORTC guidelines (1), following analysis of clinical, radiological and biological parameters. A total of 66 patients were considered: 38 hematology patients, 21 ICU patients and 7 patients from other settings (pneumology, internal medicine…). Seventy two sera assayed by GM ELISA during routine diagnostic testing have been selected: 18 GM positive and 18 GM negative for IPA patients, then considered as true positive and false negative, respectively and 18 GM positive and 18 GM negative for non-IPA patients, then considered as false positive and true negative, respectively. LFD testing of serum was performed retrospectively in a blinded fashion and visual appraisals of test positivities were conducted as described previously (2) by three independent appraisers. Test samples were compared to known antigen positive and antigen negative sera.
Results In patients identified with probable IPA according to EORTC diagnostic guidelines, 22 serum samples out of 36 were concordant by both GM ELISA and LFD (11 GM positive-LFD positive; 11 GM negative-LFD negative). Among the 14 discordant results, seven serum samples were detected by only GM ELISA and seven other by only the LFD kit. In non-IPA patients, the LFD was considered positive for 2 out of the 36 samples tested, showing superior specificity in these patients compared to the GM test (18 false positive, as chosen for the study design). The LFD results was then significant in these conditions for the IPA diagnostic ($p < 0.05$; chi^2 test) and more particularly in hematology ($p < 0.05$; Fisher's exact test).
Conclusion The LFD allows simple and rapid (under 30 minutes) testing of serum samples with a similar sensitivity for IPA detection compared to the GM ELISA, but with higher specificity. Further testing is needed to validate our findings using the fully commercialized LFD kit but, if confirmed, our results show the usefulness of the LFD as a rapid front-line test for evaluating a patient's IPA status.

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P090

Study about the incidence of yeasts of the genus *Cryptococcus* isolated from the cloacae of parrots of the genus *Amazona aestiva*

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Objective The overall objective of this study was to perform the isolation and phenotypical identification of yeasts of the complex *Cryptococcus* from the cloacae of parrots of genus *Amazona aestiva*, originating from a private bird breeding located in Jundiaí.
Methods The parrots analyzed were anesthetized and had the material collected from their cloacae using swab humidified with sterile physiological saline NaCl 0.9%. The strains were initially inoculated in Sabouraud dextrose agar with chloramphenicol 0.25%. After that, shiny colonies with a mucous look were isolated again. Using the selected colonies, several biochemical tests were performed, such as urease test, phenoloxidase test, carbohydrate assimilation test (auxanogram), sugar fermentation (zymogram), inoculation in CGB agar (L-Canavanine, glycine, 2 bromthymol blue), growth test at different temperatures and exoenzymes search (proteinase and phospholipase).
Results From isolates of genus *Cryptococcus*, 90% of the strains were of *C. albidus* var. *albidus*, and 10% of the strains were of the species *C. laurentii*. All the yeasts were urease positive and didn't present melanin production. Any yeast had fermented glycose and olnly *C. laurentii* has changed the color of the medium CGB to cobalt blue after the inoculation. In the growth test at different temperatures, any isolated could grow at 37°C. In the exoenzymes search, 80% of the isolates were phospholipase producers and 100% were proteinase producers.
Conclusions These results suggest that the yeast transmission is not restricted to the pigeon's environment and its excrements but may also be trough parrots of the genus *Amazona aestiva* that may serve as carriers of *Cryptococcus albidus* var. *albidus*. In this study, could be verified that isolated strains, even being susceptible to temperatures at 37°C, are virulence factors producers what gives them a high relevance in medical clinic. It may be found many studies about synanthropic birds, like pigeons. However, is extremely needed more researches involving wild birds that may be reservoirs and disseminators of yeasts into the environment.

P091

C. neoformans in the metropolitan area of State of Mato Grosso – Brazil

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Objective Cryptococcosis is a severe systemic mycosis caused by two species of *Cryptococcus* that affect humans and animals: *C. neoformans* and *C. gattii*. Cosmopolitan and emergent, the mycosis results of the interaction between a susceptible host and the environment. The occurrence of *C. neoformans* was evaluated in 122 samples of dried pigeon excreta collected in 49 locations in the City of Cuiabá, State of Mato Grosso, Brazil.
Method Public squares (n = 5), churches (n = 4), educational institutions (n = 3), health units (n = 8), open areas covered with asbestos (n = 4), residences (n = 23), factory (n = 1) and a prison (n = 1) were studied. Samples collected from July to December of 2010 were seeded on Niger seed agar (NSA). Dark brown colonies were identified by urease test, carbon source assimilation tests and canavanine-glycine-bromothymol blue medium. Polymerase chain reaction primer pairs specific for *C. neoformans* were also used for identification.
Results *Cryptococcus neoformans* associated to pigeon excreta was isolated from eight (6.6%) samples corresponding to six (12.2%) locations.
Conclusion *Cryptococcus neoformans* was isolated from urban areas, predominantly in residences, constituting a risk of acquiring the disease by immunocompromised as well as immunocompetent individuals.

P092

Morphologic and physiologic features of some potential toxigenic fungi *Stachybotrys* spp.

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Stachybotrys spp. are common Saint Petersburg damp buildings contaminants. They occure in 15% of explored buildings indoor. A quarter

of people which live or work at these buildings complain from headache, nausea and skin irritation. It is known that *Stachybotrys* spp. especially *Stachybotrys chartarum* can cause harmful effects at people health because of a capacity to produce toxic metabolites. One of the dangerous mycotoxins are the macrociclic trichothecene mycotoxins which are highly citotoxic and neurotoxic. The investigations in the field of toxic compounds production by *Stachybotrys* spp. in Saint Petersburg buildings are not sufficient at the present time. It is important to investigate different *Stachybotrys* strains, their morphological and physiological characteristics and metabolic activity in different nourishing media and technical material in vitro.

The objectives

– To study *S. chartarum* and *S. chlorochalonata* metabolites activity against *Paramecium caudatum* in different growth durations;

– To indicate growth and morphology distinctive features of *Stachybotrys* spp. which are the most active against *Paramecium caudatum* in media: malt-agar, potato-dextrose agar.

Materials and methods Fungi growing on the buildings and finishing materials were sampled; a microscopic identification and the DNA-sequensing method of *Stachybotrys* spp., the *Stachybotrys* strains were sowed on the potato dextrose agar (PDA), malt agar, Chapek yeast extract agar (CYA), the potato liquor with 2% dextrose and on the gypsum plasterboard with incubating at 28 °C and 37°C. At the 11, 21, 56 day incubating spores (1×10^6concentration) and cultural filtrate (1 mkl) were tested in *Paramecium caudatum*. The correlation cultural filtrate and the test-object was 1:1, 1:8.

Results 11 *S. chartarum* strains and a strain of *S. chlorochalonata* have been identified. All *S. chartarum* cultural filtrates 11 and 21 day-growing killed *Paramecium caudatum* in 0,5-69 minutes. The 11 day-growing *S. chlorochalonata* strain doesn't take the influense to *Paramecium caudatum*, the 21 day-growing *S. chlorochalonata* influense was the less compared with *S. chartarum strains* filtrates. Most 56 day-growing *Stachybotrys* spp. strains show the toxigenic properties reduction in 1:8 dilution. The metabolite activity of *Stachybotrys* spp. spores which were grown on the media was more powerful than the strains grown on the gypsum plasterboard. The specific features were detect in *S. chartarum* and *S. chlorochalonata* conidias and konidiophores growing in PDA and in malt agar. The *S. chartarum* conidiophores were simpodially and monopodially branched while *S. chlorochalonata* had only monopodially branched conidiophores. Were identified *Stachybotrys chartarum* chemotype *S* features in 7 strains growing in CYA, and *Stachybotrys chartarum* chemotype *A* – in 3 strains, *S. chlorochalonata* - in two strains.

Conclusion 12 cultures of *Stachybotrys* spp. have been determined to species over molecular-genetic method (DNA-sequencing). The studied *Stachybotrys* strains produced toxins which possessed the most biological activity to 21 days of cultivation. More toxic effect to *Paramecium caudatum* showed *Stachybotrys chartarum* chemotype *S*, composed 58% investigated by us strains.

P099

Fungiscope™ – Global Emerging Fungal Infection Registry

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Background Fungiscope™ is a global registry for emerging invasive fungal diseases (IFD) with contributors from 43 countries. The objective is to broaden knowledge on epidemiology of emerging IFD, determine the clinical patterns, describe and improve diagnostic procedures and therapeutic regimens, as well as to facilitate exchange of clinical isolates among the contributors.

Methods Fungiscope™ uses web-based data capture via www.fungiscope.net. For case enrollment, cultural, histological, antigen or molecular evidence of invasive fungal infection is required. Data collected include demographics, underlying conditions, neutrophil count, immunosuppressive medication, clinical signs and symptoms, sites of infection, diagnostic tests, pathogen identification, antifungal treatment, surgical procedures, response to treatment, and survival.

Results To date, 328 cases have been captured. Mucorales (n = 137; 42%), *Fusarium* spp. (n = 54; 17%), yeasts (n = 48; 15%), and dematiaceae (n = 34; 10%) are the most frequently registered pathogens. Chemotherapy/allogeneic stem cell transplantation for hematological malignancy were the predominant risk factors (n = 213; 65%), followed by diabetes (n = 67; 20%), intensive care (n = 68; 21%), and chronic renal disease (n = 33; 10%). Sites of infection included lung (n = 152; 46%), followed by blood stream (n = 70; 21%), paranasal sinuses (n = 56; 17%), and deep soft tissue (n = 53; 16%). For 172 (52%) patients, favorable outcome, i.e. complete or partial response to treatment of IFD was documented. All-cause-mortality and mortality attributable to IFD was 45% and 34%, respectively.

Conclusion The clinical relevance of emerging IFD is increasing. In a short time period, a wide variety of cases from Europe, Asia and North and South America was documented. Further investigators are cordially invited to contribute to Fungiscope™.

P100

New and emerging fungal pathogens in companion animals in Russia

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A number of new and emerging fungal pathogens were reported in human medical mycology in recent years. Fungi previously recognized to cause disease rarely or under specific conditions are now reported with increasing frequency. Appearance of new fungal pathogens in extrinsic geographic areas was also observed. Data concerning this subject with respect to animal mycoses is very limited but still significant due to zoonotic potential of some fungal pathogens.

Objectives To estimate the appearance of new and emergent fungal pathogens in companion animals over two time periods in Moscow Region, Russia.

Methods Retrospective case-study analysis was performed using laboratory records of animals suspected for mycoses and presented for mycological examination over two seven-year periods 1997-2004 and 2005–2012. The animal species were dogs, cats, rodents and reptiles. The mycological examinations were performed by conventional mycological methods including history, direct microscopy and fungal culture on common and selective media. The clinical significance of opportunistic pathogens was estimated in accordance with Walshe & English criteria.

Results Totally 1394 clinical veterinary samples were mycologically examined. The incidence of mycoses caused by opportunistic fungi increased from 19,3% in 1997-2004 up to 78,5% in 2005-2012. *Malassezia*-associated infections emerged from 7,4% to 52,1% with prevalence of *M. pachydermatis*. Mycoses caused by non-dermatophytic filamentous fungi increased from 11,8% to 25,3% with prevalence of *Fusarium, Alternaria, Aspergillus, Penicillium, Scopulariopsis, Chaetomium* species. Moreover several fungal pathogens previously unreported in Russia were detected in 2005–2012. Keratinophylic fungus *Chrysosporium* anamorph of *Nannizziopsis vriesii* (CANV) was isolated for the first time from *Iguana igauana* in 2007. In 2012 CANV became the prevalent fungal pathogen in reptiles (39,4%). Actinomycete *Nocardia asteroides* was diagnosed for the first time in a cat in 2008. Unusual case of allergic pneumonitis in a dog caused by inhalation of basidiospores of *Langermannia gigantea* was diagnosed in 2009.

Conclusion The dramatic emerging of opportunistic mycoses in animals was revealed in recent years (2005–2012) in comparison with 1997–2004 yy. *Malassezia* yeasts and non-dermatophytic filamentous fungi had risen in incidence. A number of new fungal pathogens extrinsic for Russia were detected. Among them *Chrysosporium* anamorph of *Nannizziopsis vriesii* (CANV) recognized as a true

pathogen of cold-blooded animals had extended extensively in very short time. The zoonotic potential of *Chrysosporium* species reported by some authors should be taken into account.

P101

Fatal break-through infection with *Fusarium andiyazi*: new multi-resistant etiological agent cross-reacting with *Aspergillus* galactomannan enzyme immunoassay

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Objectives We present the first human disseminated infection caused by *Fusarium fujikuroi* species complex (FFSC) member *Fusarium andiyazi. F. andiyazi* is so far only known as plant pathogen, but disseminated infections by members of the *Fusarium fujikuroi* species complex (FFSC) occur regularly in immunocompromised patients. We want to alert the community to this new etiological agent, which is multi-resistant and can cross-react with the *Aspergillus* galactomannan enzyme immunoassay.

Methods/results

Case report Fever, respiratory symptoms, and abnormal computerized tomography findings developed in a 65 years old man with AML, who was under posaconazole prophylaxis during his remission-induction chemotherapy. During the course of infection, two consecutive blood galactomannan values were found positive and two blood cultures yielded strains resembling *Fusarium* species according to morphological appearances.

Identification and characterization of the etiological agent The etiological agent proved to be *F. andiyazi* based on multilocus sequence typing (MLST). Sequencing of the ITS region did not resolve the closely related members of the FFSC, but additional data on partial sequences of transcription elongation factor 1 alfa did. A detailed morphological study confirmed the identification of *F. andiyazi* that had previously only been reported as a plant pathogen on different food crops. Antifungal susceptibility tests against 8 antifungal drugs, showed that the strain is multi-resistant to many currently applied drugs, including amphotericin B and voriconazole.

Conclusion *F. andiyazi* proved multi-resistant to all tested antifungal drugs, cross reacts with the *Aspergillus* galactomannan assay, and a DNA-based identification of gene sequences not currently adopted in many labs is necessary for its identification. This case of a patient under prophylactic posaconazole treatment, who was infected with this new opportunistic pathogen which caused galactomannan positivity is presented to attract attention on the matter.

P102

Syncephalastrum and *Fusarium* wound infection following a motor vehicle accident; a case with favorable outcome

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Introduction Cutaneous infections due to rare molds such as members of the order Mucorales or hyalohyphomycetes such as *Fusarium* spp. cause considerable morbidity and mortality, but early diagnosis leads to improved outcome. We report a case of proven fungal wound infection caused by the mucoralean mold *Syncephalastrum racemosum* and *Fusarium* spp. in an immunocompetent young male after a motor vehicle accident, successfully treated with early and aggressive surgery plus antifungal therapy.

Case report A 36-year-old, previously healthy man underwent an external fixation of the left leg, due to an open tibial shaft fracture following a motorcycle accident. Ten days later, a 10 × 10 cm skin necrotic lesion developed in the anterior patellar area. Fifteen days postoperatively extensive surgical debridement was performed (day 0) and the tissue was sent to Pathology and Microbiology departments. Wet tissue mount examined with KOH and Blankophor-P, demonstrated broad, nonseptate, ribbon-like hyphae with right-angle branching, characteristic of Mucorales. Thin, septate hyphae consistent with hyaline molds other than Mucorales were also observed. Tissue cultures on Sabouraud dextrose agar yielded *Syncephalastrum racemosum*, susceptible to amphotericin B (MIC = 0.25 mg/L) and a resistant strain of *Fusarium* spp. with amphotericin B and voriconazole MICs both equal to 4 mg/L (Sensititre, Trek Diagnostics). Histology examination showed intense inflammatory infiltration mainly consisting of neutrophils, and many hyaline, branched, aseptate and septate hyphae, stained with both haematoxylin-eosin (H&E) and periodic acid-Schiff (PAS). Liposomal amphotericin B (5 mg/kg/day) combined with voriconazole (200 mg twice daily) were administered intravenously (day 3). No serious side effects were observed, apart from vision changes and sensitivity to light, attributed to voriconazole. Seven successive debridements were required because both molds were repeatedly recovered from follow-up tissue cultures. A vacuum-assisted, negative pressure device was used to promote revascularization of the area. On day 25, cultures were negative; the patient showed a marked clinical improvement and trauma was evaluated to receive an autologous skin graft.

Discussion and conclusions *Syncephalastrum* has very rarely been identified as a cause of infection in immunocompromised or immunocompetent hosts. *Fusarium* spp. has rarely been involved in skin necrotic lesions in non-immunocompromised individuals. Clinical suspicion should be raised in rapidly necrotizing trauma and confirmed with tissue-based laboratory investigation. Direct examination of wet tissue mount with KOH preferably with the addition of a fluorescent whitening agent such as Blankophor, is of paramount importance for prompt diagnosis. Wide and repeated debridement of necrotic tissue in combination with systemic antifungal therapy is necessary for a favorable outcome.

P103

Lymphocutaneous sporotrichosis; first reported case from Greece

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Introduction Sporotrichosis is a subcutaneous mycosis, caused by *Sporothrix schenckii*, a dimorphic fungus rarely encountered in European countries, including Greece. We report a case of lymphocutaneous sporotrichosis in a 22-year- old male patient.

Case report The patient presented with painless nodular-ulcerated lesions along the right lateral region of the neck with locoregional lymphadenopathy. The onset of his symptoms occurred as a small painless nodule 60 days previously, during a visit to Colombia. Culture of skin scrapings yielded a fungus with the characteristic colony and microscopic morphology of *Sporothrix schenckii*. Culture of a subsequent tissue biopsy specimen yielded the same fungus. Production of "cigar-shaped" yeast cells after 7 days incubation of the fungus in

Brain Heart Infusion (BHI)-broth at 37°C confirmed the identification. Treatment with itraconazole 200 mg per-os daily for 30 days showed excellent results. The course of treatment continues with monthly clinical evaluation for a total duration of 3–6 months.
Conclusions Lymphocutaneous sporotrichosis has a characteristic "sporotrichoid pattern" with dermal and subcutaneous nodules and ulcers developing along the path of lymphatic drainage witch needs to be differentially diagnosed from an atypical mycobacterial infection, in particular with *M.marinum* but also other species (*M.chelonae*, *M.kansasii*). Topical therapy is not effective. Itraconazole 100–200 mg per day for 3–6 months is the treatment of choice and amphotericin B for severe or disseminated disease. To our knowledge this is the first documented report of sporotrichosis in Greece.

P104

First imported coccidioidomycosis in Turkey

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Background Coccidioidomycosis is caused by the dimorphic fungi *Coccidioides immitis* and *Coccidioides posadasii*, which are endemic in arid restricted geographical areas in America, mainly Arizona, New Mexico, Texas, California. Infection occurs after inhalation of conidia present in dust clouds. Coccidioidomycosis cases have been reported in nonendemic parts of the world, including Europe, by increased international travels.
Objectives We report the first culturally proven case of coccidioidomycosis in Turkey and present a brief overview of some imported cases in European countries.
Patient/methods The patient was a 41-year-old otherwise healthy Caucasian male who travelled to Texas in 2011 for three months. He returned home with severe symptoms, fever, muscle aches, initially nonproductive but then productive cough, wieght loss, and malaise. He admitted to several hospitals with gradually increasing complains during the subsequent 12 months. Clinical suspicion was histoplasmosis or blastomycosis. Empirically antifungal treatment with itraconazole was started on the third month of his return. About 12 months later from his return, a biopsy sample from his neck lesion was submitted to Cerrahpasa Medical Faculty (CMF) Deep Mycosis Laboratory and diagnosis was based on direct microscopy of two subsequent subcutaneous biopsy specimens and culture. Confirmation of the clinical isolate as *Coccidioides*sp. was performed by rDNA sequencing. Integrative medical consultations had performed in CMF by multi-disciplinary healthcare professionals to asses and fallow up the patient's condition and treatment, and he was symptomless on the last fallow up.
Results and conclusions In recent years, travel related mycosis including coccidioidomycosis have been reported outside their endemic areas. Whereas most patients with primary infections recover spontaneouly, some individuals develop severe disease. Travellers to endemic areas may be given specific advice to avoid exposure to outdoor dust and care must be taken the patient's travel history in nonendemic home countries.

P105

Phaeohyphomycosis due to *Peyronellaea zeae-maydis*: molecular and phenotypic characterization of clinical isolate

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Objectives To identify by conventional and molecular methods an unusual isolate originated from a case of cystic phaeohyphomycosis. Also, to know its *in vitro* susceptibility to antifungal agents, and characterize it as to the production of exoenzymes and the melanin type it has.
Methods A conventional characterization was performed by the inoculation of the material collected from the patient in mycosel agar medium, and, from the cultures obtained, it was performed a slide culture technique with potato dextrose agar, and for macroscopic visualization of the microorganism, it was inoculated into plate containing Sabouraud dextrose agar. The molecular identification was performed by sequencing of the ITS1-5.8S rDNA-ITS2, using ITS1 and ITS4 primers. The sequence was assembled and compared with sequences reported in GenBank using the basic local alignment search tool (BLAST) algorithm. The antifungal activity test was performed according to the document M38-A2 - CLSI.

The evaluation of the production of exoenzymes urease, gelatinase, protease, lipase, phospholipase, DNase and keratinase was verified by the inoculation of a standardized suspension of the fungus in specific media already described in the literature. The identification of the melanin type produced by the strain was verified using the specific DOPA-melanin inhibitor (L-DOPA) and specific inhibitor of DHN-melanin (tricyclazole) separately, at concentrations between 5 and 200 mg/L on Sabouraud dextrose agar.
Results In the cultural examination, filamentous colonies were observed, with initial hyaline aspect that have become dematiaceous over time. In slide culture techniques it was observed the presence of fructification with pycnidia and dematiaceous hyphae. The final identification was obtained by molecular methods, and the isolate was identified as *Peyronellaea zeae-maydis*, a phytopathogen. The microorganism showed a higher susceptibility to terbinafine (MIC = 0.125 mcg/ml), followed by itraconazole and ketoconazole (MIC = 0.25 mcg/mL) and resistance to amphotericin B (MIC = 2.0 mcg/ml) and voriconazole (MIC = 8.0 mcg/ml). Regarding exoenzymes, the isolate was able to produce urease and gelatinase. It has high production of DNase, keratinase, protease and lipase and moderate production of phospholipase. We identified the presence of DHN-melanin in the sample, and in tricyclazole concentrations from 100 mg/L, the colonies showed up completely white and tenuous, and in all concentrations of L-DOPA, the colonies were presented with dematiaceous staining similar to control.
Conclusions The fact that the patient was elderly and had diabetes may have contributed to the occurrence of infection by this microorganism, as well as their high production of exoenzymes, which are critical to the installation of the pathogen in the host. So far this microorganism has not been reported to cause phaeohyphomycosis in humans.

P106

A pooled analysis of 151 cases of mucormycosis from Turkey

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Objectives Zygomycosis is an invasive fungal disease which is rapidly progressive and often mortal. It is caused by mucor, rhizopus, rhizomucor and absidia of mucorales class. The known predisposing factors for mucormycosis are uncontrolled diabetes and immune suppressive conditions like hematologic malignancies, long term steroid usage or immunosuppressive treatments., In our study, we aimed to reviewed the published mucormycosis cases in last 17 years from Turkey in terms of data related to age, gender, co-morbidities, signs, diagnostic tools, therapeutic modalities, and mortality.
Method Published mucormycosis cases from Turkey in national and international medical literature in the last 17 years were retrieved from two national (http://uvt.ulakbim.gov.tr, http://www.turkmedline.net) and two international databases (www.ncbi.nlm.nih.gov, http://apps.webofknowledge.com). As keywords 'mukor', 'mukormikoz' and "zigomikoz" were used in national databases and 'mucor", "mucormycosis" and "zycomycosis "adding 'Turkey' in international databases.
Results Data for a total of 151 mucormycosis patients (71 female and 80 male aged 45.4) with definitive diagnosis of invasive fungal infections according to criteria of European Organization for Research and Treatment of Cancer (EORTC) were obtained from 65 reports (34 international, 31'national).We could not achieve the full texts of three reports published in international databases and three reports in national databases. In terms of common clinical findings, the most common symptom was swelling of eye and face (63%), fever (48%), followed by nasal obstruction (% 40), headache (38%), opthtalmoplegia (32%), loss of vision (31%), pitosis (30%), palatal necrosis (25%) and other neurological signs (25%). The most common co-morbidity was diabetes (49%) followed by, hematological malignancies (39.7%) and neutropenia. A total 82 of patients had mycological culture and in 51 it was positive. In radiologic imaging 126 of patients had findings in favor of fungal infection. Diagnosis was made by the help of histopathological investigation in 133 cases. Three patients had been diagnosed on autopsy. Both surgical debridement and antifungal therapy were administered in 115 patients. Four patients had received only surgical debridement and 30 only antifungal therapies. Two patients died before starting any antifungal treatment. Total mortality rate was % 54.3. Although all patients treated by only surgical debridement were alive, the mortality of the patients who surgical debridement could not be performed because of their underlying condition was high (56.7%).
Conclusion Despite new diagnostic tools and therapeutic agents mucormycosis has still very high mortality. Suspicion of mucormycosis is crucial for early diagnosis. In the management, biopsies should be done for diagnosis and combination of effective surgical debridement antifungal therapy should start as soon as possible.

[Correction added on 7 November 2013, after print publication and first online publication: The order of the author names M. Tasbakan and A. Zeka Nazli were changed in Abstract P106.]

Table I: Underlying conditions of the patients

Underlying conditions	n	%
Diabetes	74	49.0
Ketoacidosis	13	8.6
Hematologic malignancy	60	39.7
Neutropenia	53	35.1
Steroid usage	38	25.1
Renal failure	16	10.6
Aplastik anemia	8	5.3
Organ transplant	4	2.6
Low birth weight	1	0.7
Desferrioxamine therapy	1	0.7

*Patients have more than one underlying conditions.

Table II: Mycological culture results

Name	Number	(%)
Mucor spp.	19	(37.2)
Rhizopus spp.	13	(25.5)
Zygomycetes	9	(17.6)
Rhizopus oryzae	4	(7.8)
Rhizopus spp+ yeast	3	(5.9)
Rhizomucor spp	2	(3.9)
Rhizosporium spp.	1	(1.9)

P107

Surveillance of environmental fungi, with focus on *Aspergillus*, in a Portuguese Central Hospital

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Objectives Because immunocompromised patients are more prone to acquire nosocomial infections caused by fungi isolated from the environment, e.g. *Aspergillus*, this study aimed to screen the hospital environment for the presence of fungi and to understand their epidemiology in the different hospital wards analyzed.
Methods During one-year period, four seasonal samplings, i.e., air and hard surface, were performed. A total of 101 air samples and 99 surface samples were collected from the Hematology, Oncology, and Intensive Care Unit (ICU) wards of a Portuguese Central Hospital. A*spergillus* isolates were plated for growth as single colonies on malt extract agar with chloramphenicol to check the colony purity and observe colonial morphology. The universal fungal primers ITS1 and ITS4 were used to amplify DNA from all *Aspergillus* isolates, amplimers were sequenced, and isolates identified to the species-complex level. Statistical analyses were done using SPSS v15.0 program for Windows.
Results *Aspergillus* was the most frequently recovered fungal genus (20.9%), followed by *Cladosporium* (18.7%), and *Penicillium* (17.2%). Thirty-five *Aspergillus* isolates were collected from the wards with hematological patients (bone marrow transplant and hemato-

oncology wards), whereas 15 isolates were recovered from ICU. Among *Aspergillus* isolates from the hospital environment, those belonging to the species-complexes *of versicolores* (n = 26; 32.5%), *nigri* (n = 12; 15.0%), *flavi* (n = 11; 13.7%), and *circumdati* (n = 6; 7.5%) dominated. Hemato-Oncology was the ward with higher fungal counts, whereas the bone marrow transplant ward, which is protected by HEPA-filtration of the supply air, showed the lowest numbers in all sampling periods. A significant association ($p = 0.001$) was found between the season and the *Aspergillus* complexes isolated, with spring and summer having a larger number of different species-complexes detected in the hospital's air and on the surfaces. Nevertheless, air counts showed that the autumn was the season with the highest proportion of *Aspergillus* (one third of the total number of fungi detected). This could be due in part to the presence of construction work near these wards.
Conclusion The knowledge of the epidemiology of environmental fungi in each hospital may allow the establishment of preventive or corrective measures to decrease nosocomial fungal infections.

P108

A 5 year epidemiological study of superficial cutaneous in suspicious patients in Tehran (2006–2010)

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Objectives Identification of different species of dermatophytes and cutaneous mycosis agents for finding infection sources and also educating the community in order to familiarize with consequences of contacting infected people or animals will be helpful; and on this basis, the main objective of this study has been determination of distribution and dissemination ways of superficial fungal skin diseases.
Methods Records of 5500 patients suspected to cutaneous mycosis were evaluated for presence of fungal infection in a 5-year time period from April 2006 to March 2010. Skin specimens were collected from patients through skin scraping. Diagnoses were based on direct microscopy examination and culture in accordance with routine laboratory methods of mycology.
Results In total, 2271 cases (41.3%) were inflicted with cutaneous mycosis, of which, dermatophytosis, with 1279 cases (56.31%) was the most prevalent disease of this type. The remaining included 356 cases (15.68%) of tinea versicolor, 283 cases (12.76%) of erythrasma, 243 cases (10.7%) dermal candidiasis and 110 cases (4.96%) of fungal infections of nails caused from saprophyte moulds. The most common clinical form was tinea cruris with 495 cases (38.7%). Among dermatophytes isolated from cultures, Tricophyton mentagrophytis was the most prevalent agent with 198 cases (41.56%).
Conclusion This study shows that dermatophytosis is still an important fungal skin disease among cutaneous mycosis.

P109

Abstract withdrawn

P110

Congenital candidiasis: transmission of *Candida albicans* from mother to newborns documented by multi locus sequence typing

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Background A 35 years- old woman, was hospitalized for a threat of preterm delivery, at 25 weeks of gestation. Living triplets were delivered vaginally one day later, despite the initiation of a tocolytic therapy. The 3 new-borns died as a result of prematurity, aggravated by systemic congenital candidiasis. There was no maternal history of symptomatic vaginal candidiasis during the actual pregnancy.
Objectives *C. albicans* was isolated from the vaginal specimen of the mother and the 3 new-borns specimens. The aim of this work was to investigate the relatedness of these isolates.
Methods A multi locus sequence typing (MLST) was performed on the different strains of *C. albicans* recovered from the 3 new-borns and the mother (Table). The MLST analysis is based on 7 housekeeping genes: *AAT1a, ACC1, ADP1, MPIb, SYA1, VPS13, ZWF1b*. The sequences of all the PCR products were analyzed using the MLST website for *C. albicans* (http://calbicans.mlst.net).
Results The similar MLST profiles of the strains of *C. albicans* (ST 939) isolated in the mother and the newborns gave evidence for a common source, probably from vagina of the mother, inducing ascendant infection.
Conclusion MLST is a powerful tool for epidemiological purposes and demonstrated the vertical transmission of *Candida albicans* from mother to infant. This case reminds that *Candida* sp. vaginal carrying, even asymptomatic could induce preterm delivery and congenital candidiasis. Recommendations for treatment and screening of candidal vaginitis during pregnancy are scarce. Particularly, there is no consensus for asymptomatic candidal vaginitis treatment.

Table 1

Patient	Localisation	*ACC1*	*VPS13*	*ADP1*	*SYA1*	*AAT1a*	*ZWF1b*	*MPIb*	ST
Mother	Vagina	2	24	2	2	2	5	4	939
Born 1	Blood	2	24	2	2	2	5	4	939
	Skin	2	24	2	2	2	5	4	939
	Mouth	2	24	2	2	2	**20**	4	940
	Catheter	2	24	2	2	2	5	4	939
Born 2	Blood	2	24	2	2	2	5	4	939
	Skin	2	24	2	2	2	5	4	939
	Catheter	2	24	2	2	2	5	4	939
Born3	Skin	2	24	2	2	2	5	4	939
	Mouth	2	24	2	2	2	5	4	939
	Catheter	2	24	**5**	2	2	**20**	4	941

P111

Conservation Of corneal transplants: retrospective analyses of fungal contamination observed at the French eye bank (2005–2010)

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Objective To define retrospectively the incidence and characteristics of fungal contamination of cornea grafts deposited at the French Eye Bank (BFY) over a period of 6 years.
Methods For corneal preservation we used Cornea and Cornea Prep II® Max® (Eurobio, lesUlysses,France) for collecting and storing corneas. For detection of microbial contamination we inoculated corneal preservation media on different culture media: chocolate agar (GC), Schaedler broth (BS) and Sabouraud (S) (BioMérieux, Marcy l'Etoile, France).

With an average of 3 days after the arrival of cornea at the BFY (J0) the collecting medium was inoculated on BS, GC and S and the cornea transferred into the storage medium. Microbial growth could be also directly detected in corneal preservation media thanks to a pH indicator turning red upon contamination. From J12, corneas are available for transplant. Twenty-four hours before transplantation, a second storage medium control was performed (BS, GC and S). These culture media are controlled until day 30 after transplantation.
Results Among the 5535 corneas addressed to the BFY from 2005 to 2010, 1.5% (n = 85) corneas were infected by a fungus, which represented 16% (85/541) of the totality of bacterial and fungal contaminations. The fungal contaminations (84%) occurred preferentially within 10 days of graft collection. Yeasts were 91% (98/107) of fungi isolated and 79% (78/107) of the yeasts were *Candida* spp among which were *C. albicans* (41%, 31/78), *C. parapsilosis* (24%), *C. glabrata* (22%) and *C. tropicalis* (10%). The most commonly isolated filamentous fungi was *Fusarium* spp (44%, 4/9).
Conclusion Fungal contaminations involve mainly *Candida* and are not frequent occuring within 10 days of collection.

P112

Burden of serious fungal infections in Mongolia

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Introduction Mongolia has a geographically dispersed population across a large land-locked area in North East Asia. As no serious attempt has been made to estimate the number of serious fungal infections in Mongolia previously, we have attempted this, based on at risk population data and published rates.
Methods A full literature search was done to identify all epidemiology papers reporting fungal infection rates from Mongolia. We used specific populations at risk and fungal infection frequencies in the population to estimate national incidence or prevalence. The statistical yearbook of Mongolia statistics of 2011; the 2011/12HIV infection and AIDS rates from the HIV/STI Research and Surveillance Department NCCD, WHO TB statistics, 2011; COPD rates from report Prevalence and future approach for prevention and control of Respiratory Diseases; Asthma prevalence was from Vinnanen et al, Allergy, 2005.
Results The Mongolian population is about 2.8 million with 41% under 15 years of age. Estimates are: 5% of women (age 15–50 years) get 4 episodes or more of Candida vaginitis per year, a total of 40,347 annually. HIV/AIDS population is low at an estimated 127 infected people, 64% not on ARV therapy with 19 new AIDS cases annually and 17 deaths. The rate of *Pneumocystis* pneumonia (and cryptococcal meningitis) appears be very low at 1%. There were 4,256 pulmonary TB cases (all but 5 in HIV negative people) resulting in prevalence of 590 cases of chronic pulmonary aspergillosis, using a 15% annual mortality rate, perhaps 50% of the total CPA case load, estimated at 1,181 patients. The prevalence of asthma among adults is low at 1% (weighted mean) ~16,589 people and assuming 2.5% have ABPA415 cases would be expected, and 547 SAFS cases. COPD is common with an estimated 87,162 cases, and assuming a 7% admission rate, 6,101 admissions and 79 cases of invasive aspergillosis in COPD. Assuming a low rate of candidaemia of 5/100,000 141 cases are anticipated and 21 cases of candida peritonitis in surgical patients, among the 24,500 abdominal surgeries annually. Tineacapitis and fungal keratitis were difficult to estimate and probably uncommon.

Table 1

№	Infection	Number of infections per underlying disorder per year					Total burden	Rate /100K
		None	HIV/AIDS	Respiratory	Cancer/Tx	ICU		
1	Candidaemia	-	-	-	96	42	141	5
2	Candida peritonitis	-	-	-	-		21	2
3	Recurrent vaginal candidiasis (4x/year +)	40,347	-	-	-	-	40,347	2870
4	ABPA		-	415	-	-	415	15
5	SAFS		-	547			547	20
6	Chronic pulmonary aspergillosis	-	-	1,181	-	-	1,181	42
7	Invasive aspergillosis	-	-	-	87	?	87	3.1
8	Cryptococcal meningitis	?	0	-	-	-	0	0
9	Pneumocystis pneumonia	-	1	?	?	-	0	0.02
10	Histoplasmosis	?	?	?	-	-	?	?
11	Fungal keratitis	?	-	-	-	-	-	?
12	Tineacapitis	?	-	-	-	-	?	?
	Total burden estimated	40,347	1	2.143	184	42	**41,962**	

Conclusion Serious fungal infections in Mongolia are dominated by recurrent candida vaginitis and allergic and chronic aspergillosis. These basic estimates require epidemiological studies to validate or modify the substantial burden estimates.

P113

Burden of serious fungal infections in Zambia

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Background and rationale Human fungal infections contribute substantially to human morbidity and mortality. With an increase in prevalence and incidences of immunosuppressive conditions such as HIV in Zambia, the incidence is expected to be rising. To date, there is paucity of data describing the epidemiology of fungal infections in Zambia. We estimated the burden of fungal infections in Zambia based on published literature and modelling.
Methods All published epidemiology papers reporting fungal or HIV infection rates from Zambia were identified. We also extracted reported data from the WHO, UNICEF, USAID, UNAIDS, Centre for Infectious Disease Research in Zambia (CIDRZ), Central Statistical Office (CSO), Ministry of Health (MOH), Tropical Diseases Research Centre (TDRC), University of Zambia, Centre for Disease Control and Prevention (CDC). We also extracted reported data from the International Classification of Diseases (ICD) from Ministry of Health as comparators. Where no data existed, we used specific populations at risk and fungal infection frequencies in those populations to estimate national incidence or prevalence. Data for invasive mycoses, CPA, IA, ABPA, Asthma and COPD rates were made on assumptions based on incidence rates reported in the local and international literature. The denominator included the overall Zambian population, number

Table 1 Burden of serious fungal disease in Zambia.

Fungal condition	Number of infections per underlying disorder per year 2010					Total burden	Rate /100K
	None	HIV/AIDS	Respiratory	Cancer /Tx	ICU		
Oesophageal candidiasis	-	4,035	-	-	-	4,035	31
Candidaemia	-	50	-	-	500	550	4
Candida peritonitis	-	5	-	-	100	105	2
RVC (4x/year +)	174,525	-	-	-	-	174,525	2,685.
ABPA		-	22,750	-	-	22,750	175
SAFS		-	27,300			27,300	210
CPA	-	-	22,500	-	-	22,500	173.3
IA	-	-	-	?	?	?	
Mucormycosis	-	-	-	?	-	?	?
CM		920	-	-	-	920	7.1
PCP	-	2,990	?	?	-	2,990	230
Histoplasmosis	?	?	?	-	-	?	?
Fungal keratitis	?	-	-	-	-	-	?
Tinea capitis	250,000	-	-	-	-	250,000	1923
Total burden estimated.	816,271	8,000	72,550	0	600	897,421	

Table: Estimated burden of fungal disease in Zambia. ABPA=Allergic bronchopulmonary aspergillosis, CM=Cryptococcal meningitis, PCP=*Pneumocystis jirovecii* pneumonia, IA=Invasive aspergillosis, CPA=Chronic pulmonary aspergillosis, RVC=Recurrent vaginal candidiasis, SAFS=Severe asthma with fungal sensitisation,

of patients with HIV/AIDS, respiratory diseases as reported in governmental publications.

Results There are about 13 million people in Zambia (2010), Male: Female = 1:1.3. About 46.3% of the population are children (**0–14 years), 2.4% are** >65 years old (median age 16.5 yrs). HIV/AIDS – adult prevalence rate is 13.5% (~980,000). Of the 980,000 HIV+patients (2010), about 598,000 patients were not on ARVs in 2011. Assuming the number admitted to the UTH represents 25% of the total burden, we have estimated 920 CM cases annually. Assuming PCP occurs in 5% HIV+patients (not on ARVs), we estimate that about 2,990 (ARV naive HIV+) have PCP. TB prevalence (including HIV+TB) is 47,000, rate 352/100,000 population. TB incidence (including HIV+TB) is 60,000, rate of 444/100 000 populations with about 9,000 (23.6%) cases in HIV negative people. Assuming 50% of CPA cases are TB related, we estimate the 5 year period prevalence of 22,500 CPA cases (assuming 15% annual mortality) and 4,500 new cases in 2011. We estimate asthma prevalence in adults to be 910,000 (7% population) and 22,750 ABPA cases (2.5% adult asthmatics). The estimated severe asthma prevalence is 45,500 (5% asthmatics) and 27,300 SAFS cases (assuming 60% fungal sensitisation in severe asthma). Oral candidiasis 539,000 (55.1% of HIV+), vaginal candidiasis 174,525 (5% women>15 yrs). It wasn't possible to estimate burden of candidemia, IA, mucormycosis and histoplasmosis because of paucity of data.

Conclusion Using local data and literature estimates of the incidence or prevalence of fungal infections, more than 424,524 people in Zambia are estimated to suffer from serious fungal infections each year. Substantial uncertainty surrounds these estimates due to lack of enough epidemiological data. Therefore, epidemiological studies are urgently required to validate or modify these estimates.

P114

The genetic relatedness of *Candida glabrata* clinical isolates in solid-organ transplant recipients in comparison to the other group of surgical patients

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Objectives *C. glabrata* is one of the most frequently isolated non-*albicans Candida* species. In the past years, *C. glabrata* infections have increased possibly as a result of wide use of azoles that promote rapid selection of resistance. In some locations and in the presence of decreased immunity *Candida spp.* may cause systemic mycosis in patients from high risk groups, especially those undergoing induction therapy, in a course of acute myeloid leukaemia, myelodysplastic syndrome, receiving chronic total parenteral nutrition and after undergoing solid organ transplantation. The aims of the retrospective study were: to estimate the prevalence of *Candida glabrata* in liver and kidney transplant recipients compared to patients with short bowel syndrome receiving chronic total parenteral nutrition, and to determine genetic relatedness in the contexts of nosocomial infections in both groups.

Methods *C. glabrata* clinical strains isolated from patients were identified by using standard mycological procedures. The analysis of genetic relatedness of the isolated strains was conducted by using Melting Profile Polymerase Chain Reaction method.

Results The prevalence of *C. glabrata* comprised 29% of all episodes of fungal colonization and infection in solid organ transplant recipients, and of 54% in hospitalized patients receiving long-term TPN. Among 78 isolates, obtained from 55 solid organ transplant recipients and 2 organ donors, 44 different *C. glabrata* MP PCR fingerprints were observed. Forty seven organ recipients and one organ donor carried unique *C. glabrata* strains. Among 37 isolates obtained from 31 patients receiving long-term TPN, 8 different MP PCR profiles of *C. glabrata* strains were observed. Two patients carried unique *C. glabrata* strains. Most of the *C. glabrata* colonization and infections in solid-organ transplant recipients were caused by endogenic strains. Most of the *C. glabrata* colonization and infections in hospitalized patients receiving long-term TPN could result by patient-to-patient transmission.

Conclusion The prevalence of Candida glabrata was 29% in solid organ transplant recipients and 54% in patients receiving long-term total parenteral nutrition.

Most of *C.glabrata* colonizations and infections in solid-organ transplant recipients were caused by endogenic fungi.

Most of *C.glabrata* colonizations and infections in patients receiving long-term total parenteral nutrition could came from the nosocomial acquisition.

P115

Tinea capitis in Campania, Italy: a 9-year retrospective study

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Introduction Tinea capitis (TC) is a fungal infection of the scalp, hair follicles and hair shafts, especially common in the pediatric population; it is caused by dermatophyte fungi (usually species in the genera *Microsporum* and *Trichophyton*).

Table 1 Prevalence of etiological agents of tinea capitis.

	Cases, n.	Percentage
Noninflammatory		
Black dot	74	51,7
Gray Patch	29	20,3
Seborrheic dermatitis	12	8,4
Inflammatory		
Pustular	22	15,4
Kerion	6	4,2

Objectives The present work was undertaken in order to study the prevalence of TC inCampania,Italy, over a 9-year period. Also, to delineate the prevalence of the causative fungus responsible and the clinical forms of TC.
Methods This retrospective study included all the cases of TC occurring between January 2004 and December 2012 to the Mycology Laboratory at theUniversity ofNaples "Federico II" and mycologically confirmed.

Examination of the whole scalp was carried out to assess the type and extent of hair loss.

Patients were classified according to the morphological types of TC as noninflammatory (black dot [BD], gray patch [GP], seborrheic dermatitis type), inflammatory (pustular, kerion or favus). Samples for potassium hydroxide 20% mounts and fungal cultures were collected. Sabouraud dextrose agar were inoculated with the samples.
Results TC was diagnosed and confirmed by direct microscopy and culture in a total of 143 cases.

A majority of the patients (87%) belonged to the 1–18 year age group.

A slight male preponderance was seen (61%). 53% of patients gave a history of pets at home or prolonged contact with animals.

Noninflammatory TC was more common (80,4%) than inflammatory TC (19,6%). No cases of favus were seen.

Among the noninflammatory cases, black dot tinea capitis was more common than the other types. Among the inflammatory cases, pustular variant was more common than the kerion (Table 1).

M. canis was the dermatophyte most frequently isolated (64.1%), followed by *T. rubrum* (24,5%), *T. mentagrophytes* (3,3%). It has been noted a largest increase of anthropophilic dermatophytes, like *T. yaoundei* and *T. violaceum* (Figure 1).

In the 1–18 year age group, the most common dermatophyte species isolated was *M. canis;* unlike the children, *T. rubrum* was the mycetes most frequently found in adults affected by TC.

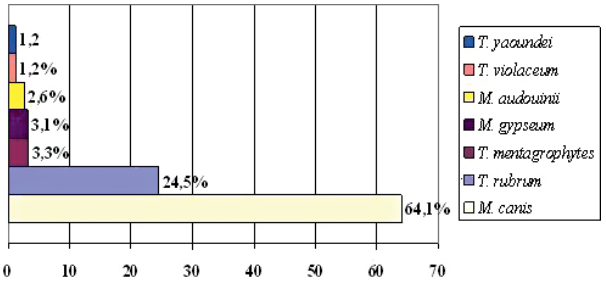

Figure 1 Prevalence of clinical forms of tinea capitis.

In adults clinical features were very variable; 51% showed typical aspects of microsporic TC; 21.5% presented typical trichophytic pattern TC; 6.5% showed kerion. In 21% of cases the clinical feature resembled other dermatitis commonly observed in adults, such as seborrheic dermatitis, folliculitis decalvans, and cicatricial alopecia. 73,2% of adults affected by TC had possible risk factors, such as immunosuppressive treatment, dermatitis on corticosteroid treatment, pet-keeping in an urban residence and family carriers.
Conclusion TC was prevalent in children aged 1–18 years old; in a high percentage of adult patients TC had atypical forms and occurred in immunocompetent patients. Noninflammatory TC was the more common type and *M. canis* was the most common dermatophyte species isolated in children, *T. rubrum* in adults. It has been noted a significant increase of anthropophilic dermatophytes that is possibly linked to the immigration from African countries.

In all cases of tinea capitis the mycological examinations are indispensable for diagnoses and treatment.

[Correction added on 7 November 2013, after print publication and first online publication: The names of the second and third authors have been changed to E. Fiammenghi and A. Patalano respectively, in Abstract P115.]

P116

Nosocomial outbreak of *Pseudallescheria boydii* infections in patients at an intensive care unit

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Objective Nosocomial outbreaks of deep fungal infections have been reported due to hospital construction works or contamination of medical devices such as dressing material. While *Aspergillus* is the most common air-borne fungal pathogen reported in outbreaks, other molds have been reported as well. Here we report on a single hospital outbreak of infections due to *P. boydii* in patients undergoing cardiac surgery.
Methods Within a period of 15 months, six patients (two female, four male, between 7 and 81 years of age) from a hospital in Southern Germany were diagnosed with a *Pseudallescheria/Scedosporium* infection (proven in 4, probable in 2). Isolates were identified by ITS-sequencing and/or specific geneprobes and conventional mycologic methods. In one patient the fungus was amplified from formalin fixed paraffin embedded tissue. Multilocus sequence typing including parts of the *actin*, *β-tubulin* (*BT2*), *calmodulin* and *second largest subunit of RNA polymerase II* (*RPB2*) genes was performed on the suspected outbreak-, and epidemiologically unrelated strains.
Results All patients were infected by *P. boydii*. Clinical isolates from five patients were available for genotyping. Four of the five patients were infected by an identical genotype of *P. boydii*. Five patients had undergone cardiac surgery due to cardiac valve failure in the same surgery unit, and one of them in a unit nearby. One isolate of a patient hospitalized for community acquired pneumonia without exposure to cardiac surgery and the unrelated control isolates showed different genotypes. Three of the six patients died.

Despite intensive screening for *P. boydii* within and outside the cardiac surgical unit, the source of infection remained obscure.
Conclusion The MLST data suggest the relatedness of the 5 isolates recovered from patients undergoing cardiac surgery at a single hospital. Recognition of the outbreak was delayed as the time from surgery to diagnosis of the infection was up to 13 months. No subsequent cases have been reported over the past six months.

P117

Epidemiology of invasive pulmonary mycoses in children and adolescents after allogeneic hematopoietic stem cells transplantation (allo-HSCT)

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Background Changing of the management in transplant practices has led to changes in patterns of invasive fungal infections. We reviewed the spectrum of invasive pulmonary mycoses (IPM) and times of occurrence of the disease in children and adolescents after allo-HSCT.

Materials and methods We prospectively analyzed the medical records of 151 allo-HSCT recipients of pediatric and adolescent units from 2009 to 2012 with lungs lesions on CT scan. All the patients underwent CT guided bronchoscopy with a comprehensive study of broncho-alveolar lavage (BAL) fluid. During the study period we performed 229 bronchoscopic procedures. We used EVIS EXERA II Olympus endoscopic video system with an external diameter of the distal end tubes of the 3.6 mm and 4.9 mm in a specialized endoscopy room or ICU. Different methods of anesthesia were performed, depending on the age and the degree of respiratory failure of patients with mandatory monitoring of vital functions of the body and oxygen levels. The samples obtained were sent immediately to the laboratory for cytology assessment, GM detection, direct microscopy, bacterial, fungal, and mycobacterial culture. Viral screening was performed by PCR.

Results Invasive pulmonary mycoses were seen in 40 (26%) patients according to EORTC/MCG 2008 criteria, 8.3% of them were proven by biopsy. The results of galactomannan (GM) tests in BAL fluid were positive in 60% of the samples. Direct microscopy and culture methods were positive in 38% of the specimens. Most of the pathogens were *Aspergillus* spp. (74%) and *Mucorales* spp. (20%). Among the rare pathogens were detected *Trichoderma* spp. and *P.jiroveci*. In 32 patients (54%) the diagnosis was established after D + 100 allo-HSCT. Overall survival within 100 days from the diagnosis was 41%. No major complications occurred during bronchoscopy and biopsy.

Conclusion - The incidence of fungal infections among children and adolescents after allo-HSCT with lung lesions on CT was 26%.

- Complex investigation of BAL fluid can reliably detect significant pathogens. The use of the indirect diagnostic tests (GM) significantly increases the diagnostic value of BAL. Culture and direct microscopy remains an important diagnostic tool allowing to reveal the local epidemiology.
- Bronchoscopy performed with modern video endoscopy equipment and adequate anesthetic can safely be used in children after allo-HSCT.
- Our data illustrate a shift towards a later occurrence of invasive pulmonary mycosis after allo-HSCT.

P118

Invasive fungal infection burden at a country level: a population-based analysis over the last decade, France, 2001–2010

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Objectives Trends of invasive fungal infections (IFI) are changing in relation with at risk populations. Benchmark estimates of the IFI burden at country level are needed to identify priority areas for improved prevention and control.

Methods The incidence, lethality, trends and risk factors (RF) of the five most frequent IFIs registered in the French hospital discharge database, during 2001–2010, were analysed.

Results There were 35,876 incident IFIs including candidemia (43.3%), *Pneumocystis jirovecii* pneumonia (Pjp: 26.1%), invasive aspergillosis (IA: 23.9%), cryptococcosis (5.2%) and mucormycosis (1.5%). The overall incidence was 5.9 cases/100,000persons/year and lethality was 27.6%. Annual incidences increased over time for candidemia, IA and mucormycosis (+7.8%, +4.4%, +7.3%) and decreased for Pjp and cryptococcosis (−8.5% and −9.8%), respectively ($P < 0.001$). Pjp incidence decreased in AIDS patients (−14.3%), but increased together with a higher lethality in seronegative cases (+13,3% and +5.6%), respectively ($P < 0.001$). Candidemia and IA annual incidences increased in patients with hematological malignancies (HM) (+5.8% and +4.3% respectively, $P < 0.001$). Candidemia, IA and Pjp annual incidence increased in patients with chronic renal failure (> +10% each, $P < 0.001$). The risk of death was significantly increased in HM-associated candidemia, IA, mucormycosis, and non-AIDS cryptococcosis; in tumors-associated candidemia, IA, and non-AIDS Pjp; and in cirrhosis-associated candidemia, IA, and non-AIDS Pjp or cryptococcosis.

Conclusion IFI now represent a major public health priority in developed countries.

P119

Species distribution of *Candida* species isolated from bloodstream infections

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Objective Although the spectrum of fungi causing bloodstream fungal infections continues to expand, *Candida* spp. remains responsible for the majority of these cases. The purpose of this study was to determine the Candida species distribution of bloodstream infections.

Methods Records from the microbiology laboratory were used to identify patients with positive peripheral blood cultures for *Candida* species from 2008 to 2012. Candidemia was defined as the isolation of *Candida* species from blood culture (one isolate per patient). A new episode was considered as the identification of same *Candida* species more than 30 days after the first positive blood culture or finding e new *Candida* species.

Candida species were identified according to convantional methods and their assimilation profiles were determined with ID32C.

Results *Candida* species were the seventh agents in blood cultures after the bacteria. The most common isolates were *C. albicans* (43.8%), and followed by *C. parapsilosis* (28.3%), *C. glabrata* (8.8%), *C. tropicalis* (8.7%), *C. krusei* (2%), *C. kefyr* (1.8), *C. guilliermondii* (1%), *C. lusitaniae* (0.9%) and other *Candida* species (2%).

Table 1 Distributions of bacteria or fungi isolated from bloodstream infections between 2008-2012.

		2008 (n)		2009 (n)		2010 (n)		2011 (n)		2012 (n)		2008-2012 (n)
1	E. coli	329	E. coli	344	Enterococ	323	E. coli	330	E. coli	343	E. coli	1663
2	Enterococ	318	Enterococ	329	E. coli	317	Enterococ	240	Enterococ	227	Enterococ	1437
3	S. aureus	242	S. aureus	215	S. aureus	182	CNS	184	CNS	190	S. aureus	967
4	A. baumannii	192	A. baumannii	196	CNS*	160	A.baumannii	179	A.baumannii	160	A. baumannii	870
5	CNS*	163	CNS*	162	K. pneumoniae	157	S. aureus	174	S. aureus	154	CNS*	839
6	K. pneumoniae	143	K. pneumoniae	130	A. baumannii	143	Candida spp	133	K.pneumoniae	154	K. pneumoniae	713
7	P. aeruginosa	102	Candida spp.	125	Candida spp.	130	K.pneumoniae	129	Candida spp	141	Candida spp.	598
8	Candida spp.	69	P. aeruginosa	102	P. aeruginosa	109	P.aeruginosa	109	P.aeruginosa	74	P. aeruginosa	496

*CNS: Coagulase-negative staphylococcus

E. coli: Escherichia coli; S. aureus: Staphylococcus aureus; A. baumannii: Acinetobacter baumannii; K. pneumoniae: Klebsiella pneumoniae; P. aeruginosa: Pseudomonas aeruginosa; Enterococ: Enterococcus faecium-E. faecalis

Table 2 Distributions of Candida Species isolated from bloodstream infections.

2008-2012		n	%
1	*C. albicans*	263	43.8
2	*C. parapsilosis*	170	28.3
3	*C. glabrata*	53	8.8
4	*C. tropicalis*	52	8.7
5	*C. krusei*	12	2
6	*C. kefyr*	11	1.8
7	*C. guilliermondii*	6	1
8	*C. lusitaniae*	5	0.9
9	*Blastoschizomyces capitatus*	8	1.3
10	*Trichosporon asahii*	3	0.5
11	Other *Candida spp*.*	12	2
12	Other non-*Candida* species**	5	0.9
TOTAL		600	100

* Other *Candida* species: *C. zeylanoides* (3), *C. norvegensis* (2), *C. famata* (2), *C. dubliniensis* (2), *C. glabrosa* (1), *C. pelliculosa* (1), *Candida* spp. (1)

** Other non-*Candida* species:*Rhodotorula* spp.(1), *Geotrichum candidum* (1), *Saccharomyces cerevisiae* (3)

Conclusion This study shows that *C. albicans* remains the predominant *Candida* species isolated from bloodstream infections in 5 years-period in our hospital.

P120

Species distribution and antifungal susceptiblity of *Candida* spp. isolated from ICUs and Febril neutropenic patient's bloodstream infections from a university hospital in Turkey

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Introduction In this study our objective was to evaluate the distribution and the resistance profile of *Candida* spp. isolated from blood cultures of candidemic patiens followed- up in intensive care and haematology/oncology unit of our hospital.

Material-methods Records from the microbiology laboratory were evaluated to identify patients with positive peripheral blood cultures for *Candida* species from January 1st 2003 to December 31st 2007, retrospectively. Clinical data were collected from the patients' available medical records to evaluate the risk factors. Antifungal susceptibility for amphotericin B, caspofungin, anidulafungin, fluconazole, itraconazole, voriconazole, and posaconazole of all *Candida* spp. was performed by E-test method according to the manufacturer's instructions and were evaluated according Clinical Laboratory Standards Institute (CLSI) criteria.

Table 1 Susceptibity rates of *Candida* spp. to major antifungals.

Candida spp. (N:124)	S (n,%)	SDD (n,%)	R (n,%)
C.albicans (36)			
AMB	36 (100)		-
FCZ	35 (97)	-	1 (3)
ITR	21 (58)	12 (33)	3 (9)
VOR	35 (97)	-	1(6)
PSC	35(97)		1 (3)
CAS	36 (100)		-
AND	36 (100)		-
C.parapsilosis (34)			
AMB	34 (100)		-
FCZ	34 (100	-	-
ITR	23 (68)	10 (29)	1(3)
VOR	34 (100)	-	-
PSC	34 (100)		-
CAS	34 (100)		-
AND	13 (62)		21 (38)
C.glabrata (22)			
AMB	22 (100)		-
FCZ	9 (41)	12 (55)	1 (4)
ITR	-	2 (9)	20 (91)
VOR	19 (86)	1 (5)	2 (9)
PSC	6 (27)		16 (73)
CAS	22 (100)		-
AND	22 (100)		-
C.tropicalis (14)			
AMB	14 (100)		-
FCZ	14 (100)	-	-
ITR	10 (71)	4 (29)	-
VOR	14 (100)	-	-
PSC	14 (100)		-
CAS	14 (100)		-
AND	14 (100)		-
C.dubliniensis (7)			
AMB	7 (100)		-
FCZ	7 (100)	-	-
ITR	7 (100)	-	-
VOR	7 (100)	-	-
PSC	7 (100)		-
CAS	7 (100)		-
AND	7 (100)		-

S: Susceptible SDD: Susceptible in a dose dependent manner R: Resistant
AMB: Amphotericin B, FCZ: Fluconazole, ITR: Itraconazole, VOR: Voriconazole, PSC: Posaconazole, CAS: Caspofungin, AND: Andiulafungin

Results One hundred candidemia episodes were identified in 100 patients. One hundred twenty- four *Candida* spp. were isolated. Twenty-three episodes (18.5%) were due to more than one *Candida* spp. The most frequent species were *C. albicans* (29%), followed by *C. parapsilosis* (24%), *C. glabrata* (18%), *C. tropicalis* (11%) and *C. dubliniensis* (6%).

Overall susceptibility to amphotericin B and caspofungin were 100% among all *Candida* species. Susceptibility to fluconazole were almost 100% among all *Candida spp.* except two strains. Although only 38% of *C. parapsilosis* were susceptible to anidulafungin, all other remaining species were susceptible.

Conclusion Our current study revealed a predominance of non albicans *Candida* spp. (71%) in our institution. *C. parapsilosis* ranked first and *C. glabrata* the second among non albicans *Candida* spp. Although more than half of the *C. parapsilosis* were resistant to anidulafungin almost all *Candida* spp. were susceptible to azoles, polyenes and caspofungin.

P121

A 5-year (2008–2012) survey of tinea capitis in the greater Athens area, Greece

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Tinea capitis, the commonest fungal infection of children worldwide, has a varying epidemiology influenced by multiple factors.

Objectives To study the incidence and epidemiological characteristics of tinea capitis in the greater Athens area, in Greece during the last 5 years (2008–2012) when the level of surveillance of the infection has considerably changed due the economic and political issues in Greece.

Methods A total of 920 cases of clinically diagnosed scalp infections have been referred to our Mycology Laboratory for investigation. All patients after having answered a detailed epidemiological questionnaire had hair shafts and scales collected with a sterile scalper and forceps or by using a sterile toothbrush. Where pus was present cotton swabs were used for the collection. Direct examination of the scales and hair was done by using 20% potassium hydroxide. All samples were cultured onto 2% Sabouraud Dextrose Agar supplemented with chloramphenicol and cyclohexamide and incubated at 26–28°C for 3 to 4 weeks.

Results Tinea capitis was confirmed in 384 patients. Out of them 362 were children aged 6 months to 18 years and 22 were adults aged 20 to 84 years.

Out of these children 59.39% were boys while adults were all women (100%). Immigrants accounted for 176 cases, 172 children and 4 adults. Inflammatory clinical forms were present in 8,6% of cases while 22,6% had concomitant lesions on the face, trunk and extremities.

The most frequent isolated dermatophyte was M.canis in greek (42,4%) and immigrant children (30,2%) as well as greek adults (10 cases). T.violaceum is the second most frequent cause in immigrant children (6%) but not greek (0,5%). T.violaceum was also the second most frequent cause in greek adults (8 cases). T.tonsurans which, according to our findings for the same geographical area, was practically absent in the years 1996–2007 (only 2 cases) accounted for 5.2% in immigrant and 0,5% in greek children. T. soudanense is a rare finding concerning only immigrant children (2,08%).

Conclusion Our results indicated that the epidemiology of tinea capitis in the greater Athens area is changing during the last 5 years.

The zoophilic M.canis remains the main case both in greek and immigrant population, but anthropophilic dermatophytes are emerging probably as a result of the lack of surveillance in the immigrant communities who live in very low socioeconomic conditions.

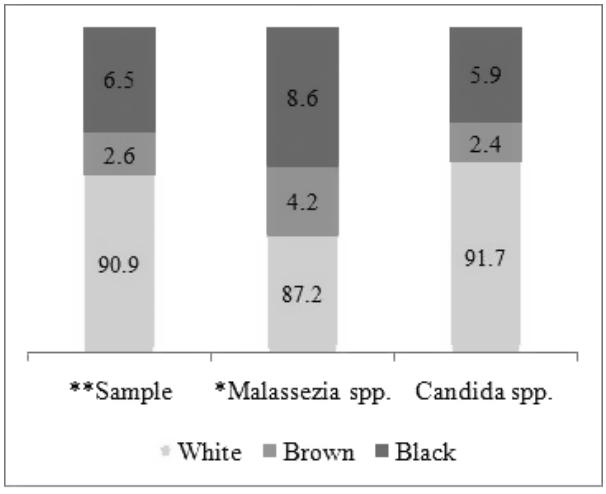

Figure 1. Ethnic proportion found in comparison with the sample proportion (%).
* p-value <0.05
** ethnic sample proportion, considering all subjects submitted to mycological direct examination

Table 1: Positive associations between the sites affected and fungi (%).

Genus	Sites with positive associations (%)
Malassezia spp.	Trunk (73.4%)
	Arms (14.2%)
	Face (5.6%)
Candida spp.	Fingernails (72.4%)
	Oral mucosa (1.1%)
	Hands (5.4%)

P122

Genera *Candida* and *Malassezia*: 16-year study in a metropolitan area, Southern Brazil

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Objective To determine the prevalence of genera *Candida* and *Malassezia* in the metropolitan area of Porto Alegre, Brazil, and to compare genera based on patients' data.

Methods A cross-sectional study was developed using the data of direct mycological examination from all patients attended at Department of Dermatology of Complexo Hospitalar Santa Casa de Porto Alegre, southern Brazil, from January 1996 to December 2011. The statistical analyses performed for each specific objective were: Simple linear regression (to determine the prevalence of behavior over the years), Chi-square (to compare prevalence of fungi between the genders), Mann-Whitney U (to compare patients' age between the genders), Kruskal-Wallis (to compare the ages of patients among the genera); Chi-square corrected by Bonferroni (to compare ethnic proportion of cases affected by each genera with the sample proportion), and Fisher's Exact Test/residues analysis (to determine the differences among the anatomical sites affected by fungi). In all analyses, $\alpha = 0.05$ was considered.

Results The total sample was 71463, and 53.9% (38520) were positive for fungi, *Candida* spp. was found in 12.5% (4815) and

Malassezia spp. was positive in 5.8% (2241). For both genera, there was a decrease over the years when compared with other results of direct examination. The angular coefficients (b) were −0.3 and −0.7%/ year for *Candida* and *Malassezia*, respectively. Women infected by *Candida* spp. were older than men (54 and 47 years, respectively), while there was no difference between the ages (31 years for both genders) for infections caused by *Malassezia* spp. The genus *Malassezia* was more frequent in men than in women (7.1 and 5.1% of all positive results of direct examination, respectively). As to the genus *Candida*, it was more prevalent in women (15.9% of women were affected by this genus versus 5.8% of men). The median age of patients infected by *Malassezia* spp. is below the age of those infected by *Candida* spp. (31 and 53 years, respectively). *Malassezia* spp. affected more browns and blacks and less whites than the expected (Figure 1). For the genus *Candida*, there was no difference between the ethnic groups. There was difference ($p < 0.001$) among the sites infected by fungi, showing a preference for certain regions of the body, being the trunk the region most affected by *Malassezia* spp., and fingernails the most affected by the genus *Candida*. (Table 1), and for genera *Malassezia* and *Candida*, the nails and skin of the feet were the areas with the largest negative associations.

Conclusion For the genus *Candida*, women were more affected by the yeast, and with the age being higher than men. The largest positive association occurred with the fingernails, and there was no difference between ethnic groups. As to the genus *Malassezia*, it showed strong positive association with the trunk, and there was no difference in age between men and women, but it affected men and brown and black ethnic groups more often. In this study, it was observed there was a decrease in the frequency of these fungi over of sixteen years studied.

P123

Dermatophytosis: a 16-year study in a metropolitan area, Southern Brazil

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Objective To determine the prevalence of dermatophytes in the metropolitan area of Porto Alegre, Brazil, and to compare species based on patient data.

Table 1. Prevalence of dermatophytes between genders in the metropolitan area of Porto Alegre, Brazil (1996-2011)

Species	Infection in gender (%)		p<0,05
	Male (n= 5117)**	Female (n= 9067)**	
T. rubrum	51.5	30.4	*
T. interdigitale	23.0	19.9	*
T. tonsurans	0.6	0.6	
M. canis	1.6	1.7	
M. gypseum	0.9	0.8	
E. flocossum	1.7	0.5	*
Total	79.3	54.9	*

* There is statistical difference in the prevalence of infection between the genders.

** Refers to cases positive for fungal in culture examination.

Table 2. Age (median) in general and gender in dermatophytosis cases in the metropolitan area of Porto Alegre, Brazil (1996-2011)

Species	Age (years) Median (quartiles 25 / 75)			
		Gender		
	Total (n= 8619)	Male (n= 3842)	Female (n= 4774)	p<0.05
T. rubrum	40 (29 / 53)	38,5 (28 / 52)	41 (29 / 54)	*
T. interdigitale	43 (30 / 55)	40 (28 / 50)	45 (31 / 56)	*
T. tonsurans	40,5 (24 / 52)	38 (24 / 54)	45 (26 / 52)	
M. canis	11 (6 / 33)	7 (4 / 13)	20 (8 / 37)	*
M. gypseum	23 (6 / 46)	7 (3 / 26)	32,5 (15 / 48)	*
E. flocossum	37 (28 / 50)	34 (27 / 42)	45,5 (34 / 62)	*

* There is statistical difference in age between genders ($\alpha = 0.05$)

Methods A cross-sectional study was done using the data from all patients' direct mycological and/or culture examinations, attended at Department of Dermatology of Complexo Hospitalar Santa Casa de Porto Alegre, south of Brazil, from January 1996 to December 2011. The statistical analyses performed for each specific objective were: Simple linear regression (to determine the prevalence of behavior over the years), Chi-square (to compare prevalence of fungi between the genders), Mann-Whitney U (to compare patients' age between the genders), Kruskal-Wallis (to compare the ages of pacients among species of dermatophytes); Chi-square corrected by Bonferroni (to compare ethnic proportion of cases affected by each species with the sample proportion), Fisher's Exact Test/Analysis waste (to determine differences between anatomical sites affected by fungi). In all analyses, $\alpha = 0.05$ was considered.

Results Were obtained 14,214 cases mycological culture-positive, being 9,048 cases positive for dermatophytes, making this work the largest epidemiological study of dermatophytosis in the country. *Trichophyton rubrum* occurred in 59.6% of cases, followed by *T. interdigitale* (34%), *Microsporum canis* (2.6%), *Epidermophyton floccosum* (1.5%), *M. gypseum* (1.3%), *T. tonsurans* (0.9%) and *T. violaceum* (1 case). The slopes of the linear regressions, for *T. interdigitale, E. floccosum, T. rubrum* and *M. canis*, were +1.119, +0.211, −0.826 and −0.324% per year, respectively. Males presented higher prevalence of infection (79.3% versus 54.9%), (Table 1) but women were older than men. (Table 2) Patients with *M. canis* and *M. gypseum* were younger, and patients with *T. rubrum* were younger than patients with *T. interdigitale*. (Table 2) *T. interdigitale* and *M. canis* were most prevalent in Caucasians and *T. rubrum* was less prevalent in brown people than expected. *Tinea unguium* was more prevalent of the dermatophytosis (48.5%) being toenails more affected than fingernails (94.4% vs. 4.1%), followed by *tinea pedis* (33.1%), *corporis* (6.8%), *cruris* (5.9%) *manuum* (2.4%), *facie* and *capitis* (1.5% each one) and *barbae* (0.07%). *T. rubrum* was the predominant fungus in all regions of the body except in the scalp where *M. canis* was the responsible for 75% of the cases. The species with the highest positive associations were: *T. rubrum* (groin); *T. interdigitale* and *E. floccosum* (skin of the feet); *M. canis* and *T. tonsurans* (scalp); *M. gypseum* (face).

Conclusion This study corroborates other similar studies in the region related to the distribution of dermatophytes, being *T. rubrum* the most common species followed by *T. interdigitale,* and being *T. tonsurans* the one that presents a very low prevalence, unlike other Brazilian states. However, this study showed gender differences in relation to age and prevalence of the infection by dermatophytes. Moreover, we observed a decrease in the prevalence of *T. rubrum* and *M. canis* and an increase of *T. interdigilate* and *E. floccosum*. In this sense, the continuation of epidemiological studies in the region is necessary for monitorring and controlling the evolution of dermatophytosis.

P124

DNA fingerprinting using DiversiLab system for genotypic characterization of *Microsporum audouinii* and *Trichophyton violaceum* isolates in the Belgian population: preliminary study

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Objectives To investigate the epidemiological determinants responsible for the high number of anthropophilic dermatophytes received by the National Reference Center for Mycosis of Liege (NRCL) during the year 2012. To perform a genotypic characterization by the Diversilab® system focusing on the two main isolated species, *Microsporum audouinii* and *Trichophyton violaceum*. To present a preliminary study preceding the national survey launched in 2013.
Methods A total of 51 strains of *M. audouinii* (50 clinical+1 reference (ref.) strains) and 15 strains of *T. violaceum* (14 clinical+1 ref. strain) originating from different locations through Belgium were included in the study. The fungal strains were first cultivated on Malt agar, then sub-cultured in Sabouraud liquid medium (Fluka). The grown mycelium was processed for DNA extraction following recommendations of the manufacturer (Ultra Clean® DNA Microbial isolation kit, MoBio laboratories). Genotypic analysis was performed using the DiversiLab® system (BioMérieux) for DNA fingerprinting and analysis.
Results Regarding *M. audouinii*, four different genotypic groups of strains were separated. Group 1 includes 11 strains and is only found in the Liège surroundings. Group 2 includes only one strain with little differences compared to group 1 and collected from the Liège area. These two groups may be related to each other. Group 3 contains 36 strains and the reference strain. This genotype is distributed in different Belgium locations. The last group, group 4, contains only 3 isolates sharing low similarities in comparison with the 3 other groups. Concerning *T. violaceum*, 6 different genotypic groups with a mixed geographical distribution were determined. Group 1 includes 8 clinical isolates and the ref. strain. The other five isolates are all different and seem not to be related to each other.
Conclusion The automated typing DiversiLab® system proved to be an easy and efficient method to investigate the molecular epidemiology of dermatophytes infections. Preliminary results of the study show that, through Belgium, several groups of isolates co-exist for *M. audouinii* and *T. violaceum* providing evidence of genetic heterogeneity. This variation can be related to acquired mutations due to environmental adaptation. Further investigations are necessary to better understand the impact of this genotypic variation.

P125

Occurrence of fungemia due to *Candida albicans* and *Candida non-albicans* in children's hospital in the city of São Paulo, Brazil from 2007 to 2010

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Objectives In the last two decades, the epidemiology of candidemia has changed with increased episodes caused by C. non-*albicans* species. This study aimed to evaluate, during four years, the frequency of *Candida* species isolated from blood of pediatric patients in a public hospital in São Paulo, Brazil.
Methods Were studied in the period 2007–2010, yeast strains isolated from blood of 107 patients represented by children from 0–11 years. The identification of all isolates was performed using traditional phenotypic methods. For identification of complex C. parapsilosis was also used molecular techniques, as well as for differentiation between *C. albicans* and *C. dubliniensis*.
Results The total sample was represented by the following species: *C. albicans* (39/107), *C. tropicalis* (25/107), *C. parapsilosis* (23/107), *Pichia anomala* (6/107), *C. guilliermondii* (5/107), *C krusei* (3/107), *C. orthopsilosis* (2/107), *C. glabrata* (2/107), *C. metapsilosis* (1/107) and *C pararugosa* (1/107). During the study period was observed a higher frequency of isolation of *C.* non-*albicans* (63.55%).
Conclusion Non - *albicans* species were more frequent in the course of this study, and this fact proves to be important, especially in the pediatric population, because different *Candida* species are associated with varying degrees of disease severity. We also emphasize that a correct identification of the species is recommended, for this to be reversed in a rapid diagnosis and effective treatment.

P126

The prevalence of candidaemia in a developing african country using manual methods of detection

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Objectives This study aimed to provide information on the prevalence of Candidaemia and the phenotypic epidemiological pattern of Candida species isolated from blood samples of patients at risk in Ahmadu Bello University Teaching Hospital Zaria which is sited in Northern Nigeria, the most populous country in Africa.
Methods A Cross sectional study was carried out and using a calculated minimum sample size of 385,390 patients were recruited into the study. The study population comprised of patients with septicaemia with identifiable risk factors for candidaemia namely Immunosupression, malignancy, low birth weight, Prematurity, broad spectrum antibiotics use (>3/52), ICU admission and post-surgical patients.

Blood samples 2–5 mls and 5–10 mls from consenting paediatric and adult patients respectively was collected aseptically and processed manually. The samples were inoculated into Brain Heart Infusion broth (BHI) as the primary inoculation media at a ratio of 1:10.

The blood culture bottles were vented using sterile 22 gauge needles stopped with sterile cotton wool and then incubated aerobically at 30°C in a humidified dark chamber.

Cultures were examined daily and blind sub culturing was done after 24, 72 and 120 hours of incubation onto Sabourauds dextrose agar + Chloramphenicol, 7% Sheep blood agar and MacConkey agar.

The Isolates were examined microscopically using lactophenol cotton blue and Gram's stain.

Germ tube test and urea test was performed on yeast isolates. The isolates were then sub cultured onto CHROMagar Candida (Oxoid) for speciation.

24 hour old cultures were stored in sterile physiologic saline at room temperature for further identification and testing.

Data obtained was analysed using SPSS 17.

Results Germ tube test was positive for only one of the isolates (5.9%) and all isolates were urease negative.

Of the 390 patients studied, the prevalence of Candidaemia was 4.4% and non-albicans species was more common 16 (94.1%).

There were 223(57.2%) males and 167(42.8%) females with a male female ratio of 1.3:1 and the age range was from 2 days to 90 years.

The various species isolated were *C. parapsilosis* 8 (47.1%), *C. glabrata* 4 (23.5%), *C. tropicalis* 3 (17.6%), *C. krusei* 1 (5.9%) and *C. albicans* 1 (5.9%).

Conclusion Candidaemia is also a problem in Developing countries and in the absence of automated blood culture systems, manual methods of detection can be utilized in diagnosis and management of patients at risk.

The higher prevalence of non-albicans species though uncommon, highlights the need for susceptibility testing since non-albicans species is more implicated in antifungal resistance.

P127

Chronic pulmonary aspergillosis complicating treated pulmonary tuberculosis in Gulu, Uganda

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Objectives 15–25% of Africans treated appropriately for pulmonary tuberculosis (PTB) die within a few years of completing treatment. Chronic Pulmonary Aspergillosis (CPA) may be responsible for many of these deaths. CPA is a progressive condition leading to prolonged fatigue and breathlessness then death from respiratory failure or sudden massive haemoptysis. A recent controlled trial in India demonstrated treatment with generic fixed dose Itraconazole is well tolerated and leads to stabilization or improvement in 76% of patients.

In 1970 36% of 399 British patients with residual cavities after treated PTB were found to have precipitating antibodies to *Aspergillus*. Half of these developed an aspergilloma within a 2 year follow up period. Our group has recently estimated the global annual incidence of CPA at 372,000 cases, with a global five year period prevalence of 0.8 to 1.3 million cases and 43 cases per 100,000 in a representative sub-saharan country (DR Congo). This estimate was based on the results of the 1970 survey and current published data on the frequency of residual cavitation after completing PTB treatment. It does not take account of the possibility of either increased susceptibility or reduced chronic inflammation with fibrosis due to HIV/AIDS.

Methods We aim to measure the prevalence of CPA in Gulu, Uganda. Diagnosis requires a combination of a) chronic respiratory symptoms, b) aspergilloma, pleural thickening or progressive cavitation on chest x-ray and c) *Aspergillus* specific antibodies. We recruited 400 adult patients (200 HIV positive and 200 HIV negative) who completed PTB treatment within the last 7 years, plus 300 healthy controls, between October 2012 and January 2013. Smear negative cases were accepted only if there was a complete resolution of symptoms with treatment.

Results Chronic respiratory symptoms were present in 238 patients (59%). This included haemoptysis in 9 (2%), cough in 77 (19%), fatigue in 150 (37%) and breathlessness in 149 (37%). Chest x-ray demonstrated cavitation in 97 (24%), pleural thickening in 69 (17%) and aspergilloma in 12 (3%).

Mean CD4 count in the HIV positive group was 415 cells/μL and 30 (15%) of the HIV positive patients had CD4 < 200 cells/μL. There was no statistically significant difference in symptoms between HIV positive and negative groups. Cavitation on chest x-ray was more common in HIV negative patients than HIV positive patients (31% vs 18% p = 0.004).

Overall 60 (15%) of patients had both chronic symptoms and x-ray changes consistent with CPA. There was no statistically significant difference in this rate between HIV positive and negative groups.

Conclusions These initial results suggest that CPA may well be a common complication of treated pulmonary tuberculosis. Serum has been taken from patients and will be screened for antibodies to *Aspergillus*. We plan to perform a re-survey of this cohort in 2014 with repeat chest x-ray and serology. This will allow us to identify progression of cavitation. We will then be able to state the frequency of CPA as a complication of PTB in this African population.

Table 1 –Symptoms in 400 patients previously treated for tuberculosis in Gulu, Uganda

Finding	All patients N = 400	HIV negative N = 200	HIV positive N = 200	p-value
Mean time since TB treatment initiation	44 months	42 months	46 months	0.55**
Cough	77 (19%)	31 (15%)	46 (23%)	0.057
Haemoptysis	9 (2%)	4 (2%)	5 (2%)	1.000*
Fatigue	150 (37%)	75 (37%)	75 (37%)	1.000
Breathlessness	149 (37%)	75 (37%)	74 (37%)	0.918
Chest pains	166 (41%)	76 (37%)	90 (45%)	0.155
One or more chronic symptoms	238 (59%)	115 (57%)	123 (61%)	0.415

Note – p-value for difference between HIV positive and negative cases calculated by chi-squared except for rows marked * where Fishers exact test was used and rows marked ** where t-test was used to compare means.

Table 2 Chest X-ray findings in 400 patients previously treated for tuberculosis in Gulu, Uganda

Finding	All patients N = 400	HIV negative N = 200	HIV positive N = 200	p-value
Pleural thickening on CXR	69 (17%)	45 (23%)	24 (12%)	0.006
Cavities on CXR	97 (24%)	61 (31%)	36 (18%)	0.004
Single	22 (6%)	11 (5%)	11 (6%)	0.990
Multiple	75 (19%)	50 (25%)	25 (13%)	0.001
Aspergilloma on CXR	12 (3%)	5 (3%)	7 (4%)	0.507
Possible	9 (2%)	5 (3%)	4 (2%)	1.000*
Probable	3 (1%)	0	3 (2%)	0.123*
Chronic symptoms and x-ray changes	60 (15%)	36 (18%)	24 (12%)	0.093

Note – p-value for difference between HIV positive and negative cases calculated by chi-squared except for rows marked * where Fishers exact test was used.

P128

Fulminant rhinocerebral zygomycosis in a non-immunocompromized thalassemic patient, with severe iron overload

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Objectives To describe a case of rhinocerebral zygomycosis, occurring in a non-immunocompromized thalassemic patient, with severe iron overload, and emphasize the role of iron in the pathogenesis of this mycosis.

Methods The course of a 38-old female transfusion-dependent, but inadequately iron-chelated, diabetic thalassemic patient, exhibiting a fulminant form of zygomycosis is presented, and relevant literature is reviewed.

Results The patient was diagnosed with homozygous β-thalassemia and started regular transfusion program at the age of 14 months. On 1979 she underwent splenectomy and on 1984 she started iron chelation treatment with Desferrioxamine (DFO). Iron chelation was ineffective/insufficient and serum ferritin averaged 2500 ng/ml. She gradually developed diabetes mellitus (DM), hypothyroidism, hypoparathyroidism and hypogonadism. On 1991 she was found HCV positive and received interferon-α treatment. DFO-Deferiprone combination was not afforded, due to co-administration of interferon-α, which produced mild neutropenia. Since 2007 she manifested recurrent episodes of leg arthritis, NSAIDs were unsuccessful, but the combination of 12.5 mg prednisolone daily, plus 10 mg of methotrexate weekly for two months (1.5.2009–30.6.2009), resulted in substantial improvement. On 10.9.2009, while on 7.5 mg prednisolone maintenance, she was presented in another hospital with low-grade fever and right facial pain. She was admitted and treated as sinusitis,

with antibiotics. Three days later her condition worsened. A facial CT scan revealed extensive soft tissue lesions in her right maxillary and frontal sinuses, yet sparing the bone areas. On 15.9.2009 she was referred to our department with uncontrolled pain and signs of left orbital protrusion. BP was 190/100 mmHg, pulse rate 105/min and T 39.3 C. WBC were 18.5/μl (69% neutrophils, 17% lymphocytes, 10% monocytes), fasting glucose 189 mg/dl, without ketoacidosis, but HbA1c was 7.8%. Serum CRP was 38.5 mg/dl, total protein 79 g/l, albumin 39 g/l, γ-globulins 16.1 g/l, iron 221 μg/dl, TIBC 328 μg/dl (saturation 67.4%) and ferritin 3371 ng/ml. The remaining of laboratory work-up was unremarkable. A facial MRI demonstrated extension of the lesions to the right retro-orbital area and the left maxillary and sphenoidal sinus.She was immediately operated by the ENT surgeons and abundant dark-red necrotic-appearing material was removed. Histological examination confirmed the diagnosis of mucormycosis. Although she immediately started treatment with Liposomal AmphotericinB 7 mg/kg/d, her condition continued to deteriorate. On 19.9 she reported loss of vision from her right eye and a new CT revealed two lesions in critical areas of the CNS (pont-cerebellar junction). Ambisome dose increased to 10 mg/kg, but she gradually started to drop her level of consciousness until she progressed to coma on 22.9.2009. She was intubated but succumbed two days later.

Discussion-conclusions Less than 5 cases of zygomycosis, occurring among thalassemic patients have been described in the literature. Although our patient was diabetic and had been exposed to low-dose prednisolone-methotrexate treatment a few weeks prior to the manifestation of zygomycosis, her dominant feature was the poorly controlled iron overload status. We therefore believe that diabetic thalassemic patients, particularly those inadequately chelated or under DFO treatment should be considered as high-risk for the manifestation of invasive zygomycosis.

P129

The ROCANET study: preliminary results from a prospective observational survey of candidaemia in the Rome city

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Objectives *Candida* species are leading causes of nosocomial bloodstream infections and are associated with substantial morbidity, mortality and costs. While *Candida albicans* was by far the predominant species, recent data highlight the emergence of non-*albicans Candida* species, such as *Candida glabrata*, *Candida krusei* and *Candida parapsilosis*. While the antifungal susceptibility pattern is closely linked to the species, it is yet greatly important to understand and monitor the local species epidemiology as well as the antifungal resistance rate. The ROCANET (ROme CAndida NETwork) was established in December 2012 in order to perform prospective surveillance of all candidaemias among patients hospitalized at selected medical centres in the Rome city area. Its aim is to improve the knowledge of the burden of candidaemias in different groups of patients, better define patients at risk and understand the epidemiology, species distribution, antifungal susceptibility and outcomes of candidaemias from the study sites.

Methods All the patients admitted to 10 large hospital medical centres of Rome (Italy) from January 2013 to December 2014 and diagnosed with candidaemia will be studied. Multiple candidaemia will be recorded for patients with recurrent or subsequent episodes of infection. Age, gender, APACHE II and SAPS scores, the Charlson's score as a composite index of comorbidities, primary diagnosis, presence of known risk factors for candidiasis, abdominal surgery, type of infection, microbiological parameters (*Candida* species type, antifungal susceptibility profile, genotype), antifungal treatment and outcome will be recorded. All the *Candida* isolates from the study patients will be collected and maintained at the Clinical Microbiology Laboratory of the Università Cattolica del Sacro Cuore of Rome. The isolates will be re-identified by the MALDI-TOF MS method and sequence identified using the ITS gene region, whereas susceptibility testing will be performed against 7 antifungal agents (anidulafungin, caspofungin, micafungin, fluconazole, itraconazole, posaconazole, voriconazole) using CLSI and EUCAST methods. In the case of antifungal drug-resistant isolates, underlying molecular resistance mechanisms will be assessed, as well all the isolates will be genotyped using multilocus sequence typing, as appropriate.

Results From January 2013 to May 2013, a total of 101 isolates of *Candida* species were studied, including 54 *C. albicans*, 25 *C. parapsilosis*, 8 *C. glabrata*, 7 *C. tropicalis*, 2 *C. guilliermondii*, 1 *C. krusei*, 1 *C. dubliniensis*, 1 *C. pelliculosa*, 1 *C. inconspicua* and 1 *C. lipolytica*. Overall, resistance to the echinocandins was very low, with *C. albicans* (1 isolate) and *C. glabrata* (1 isolate) being resistant to anidulafungin, caspofungin or micafungin and shown to have *fks* mutations. Resistance to fluconazole was low among isolates of *C. albicans* (1 isolate) and *C. tropicalis* (1 isolate), whereas 2 isolates of *C. glabrata* were found to be resistant to fluconazole. Voriconazole and posaconazole were active against all *Candida* species except *C. glabrata* (1 isolate was cross-resistant).

Conclusions Overall, echinocandin and triazole resistance were uncommon. Although no fluconazole and echinocandin co-resistance among *C. glabrata* isolates was observed, continued close surveillance is locally warranted.

P130

Species distribution and voriconazole, posaconazole susceptibility of 840 Candida strains isolated in a Turkish university hospital over a 11 year period

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Background It is important for clinicians to be aware of long term trends in the species distribution and susceptibility of overall *Candida* isolates for every large medical center.

Objectives The aim of this study was to identify retrospectively trendsin species distribution and to determine susceptibility profile to voriconazole and posaconazole of *Candida*species isolated in Cerrahpasa Medical Faculty, Deep Mycoses Laboratory from a variety of clinical sources over a 11 year period.

Methods A total of 840 clinical *Candida* isolates recovered from deep mycosis suspected patients' materials betweenJanuary 2000 and end 2011. Each isolate was obtained from a different patient. All isolateswere identified to the species level by classical morphological and physiological tests including germ tube formation in human serum at 37°C for 3 h, blastoconidia, pseudohyphae, true hyphae and chlamydoconidia formation in Dalmou plate culture on corn meal agar-Tween 80, growth at various temperatures, fermentation of carbon sources, and carbohydrate assimilation patterns. Susceptibility testing was performed by the reference method using the M27-A2 document of the CLSI. Standard antifungal powders of voriconazole (VRZ, Pfizer, Istanbul, Turkey), and posaconazole (PSZ, Schering-Plough, Istanbul, Turkey) were obtained from their respective manufacturers. Recently described species-specific clinical breakpoint (CBP) values were used to categorize the minimum inhibitory concentrations (MICs) of voriconazole as susceptible (S), intermediate (I) and resistant (R) against the strains of *Candida.*

Results Overall, the leading species was *C albicans* (49.0%), followed by *C parapsilosis* (18.0%), *C glabrata* (10.4%), *C tropicalis* (7.8%), *C guilliermondii* (5.8%), *C krusei* (2.7%), *C kefyr* (2.4%), *C lusitaniae* (2.1%) and *C lypolitica* (1.8%). Both VRZ and PSZ demonstrated

excellent *in vitro* activities to all *Candida* isolates with very low (0.4%) resistance rate.
Discussion It is important to obtain information on the species distribution and susceptibility of overall *Candida* isolates for every large medical center. This study provides epidemiological information on the spectrum of *Candida* species isolated in the deep mycosis laboratory of a large Turkish university hospital over a 11 year period. The newer antifungal agents VRZ and PSZ demonstrated excellent *in vitro* activity against all *Candida* isolates.

P131

Evaluation of nosocomial *Candida* infections

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Objective In recent years hospitalization rate is increased due to severe underlying disease and surgical interventions. In hospitals, particularly in intensive care units because of increase in usage of antibiotics, immunosuppressive therapies, central venous catheters and total parenteral nutrition nosocomial fungal infections become an important problem. In this study, nosocomial Candida infections of the last four years were evaluated.
Methods Hospital nosocomial infections data was investigated retrospectively. The characteristics of nosocomial Candida infection cases hospitalized at Celal Bayar University hospital from January 2009 to January 2013 were recorded by SPSS 15.0 program.
Results In the last four years 220 Candida infection episodes occurred in 204 patients. The mostly found risk factors were hospitalization in intensive care unit (72%), urinary catheters (64%) and being more than 65 years old (40%). The mostly seen Candida infections were urinary tract infections (62%) and 67% had urinary catheters. Candida species identification was done in 73% of the cases and the *C.albicans* was the mostly isolated (37,7%). Non-albicans Candida species increased by years. The mortality rate among years was changed between 35–62%. The mostly selected empiric treatment for the fungal infections was fluconazole but empiric treatment was changed into caspofungin when candidemia was diagnosed.
Conclusion In recent years, with the increase in infections caused by Candida species, changes have began to be seen in the diversity of these species. Non albicans-Candida species were also be increased among years in our hospital. This change can be a problem for the treatment response over time and influence our empiric treatment selection. In conclusion, nosocomial Candida infections have to be thought when an infection is determined in patients with various risk factors and especially in intensive care hospitalization and adequate diagnosis and treatment interventions should be performed.

P132

Non-albicans Candida species in blood stream infections

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Objective During recent years, there has been a change in the epidemiology of Candida infections, characterized by a progressive shift from a predominance of Candida albicans to non-albicans Candida species. This study was undertaken to analyze the change in the epidemiology of candidemia in Celal Bayar University hospital, Manisa, Turkey.
Methods A retrospective review of candidemia between 2004 to 2007 and 2010 to 2013 were investigated and the demographic characteristics of patients, underlying diseases, risk factors, isolated Candida species, antifungal treatment and outcome of patients were recorded. The analysis of the data was performed by SPSS 15.0 program.
Results Between 2004-2007, 82 candidemia cases were diagnosed and the mean age of the patients were 43,21 ± 28,11 (1 month-82 years). In 2010 to 2013 period, 67 candidemia cases were found and the mean age was 43,55 ± 26,07 (1 month-84 years). While in the first study period hospitalization in intensive care unit was 42,7%, this increased to 86,6% between 2010 to 2013. In 2004–2007 period C.albicans was isolated in 62,2% of the candidemia cases, in the second period there was a significant increase in non-albicans Candida species. Mortality rate was 46,3 in the first study period and it increased to 59,7% between 2010–2013. There was a significant relation between the mortality and age, urinary catheter presence, mechanical ventilation, total parenteral nutrition and hospitalization in intensive care.
Conclusion As candidemia was an important cause of mortality, empirical antifungal therapy should be thought in patients with risk factors and underlying conditions for mortality. In the selection of empirical treatment, infections caused by non-albicans Candida species has to be considered.

P133

Candida auris, emerging yeast causing candidemia in intensive care units; a multicentre study

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Objectives Epidemiological surveillance of *Candida auris* causing blood stream infections in ICU patients.
Methods During a multicentre candidemia surveillance study, a large number of unusual yeasts were isolated from blood cultures of patients admitted in ICUs. All the isolates were phenotypically identified by commercial system (vitek 2, biomerieux). For the yeast showing concordance <98%, the identity was confirmed by sequencing of D1/D2 region of the 28S ribosomal subunit and Internal transcribed spacer region of rDNA. The phenotypic characters of *C. auris* isolates were analysed. Molecular typing of *C. auris* isolates was performed by Amplified length polymorphism (AFLP). The antifungal susceptibility to a panel of eight antifungals was determined by broth microdilution method as per M27 -A3 protocol of CLSI.
Results Out of total 55 isolates identified by Vitek 2 as *C. haemulonii*, 53(96.36%) were identified and confirmed as *C. auris* by sequencing. The percentage identity on sequence blast search was 99 -100%. The remaining two isolates (3.64%) were identified as *C. haemulonii.* All the *C. auris* isolates did not produce pseudohyphae or true hyphae on CMA. They fermented glucose, maltose and sucrose, whereas *C. haemulonii* failed to ferment maltose. The pattern of carbon assimilations against a panel of 14 sugars showed different profiles for the two species. *C. auris* isolates were able to grow upto 42°C but not in presence of 0.01% cycloheximide. Majority of the isolates showed elevated minimal inhibitory concentrations for amphotericin B (MIC50, 1 μg/mL; MIC 90, 2 μg/mL) and resistance against fluconazole (MIC50, 64 μg/mL; MIC90, 128 μg/mL). Against caspofungin the isolates had high minimal effective concentrations (MEC50, 0.5 μg/mL; MEC90, 8 μg/mL). All other antifungals had good in-vitro activity including voriconazole (MIC50, 0.5 μg/mL, MIC 90,

2.0 μg/mL), itraconazole (MIC50, 0.12 μg/mL, MIC90, 0.5 μg/mL), posaconazole (MIC 50, 0.06 μg/mL, MIC90, 0.5 μg/mL), micafungin (MIC 50, 0.5 μg/mL, MIC 90, 2.0 μg/mL) and anidulafungin (MIC 50, 0.5 μg/mL, MIC 90, 2.0 μg/mL). AFLP analysis showed very close similarity between the isolates of *C. auris* and could be differentiated from phylogenetically close member, *C. haemulonii.*

Conclusion *Candida auris* is emerging *Candida* species causing candidemia in ICU settings in India and requires further epidemiological study. Identification to species level is important as it shows high MIC to certain antifungal agents.

P134

The burden of serious fungal infections in Sri Lanka

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Objectives Estimates of fungal infection caseloads are required to plan and implement healthcare policies. Most Sri Lankan health care is free of charge with a few private hospitals. In each district a specialized medical facility is available. With the increase in elderly population and medical developments, increased numbers of those with fungal diseases are expected. This necessitates a new vision for fungal diseases in Sri Lanka, a tropical country with a population of 20 million. We have estimated the national fungal infection caseloads from epidemiological datasets.

Methods We searched national data available from the Sri Lankan department of census & statistics, Ministry of Health, WHO & Faculty of Medicine, Colombo, as well as surveillance studies published by us and other authors for relevant disease terms. Locally collected incidence data were available for candidaemia, fungal keratitis, cryptococcosis, ABPA& SAFS. Generally, disease estimates were conservative as they assumed the lowest incidence rates reported in the literature and focused only on well-defined risk populations.

Results Sri Lankan population in 2012 was 20.2 M (51.5% female, 25.5% children). The adult HIV prevalence is <0.1%. In 2009, 1,196 HIV/AIDS persons were detected and 40.6% were on anti-retroviral therapy. There were 16 deaths. Prevalence of all forms of TB was 21,000 (2010). According to TB and other respiratory death rates the prevalence of chronic pulmonary aspergillosis post TB (1,443) and all forms of chronic pulmonary aspergillosis (2,886) were estimated. Annual incidence of cryptococcal meningitis is 13, candidaemia 507 and mucormycosis 41. Based on an AML incidence of 3/100,000 and over 500 renal transplants, we estimated 229 cases of invasive aspergillosis. Based on candidaemia and immunocompromised patients, 76 candida peritonitis, 320 oral candidiasis and 97 oesophageal candidiasis and 25,750 recurrent vaginal candidiasis cases were estimated. Asthma affects 414,000 adults 2.75% of the adult population, and assuming ABPA prevalence is 2.5% 10,344 persons, and 33% of the worst 10% of asthmatics have SAFS 13,654 persons are estimated. Fungal keratitis is documented in 1,277 patients and tinea capitis in 50 children. Pneumocystis incidence could not be estimated. The total estimated annual serious fungal disease burden in Sri Lanka is 56,687.

Conclusion Our estimates suggest that candidaemia and invasive aspergillosis are the leading causes of fungal-associated deaths. Fungal related morbidity is mainly attributed to SAFS, ABPA and CPA. More precise data from the whole country is needed for healthcare policymaking.

P135

Molecular characterization and antifungal susceptibility profile of *Candida* species isolated from vulvovaginal candidiasis patients in a Std Centre, New Delhi, India

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Objectives This study was carried out to determine the prevalence and antifungal susceptibility profile of *Candida* species isolated from high vaginal swabs of patients with vulvovaginal candidiasis (VVC) in a regional STD Centre of New Delhi, India.

Methods A total of 264 *Candida* strains isolated from vaginal swabs of 247 women with clinical signs and symptoms of VVC were investigated during 2012–2013. Candidiasis was confirmed by presence of yeast and/or pseudohyphae on direct microscopy followed by culture. Preliminary identification was done by conventional phenotypic methods and carbohydrate assimilation using VITEK2 compact. All presumptive germ tube positive *Candida* isolates were subjected to PCR using *hwp1* gene specific primer to differentiate *C. albicans*, *C. dubliniensis* and *C. africana*. Among non-*albicans Candida* species, all presumptive *C. glabrata* species complex were subjected to PCR using *RPL31* gene specific primer. All of the *C. nivariensis* and *C. africana* isolates were confirmed by ITS and D1/D2 sequencing. *In vitro* antifungal susceptibility testing was determined, using the CLSI microbroth dilution method M27-A3. The tested drugs included fluconazole, itraconazole, voriconazole, posaconazole, clotrimazole, miconazole and ketoconazole.

Results Overall Among 264 *Candida* isolates, 48.1% (128) were *C. albicans,* followed by 45.1% (119) *C. glabrata*, 1.9% (5) *C. nivariensis*, 1.5% (4) each of *C. tropicalis* and *C. africana*, 0.8% (2) *C.krusei* and 1 (0.4%) each of *C. kefyr* and *C. parapsilosis*. Of 264 *Candida* isoaltes 132 were germ tube positive. Of these 128(96.9%) formed chlamydospores and remaining 4(3.1%) were negative and confirmed as *C. albicans* and *C. africana* respectively by *hwp1* PCR amplification. Of the remaining 132 *Candida* isolates, 124 confirmed as *C. glabrata*, 5 *C. nivariensis* by *RPL31* PCR amplification. Isolates of *C. albicans*, were susceptible to fluconazole (MIC_{90}, 4 μg/ml), itraconazole (MIC_{90},0.25 μg/ml), voriconazole (MIC_{90},0.06 μg/ml), posaconazole (MIC_{90},0.125 μg/ml), ketoconazole (MIC_{90},0.125 μg/ml), clotrimazole (MIC_{90},0.06 μg/ml), and miconazole (MIC_{90},4 μg/ml). *C. glabrata* revealed increased MICs of fluconazole (MIC_{90}, 32 μg/ml) and was susceptible to itraconazole (MIC_{90}, 0.5 μg/ml), voriconazole (MIC_{90}, 0.25 μg/ml), posaconazole (MIC_{90}, 1 μg/ml), clotrimazole (MIC_{90}, 0.5 μg/ml), ketoconazole (MIC_{90}, 0.25 μg/ml) and miconazole (MIC_{90}, 0.25 μg/ml). All the patients with *C. albicans* VVC were treated with 150 mg OD oral fluconazole once a week for 4 weeks where as those with *C. glabrata* VVC were administered oral voriconazole 200 mg BD or local amphotericin liposomal gel for 2 weeks. Twenty four percent of the patients had multiple positive cultures due to recurrent infections. *C. glabrata* found to be the most common in multiple positive cultures.

Conclusion *Candida albicans* and *C. glabrata* were the predominant causes of vulvovaginal candidiasis. Molecular techniques identified cryptic species such as *C. nivariensis* and *C. africana* as rare agents of VVC.

P136

Skin, nail and hair mycoses prevalence in residents of the Altay region, Russia

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Objectives To explore the prevalence of skin, nail and hair mycoses in the Altai region, Russia. To determine the relationship between the prevalence of dermatomycoses and risk factors.
Methods The prospective study included people aged 0–80 examined during active preventive medical examinations at various enterprises of the Altai region (industry, agriculture, social services). To assess the significance of differences between compared groups, criteria for statistical significance $\chi 2$ and λ were used. For visual evaluation, the method of self-organized feature maps (Kohonen maps) was used.
Results 9736 people were examined: 3856 men and 5880 women. 3070 people were urban residents, 6797 - rural residents.

The prevalence of skin, nail and hair mycoses in the population of the Altai region was 21,94 ± 0,83 per 100 people, but it was different in various medico-geographical areas: the maximum - in Barnaul area (39.13), medium (22.12 19,9–14,3) - in Aleiskaya, Biyskaya, Rubtsovskaya, Slavgorodskaya areas and Kamen-na-Obi, the minimum - in Zarinskiy zone (5.2).

High prevalence rate of feet mycosis (7,24 ± 0,52%) and onychomycosis (8,82 ± 0,57%) was registered. For people older than 60 years it exceeded 25 cases per 100 people. Diseases were common for cooks, laborers, mechanics and livestock breeders.

Hands mycosis and onychomycosis were rarely revealed (0,61 ± 0,16%; 0,72 ± 0,17%), mainly among middle-aged and young people. Cooks and recruits had hands onychomycosis 4–5 times more often.

Mycosis of smooth skin was most often identified among children of 6–10 years (8,91 ± 5,67%) and young people aged 18-25 (5,62 ± 1,36%). The disease was common for salesmen and laborers.

High prevalence rate of hair mycosis was revealed among children under 6 years - 7,79 ± 6,11; for those aged 6–10 it was 3 times lower - 2,97 ± 3,38%, for other age groups the infection was rare. Among the adult population nurses were at risk.

For people with concomitant somatic diseases, the prevalence rate reached 33 per 100 people.

Among people with diseases of musculoskeletal, nervous and cardiovascular systems, as well as disorders of gastrointestinal tract mostly often onychomycosis and mycosis of feet were detected, more rarely - skin mycosis, and hand mycosis and onychomycosis.

Among patients with broncho-pulmonary disease the most common was onychomycosis of feet (10 ± 4,6%), mycosis of feet (5,88 ± 3,61%) and skin mycosis (4,12 ± 3,05%).

For patients with endocrine diseases dermatomycoses were registered in 64,63 ± 5,58% cases. Skin mycosis was found more frequently, feet mycosis and onychomycosis - less often.

Conclusions

1 Skin, nail and hair mycoses were identified among 21,94 ± 0,83% residents of the Altai region.
2 In various medico-geographical areas maximum and minimum prevalence rates differ by 8 times.
3 The most common dermatomycoses were feet mycosis and onychomycosis.
4 Prevalence of mycosis depends on profession and concomitant somatic diseases.

P137

Burden of serious fungal infections in Jamaica

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Background and rationale The incidence and prevalence of fungal infections in Jamaica is unknown. The first case of conidiobolus was reported from Jamaica in 1965. *Micrsporum gypseum* has been found frequently in the soil in Jamaica (50% samples), cases of histoplasmosis are reported as are several cases of mycetoma. In addition the mostly black ancestry predisposes to tinea capitis in children. We estimated the burden of fungal infections in Jamaica from published literature and modelling.
Methods All published epidemiology papers reporting fungal or HIV infection rates from Jamaica were identified. We also extracted reported data from the WHO STOP TB program and UNAIDS. Where no data existed, we used specific populations at risk and fungal infection frequencies in those populations to estimate national incidence or prevalence. CPA, ABPA, SAFS rates were based on asthma and TB rates. Asthma rates (doctor diagnosed asthma) in 13-14 year olds were taken from Kahwa et al, BMJ Open, 2012 and assumed to relate to adulthood. Other assumptions were based on incidence rates reported in the local and international literature. The denominator included the overall Jamaican population, number of patients with HIV/AIDS and respiratory diseases.
Results The Jamaican population was estimated to be 2,719,000 million people (2009), of whom 29% are children (0–14 years) and 11% are >60 years old. The adult asthma population was estimated at 522,000. Using a 2.5% rate of ABPA based on other studies (see Denning et al, Med Mycol, 2013), Jamaica has 13,000 ABPA cases and 17,225 SAFS cases (480 and 633/100,000 respectively). Only 98 cases of pulmonary TB were reported in 2011, so chronic pulmonary aspergillosis is probably rare with an estimated prevalence of 14 cases after TB (1/100.000), perhaps 15% of the total CPA caseload. An estimated 42,885 women have 4 or more attacks of vaginal

Table 1 Burden of fungal infection in Jamaica.

Fungal condition	Number of infections per underlying disorder per year					Total burden	Rate /100K
	None	HIV/AIDS	Respiratory	Cancer /Tx	ICU		
Oesophageal candidiasis	?	2,100	-	?	-	2,100	77
Candidaemia	-	-	-	95	41	136	5
Candida peritonitis	-	?	-	-	20	20	0.75
RVVC (4x/year +)	42,885	-	-	-	-	42,885	3,154
ABPA		-	13,050	-	-	13,050	480
SAFS		-	17,225			17,225	633
CPA	-	-	82	-	-	82	11.2
IA	-	-	-	?	?	?	
Mucormycosis	-	-	-	?	-	?	?
CM	?	?	-	-	-	?	?
PCP	-	1.890	?	?	-	1,890	70
Histoplasmosis	?	?	?	-	-	?	?
Fungal keratitis	?	-	-	-	-	-	?
Tinea capitis	?	-	-	-	-	?	?
Total burden estimated.	**42,885+**	**3,990+**	**30,360**	**95+**	**61+**	**77,391+**	

ABPA=Allergic bronchopulmonary aspergillosis, CM=Cryptococcal meningitis, PCP=*Pneumocystis jirovecii* pneumonia, IA=Invasive aspergillosis, CPA=Chronic pulmonary aspergillosis, RVVC=Recurrent vaginal candidiasis, SAFS=Severe asthma with fungal sensitisation

candidiasis annually (6% women >15 yrs, based on a Nigerian rate) (3,154/100,000 females). Using a common international figure for candidaemia incidence of 5/100,000, 136 cases of candidaemia occur each year, and 20 cases of *Candida* peritonitis in surgical patients. The burden HIV/AIDS is estimated to be 27,000 + patients, 1.6% of adults of whom 14,000 are not receiving antiretroviral therapy (CD4 counts <350/uL). Assuming 50% of these patients develop either oral or oesophageal candidiasis annually, 6,300 and 2,100 cases respectively would be expected annually. Assuming 20% of those not on ARVs progress to a life-threatening opportunistic infections each year, and that the rate of PCP is 27%, 1,890 PCP cases would be expected in AIDS annually. It wasn't possible to estimate the burden of histoplasmosis, invasive aspergillosis, mucormycosis, cryptococcal meningitis and fungal keratitis caseload through paucity of data.

Conclusion Using local data and literature estimates of the incidence or prevalence of fungal infections, over 75,000 people in Jamaica probably suffer from serious fungal infections each year, most related to asthma, recurrent vaginal thrush and HIV infection. Local epidemiological studies are urgently required to validate or modify these estimates.

P138

Burden of serious fungal infections in Trinidad and Tobago

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Background and rationale The incidence and prevalence of fungal infections in the Carribean is not known, but is likely to be high because of substantial rates of asthma and HIV infection. As most people are black, tinea capitis in children likely to be common. *Histoplasma capsulatum* is endemic on the islands with a 69% skin positivity rate in those <60 years old (1981). We estimated the burden of fungal infections in Trinidad and Tobago (T&T) from published literature and modelling.

Methods All published epidemiology papers reporting fungal or HIV infection rates from T&T were identified. We also extracted reported data from the WHO STOP TB program and UNAIDS. Where no data existed, we used specific populations at risk and fungal infection frequencies in those populations to estimate national incidence or prevalence. Data for invasive mycoses, CPA, IA, ABPA, Asthma and COPD rates were made on assumptions based on incidence rates reported in the local and international literature. The denominator included the overall T&T population, number of patients with HIV/AIDS and respiratory diseases.

Results The T&T population was estimated to be 1,339,000 million people (2009), of whom 21% are children (0–14 years) and 10% are >60 years old. Asthma prevalence (wheezing in the last 12 months) is 13.2% in adolescents (11–19 years old) and assumed to be the same throughout adulthood; an estimated adult asthma population of 139,631. Using a 2.5% rate of ABPA based on other studies including one from South Africa, T&T has 3,491 ABPA cases and 4,608 SAFS cases. In contrast, chronic pulmonary aspergillosis is uncommon with an estimated prevalence of 27 cases after TB (2/100.000), as few cases of TB are found on T&T, perhaps 25% of the total CPA caseload. An estimated 23,763 women have >4 attacks of vaginal candidiasis annually (6% women >15 yrs, based on a Nigerian rate). Using a common international figure for candidaemia incidence of 5/100,000, 87 cases of candidaemia occur each year, and 10 cases of *Candida* peritonitis in surgical patients. An estimated 14,00 people are infected with HIV in T&T, an adult prevalence rate of 1.1%., of whom at least 5,000 are not on antiretroviral therapy (<350 CD4/uL). We estimate that 2,250 and 750 patients develop either oral or oesophageal candidiasis annually, assuming 50% of those not on ARVs are affected. Assuming 20% of those not on ARVs progress to a life-threatening opportunistic infection each year, and that the rate of PCP is 27%, 486 PCP cases would be expected in AIDS annually. It wasn't possible to estimate the burden of histoplasmosis because of paucity of data, but it certain exists as several small series have been reported. Likewise invasive aspergillosis, mucormycosis, cryptococcal meningitis and fungal keratitis caseload could not be estimated.

Conclusion Using local data and literature estimates of the incidence or prevalence of fungal infections, over 33,000 people in T&T are estimated to suffer from serious fungal infections each year. Substantial uncertainty surrounds these estimates and local epidemiological studies are urgently required to validate or modify these estimates.

Table 1

Table Burden of serious fungal infections in Trinidad and Tobago

Fungal condition	Number of infections per underlying disorder per year 2010					Total burden	Rate /100K
	None	HIV/AIDS	Respiratory	Cancer /Tx	ICU		
Oesophageal candidiasis	?	750	-	?	-	750	56
Candidaemia	-	-	-	47	20	67	5
Candida peritonitis	-	?	-	-	10	10	0.75
RVVC (4x/year +)	23,763	-	-	-	-	23,763	3550
ABPA	-	-	3,491	-	-	3,491	260
SAFS	-	-	4,608			4,608	344
CPA	-	-	110	-	-	110	8.2
IA	-	-	-	?	?	?	
Mucormycosis	-	-	-	?	-	?	?
CM	?	?	-	-	-	?	?
PCP	-	486	?	?	-	486	36
Histoplasmosis	?	?	?	-	-	?	?
Fungal keratitis	?	-	-	-	-	-	?
Tinea capitis	?	-	-	-	-	?	?
Total burden estimated.	**23,763+**	**1,236+**	**8,208**	**55+**	**30+**	**33,292+**	

ABPA=Allergic bronchopulmonary aspergillosis, CM=Cryptococcal meningitis, PCP=*Pneumocystis jirovecii* pneumonia, IA=Invasive aspergillosis, CPA=Chronic pulmonary aspergillosis, RVC=Recurrent vaginal candidiasis, SAFS=Severe asthma with fungal sensitisation.

P139

Burden of serious fungal infection in Iran

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Objectives Iran is a Middle East country with a diverse range of climatic conditions in different areas. In last two decades, Iran has made significant improvements in the health system including applying advanced procedures in management and diagnostic methods. There are many transplants, cancer and intensive care unit centers. Nowadays, fungal disease diagnosis is not confined to cutaneous mycoses but includes life threatening mycoses because of their importance for survival. There are many reports on serious fungal diseases in Iran which we reviewed comprehensively.

Table 1 Burden of fungal infection in Iran.

Type of disease	Predominant groups at risk	Risk population size	Estimation for burden (%)	Total burden	Most mean age estimates for each disease
ABPA	Asthma	2500000	1.36%	34166.67	<15
IA	Cancer	74791	0.33%	251	15-45
Recurrent vaginal candidiasis	use of antibiotics , Diabetes	36710968	20.3%	2188323	30-45
Oral candidiasis	None	59988772	133.6/100000	23703	>15
Tinea capitis	Poor hygiene	10973035	13.36%	175369	<10
Pneumocystosis	AIDS	24735	5.9%	1472	>45
Total burden estimated				2423489.67	

Method To identify the literature on fungal diseases in Iran, we searched Medline and several national databases of Iran. We searched meeting abstracts, too. We excluded case reports. We used specific populations at risk and fungal infection frequencies in the population to estimate national incidence or prevalence. Population statistics were derived from the Statistics National Organization of Iran.
Results Of Iran's 72,874,050 populations, 50.9% are male and 49.1% are female. Estimates are: 2,188,323 Iranian women get recurrent vaginal thrush; 175,569 children have tinea capitis. Estimation for burden of oral candidiasis was 133.6 per 100,000 populations at risk. Total burden and estimation for burden of IA in cancer patients were 251 and 0.33%, respectively. The estimation for burden of ABPA in asthma patients was 1.36%. Based on the 24,735 cases of HIV infection reported, an estimated 1,472 cases of Pneumocystosis is estimated. Based on our review, *Aspergillus flavus* was the most common isolated species among *Aspergillus* spp. in IA patients. *Candida albicans* was the prevalent species of *Candida* in candidiasis. Mucormycosis cases were only confirmed by histopathological method and etiologic agents were not identified by culture.
Conclusion The reported incidence of invasive mycoses among immunocomporomised patients from Iran is considerably high in comparison with those of developed countries. Interestingly, *A. flavus* is the most prevalent specie of *Aspergillus* in IA patients which is different with the findings of the most other studies from different countries. Substantial uncertainty surrounds these estimates and epidemiological studies are urgently required to validate or modify these estimates.

P140

Burden of serious fungal infections in Dominican Republic

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Background and rationale The incidence and prevalence of fungal infections in Dominican Republic (DR) is unknown. Extraordinarily little is known about fungal infections in DR, other than *Microsporum audouinii* and *Trichophyton tonsurans* are the leading cause of tinea capitis in children and that histoplasmosis is endemic. We estimated the burden of fungal infections in Jamaica from published literature and modelling.
Methods There were no epidemiology papers reporting fungal infection rates from DR, so every estimate is based on modelling. We extracted data from the WHO STOP TB program and UNAIDS annual reports. We used specific populations at risk and fungal infection frequencies in those populations to estimate national incidence or prevalence. ABPA and SAFS estimates were based on a 9.97% adult clinical asthma rate (To et al, BMC Pub Health 2012) and CPA on TB rates. Other assumptions were based on incidence rates

Table 1 Burden of fungal infection in Dominican Republic.

Fungal condition	Number of infections per underlying disorder per year					Total burden	Rate /100K
	None	HIV/AIDS	Respiratory	Cancer /Tx	ICU		
Oesophageal candidiasis	?	3,805	-	?	-	3,805	36.7
Candidaemia	-	-	-	353	151	136	5
Candida peritonitis	-	-	-	-	76	76	0.75
RVVC (4x/year +)	158,134	-	-	-	-	158,134	3,134
ABPA	-	-	25,149	-	-	25,149	249
SAFS	-	-	33,197			33,197	329
CPA	-	-	1,374	-	-	1,374	52
IA	-	-	-	61	?	?	0.8
Mucormycosis	-	-	-	20	-	?	0.2
CM	?	?	-	-	-	?	?
PCP	-	1,296	?	?	-	1,296	12.8
Histoplasmosis	?	?	?	-	-	?	?
Fungal keratitis	?	-	-	-	-	-	?
Tinea capitis	?	-	-	-	-	?	?
Total burden estimated.	**158,134 +**	**5,101+**	**59,720**	**434+**	**227+**	**223,616 +**	

ABPA=Allergic bronchopulmonary aspergillosis, CM=Cryptococcal meningitis, PCP=*Pneumocystis jirovecii* pneumonia, IA=Invasive aspergillosis, CPA=Chronic pulmonary aspergillosis, RVVC=Recurrent vaginal candidiasis, SAFS=Severe asthma with fungal sensitisation

reported in the local and international literature. The denominator included the overall Jamaican population, number of patients with HIV/AIDS and respiratory diseases.
Results The DR population was estimated to be 10,090,000 million people (2009), of whom 31% are children (0–14 years) and 9% are >60 years old. The adult asthma population was estimated at 1 million. Using a 2.5% rate of ABPA based on other studies (see Denning et al, Med Mycol, 2013), DR is estimated to have 25,150 cases of ABPA and 34,000 SAFS cases (250 and 529/100,000 respectively). 3,300 cases of pulmonary TB were reported in 2011, which is estimated to lead to 145 new cases of chronic pulmonary aspergillosis annually and a 5 year prevalence of 458, assuming a 15% death anual death rate. If TB accounts for 33% of the cases then the total CPA prevalence is 1,374 cases in DR. Invasive aspergilosis in haematoliogical malignancy is estimated to affect 61 patients, assuming a 10% attack rate. If the incidence of candidaemia is 5/100,000, 505 cases of candidaemia occur each year, and 76 cases of *Candida* peritonitis post-surgery. An estimated 158,134 women have >4 attacks of vaginal candidiasis annually (6% women >15 yrs). The burden of HIV/AIDS is estimated to be 62,000 + patients, 1.1% of adults of whom 24,000 have CD4 counts <350/uL and are not being treated Assuming 50% of these patients develop either oral or oesophageal candidiasis annually, 5,400 and 3,800 cases respectively are estimated annually. Assuming 20% of those not on ARVs progress to a life-threatening opportunistic infections each year, and that the rate of PCP is 27%, 1,296 PCP cases are anticipated annually. It wasn't possible to estimate the burden of histoplasmosis, cryptococcal meningitis or fungal keratitis, and some estimates are incomplete, notably invasive aspergillosis, as relevant data are not available.
Conclusion We estimate that over 220,000 people in DR probably suffer from serious fungal infections each year, most related to asthma, prior TB, recurrent vaginal thrush and HIV infection.

P141

Burden of serious fungal diseases in Republic of Korea

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Objectives Republic of Korea is a member of the Organization for Economic Cooperation and Development (OECD) with a population of 50.7M in 2011 (49.9% female, 9.2% < 10 years, 11.2% ≥ 65 years) and GDP of $30,370 in 2009. Although advanced health surveillance systems are being used in Korea, there have been no nationwide data for serious fungal diseases to date. The objective of this study is to estimate the burden of serious fungal diseases in Republic of Korea.
Methods The bases for the computations have been adopted from previously published literature (Denning et al, Bull World Health Organ. 2011;89:864–72 and Denning et al, Med Mycol. 2013;51 (4):361–70). Population and hospital data were obtained from Korean Statistical Information Service (KOSIS). HIV/AIDS data were obtained from World Health Organization (WHO), Korea Centers for Disease Control and Prevention (KCDC), and the KCDC Cohort Study in HIV/AIDS patients. Transplant data of 2011 were obtained from KCDC and Korean Society of Blood and Marrow Transplantation.
Results Current burden of serious fungal diseases in Republic of Korea was estimated at 961,417 cases every year (1.9% of Korean population; details shown in Table). Based on the 888 newly diagnosed HIV/AIDS patients in 2011 with the assumption that 1.1% of HIV/AIDS patients present with cryptococcal meningitis, the burden of cryptococcal meningitis was estimated at 10 cases, plus with assumption that 10% present with *Pneumocystis jirovecii* pneumonia, the burden of PCP was estimated at 89 cases yearly in this population. Oesophageal candidiasis has been estimated to affect 135,177 patients every year, including 10,510 patients with cancer. Assuming the prevalence of asthma in adults is 4.57%, the prevalence of ABPA was estimated at 94.8/100,000 and SAFS at 125.2/100,000. The rate of candidemia was estimated at 5/100,000 population with 2,537 cases per year. Assuming 5% of adult women have recurrent *Candida* vaginitis, *Candida* vaginitis affected 689,214 women. Invasive aspergillosis in immunocompromised patients was estimated at 813 patients and in COPD admissions 1,215 cases with a combined rate of 3.5/100,000. Apart from serious fungal infections, there were 2,384,446 patients (4.7% of population) with dermatophytoses in 2009, including 40,700 children (0–9 years) and 116,384 older children (10–19 years). Prevalence of tinea capitis in children was unable to be identified.
Conclusion Based on local data and estimates of this investigation, 1.9% of South Koreans have serious fungal diseases. Considering that nationwide survey of fungal infections in susceptible populations are lacking and invasive fungal infections in cancer or HIV/AIDS patients tend to be under-reported by medical record/health insurance systems, the true number of serious fungal infections in Korea should be higher than our estimates.

Table 1 Burden of serious fungal diseases in Korea.

Burden of Fungal Infection	Number of infections per underlying disorder per year					Total Burden	Rate/100K
	None	HIV/AIDS	Respiratory	Cancer/Immunocompromised	ICU/ abdominal surgery		
Cryptococcal meningitis	33	10	-	33	-	76	0.15
Pneumocystis pneumonia	-	89	-	404	-	493	0.97
Invasive aspergillosis	-	-	1,215	559	-	1,774	3.50
Chronic pulmonary aspergillosis	-	-	20,140	-	-	20,140	146.11
Allergic bronchopulmonary aspergillosis (ABPA)	-	-	48,110	-	-	48,110	94.83
Severe asthma with fungal sensitization	-	-	63,505	-	-	63,505	125.17
Candidemias	-	-	-	1,776	761	2,537	1.50
Candida peritonitis	-	-	-	-	381	381	0.75
Oral candidiasis	-	-	-	-	-	-	-
Oesophageal candidiasis	117,502	427	6,738	10,510	-	135,177	266.44
Recurrent Candida vaginitis (> 4/year)	689,214	-	-	-	-	689,214	2,716.90
Mucormycosis	-	-	-	10	-	10	0.02
Histoplasmosis	-	-	-	-	-	0	0.00
Coccidioidomycosis	-	-	-	-	-	-	-
Blastomycosis	-	-	-	-	-	-	-
Fungal keratitis	-	-	-	-	-	-	-
Tinea capitis	-	-	-	-	-	-	-
Total serious fungal infection burden	806,716	677	138,493	12,855	2,357	**961,417**	

P142

Abstract withdrawn

P150

A complex study of invasive fungal infections in a Hungarian University Hospital

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Objectives Invasive fungal infections (IFIs) have high mortality, and in many cases can be recognised post mortem. These patients are usually hospitalized in Intensive Care Units (ICUs). Various symptoms and signs may suggest the suspicion of this infection, but these are non-specific. Aims of our study are: 1) monitoring the clinical parameters of (adult) patients with possible IFI to achieve correct and earlier diagnosis, 2) improving the microbiological background to help the work of clinicians, 3) choosing adequate clinical and laboratory parameters for decision of optimal antifungal treatment (agent, duration, adverse effects etc.), 4) developing a combined (clinical and laboratory) algorithm to diagnose and to treat adequately IFIs in our institution.
Methods We prospectively monitor clinical and (chemical) laboratory signs of adult patients with suspected IFIs (basic illness, risk factors, symptoms, levels of procalcitonin and C reactive protein etc.). According to these findings, if deep suspicion of IFI exists, we start microbiological investigations for the detection of fungal colonization (s) and/or infection(s). We initiate microbial culturing of specimens originated from different body sites (endotracheal aspirate, throat, urine, invasive devices, blood etc.), and we carry out molecular biological method (PCR) for detection of fungi in blood samples. We calculate Candida Score and Colonisation Index based on these results. In the laboratory, we identify fungal strains with our laboratroy methods used in daily routine (germ tube production, micromorphology on rice agar, biochemical identification with AUXACOLOR 2 and PNA-FISH), and we also identify them by MALDI-TOF Biotyper device. We perform sequence analysis from every isolate belonging to different species in every patient. Pulse-field gel electrophoresis will be done from all isolates of the same species originated from different body sites of the patient.
Results Since 1st January, 2013 ten patients have been involved in this study from. Age distribution was 35–84 years (mean 66 years). APACHE II scores were between 16 and 49. Calculated Candida Scores were 3 and 4 for every patient, and Colonisation Index varied between 0.33 and 1.0. *Candida* species were cultured from 4 or 5 (non sterile) body sites in 5 patients. In most cases, 2 or 3 different species were cultured from different body sites, 2 patients were infected/colonised by the most frequently isolated species, *C. albicans* solely. In 3 patients, *Candida* species were found in blood cultures, fungal DNA could be detected in blood samples and/or sera by PCR (without blood culture positivity) in 4 patients. Results of molecular typing methods and sequencing are in progress.
Conclusion According to our preliminary results, monitoring of different markers of suspected IFI is important, however there are no specific signs, so the clinicians have to evaluate these informations (risk factors, chemical laboratory results, testing of colonisation, blood cultures, molecular biologic detections) in totality. Waiting for the results of blood cultures is not adequate, because of low positivity rate. Postive result of the PCR for detection of fungal DNA does not represent IFIs in every case.

P151

A prospective follow-up of patients with Candiduria in the Intensive Care Unit: it's effect on mortality and risk factors

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Objective Detection of *Candida* spp in urine specimens of urinary catheterized patients hospitalized in Intensive Care Units (ICU) is a common problem. However the clinical importance of this situation is not clear. In this study we aimed to evaluate risk factors and mortality rates of patients with candiduria.
Methods All adult patients hospitalized more than 48 hours in Medical ICU were included in the study. The patients were followed until discharge or death without intervention. Data including demographics, co-morbidities, invasive procedures, antibiotic and antifungal usage, hospital length of stay, and death within the 30 days after candiduria were recorded by using a case report form. Statistical Package for the Social Sciences (SPSS) Version 15.0 was used for statistical analyses. Categorical variables were evaluated using the chi-square and 2-tailed Fisher's exact tests. For continuous variables t-test was used. Multivariable, backward stepwise, logistic regression analysis was used for the identification of independent risk factors for candiduria.
Results Total 159 patients were observed during the study period. Candiduria were detected in 59 (37.1%) patients. Mean hospitalization time until candiduria occurred was 28,92 ± 28,1 (min 1-max 114 days). In candiduria group 15 (25,4%) patients and in control group 32 (32%) patients were died (p = 0,37). Characteristics of all patients were given in the table. Antifungal therapy was used in 27 (45,7%) patients in candiduria group and 18 (18%) patients in control group (p = 0,0001) however mortality rate was not changed with antifungal therapy (p = 0,93). In multivariate analysis diabetes mellitus (p = 0,01), cerebrovascular accident (p = 0,011), erythrocyte transfusion (p = 0,032), piperacilline/tazobactam and cephaperazone/sulbactam (p = 0,008), and carbapenem use (p = 0,023) were found as risk factors for candiduria.
Conclusion Candiduria is a common and confusing problem and causes unnecessary antifungal usage. No significant effect of candiduria on mortality was found in this study. Therefore this situation should be managed together with clinical findings carefully.

Table 1 Characteristics of patients.

	Cases n= 59(%)	Controls n=100(%)	P value
Gender (Male)	28(47,4)	57 (57)	0,24
Age (mean)±SD	69,4±15,7 (17-91)	67,9±17,7 (18-91)	0,60
Lenght of stay (days-median)	14 (3-90)	10 (3-156)	0,08
Diabetes Mellitus	25 (45,3)	21 (21)	**0,004**
COPD*	9 (15,2)	16 (16)	0,90
Cerebrovascular accident	18 (30,5)	14 (14)	**0,01**
Steroid use	4 (11,8)	14 (14)	0,70
Haematological malignancy	13 (22,0)	22 (22)	0,99
Hemodialysis	13 (22,0)	29 (29)	0,30
Erythrocyte transfusion	53 (89,8)	68 (68)	**0,001**
Total parenteral nutrition	19 (32,2)	25 (25)	0,32
Mechanical ventilation	46 (77,9)	71 (71)	0,38
Surgical operation	12 (20,3)	7 (7)	**0,01**
Piperacilline tazobactam/Cephaperazon sulbactam use	45 (76,2)	46 (46)	**0,0003**
Carbapenem use	40 (67,7)	45 (45)	**0,009**
Aminoglycoside use	24 (40,6)	18 (18)	**0,002**
3rd generation cephalosporin use	19 (32,2)	16 (16)	**0,02**
Candidemia	7 (11,8)	8 (8)	0,42
Mortality (within 30 days)	15 (25,4)	32 (32)	0,37

*COPD: Chronic obstructive pulmonary disease

P152

Clinical risk factors and bronchoscopic features of invasive aspergillosis in Intensive Care Unit patients

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Objective Invasive aspergillosis (IA) is an important cause of morbidity and mortality in immunocompromised patients. During recent years, a rising incidence of IA in ICU patients has been reported. The patterns of IA related infection may differ according to the type of underlying disease. Unfortunately little is known about the characteristics of IA in ICU patients. In the present study we assessed IA related clinical and bronchoscopy findings in ICU patients.
Methods This study was performed at the ICU units in Sari, Mazandaran from August 2010 through September 2011. We retrospectively analysed 43 ICU patients with underlying predisposing conditions. Bronchoalveolar lavage (BAL) samples were collected by bronchoscope twice a weekly. The samples were analyzed by culture and non-culture based diagnostic methods for the occurrence of IA. Patients were assigned a probable or possible diagnosis of IA according to the consensus definition of the EORTC/MSG.
Results Out of 43 suspected patients to IA, 13 (36.1%) cases showed IA. According to criteria presented by EORTC/MSG, they were categorized as: 4 cases (30.8%) of possible IA and 9 (69.2%) of probable IA. The observed mortality was 61.5%. The main underlying predisposing conditions were neutropenia(53.8%), COPD(30.8%) and hematologic malignancy (15.4%). The macroscopic finding in bronchoscopy included of Prulent secretion (46.6%), Mucosal bleeding (33.3%), Mucosal erythema (26.6%), Trachobronchomalasia (13.3%).
Conclusion The diagnosis of IA in patients with critical illness in ICU is even more difficult. The clinical diagnostic process is often dependent on indirect circumstantial data enhancing the probability of IA. Bronchoscopy with inspection of the tracheobronchial tree, sampling of deep airway secretions and BAL can be helpful.

P153

Six-year trend analysis of nosocomial candidemia and risk factors in two intensive care hospitals in Mato Grosso, Midwest region of Brazil

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Objective Infections caused by Candida species are among the leading causes of bloodstream infections in hospitals and have high mortality rates.
Methods We conducted a cross-sectional study, laboratory-based and analysis of secondary data (medical records and other medical records) from January 2006 to December 2011 in two tertiary teaching hospitals in Cuiabá/MT.
Results 130 episodes of candidemia in 124 patients showed clinical and microbiological confirmation of a total of 72,767 patients treated between. The prevalence of both hospitals candidemias was equal to 1.8 per 1,000 admissions cases, the death rate of 0.9 per 1000 admissions and lethality equal to 49.2%. Of the total patients, 58.5% belonged to the female and 41.5% were male. Of these, 61.5% were

adults, 13.1% pediatric and 25.4% neonates. The intensive care units received 69.2% of patients. The average hospital stay for patients with candidemia was equal to 53.5 days (SD = 45.5, 95% CI = 45.6 to 61.4). The main risk exposures were: antifungals (85.4%), hospital stay ≥ 21 days (82.3%), central venous catheter (77.7%), H2 blockers (71.5%), parenteral nutrition (70.8%) and mechanical ventilation (63.8%). More antifungal agents were used: fluconazole (50.4%) and Amphotericin B (47.3%). *Candida parapsilosis* (38.5%), *C. albicans* (34.6%) and *C. tropicalis* (18.5%) were the most prevalent etiologic agents. Of the variables that were significant in univariate analysis, only central venous catheter, parenteral nutrition and age proved to be independent exhibitions (stepwise multivariate analysis) and also related to the outcome of death. By the same method, but evaluating the association between risk exposures and the isolated species, it was found that neutropenic patients contained higher chance episodes of candidemia by *C. albicans* (p=0.05; OR=2.4), it was also observed between sex and *C. parapsilosis* (p<0.01; OR=3.0) and parenteral nutrition, and *C. tropicalis* (p<0.05; OR=3.0). *C. parapsilosis* was more frequent in intensive care units in relation to *C. albicans*.
Conclusion The Candida yeasts for the non-albicans were the most prevalent (65.5%) in this population. The species *C. parapsilosis* emerged as the main etiologic agent of hematogenous infections caused by Candida species. The prevalence of candidemias observed in this study was similar to data published by other researchers in Brazil and other countries.

P154

Candidemia following cardiovascular surgery within 180 days; Aortic Aneurysm Surgery may be the risk factor

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Objectives Candidemia remains a major cause of morbidity and mortality in critically ill patients. Previously diabetes mellitus, mechanical ventilation for more than 10 days, blood stream infection, cardiopulmonary bypass ongoing for more than 120 minutes were recognized as independent predictors for candidemia. Our purpose is to show the clinical features of candidema in patients who had cardiovascular surgery within 180 days and estimate risk factors and diagnostic management including serum 1,3-βD-Glucan assay.
Methods All episodes of Candidemia in patients who had cardiovascular surgery within 180 days, 18 years of age or older, were evaluated from April 2005 to March 2013 in Tokyo Women's Medical University Hospital (1423-bedded university hospital). Candidemia was defined by blood culture positive for Candida species. Canidemia in patients who had cardiovascular surgery over 180 days, solid organ tumor, and hematological disorders were excluded. Information on the patient's age, gender, underlying cardiovascular disease, type of surgery, time period from surgery to onset of candidemia, type of candida species from blood culture, clinical outcomes and White Blood Cell count (WBC), serum C-reactive protein (CRP), and Serum 1,3-βD-Glucan assay were collected.
Results Twelve out of 207 (5.8%) candidemia were included in this study. The incidence of nosocomial Candidemia was 0.066 cases per 1000 patient admissions year and the incidence of candidemia following cardiovascular surgery was 2.89 cases per 1000 cardiovascular surgery in this study period. The mean age was 62 years (29 years to 77 years). Nine out of 12 patients (75%) were male. Six (50%) had diabetes. Seven (58%) had Aortic Aneurysm Surgery (AAS). Three out of seven (42.9%) in AAS had additional partial colorectomy due to ischemic colitis caused by dissecting aortic aneurysm. Six (50%) had candidemia due to *Candida albicans*. The time period from surgery to onset of candidemia ranged from 7 to 126 (44.3 ± 35.3) days. Eleven (91.7%) had intravenous catheter with total parental nutrition (TPN). Ten (83.3%) had prosthetic materials such as prosthetic valve, synthetic blood vessel graft. WBC ranged from 1730/μl to 27900/μl (9482.5 ± 7496.2/μl), serum CRP ranged from 2.1 mg/dl to 20.7 mg/dl (10.21 ± 5.74 mg/dl), Serum 1,3-βD-Glucan assay ranged from 8 pg/ml to 5244.9 pg/ml (654.4 ± 1478.9 pg/ml) at diagnosis of candidemia. The serum 1,3-βD-Glucan assay was less than cut-off level (8 < 20 pg/ml) in one (0.083%) patient with candidemia due to *C.albicans*. The 90-days survival rate was 75%.
Conclusion Our results suggest that AAS may be one of risk factors for postoperative candidemia within 180 days. Dissecting aortic aneurysm and AAS may cause ischemic colitis. As a result, broad spectrum antibiotics and TPN, and central venous catheter, which are generally recognized as risk factors for candidemia, were used for long time period following cardiovascular surgery. The clinical value of 1,3-βD-Glucan assay is still controversial in diagnosis of postoperative candidemia. Further investigation is needed.

P155

Susceptibility testing of invasive candidosis pathogens to fluconazole by CLSI M27-A3 method

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Objectives To determine susceptibility of invasive candidosis pathogens to fluconazole by CLSI M27-A3 method.
Methods A total of 62 clinical isolates of patients with invasive candidosis in Russia in 2011–2012 were studied. Species identification was made by AUXACOLOR2 (BioRad, USA). Isolates included 6 species: *C.albicans* (51.7%), *C. glabrata* (17,7%), *C.krusei (11.4%), C.parapsilosis (9.6%), C.tropicalis (8%), C.lipolytica (1.6%).* CLSI M27-A3 method (microdilution) was used for susceptibility testing of *Candida* spp. to fluconazole. We used RPMI 1640 medium, an inoculum of $0,5 \times 10^3$ to $2,5 \times 10^3$ cells/ml, and incubation at 35 °C, 48 hours. MICs were determined visually as the lowest concentration of drug that caused a significant (50%) inhibition of growth below control levels. Quality control was performed by reference strain *C. parapsilosis* ATCC 22019. Interpretation criteria based by CLSI M27-S4 (December, 2012) for fluconazole: susceptible (S), MIC 2 μg/ml (for *C.albicans, C.parapsilosis, C.tropicalis*); susceptible dose dependent (SDD), MIC 4 μg/ml (for *C.albicans, C.parapsilosis, C.tropicalis)* and 32 μg/ml (for *C. glabrata*); resistant (R), MIC >8 μg/ml (for *C.albicans, C.parapsilosis, C.tropicalis)* and >64 μg/ml (for *C. glabrata)*. *C.krusei* is naturally resistant to fluconazole.
Results 58% (36) strains were susceptible, SDD - (14) 22,6% and R-(3) 4.8%. Resistant category did not include C.krusei strains. In Candida albicans 29/32 (90.6%) were susceptible, SDD -1(3.1%) and R - 2 (6.3%). In Candida non-albicans susceptibility was found only in 23.3% (7) strains. SDD strains were 13 (43.3%) and R - 1 (3,3%). All C.glabrata strains (11) were susceptible dose dependent. One C.parapsilosis strain was identified as resistant, S- 3 strains, SDD- 2. Among C.tropicalis strains: S were 4 strains, SDD - 1 strain. For 1 *C.lipolytica* strain MIC was 8 μg/ml.
Conclusion Using new interpretative criteria of CLSI M27-A3 we found that 58% *Candida* spp. strains from patients with invasive candidosis in Russia were susceptible to fluconazole.

P156

Species-specific Prevalence of vaginal candidiasis with type 1 and type 2 diabetes mellitus among women in Sana'a City

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Vaginal candidiasis (VC) is a fungal or yeast infection of the vulva and/or vagina. It causes a smelly, thick, white-yellow discharge that might be accompanied by itching, burning and swelling. Vaginal

candidiasis is the second most frequent infection of the female genital tract. Diabetes mellitus is a chronic, insidious disease that can affect any organ of the body. One of the problems associated with this condition is infection patients with diabetes mellitus are at increased risk of vaginal candidiasis. The purpose of this study was to determine the prevalence and species distribution of *Candida* species isolated from vaginal candidiasis infection in diabetic and non-diabetic women in Sana,a City. This descriptive - analytic study was performed on 150 diabetic women referred to Al-Thaourah and AL-Gmohore hospitals in Sana,a City. All specimens were diagnosed or examined under direct microscopy and cultured on Sabouraud dextrose agar (SDA) or (YEPDA) media. Complimentary tests such as germ tube test, corn meal agar media, CHROM agar test and sugar fermentation test were carried out to differentiate between the *Candida* species. Minimum inhibitory concentration (MIC) test was performed for positive cases. Patient's clinical history information was collected by a questionnaire. Statistical analysis was performed using the Chi-square test. Of 150 samples tested under all biochemical experiments and culture methods, 62 out of 150 with percentage 42% were infected to vaginal candidiasis. The frequencies of the isolated *Candida* species include *Candida albicans* 33 species with 53%, *Candida glabrata* with 19 species with 31%, *Candida tropicalis* 6 species with 10% and *Candida krusei* 4 species with 6%. Vaginal candidiasis was more prevalent in women infected with diabetes mellitus than ones with non diabetes mellitus women. 11patients out of 150 patients with percentage 8% of the patients had type I diabetes mellitus and 51 patients out of 150 with percentage 34% of them type II with ratio 18:82 from totally of 62 out of 150 with 42% vaginal candidiasis patients had infected. *C. albicans* was, by far, the most predominant yeast isolates. The culture technique of vaginal discharge not be warranted so we used the microscopic and biochemical tests because are more sensitive in identification of *Candida* species than culture methods. But vaginal culture is indispensable to confirm the diagnosis by microscopic examination.

P157

Outbreak of candidemia caused by multiple *Candida non-albicans* in pediatric hematology/oncology patients due to external contamination: clinical and mycological findings

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Introduction Outbreaks of candidemia in pediatric hematology/oncology departments are rare, and if occur require rapid search of common source. We describe an outbreak comprising 10 cases of candidemia caused by *Candida* non-albicans.
Patients and methods Thirty-four patients with various underlying diseases from 3 different oncology/hematology departments of the large pediatric tertiary center developed candidemia during 2002–2009. In 10 pts of one ward (6 boys, 4 girls, median age 11, range 0.7 - 16, years) candidemia occurred within 1 week in 2010., fitting the definition of epidemics.
Source Intravenous KCl 4% solution, contaminated with *Candida* was identified as a source of candidemia. This solution was manufactured by pharmaceutical factory, not by local hospital pharmacy.
Clinical characteristics In all 10 patients candidemia was caused by *C.* non-*albicans* and agranulocytosis <500/mm^3 was detected in only 4 (40%). At time of recovery of *Candida* from blood 9/10 patients were receiving systemic antifungals: fluconazole - 6, voriconazole - 2, voriconazole and caspofungin - 1. *C. guilliermondii* was isolated in 4 (in one case concomitantly with *C. colliculosa*), *C. parapsilosis* in 3 and *C. pelliculosa* in another 3 patients. Clinical manifestation of candidemia were fever >38.5°C in all patients and pneumonia in 2. *C. guilliermondii* was recovered from 2 vials of the same batch of 4% KCl solution.
Treatment In all patients systemic antifungal therapy was escalated; 5 received caspofungin, 3 -combination of caspofungin and other antifungal and 2 - combinations of 3 drugs. All 4 patients with granulocytopenia received G-CSF for a median of 9 (range 1–17) days upon ANC recovery >1500/mm^3. Central venous catheter was removed in 4 patients, after a median of 3 (range 1–7) days from the day of blood culture positivity for *Candida*.
Outcome Resolution of clinical symptoms and clearance of candidemia was achieved in all 10 patients, none of them died. Median duration of candidemia was 2.5 (range 1–28) days. Secondary antifungal prophylaxis was used in all 9 patients, who needed further chemotherapy but in 2 (both with CVC, which were not removed during first episode) candidemia relapsed during the period of marrow aplasia. No new cases of candidemia were detected after withdrawal of the contaminated batch of KCl.
Conclusion In patients in whom risk factors for invasive fungal infections are absent or rapidly resolved candidemia caused by a direct pathogen inoculation in the bloodstream with contaminated infusion media, has a favorable outcome provided that appropriate systemic antifungal therapy is promptly given. Removal of CVC contributes to reducing risk of candidemia relapse even in such an "artificial"model of candidemia caused by rare fungal species.

P158

Pulmonary mucormycosis in hematological and oncologycal patients in Saint-Petersburg, Russia

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Objectives To analyze risk factors, clinical symptoms, etiology and treatment in onco-hematological patients with pulmonary mucormycosis in St. Petersburg, Russia.
Methods The prospective study was conducted during the period 2004–2012 y.y. Diagnosis of mucormycosis was made according to EORTC/MSG criteria (2008).

Results. We observed 22 onco-hematological patients with pulmonary mucormycosis. The mean age of patients was 21 y (range 5–55), 10 were children (46%), 14 males and 8 females. Main underlying conditions: acute myeloid leukemia - 41%, acute lymphoblastic leukemia - 22%, Hodgkin's lymphoma - 9%, neuroblastoma- 5%, myeloid sarcoma - 4%, aplastic anemia - 4%, anemia Fankoni - 4%, myelodysplastic syndrome - 4%, chronic myeloid leukemia - 4%, multiple myeloma- 4%, non-Hodgkin lymphoma - 4. More than two organs were affected in 33% of patients. Diagnosis was confirmed by directmicroscopy and histology in all patients (at autopsy - 9%). In 14 patients cultures of clinical materials were positive: *Rhizopus* spp. (50%), *Rhizomucor pusillus* (21%), *Rhizomucor* spp. (14%), *Lichtheimia corymbifera* (7%), *Rhizopus microsporus* (7%). Antifungal therapy was performed in 15 patients (7 patients died before the beginning of specific antimycotic therapy.Amphotericin B deoxycholate was usedfor 50% patients, amphotericin B lipid form - 50%, posaconazole - 60%, caspofungin - 33%. Combination therapy was used for 50% of patients (amphotericin B+caspofungin, amphotericin B+posaconazole). Duration of treatment was 3–224 days (median - 50). 27% of patients were treated by surgery. In patients who received treatment 12 week overall survival was 50%.
Conclusion The main underlying diseases in onco-hematological patients with pulmonary mucormycosis were acute leukemia (63%),

main aetiologyagentswere*Rhizopus* spp. (50%). Amphotericin B deoxycholate and posaconazole werethe main antifungalagents. Twelve week overall survival was 50%.

P159

Invasive candidemia/candidiasis on hematological wards in 2000–2012 – a results from FIND – *Candida* project

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Objective To collect and analyze the data of patients with invasive candida infections (IC) at hematological departments in Czech and Slovak republics entered to the database FIND Candida.
Methods 118 patients from 12 centers were enrolled to this restrospective observational study covering 13 year's period. Data about underlying disease, risk factors, clinical signs, prophylaxis and therapy was collected. Clinical, microbiological results and outcome (according to EORTC/MSG criteria 2008) were analyzed. Candida score (Leon, 2006) was calculated to assess its validity in this population of patients. Frequency tables and standard descriptive statistics were used for summation of the patient characteristics, the probabilities of overall survival were estimated using the Kaplan-Meier method, and a comparison of survival in the groups of patients was performed using a log-rank test.
Results 72 male/46 female patients with median age of 40 years (1–79) hospitalized on hematological wards were included into the study. In 90 (76%) patients hematological malignancy was present. Invasive candidemia was documented in 102 patients, 16 patients had disseminated candidiasis. *C. albicans* was isolated in 41/118 (34.7%) patients. 64% of patients were neutropenic at the time of diagnosis of IC and only 22% were in complete remission of their underlying disease. Central venous catheter was present in 85% of patients and 92% received broad spectrum antibiotics. 38 patients (32%) underwent hematopoietic stem cell transplantation (23 allogenenic, 15 autologous) and 61 patients (52%) were hospitalized on ICU with median duration of 20 days (3–181). Antifungal prophylaxis before the episode of IC was administered in 57 (48%) patients, the most common drug used was fluconazole. 107 patients received treatment for IC; at the end-of-treatment (EOT) 69% clinical response rate was documented with 63% eradication rate. Mortality at EOT was 27%. Median survival was 13.6 months from the diagnosis of IC with median follow-up 44.1 months. Comparison of clinical effect according to pathogen showed that favorable clinical response was documented in 51% and 70% ($p = 0.042$) in patients with IC caused by *C.albicans* and non-albicans candida, respectively, with mortality 63 and 56%(NS), respectively. Mortality of patients with IC hospitalized on ICU vs. non-ICU patients was 67% and 49% ($p = 0.046$), respectively. Clinical response was 71% in patients with HSCT and 60% (NS) in non-HSCT patients, mortality was 47% and 64% (NS), respectively. Patients in remission of their underlying disease had better clinical response than patients without remission (81% vs. 59%; $p = 0.039$) and lower mortality (35% vs. 65%; $p = 0.005$). Overall average Candida score was 1.6 (median 1) and 2.5 (median 3) in patients who died at EOT.
Conclusion IC represents a less common but serious complication in patients on hematological wards with early mortality of 27%. Candida score played no significant role in this population of patients with IC. Outcome was significantly better in patients with remission of underlying disease, in patients with HSCT, non-ICU patients and in patients with IC caused by non-albicans candida.

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P160

Persistent cryptococcal meningitis post renal transplant

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Backround Fungal infection in renal transplant recipients are less common than bacterial infections but pose significant problem due to immunosuppressive agents and nephrotoxicity pose by antifungal agents.
Case report We report here a 17 year old boy post renal transplant in 2007 for Focal Segmental Glomerulonephritis who presented with fever and headache only 5 months following transplant.He was on tacrolimus,mycophenolate mofetil and prednisolone.Physical examination showed a relatively well child with fever,no respiratory distress with no evidence of skin lesions or sinusitis.CSF was positive for indian ink with cryptococcal antigen titre (CRAG) of 1:>512 and positive culture of *Cryptococcus neoformans*.He was induced with conventional amphotericin B for 1 week which was changed to Fluconazole when creatinine doubled.Despite being on oral Fluconazole,his CRAG remain persistently high with him being asymptomatic and 10 months later his indian ink became positive again.He was reinduced with amphotericin B colloid dispersion with total dose of 2325 mg with trial of oral voriconazole.Examination to look for foci of infection only showed retinitis with fungal nail infections.
Results
MRI Brain and sinuses: Normal:Echocardiogram: normal CRAG (CSF): 1:>512 (0), 1:>512(3 months),1:>512(5 months),1:>512 (10 months),1:512(11 months) with negative cultures.HIV serology: negative; T&B cell enumeration test:normal
Discussion He is now on 3 years of antifungal therapy and CRAG (serum) remain positive.Investigation to look for other causes of immunodeficiency were negative and he remain aymptomatic.Whether persistent CRAG is due to other sanctuary remain to be seen.
Conclusion In renal transplant recipients,cryptococcus meningitis can exist in subclinical form and we need to look evidence of active disease and use appropriate antifungal to sterilise CSF before maintenence therapy started.

P161

Quantification of chemokine and cytokine levels after standardized in vitro stimulation of peripheral blood mononuclear cells from patients with haematological malignancies

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Objectives Invasive aspergilllosis (IA) remains to be a major complication of patients with haematological malignancies (HM) and after allogeneic stem cell transplantation (SCT). In these patients, IA is the most common cause of mortality due to infection. Earlier diagnosis of *Aspergillus* infections would facilitate more effective management and prevent progression to invasive disease which typically has a poor

response to antifungal drugs. Easily detectable biomarkers for early detection or confirmation of IA are heavily needed.

Therefore, we have consecutively isolated peripheral blood mononuclear cells (PBMC) from patients with haematological malignancies. PBMCs were incubated with 2 different fungal stimuli, respectively and chemokine and cytokine levels were quantified in comparison to healthy controls.

Methods Since 2011, 1 ml of peripheral blood was consecutively collected 3 times following a positive galactomannan result (GM, Platelia, Bio-Rad). As controls blood from healthy volunteers and from HM patients without positive GM assay was achieved. All patients underwent chemotherapy or allogeneic SCT and were monitored for invasive fungal diseases (IFD) using the current consensus criteria of the European Organization for Research and Treatment of Cancer / Mycoses Study Group. Blood samples were drawn bedside into TruCulture tubes (TCT, Myriad RBM) and incubated at 37°C for 24 h. TCT stabilizes immune cells under standardized conditions, thereby facilitating interaction and stimulation of PBMCs *in vitro*. Zymosan, a fungal cell wall component, and inactivated *Aspergillus fumigatus* germ tubes were used as a stimulus. Culture supernatants were harvested and analyzed in a multiplex ELISA assay (Bio-Rad) quantifying n = 27 different human chemokines and cytokines.

Results PBMCs were obtained from 4 healthy volunteers, 5 unclassified patients (controls, 20 samples), and 1 possible and 6 probable IA patients (cases, 19 samples). All blood specimens were incubated in TCT with either zymosan or germ tubes or left untreated. Analysis of the supernatants by multiplex ELISA revealed that 10 / 27 chemo- and cytokines were not induced by either stimulus (IL-5, IL-7, IL-12p70, IL-13, IL-15, Eotaxin, IP-10, PDGF-bb, RANTES and VEGF). In contrast, 15 / 27 analytes (IL-1b, IL-1ra, IL-4, IL-6, IL-8, IL-9, IL-17, basic FGF, G-CSF, GM-CSF, IFN-g, MCP-1, MIP-1a, MIP-1b, TNF-a) were induced by both stimuli, while IL-2 and IL-10 were induced by zymosan only. In general, stimulation with zymosan led to higher levels of cytokine and chemokines, with IL-1b (37000-fold), TNF-a (7400-fold) and MIP-1a (5500-fold change) showing maximal induction.

Conclusion In this pilot study, we were able to demonstrate that prospectively collected human PBMCs showed cytokine and chemokine release after stimulation with zymosan and *A. fumigatus* germ tubes *in vitro*. Although studies with higher number of patients are necessary, we were able to demonstrate specific release of chemokines and cytokines after PBMC stimulation. These markers might become a potential tool for early and confirmative biomarker of IA.

P162

Pneumocystis jirovecii pneumonia in immunosuppressed HIV-negative patients

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Objectives Pneumocystis pneumonia (PCP) is a severe and life-threatening opportunistic infection caused by the fungus *Pneumocystis jirovecii*, occurring in immunocompromised patients. The study investigated predisposing factors, clinical features and outcome of PCP in HIV-negative patients.

Methods The study was retrospective. The records of patients cared for at the department of Medicine of the University Hospital of Heraklion, Crete, Greece, with the diagnosis of PCP from January 2004 through May 2013 were reviewed.

Results Sixty two immunosuppressed patients developed PCP during the study period. Thirty one (50%) had hematological malignancies (HM), 16 (26%) solid tumor (ST) and 15 (24%) chronic inflammatory disease. Only 14 (22.5%) had received long-term systemic corticosteroids. All patients had signs and symptoms of pneumonia upon admission, while 13 (21%) were suffering respiratory failure. Twenty one (34%) had received trimethoprim-sulfamethoxazole (TMP-SMX) prophylaxis before the PCP onset. Nine were admitted to the ICU. Mortality attributable to PCP reached 29%. Mortality rate was higher in patients with ST as compared to those with HM or chronic inflammatory diseases (72% vs. 30% vs. 13% respectively, p-value = 0.012)

Conclusion PCP mortality was higher in patients with ST. Prophylaxis with TMP-SMX did not protect a significant portion of the present patients. Hence, PCP should be included in the differential diagnosis in immunosuppressed patients with symptoms from the respiratory tract even in the presence of prophylaxis.

P163

Pneumocystis jirovecii diversity in French Guiana

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Objectives Until now, French *Pneumocystis jirovecii* (*P.jirovecii*) isolates that have been characterized by genotyping are those obtained from Metropolitan France, while data on genomic characteristics of *P.jirovecii* from French Guiana are scarce. Indeed, to our knowledge genotyping of *P.jirovecii* from a Guianese patient has been reported in only one instance. The present study reports multilocus typing of *P.jirovecii* organisms from AIDS patients living in this overseas French territory.

Methods Seven AIDS patients monitored at the Andrée Rosemon Hospital (Cayenne, French Guiana) and initially diagnosed with *Pneumocystis* pneumonia (PCP) were retrospectively enrolled in the study. Seven archival *P.jirovecii* isolates were examined at: i) the dihydropteroate synthase (DHPS) locus using a PCR-Restriction Fragment Length Polymorphism technique, ii) the internal transcribed spacer (ITS) 1 and ITS 2 of the rRNA operon and the mitochondrial large sub unit rRNA (mtLSUrRNA) gene using PCR, cloning and sequencing.

Results Six patients were infected with a wild DHPS type (A^{165} C^{171}). The seventh patient was infected with a double mutant (G^{165} T^{171}). Eight different ITS haplotypes were identified: Eg (3 patients), Eu (2 patients), Gg, Eo, No, Ne, Eh, Ea (one patient each). Five patients were infected with only one ITS haplotype. One patient was infected with 2 ITS haplotypes (Eg, Eu) and another patient was infected with 4 ITS haplotypes (Eo, No, Ne, Eh) suggesting occurrence of mixed infections in these two patients. Eg is one of the most frequent haplotype that have previously been observed worldwide including Metropolitan France. Eu has been reported as an infrequent haplotype in South Africa and has not been reported in Metropolitan France. Three different mtLSUrRNA types were identified: "T^{85} C^{248}"in 4 patients, "A^{85} C^{248}"in 3 patients, and "A^{85} T^{248}"in one patient. Six patients were infected with only one mtLSUrRNA type whereas one patient was infected with 2 mtLSUrRNA types ("A^{85} T^{248}", "A^{85} C^{248}") suggesting again mixed infection occurrence. Type "T^{85} C^{248}"has been reported as the major type in Cuba and Zimbabwe, as a less frequent type in the United Kingdom, Spain, and Metropolitan France.

Conclusion PCP involving *P.jirovecii* DHPS mutants occurs in French Guiana. A high diversity of ITS haplotypes and mtLSUrRNA types is observed. There is a partial commonality of *P.jirovecii* types in both Metropolitan France and this overseas territory. Nonetheless, the results suggest that some *P.jirovecii* types may represent peculiar geographic characteristics of the fungus in French Guiana.

P164

Mucormycoses in patients with hemato-oncologic diseases

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Background Invasive fungal infections (IFI) are important causes of morbidity/mortality in immunocompromised patients with hematologic malignancies. While *Aspergillus* and *Candida* species still account for the majority of these infections, agents belonging to the class of the zygomycetes (particularly *Mucorales*) have emerged as increasingly relevant and highly lethal causes of IFI (reaching 90% mortality in some series).
Objectives Retrospective analysis of Mucormycoses at our centre in the last ten years.
Methods We studied data from 6 patients who were diagnosed with Mucormycoses between 2002 and 2011.
Results The patients were predominantly female (5/6), median age 48 years [18;64], diagnosed with acute myeloid leukemia (4) and aplastic anemia (2). The diagnosis of Mucormycoses was established after a median of 21 days of neutropenia [3;57], during induction (3), consolidation (2) and salvage therapy (1) of current chemotherapy protocols. Two cases presented with pulmonary involvement and 4 with rhinosinusal. Computed tomography was performed after prolonged febrile syndrome with symptoms suggestive of fungal infection. Histological confirmation of the diagnosis was achieved in all patients, while mycological culture was always negative. As part of reduction of the infected mass and prevention of extension to adjacent structures, patients were submitted to surgical debridement (those with rhinosinusal involvement) or thoracic surgery (right lower lobe/ upper left lobectomy, in those with lung involvement); one patient required a second surgery with lobectomy and partial hepatectomy due to diaphragmatic extension of the fungal infection and consequent liver involvement. Liposomal amphotericin B (5 mg/kg/day) was started empirically and maintained for a median of 45 days [13;80], followed by a period of secondary prophylaxis also with liposomal amphotericin B in a tapered low dose scheme, for a median of 3 months [1;6]. Median follow-up was 20.5 months [3;134]. Four patients died, only one attributable to the fungal infection (or concomitant bacterial infection by multirresistant *Enterobacter*); the other three died later on, due to progression of the underlying disease, although they had resolution of Mucormycoses.
Conclusions Managing mucormycoses is a challenge on account of its unspecific clinical presentation and lack of better and faster diagnostic tests, so as to initiate appropriate treatment as early as possible. High-dose antifungal therapy, control of the underlying predisposing condition and aggressive surgical approach are still the best options for treating these often fatal infections.

P165

Invasive aspergillosis in Saint Petersburg, Russia: analysis of 445 proven and probable cases

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Objective Analysis of demographic parameters, underlying diseases, risk factors, aetiology, clinical features, treatment and survival rates in patients with invasive aspergillosis (IA) in Saint Petersburg, Russia.
Materials and methods Prospective study in 1998–2013 yy. We used criteria EORTS/MSG, 2008 for the diagnosis of proven and probable IA. For identification of prognostic factors multifactorial analysis was used.
Results We observed 445 patients with proven (7%) and probable (93%) IA from 15 hospitals in St. Petersburg, 356 adults (80%) and 89 children (20%). The average age of patients was 36 years (range 1–83), males - 55%, females - 45%. Main underlying diseases were hematological malignancies (88%): acute myeloid leukemia - 30%, acute lymphoblastic leukemia - 22%, non-Hodgkin lymphoma - 10%, Hodgkin lymphoma - 7%, chronic lymphocytic leukemia, chronic myeloid leukemia, and multiple myeloma - 4% each, myelodysplastic syndrome - 3%, aplastic anemia - 2%, and other hemoblastosis - 2%. Non-hematological underlying diseases were: COPD - 2,5%, oncological malignancies - 2%, chronic sinusitis - 2%, drug-induced agranulocytosis - 1,5%, chronic renal failure - 1%, connective tissue diseases - 1%, tuberculosis 0,5%, immunodeficiencies - 0,5%, other - 1%. The risk factors: prolonged neutropenia - 64%, steroid therapy - 45%, allogeneic hematopoietic stem cells transplantation - 28%, graft versus host disease - 18%, surgery - 6%, organ transplantation - 1%.

The main sites of infection were lungs - 86%, sinuses - 12%, central nervous system - 4%. Two or more organs were affected in 6% of patients.

Galactomannan test in serum or bronchoalveolar lavage fluid (BAL) was positive in 62% cases. Direct microscopy of BAL or sputum was positive in 20% of cases. *Aspergillus* spp. were isolated in culture in 31% cases. The main aetiological agents were: *A.fumigatus* - 42%, *A.niger* - 33%, *A.flavus* - 21%, *A.versicolor* - 2%, *A.nidulans* - 1%, and *A.terreus* - 1%. Mixed *Aspergillus* infection was found in 12% cases.

Antifungal therapy was received by 98% patients: voriconazole - 55%, amphotericin B deoxycholate - 34%, itraconazole - 29%, caspofungin - 17%, posaconazole - 5%. Two and more antifungal drugs were used for 62% patients, surgery - 4%.

Overall survival rate in twelve weeks was 83%. Positive prognostic factors of 12th week survival were antifungal treatment with voriconazole ($p = 0.03$) and secondary antifungal prophylaxis ($p = 0.0003$).
Conclusion IA mainly develops in hematological patients (88%). The main underlying diseases were acute myeloid leukemia (30%) and acute lymphoblastic leukemia (22%). Aetiological agents were: *A.fumigatus* (42%), *A.niger* (33%), *A.flavus* (21%). Lung involvement develops in most cases of IA (86%). Twelve week overall survival was 83%. Positive prognostic factors of 12th week survival were treatment with voriconazole and secondary antifungal prophylaxis.

P166

Voriconazole and its major circulating metabolite are not phototoxic in the *in vitro* 3T3 neutral red uptake dye assay

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Objectives Photosensitivity skin reactions have been reported in patients receiving voriconazole. The mechanism for these skin reactions is unknown. This study evaluated the phototoxic effects of voriconazole and *N*-oxide voriconazole, its major circulating metabolite, using the Organisation for Economic Co-operation and Development (OECD) 432 *in vitro* 3T3 neutral red uptake phototoxicity assay. The 3T3 neutral red uptake assay measures the toxicity of a compound in the presence and absence of a non-cytotoxic dose of ultraviolet (UV) light. Under normal conditions, the neutral red dye penetrates 3T3 cell membranes in an active manner, accumulating intracellularly. However, with UV stimulation, phototoxic compounds will

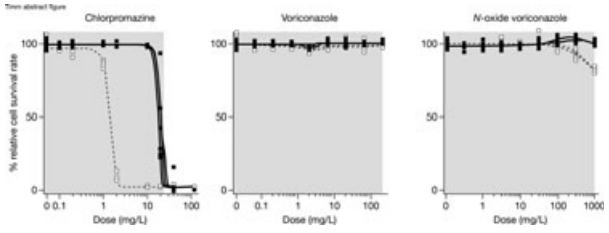

Figure 1 Concentration/response curves in the presence (open rectangles) and absence (solid rectangles) of UV radiation.

disrupt 3T3 cell membranes leading to cytotoxicity. This results in a decrease in the uptake of dye, which can be measured quantitatively.
Methods Voriconazole and *N*-oxide voriconazole dilutions ranging from 0.065 to 206.5 mg/L and 0.313 to 1000 mg/L, respectively, were prepared in vehicle (1% dimethyl sulphoxide in Dulbecco's Phosphate Buffered Saline). Cells were exposed to 5 J/cm^2 of UVA radiation and to 19 to 22 mJ/cm^2 of UVB radiation using a xenon arc solar simulator. Cytotoxicity was assessed by measuring the reduction in neutral red dye uptake in 3T3 cells exposed to voriconazole, *N*-oxide voriconazole or chlorpromazine (positive control compound) in the presence and absence of UV radiation.
Results Neither voriconazole nor *N*-oxide voriconazole affected cell survival in this assay (Figure), indicating that neither compound was phototoxic. All OECD 432-recommended assay validation criteria were met, including high rates of cell death in chlorpromazine-treated positive controls and the viability of 3T3 cell cultures (via measurement of neutral red uptake dye optical density at 540 nm in cells grown in control medium).
Conclusion The photosensitivity skin reactions reported in some patients receiving voriconazole are unlikely to be the result of a direct phototoxic reaction caused by voriconazole or *N*-oxide voriconazole.

P167

Risk factors and prevalence for candidemia in liver transplant recipients without antifungal prophylaxis, 10-year follow-up

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Objectives Candidemia rates in liver transplant recipients (LTRs) differs from center to center, geographic locations and type of solid organ transplantation. We aimed to investigate the risk factors and prevalence of candidemia in adult LTRs in our center.
Methods Between January 2003 and December 2012, microbiological and clinical data of adult (>18 years) LTRs were searched from mycology laboratory and patients' records retrospectively. The presence of candidemia was defined by determining at least one positive blood culture. Cases were followed at least 150 days after transplantation (150 days–10 years). The risk factors were investigated in preoperative, intraoperative and postoperative periods. The data collected including age, gender, body mass index, primary etiology, Model For End - Stage Liver Disease (MELD) score, preoperative albumin and creatinin levels, diabetes mellitus, the presence of ascites and encephalopathy, intraoperative glucose levels, operation and cold ischemia time, type of donor, the number of red blood cell, fresh frozen plasma, the use of vascular graft, the level of intraoperative blood loss, the type of biliary anastomosis, duration of antibiotic prophylaxis, biliary complication, hospitalization in intensive care unit, reoperation, the presence of bacterial infection (the infections of surgical site, blood stream and pulmonary) and preoperative and postoperative length of stay. None of the patients received antifungal prophylaxis. Statistical Package for the Social Sciences (SPSS) Version 15.0 was used for statistical analysis. Multivariate, forward stepwise, logistic regression analysis were used for the identification of independent risk factors for candidemia
Results In the study period, 388 of 472 patients undergoing liver transplantation were included. Candidemia was detected in 10 (2.6%) patients. The most important risk factor is albumin level below 2.8 mg/dL in preoperative period (p-value = 0.022). For the intraoperative period, the use of vascular graft is found to be significant (p-value0.025). In postoperative period, the presence of biliary complication, hospitalization in intensive care unit (>6 days), reoperation, the presence of surgical site infection and postoperative length of stay are detected as significant risk factors (p-values = 0.000, p = 0.002, p = 0.026, p = 0.011, p = 0.000 respectively).

The most common pathogen was *C. albicans* (6 patients) followed by *C. guilliermeondii* (1 patients) and *C. lusitania* (1 patient), in 2 patients both albicans and nonalbicans strains were detected. Antifungal therapy was started in all patients except one patients with candidemia because of delayed diagnosis. Five (50%) patients with candidemia died. Attributable mortality of candidemia was 60, 0% (3/5 patients). All of these 5 cases, *C albicans* was responsible for candidemia. The presence of candidemia increases the mortality rate (p-value = 0.023)
Conclusion Candidemia should be diagnosed as early as possible. In our study population without antifungal prophylaxis, candidemia ratio is not higher than the other studies reported formerly. But mortality of infections especially due to *C albicans* was high. A lower rate of candidemia can be achieved through good patient selection, meticulous surgical technique, use of biological rather than synthetic vascular graft and early discharge after transplantation.

P168

Candida species colonization and infection in patients with neoplasia

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Objectives Over the last three decades *Candida* infections have increased dramatically in susceptible hosts and a significant shift in the type of species has been observed. The present study reviewed the *Candida* epidemiology of colonization and infection in patients with hematological malignancies (HM) and solid tumors (ST) cared for at the University Hospital of Heraklion, Crete, Greece.
Methods The medical records of hospitalized patients with ST and HM with fever and possible *Candida* species infection of the 2000–2013 period were retrospectively reviewed.
Results Of the 115 episodes with fever and positive cultures for *Candida* species, only 49 (43%) represented true infection, occurring in the same number of patients. In the rest 66 patients fever was attributed to other causes and the presence of *Candida* species has been considered as colonization. Sixty four patients out of the totally 115 (56%) were males. The median age was 69 (range: 30–93) years. *Candida* species were isolated from different clinical samples of the 115 patients, more frequently from urine (46; 40%), sputum (27; 23%) and blood (17; 15%). Among the 66 colonized patients, 38 (58%) suffered HM and 28 (42%) ST. *C. albicans* was the predominant species isolated from colonized patients, being the colonizer in 28/38 (72%) patients with HM and 25/28 (89%) with ST. *C. glabrata was* the most frequent non-*albicans* colonizer isolated in 8/38 (20%), and 2/28 (7%) patients respectively. Among the 49 patients with *Candida* infection, 39 out of 49 (80%) had ST and 10 (20%) HM, with *C. albicans* being the predominant cause of infection in 26/39

(67%) and 6/10 (60%) patients respectively. *C.tropicalis* was the most frequent non-*albicans Candida* causing infection in 8 /39 (20%) patients with ST, followed by *C. glabrata (*3 /39; 8%). More patients with HM and *Candida* species infection than patients with ST had neutropenia (6; 60% vs. 6; 13.4%, $p < 0.01$) and prior steroid use (9; 90% vs. 18; 46.2%, $p = 0.01$) as expected. No statistically significant difference in duration of hospitalization between patients suffering of ST [22.5 days (4–55)] and those with HM patients [27 (8–126)] have been observed. Thirteen patients died during hospitalization, representing all causes mortality. Seven deaths (18%) occurred in patients with ST and 6 (60%) in those with HM ($p = 0.01$).

Conclusion The study has shown that a substantial proportion of patients with neoplasia are colonized with *Candida* species, a condition predisposing to subsequent infection. Although a shift towards to other *Candida* species has been reported in many countries, in the present population *C. albicans* remains the predominant colonizer and the most frequent cause of infection as well.

P169

Immunological features of invasive aspergillosis in hematological patients

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Introduction Invasive aspergillosis (IA) often complicates hematologic diseases. The immunological features in immunocompromised patients are not well understood.

Objective To study immunological features in 63 hematological patients with IA.

Materials and methods We observed two groups. Group I included 26 patients after allogeneic hematopoietic stem cells transplantation (allo-HSCT), mean age of patients was 23 years (range 6–59), males - 73%. Group II: 37 hematological patients after cytotoxic therapy (CT), mean age - 44 years (range 6–65), males - 46%. In the allo-HSCT group 100% of patients had the probable IA according to EORTS/MSG, 2008 criteria, in the CT group - 97% probable and 3% proven IA. Immunological parameters were evaluated within 2–4 weeks after IA diagnosis.

Results In these groups, the prevailing underlying diseases were: acute myeloid leukemia - 17% in each group, acute lymphoblastic leukemia - 11% and 16%, respectively, chronic leukemia - 6% in each group. Other hematological malignancies (lymphoma, myelodysplastic syndrome, and aplastic anemia) were rare. Patients after allo-HSCT have unrelated donors - 42%, HLA-matched - 31% and HLA-mismatched donors - 27%. All patients received immunosuppressive therapy. Graft versus host disease (GVHD) was observed in 88% of cases. After allo-HSCT the IA was diagnosed between 18 and 270 days with the median of 33 days, after CT - between 5 and 50 days, median - 36 days.

12th weeks overall survival was 66% and 92% in the both groups, respectively.

We identified significant immunological defects in all patients. In both groups we found: lymphocytopenia ($<1.0 \times 10^9$/L) ($p = 0.014$; $p = 0.006$), a reduction in the absolute number of T-helper CD4 + ($<0.680 \times 10^9$/L) ($p = 0.001$; $p = 0.018$), and low number of lymphocytes with activation markers - receptors for IL-2 (CD25 +). Level of cytotoxic T-cells CD8 + was increased ($>0.700 \times 10^9$/L) compared to baseline in allo-HSCT patients ($p < 0.001$).

Humoral immune response: the number of B-cells (CD20 +), IgG (<7.0 g/l), and IgM (<0.4 g/l) was reduced in both groups.

The decline of the killing activity of neutrophils and increased phagocytic activity was found in 62% and 70%, respectively, along with significant reduction in the production of proinflammatory cytokines IFN-γ, TNF-α, IL-8, G-CSF, IL-10. Low production of IL-17 ($p = 0.003$) and IgA ($p = 0.014$) was found in allo-HSCT as compared to the patients receiving CT.

Conclusion Invasive aspergillosis developed in hematological patients with decrease in the absolute number of lymphocytes subsets, immunoglobulin levels, imbalance neutrophil functional activity, and significant inhibition of cellular immune response key cytokine production: IFN-γ, TNF-α, G-CSF, IL-8. Special features of the immune response in allo-HSCT patients were low production of IL-17 and IgA as compared to the patients after cytotoxic therapy.

P170

Positive reaction of galactomannan in a *Fusarium* infection from allogenic transplant patient in ICU

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Fusarium spp is the third most common cause of invasive mould infection in patients with hematologic malignancies. A positive reaction to Galactomannan (GM) assay test could be a sign of a possible *Aspergillus* infection. However, cross-reactions of the monoclonal antibody (MAb) EB-A2 used in Platelia *Aspergillus* EIA test (Bio-Rad) have been described with other fungi, such as *Fusarium.*

Objectives To present a case of fusariosis in an acute myeloid leukemia (AML) patient/hematopoietic stem cell transplantation (HSCT) recipient with positive GM as an earlier diagnosis of fungal infection in a high risk patient.

Methods and Results A 42-year-old man with AML-M6 was submitted to an allogenic HSCT in January 2011 in Portuguese Oncology Institute of Porto. He seHeHtarts on corticosteroids and cyclosporine A due to chronic graft versus host disease (GVHD) (involving the skin, liver and gastro-intestinal tract) and develops iatrogenic type 2 diabetes. Under GSCF because of prolonged aplasia. Post transplantation period complicated with hemorrhagic cystitis due to adenovirus and reactivation of CMV. Later he develops enterocolitis and bacteremia by *Enterococcus faecium* and starts empirical treatment with meropenem, vancomycin and acyclovir (while on prophylaxis with posaconazole and atovaquone). Admitted in intensive care unit (ICU) with septic shock (Apache II 32, SAPSII 73) due to pneumonia and starts piperacilin/tazobactan, daptomicin, cidofovir, caspofungin and atovaquone. Blood cultures, traqueal aspirate and wound sacral swab were investigated and vancomycin resistent *Enterocccus faecium* (VRE) was isolated in all samples. He develops renal failure and starts on hemodialysis. Bronchoalveolar lavage (BAL) was performed and fungal hyphae were observed in cytological exam. The microbiological findings were: VRE in BAL culture, DNA CMV positive (7800 copies/ml), DNA adenovirus positive, GM Platelia *Aspergillus* EIA positive (GMI = 3,8) and PCR *Aspergillus spp* was negative with a Sabouraud Gentamicin Cloranfenicol (SGC) culture positive for *Fusarium oxysporum.* Secondary skin lesions with necrotizing cutaneous papules appeared during the ICU stay. After eleven days of treatment the patient died by septic shock refractory to treatment.

Conclusion Platelia *Aspergillus* test kit detects GM, not *Aspergillus.* We can obtain a positive reaction to any GM, regardless of the source. So, GM assay test can be useful for early diagnosis of others fungal infections besides Aspergillosis.

P171

Successful treatment of combination of invasive candidiasis (*C.krusei*) and aspergillosis (*A.flavus*) in haematological patient

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Objectives Invasive candidiasis (IA) and aspergillosis (IA) are leading causes of morbidity and mortality in haematological patients. Publications about combination of iC and IA in haematological patients are limited.
Methods EORTS/MSG criteria (2008) of diagnosis of IFD and treatment response were used.
Results 58 years old male was hospitalized in the City hospital '31, Saint-Petersburg, at 06.08.2012 with non-Hodgkin's lymphoma, IVB. He received cytostatic chemotherapy R-DA-EPOCH. The refractory to antibacterials febrile neutropenia started after a week of chemotherapy. *C krusei* was identified in cultures of peripheral blood and central venous catheter (CVC) samples. Treatment with amphotericin B 50 mg/day was started, CVC was replaced. At the same time the necrotic changes of nasal soft tissues developed. On computerized tomography (CT) scans there were symptoms of rhinosinusitis and pulmonary infiltrates in the upper lobes of the lungs. Necrosectomy of nasal soft tissues was performed. In microscopy of tissue specific hyphae were seen. *A.flavus* was revealed in culture of this tissue and bronchoalveolar lavage fluid. The treatment was changed to caspofungin 50 mg/day and voriconazole 400 mg/day.

A complete effect of the treatment of invasive candidosis (*C.krusei*) and invasive aspergillosis (*A. flavus*) was achieved. Duration of antifungal therapy was 98 days. There were no side effects of caspofungin and voriconazole.
Conclusion Combination therapy with caspofungin and voriconazole can be successful in the treatment of combination of invasive candidiasis and aspergillosis in haematological patients.

P172

Correlation of galactomannan antigen levels with neutrophil counts and iNOS activity in hematogical patients at high risk for invasive aspergillosis

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Objectives The detection of galactomannan (GM) antigen in serum samples is an important step helping the diagnosis of invasive aspergillosis (IA). It is known that polymorphonuclear leucocytes (PMN) attack hyphae dependinding on the release of reactive oxygen intermediates. We hypothesized that inducible nitric oxide synthase activity (iNOS) levels or PMN counts can be identified as prognostic factors of IA besides GM index (GMI). The objective of this study is to investigate the correlation of PMN counts and iNOS with GMI in hematological patients with possible IA.
Methods Totally 68 neutropenic patients who were expected to be neutropenic at least 10 days and acute leukemia and aplastic anemia patients, were routinely screened twice weekly for GMI. Seventy seven serum samples that was GMI ≥0.5 were included in this study. All the data concerning antibiotics and systemic antifungal treatment were collected at the time of of serum sampling for the GM assay. GMI was measured by ELISA (Platelia Aspergillus, Biorad, France). According to the neutrophil counts, the patients with possible IA were grouped as group 1 (<100 PMN/mm^3), group 2 (100–500 PMN/mm^3) and group 3 (>500 PMN/mm^3). iNOS levels were measured by ELISA (Eastbiopharm, China).

Table 1 Characteristics of patients.

Table. Clinical charecteristics, galactomannan (GM) antigenemia indices and treatments at the time of the GM result in patients with possible invasive aspergillosis as related to the three groups defined according to the polymorphonuclear neutrophil (PMN) counts.

	Group 1 (n=33) <100 PMN	Group 2 (n=21) 100-500 PMN	Group 3 (n=23) >500 PMN	Total (n=77)
Sex ratio, M/F	30/3	13/8	17/6	60/17
Age,years (Mean)	50.0±17.6	44.5±17.2	42.7±16.2	46.3±17.2
Underlying diseases, no				
Acute myeloid leukaemia	22	15	9	46
Acute lymphoblastik leukaemia	4	4	4	12
Lymphoma	3	2	4	9
Other	4	--	6	10
Antibiotics as potential source of false GM positivity[a], no. (%)	16 (80.0)	13 (86.6)	16 (88.8)	45 (84.9)
Antifungal drugs[b], no. (%)	30 (90.0)	12 (57.1)	14 (60.8)	46 (59.7)
GM index ≥ 1, no. (%)	23 (69.7)	10 (47.6)	13 (56.5)	46 (59.7)
GM index ≥ 0.7, no. (%)	5 (15.2)	6 (28.6)	5 (21.7)	16 (20.8)
GM index ≥ 0.5, no. (%)	5 (15.2)	5 (23.8)	5 (21.7)	15 (19.5)
iNOS (<35 U/L), no	20	17	17	54
iNOS (>35 U/L), no	13	4	6	23

[a]Piperacillin-tazobactam, amoxycillin, amoxycillin-clavulanate

[b]Caspofungin, voriconazole, amphotericin B deoxycholate or liposomal formulation

Results The demographic features like age, sex, underlying hematological disorders, treatments, GMI and iNOS levels of patients were shown (Table). Although the highest GM indices were obtained in group 1 compared to group 2 and group 3, there was no statistically significant difference among three groups. In patients with iNOS level <35 U/L (n = 54), 34 of them had GMI ≥1 and 35 of them had PMN counts >100 (group 2 and 3). There was no statistical difference between iNOS levels and PMN counts or GMI. The proportion of patients receiving antibiotics possibly responsible for false-positive GM results were not statistically different among groups. However, most of the patients using especially piperacillin-tazobactam had GMI ≥1 (p = 0.03). GMI of patients receiving antifungal therapy did not differ among three groups. Antifungal therapy did not significantly affect GM results.
Conclusion The GMI is higher in severely neutropenic patients (<100 PMN) than in other (≥100 PMN) patients. However, high GMI may be false-positive due to antibiotic usage. In patients with ≤100 PMN counts, iNOS levels would also be expected low. This probably means that iNOS levels are increasing in order to fight with fungal conidia if there is a progression to IA. Therefore, PMN counts correlated with iNOS activity should be kept in mind in routine use of GM assay.

P173

Evaluation of fungal risk for immunocompromised patients alternatively hospitalized in haematology ICU and at home

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Objectives Despite chemoprophylaxis and all standard preventive measures, Invasive Fungal Infection (IFI) incidence remains high in hematology patients. In France, quarterly controls are recommended in haematology intensive care units (ICU). However, little is known about the indoor environment of haematology patients when they return home between two hospitalization periods, when they can be

considered as chronically immunosuppressed. The aim of our study was to assess the fungal exposure of these patients at home and to compare it with that of the hospital, especially when IFI was diagnosed. We sought to demonstrate the interest of also measuring fungal exposure at home to assess the overall fungal risk in such patients.
Methods Fifty-three patients from a haematology ICU were included in the study. Fungal exposure was assessed by quantifying opportunistic molds at hospital during hospitalisation and in patients' homes. Environmental surveillance was performed weekly in the haematology ICU with five air samples using the MAS100^{TM}Impactor in the corridor and four air samples in patients' homes after hospitalization. Cultures were carried out on DG18 media. Clinical, biological and radiological data were collected according to consensual criteria of EORTC/MSG for IFI classification. In addition to usual risk factors such as severe and prolonged neutropenia (<500 polynuclear neutrophils/mm^3 of blood and >15 days), mild neutropenia (0.5G/L-1G/L), corticoid therapy and all situations which could result in mold exposure in the 3 months prior to IFI diagnosis were also analyzed.
Results IFI was diagnosed for 14/53 patients. In hospital, 80% of weekly controls were negative for opportunistic species, 17.4% detected low level of opportunistic species and 2.4% showed uncommon concentrations of *A.fumigatus* spores in corridor air (9, 14 and 25 CFU/m^3). Some patients who developed IFI were hospitalized during these peaks. The air in most homes (34/53) had a low level of total mold (less than 170 CFU /m^3) but opportunistic molds (*A.fumigatus, A. flavus, Mucor* spp., *Lichtheimia* spp., *Rhizomucor* spp. and *Rhizopus* spp.) were found in 69.8% of homes with *A. fumigatus* identification in 58.4% of cases (31/53). Twelve homes out of 53 had a ratio of opportunistic mold to total mold as high as 10% and 4 homes had more than 20% of opportunistic mold.

Our study established that 6 IFI patients/14 could have been exposed to opportunistic molds at home and in hospital, 3/14 only in hospital and 5/14 only at home. These last 5 patients were living in homes among the 25% having the highest rate of opportunistic molds. The percentage of opportunistic molds at home was a significant predictor variable for the development of IFI in this study.
Conclusion This study emphasizes the fact that preventive measures should be aimed not only at the hospital but also the home environment. Monitoring fungal contamination in homes of immunosuppressed patients, focusing on opportunistic molds, could contribute to detecting IFI risk and would be a relevant continuation in patient surveillance.

P174

Secreted effectors of *Aspergillus fumigatus* influence platelet activity of the human host

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Objectives *Aspergillus fumigatus* is a ubiquitous fungus colonizing soil and decomposing organic matter. It produces small conidia that are distributed by the air and reach the alveoli of the human lung when inhaled. In immunocompromised patients this opportunistic pathogen can cause several forms of diseases, the most severe form being Invasive Aspergillosis (IA). IA and also bronchopulmonary forms of aspergillosis can be accompanied by local tissue damage as well as local bleedings and thrombus formations at the site of infection. This could be the result of potentially exaggerated and uncontrolled cells of the specific immune system, mechanical destruction of lung tissue caused by steadily growth of the fungus during invasion, or alteration of the local haemostasis by *Aspergillus* at the site of infection.
Methods To elucidate the influence of *A. fumigatus* on haemostasis, platelets from healthy donors were isolated by centrifugation and collagen- or ADP- induced aggregation was analysed. Platelets were coincubated with conidia, swollen conidia, germtubes and hyphae of the wild type isolate ATCC46645 as well as with native and concentrated culture filtrates from germtubes and hyphae. In addition, protease-containing culture filtrates of ATCC46645, of the *prtT*-knockout strain AfS61 as well as the reconstituted strain AfS62 were used. Platelets were also treated with decreasing concentrations of the mycotoxin gliotoxin. Results were confirmed by flow cytometry (platelet-activation markers CD62P and CD63). Here also a time-kinetic experiment was performed by coincubation of platelets with gliotoxin (5, 15, 30 min). In addition, activated thromboplastine time (PTT), prothrombin time (TPZ) and the levels of fibrinogen and of D-dimers of platelet-poor plasma was analysed after coincubation with the above mentioned effectors.
Results The analysis of coagulation parameters revealed no influence of morphologies, culture filtrates or gliotoxin on coagulation. Aggregation assays of human platelets coincubated with different *A. fumigatus* morphologies showed an increased aggregation of platelets conincubated with hyphae or its concentrated culture supernatant. In addition, the coincubation of protease-containing culture filtrates of ATCC46645 and AfS62 appeared to activate resting platelets whereas the AfS61 culture filtrate had no significant effect. Heat inactivation as well as protease-inhibitors could not reverse the activating effect to full extent, which was validated by flow cytometry. Furthermore, purified gliotoxin was used and a concentration-dependent inhibition on collagen- or ADP-induced platelet aggregation could be shown. This inhibition was confirmed by flow cytometry. Time-kinetic experiment of platelets coincubated with gliotoxin revealed a decrease in CD62P-expression on resting and ADP-activated platelets over time.
Conclusion Taken together, these data imply an effect of *A. fumigatus* secreted effectors and secondary metabolites on host haemostasis. The discovery of the targets and the mode of action for these effectors and metabolites may lead to new insights into the mechanisms of *A. fumigatus* pathogenicity to support antifungal therapy.

P175

Invasive aspergillosis due to a resistant strain of *Aspergillus fumigatus* with TR4/L98H mutation in an haematology patient in France

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Objective Azole resistance in *Aspergillus fumigatus* has been increasingly reported in recent years. The primary acquisition of environmental-resistant isolates is characterized by the dominance of a single resistance mechanism (TR34/L98H mutation). Only a few cases of invasive aspergillosis due to resistant strains with the TR34/L98H mutation have been described thus far (8 cases in the Netherlands, one case in Germany, one case in Spain). We describe the first case of invasive aspergillosis in France due to a multi-azole resistant strain of *Aspergillus fumigatus*, with mutation TR32/L98H.
Patient-Methods Mr X, a 63-year-old farmer in Jura, France, was diagnosed with severe aplastic anemia in 1997. An allogenic hematopoietic stem cell transplant was done in January 2011. The patient presented reactivation of Graft versus Host disease at D130 and D 180, which required increasing the dose of corticoid therapy and cyclosporin, then tacrolimus, then rituximab in October 2011. Antifungal prophylaxy were given during the entire post graft period, with voriconazole (Jan–April 2011) then posaconazole (200 mg 3x/day) (from May 2011). On April 26, 2012, the patient was diagnosed with probable invasive aspergillosis. Mycological examination showed positive *Aspergillus* antigen in serum and positive culture of *Aspergillus*

fumigatus from sputum. As the patient had received azole antifungal prophylaxy for 16 months, the antifungal therapy was started with a combination of liposomal amphotericin B and caspofungin. Though a second *Aspergillus fumigatus* isolate was obtained from a sputum sample 15 days later, clinical improvement was obtained. An environmental survey was conducted at the patient's home (14 air samples). We also collected samples from the outdoor environment around his home (27 samples from soil, cereal fields, hay), as he reported the use of fungicides (prothioconazole- epoxiconazole) in his work

Results The two clinical isolates showed the same resistant pattern (EUCAST method: resistance to itraconazole (Minimal Inhibition Concentration (MIC) >8 μg/mL) and to voriconazole (= 4 μg/mL)). Sequencing the complete CYP51A gene revealed the presence of the 34 bp tandem repeat and the L98H mutation in both isolates. All environmental samples were seeded on DG18 medium and on malt agar supplemented with 4 mg/L of itraconazole. From the 41 environmental samples, 145 isolates of *Aspergillus fumigatus* grew on DG 18 media, and one isolate grew on itraconazole-containing malt agar. MIC determination and gene sequencing of environmental isolates are ongoing.

Conclusion Our observation provides additional arguments in favor of recommending active sampling (sputum, bronchoalveolar lavage) to isolate the *Aspergillus* strains and perform susceptibility testing in patients with suspected invasive aspergillosis. It also raises the question of reconsidering voriconazole as first-line therapy in patients with invasive aspergillosis in specific situations (rural, farmer, …) where contact with strains from fields treated with fungicides is likely.

P176

Imported African histoplasmosis by *Histoplasma capsulatum* var. *duboisii* in an HIV2 infected patient

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Objectives African histoplasmosis caused by the fungus *Histoplasma capsulatum* var. *duboisii*, is a rare endemic mycosis occuring in western and central regions of sub-Saharian Africa. For unknown reasons, although HIV infection and *H. capsulatum* var. *duboisii* coexist in Africa, this coinfection remains rare. In Europe, diagnosed cases of African histoplasmosis are all imported.

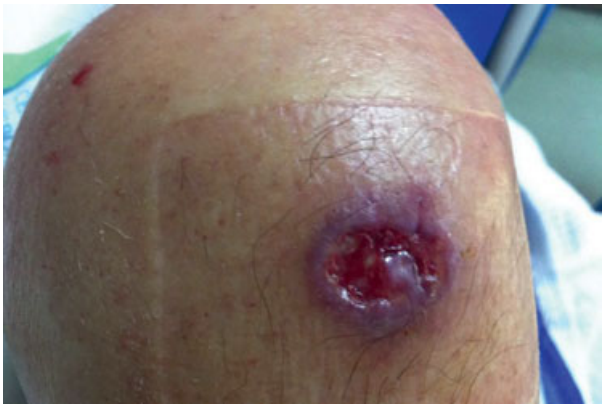

Figure 1

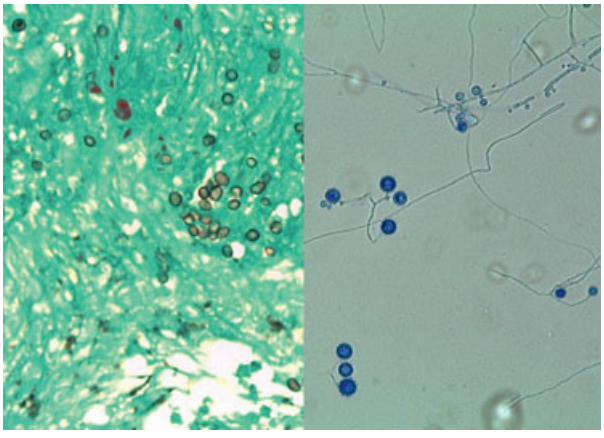

Figure 2

We describe a case of African histoplasmosis on a Portuguese war veteran co-infected with HIV-2 who fought in Guiné-Bissau in 1963–65 and Angola in 1972.

Methods We report a case of a 76-year-old man, diagnosed with HIV-2 infection in the previous year (under combined antiretroviral therapy) presenting an ulcerated skin lesion on the right tight (image 1), just above the knee. He was diagnosed pulmonary tuberculosis the year before and was finishing one year treatment. The solitary skin lesion begun as a small non-pruriginous eritematous papule, evolving in 6 month to a painless 3–4 cm ulcer with raised borders surrounded by a hiperpigmented halo. There were no adenopathies or bone lesions. Respiratory samples and blood cultures were systematically negative for *Histoplasma capsulatum*. He was treated with IV liposomal Amphotericin-B for one month, followed by oral itraconazol (now on the first month), with a favourable clinical outcome.

Results Histopathology of skin biopsy revealed a superficial ulceration with underlying granulomatous infiltrate with many giant cells, where numerous round mononucleated yeasts measuring 7–8 μm were evident and highlighted with PAS and Grocott stain (image 2, left side).

Skin biopsy was observed on a wet mount with KOH and revealed numerous round yeasts that were also seen on Gram stain, measuring 7–8 μm.

Culture of skin biopsy on two Sabouraud dextrose agar (with and without cicloheximide) showed growth of a filamentous fungus compatible with *Histoplasma capsulatum* (image 2, right side), with large thick-walled spherical macroconidia with finger-like projections (tuberculate conidia) that arise from short conidiophores, and small oval microconidia arising on short stalks from undifferentiated hyphae. Reversion to the yeast fase has not been succeed yet.

Identification was further confirmed by sequencing of genomic DNA fragments using the universal fungal primers ITS1 and ITS4. The sequences obtained were compared with sequences deposited in the GenBank and the result was: *Histoplasma capsulatum* var. *duboisii* (99% homology).

Conclusion With banalization of business or leisure trips, endemic mycosis are becoming frequently diagnosed in countries outside their natural geographic endemic areas and only a high index of suspicion makes the diagnosis possible. Apart from trips, nowadays in Portugal 4% of the resident population is immigrant, mostly from Brasil (25.5%) but also from Angola and Guiné-Bissau (9.2%), being the former an endemic country of American histoplasmosis and the later of both American and African histoplasmosis. We consider histoplasmosis a probable underdiagnosed disease that should be suspected mainly in immunodeficient HIV positive individuals with a past history of travel or residence in an endemic area.

P177

The utility of 1,3-β-D-glucan as a screening method for invasive fungal disease in adult high risk hematological patients in Sweden

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Background Early diagnosis of Invasive Fungal Diseases (IFD) still poses a significant problem in the management of hematological patients.

Objectives To retrospectively study two years' experience of using detetction of fungal cell wall antigen 1,3-β-D-glucan (Betaglucan) as a screening method for IFD in adult severely immunocompromised hematological patients in Sweden regarding 1) diagnostic accuracy and 2) possible confounding factors in the interpretation of positive test results.

Methods All patients (n = 167) at the Dept. of Hematology ward who had been tested for betaglucan (Glucatell® kit, Cape Cod Ass.) between Nov 2009 - Nov 2011 were investigated. The medical records were examined to determine if patients had contracted Invasive Fungal Disease according to the 2008 EORTC/MSG-criteria. ROC-curves were made to determine optimal cut-offs for the betaglucan test in this patient cohort. A subgroup analysis of patients (n = 58) without IFD (true negative) but who had at least one betaglucan test result >50 pg/ml out of at least two consecutive serum samples was made to identify possible confounding factors, *e.g.* i.v. antibiotics, i.v. plasma or albumin, i.v. immunoglobulins, antifungal treatment, total parenteral nutrition, ongoing chemotherapy, fungal mucosal colonization, bacteremia and dialysis. The multivariate method of pattern recognition Orthogonal-Projection to Latent Structures (O-PLS) was used.

Results A total of 167 patients (median age 54,range 15–85, 40% female) with hematological malignancies were studied, of whom 43% had undergone allogeneic hematopoietic stem cell transplantation. The patients had been tested a median of 6 times for the presence of betaglucan during this time period, (serum samples n = 1449) and 15% were positive (>80 pg/ml). Fifteen patients (9%) had probable or proven IFD, and all had positive betaglucan tests. The mean of the highest Betaglucan level detected the week after diagnosis in 12 of the patients with probable or proven IFI was 420 pg/ml (excluding the one patient with Zygomycosis and the two patients whose diagnosis was dependent on the Betaglucan value). In contrast, the mean serum betaglucan level was 64 pg/ml in patients with no IFI (excluding all possible IFI) ($p < 0.001$). A minority of the patients (13%) with only a single serum sample with betaglucan >80 pg/ml had IFD, while 50% of the patients with at least three consecutive betaglucan tests >80 pg/ml had IFD. 78% of the patients with at least three consecutive betaglucan tests >300 pg/ml had IFD. Multivariate analyses indicated that i.v. administration of albumin or plasma, but not of antibiotics or total parenteral nutrition, was associated with positive betaglucan tests in serum. The mean betaglucan level in the group without IFD receiving albumin or plasma was 148 pg/ml (±37) compared to 50 pg/ml (±2.6) for those not receiving this treatment ($p = 0.0176$).

Conclusion Our findings indicate that Betaglucan testing is a valuable complementary diagnostic tool for IFD in adult patients with hematological malignancies also in Sweden, in particular when using repeated testing. Plasma and albumin therapy may give rise to false positive reactions.

P178

Real life performance of serum GM for invasive aspergillosis in allogeneic HSCT recipients

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Objectives Galactomannan (GM) is a well-established non-culture-based assay for the diagnosis of invasive aspergillosis (IA) in high risk patients. Among others, it's performance is influenced by the type of the underlying disease and the presence of neutropenia.

The aim of this study was to evaluate real life performance of serum GM in allogeneic HSCT recipients in our center, 15 years after its introduction into clinical practice.

Methods Allogeneic HSCT recipients are routinely screened for serum GM: twice weekly during the first 100 days after transplant, than once weekly or when clinically indicated.

The clinical records and GM results of all the patients who received allogeneic HSCT between 01/01/2007 and 30/06/2012 were reviewed. For patients with IA, only samples from the period of infection were considered.

Patients were classified as proven, probable, possible or no IA according to 2008 EORTC/MSG criteria. Patients with proven or probable IA were included as cases, those with no aspergillosis were considered controls. Subjects diagnosed with IA but with radiological findings that were not typical according to 2008 EORTC/MSG criteria were excluded. Early IA was defined as occurring within 40 days after HSCT.

Testing for GM was performed with Platelia Aspergillus assay and samples with the optical density index (ODI) ≥0.5 were considered positive.

Results During the observation period 501 allogeneic HSCT were performed and over 19.000 serum samples were tested for GM.

Sixty six patients were diagnosed with proven/probable IA, in median 22 days after HSCT (range: -29, +495). Among them, 41 had early IA. Fifteen 15 patients with IA and atypical radiological findings were excluded, while the remaining 420 patients had no IA.

The results of the GM performance are shown in Table 1. False negative results were diagnosed mainly with GM in BAL, and only in one case with sputum culture.

Table 1
The performance of serum GM for the diagnosis of IA and early IA in allogeneic HSCT recipients.

	Sensitivity, % (95%CI)	Specificity, % (95%CI)	PPV, % (95%CI)	NPV, % (95%CI)
IA				
Single sample ODI ≥ 0.5	85.9 (73.9-92.5)	76 (71.6-80)	35.7 (28.2-43.7)*	97 (94.5-98.5)
Single sample ODI ≥ 0.7	69.7 (57.1-80.4)	88.1 (84.6-91)	47.9 (37.6-58.4)*	94.9 (92.2-96.8)
2 consecutive samples ODI ≥ 0.5	74.2 (62-84.2)	94.1 (91.3-96.1)	66.2 (54.3-76.8)*	95.9 (93.5-97.6)
Early IA				
Single sample ODI ≥ 0.5	87.8 (73.8-95.9)	86.2 (82.5-89.3)	38.3 (28.5-48.9)**	98.6 (96.9-99.5)
Single sample ODI ≥ 0.7	73.2 (57.1-85.7)	92.9 (90-95.1)	50 (36.8-63.2)**	97.3 (95.1-98.6)
2 consecutive samples ODI ≥ 0.5	75.6 (59.7-87.6)	96.2 (93.9-97.8)	(50.7-79.1)**	97.6 (95.6-98.8)

ODI, optical density index.
*For the disease prevalence of 14%; ** For the disease prevalence of 8.9%.

Conclusions GM is fundamental for the diagnosis of IA in allogeneic HSCT recipients. Its performance is better during the early post-transplant phase, i.e. primary neutropenia. The performance of two consecutive samples with ODI ≥0.5 is superior to a single sample with a higher positivity (ODI ≥0.7). Although single false positive results are not infrequent during long-time screening, the specificity and NPV of two consecutive positive samples is high confirming its utility in screening in high risk patients.

P179

Multi-modality guided treatment with resolution of long lasting invasive aspergillosis and hormografiella species within immunocompromised host; a case history

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Objectives Invasive Hormografiella species in immunocompromised patients are very rare and difficult to treat. The prognosis of these patients is almost always dismal (90% mortality in literature). We treated a patient with this type of fungal disease and used plasma antifungal levels, inflammation markers (CRP), and imaging techniques like PET-CT scans to guide the response of treatment. Furthermore, these parameters and techniques played an important role in treatment changes. The objective of this complex case is to show how different diagnostic tools can guide the clinician and provide a higher chance of successful treatment of these patients.

Methods and results We treated a 58 years old male patient with hairy cell leukemia (HCL) with weekly cladribine under adequate antibacterial, antiviral, and antifungal prophylaxis. After a prolonged period of pancytopenia, he developed neutropenic fever with coughing and dyspnea with pleuritic chest pain at the left side for which broad spectrum antibiotics were given. A chest x ray and subsequently high resolution ct scan revealed several lung nodules with halo signs and pleural effusion. A galactomannan (GM) was positive in serum (3.44), BAL fluid (lingual; 9.24), and pleural fluid (12.7). Aspergillus PCR was positive and culture of BAL fluid revealed Hormografiella spp., which is a filamentous non-Aspergillus mould belonging to the class of Basidiomycetes (in this case: anamorph of Coprinellus xanthothrix). This species was susceptible for all antifungals, except for flucytosine. Voriconazole was started initially, subsequently ambisome, caspofungin, and also flucytosine were added with adequate blood levels because of progressive fungal disease and symptoms. Also G-CSF was added to improve neutrophilic counts, which took about one month. Afterwards he was still moderate to severe lymphocytopenic for almost 5 months. All therapeutic changes were guided by symptoms, CRP, GM levels, and PET-CT scans in time, as also masses were found in the thyroid gland and between the left liver lobe and the spleen. After 5 months he was put on posaconazole with adequate plasma levels and was discharged from the hospital. Eventually his GM and CRP normalized after 7 months of treatment. Unfortunately, his abdominal mass grew on PET-CT scan, whereas the other masses decreased in size and activity or resolved. An operative procedure with resection of this mass and splenectomy was performed; a granulomatous inflammation with hyphae was found, whereas the cultures were negative. After 2.5 years his antifungal treatment was stopped after his PET scan became negative, and his IgG levels and CD4 counts had resolved. His HCL is still in remission.

Conclusion Multi-modality diagnostics, especially PET-CT scanning, have important impact in guidance of treatment of complex, long lasting fungal disease, especially in prolonged immunocompromised conditions

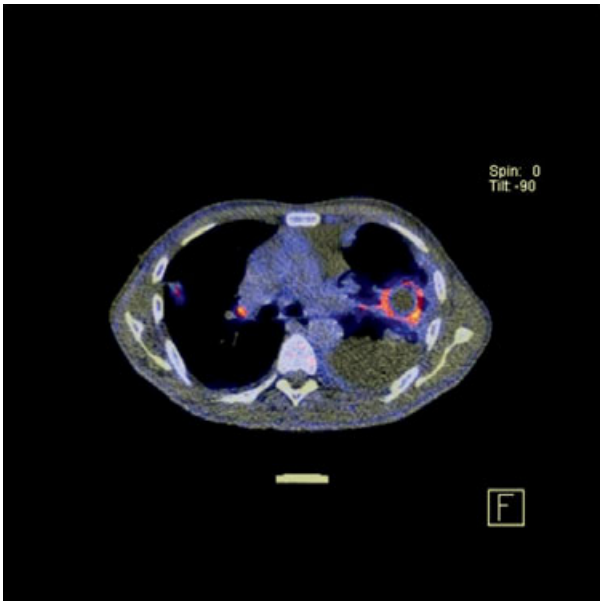

Figure 1

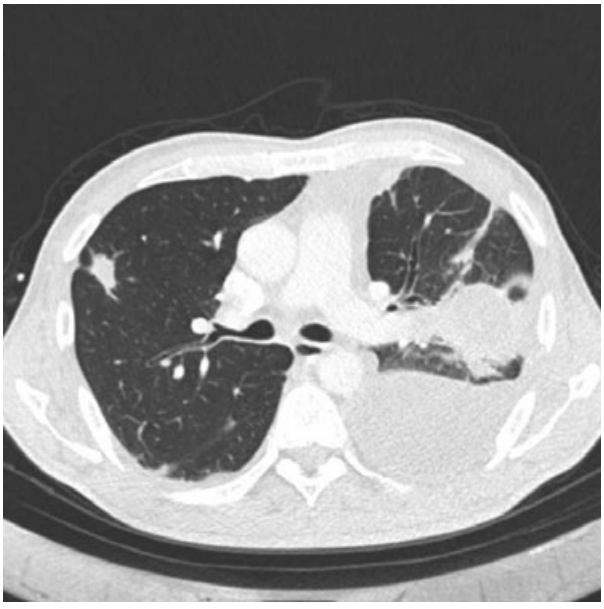

Figure 2

P180

The efficacy of the prophylaxis with amphotericin B lopisomal formulation (AnfoBlip) in the prevention of invasive fungal infections after liver transplantation (LT) in high-risk patients

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Objective Evaluate the efficacy of the prophylaxis with Amphotericin B lopisomal formulation (AnfoBlip, 100 mg/day) in the prevention of invasive fungal infections after liver transplantation (LT) in high-risk patients

Design retrospective, non-interventional study, with a 3-month follow-up

Methods and Patients All patients who underwent LT between January 2008 and December 2012 (n = 490; mean age 47.4 ± 12.4 years, 68.5% male, mean MELD 15.1 ± 4.1, mean CTP score 7.7 ± 3.3) were included. The high risk population was

defined as: urgent LT, LT in patients undergoing hemodyalisis, retransplantation, early urgent surgical complications after LT (including vascular), patients needing ≥40 units of blood cell components or derivatives. All documented invasive fungal infections were considered. Patients who died intraoperatively or within 1 week after LT were excluded.
Results Of all the patients included in the study, 26 died intraoperatively and 21 patients during the follow-up period. Of the remaining, 99 (20%) were considered to be at high risk for fungal infection. These patients presented a higher MELD score (19.5 vs 14.2, $p < 0.001$), higher CTP score (12.1 vs 6.9, $p > 0.001$), more frequent acute kidney injury (AKI≥2 21% vs 10%, $p < 0.001$) and greater need for vasopressors intraoperatively (67% vs 14%, $p < 0.001$).
61 of them (61.6%) received prophylaxis with AnfoBlip. 8 fungal infections were detected (7 Candida albicans and 1 Candida tropicalis), 5 in the high risk group without prophylaxis and 1 in the high risk group with prophylaxis. In one patient AnfoBlip was discontinued due to worsening renal function.
Conclusion The introduction of prophylaxis with AnfoBlip after LT in selected patients decreases the incidence of invasive fungal infections. Nonetheless we observed that not all patients at higher risk received this prophylaxis.

P181

Evaluation of the pattern of antifungal therapy prescription after liver transplantation

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Objective To evaluate the antifungal therapy prescription after liver transplantation (LT) Design: retrospective, non-internventional study, with a 1-year folow-up.
Patients and Methods All patients who underwent LT during January 2008 and December 2012 (n = 490; mean age 47.4 ± 12.4 years, 68.5% male, mean MELD 15.1 ± 4.1, mean CTP score 7.7 ± 3.3) were included for enrollment. Patients were further separated in prophylaxis group, considered at high risk and receiving antifungal therapy within the first 10 days after LT, and patients receiving any other antifungal therapy afterwards, up to the end of the follow-up period. All documented invasive fungal infections were considered. Patients who died intraoperatively or within 1 week after LT were excluded.
Results From the total of enrolled patients, there were 26 intraoperative deaths and another 32 patients died during the follow-up period. Ninety nine were considered at high risk for fungal infection and, of them, 61 (61.6%) received prophylaxis with AnfoBlip, at a standard dose of 100 mg/day. During this period, a total of 8 fungal infections were detected (7 by Candida albicans and 1 by Candida tropicalis), 5 in the high risk group without prophylaxis and 1 in the high risk group who received prophylaxis period. After this period antifungals were prescribed to other 19 patients. The most prescribed antifungal class was azoles (15), followed by liposomal Amphotericin B and echinocandins (2). Prescriptions were mostly performed during readmissions (14) or in outpatient follow-up. In this period, only 3 fungal agents were detected, all Candida albicans.
Conclusion Initial prophylactic treatment of antifungal invasive infection after LT in high risk patients during the study period covered only 61% of the eligible patients; after this period, the prescriptions are performed empirically, mostly not supported by microbiological results but upon a higher clinical suspicion of the in-charge physician.

P182

Invasive aspergillosis in patients with hematological malignancies in Czech and Slovak Republics: fungal infection database (find) analysis (2001–2011) – an update

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Objectives "Fungal InfectioN Database" (FIND) represents international database of invasive fungal infections in Czech and Slovak hematooncological departments. FIND - *aspergillus* covers all case of invasive aspergillosis (IA) in participating centers since 2001.
Methods The goal of our retrospective analysis was to evaluate incidence, early diagnostic procedures and effect of antifungal therapy in proven and probable IA that occurred in 16 institutions participating in FIND database between 2001–2011. Till 2009 we followed EORTC/MSG 2002 and from 2010 EORTC/MSG 2008 criteria in evaluation of IA diagnosis and therapy response.
Results 256 probable and 58 proven IA (91% isolated pulmonary IA, IPA) have been documented. Prolonged and profound neutropenia (61%) and long-term use of corticosteroids (28%) were identified as the major risk factors of IA. 68% pts. had consecutive positivity of serum-galactomannan (S-GM) (OD index >0.5). 83% pts. with IPA and bronchoalveolar lavage (BAL) had positive GM in BAL fluid (OD index >0.5). In pts. with IPA only 7.6% BAL fluids and 8.4% sputum samples had positive microscopic result for filamentous fungi and 2.2% BAL fluids and 1.8% sputum samples had positive culture for *Aspergillus* spp. The primary antifungal therapy of IA was used in 89% pts. - 41% voriconazole (VORI), 7% echinocandins (ECHINO), 23% VORI+ECHINO, 7% amphotericine B deoxycholate (C-AMB) and

9% lipid-based AMB (LBA). Overall RR to primary therapy of IA was 46% - VORI 55%, VORI+ECHINO 54%, C-AMB 35%, LBA 39%, ECHINO 25%. There was a statistically significant difference in overall RR to targeted therapy in pts. with neutrophil count <0.1 and >1.0 x10^9/l at the end of therapy (21% vs. 71%). The overall mortality rate was 61%, with 39% attributable to IA.

Conclusion On the basis of our analysis we confirm typical risk factors for IA and critical role of S-GM and CT for early diagnosis and prompt start of antifungal therapy of IA. A reasonable treatment response was achieved using VORI, VORI+ECHINO or LBA in primary therapy of IA. We have confirmed neutropenia at the end of antifungal therapy as the major predictive factor for therapeutic response.

On behalf of CELL - The Czech leukemia study group for life.

P183

Galactomannan determination for diagnosis of invasive aspergillosis in different groups hematooncological patients in Saint-Petersburg, Russia

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Objectives To analyze the utility of galactomannan (GM) test for the diagnosis of invasive aspergillosis (IA) in patients with hematological malignancies in St. Petersburg, Russia.

Methods The study included 728 samples: 480 serum, 164 bronchoalveolar lavage fluid (BAL) and 15 cerebrospinal fluid (CSF) from 351 hematooncological patients with IA in 19 hospitals in St. Petersburg in 1998–2012 yy. Diagnosis of IA was based on EORTC/MSG 2008 criteria. Detection of GM was performed with "Platelia *Aspergillus* EIA"(Bio-Rad). The test was considered as positive with a cut-off of ≥0.5 (serum, CSF) and ≥1.0 (BAL). BAL and CSF were examined by direct microscopy with calcofluor white and culture.

Results Test "Platelia *Aspergillus* EIA"was positive in serum of 65,0% of patients invasive aspergillosis: in 62,8% - pulmonary aspergillosis, 57,7% - *Aspergillus* synusitis and 73% - central nervous sustem aspergillosis. Direct microscopic examination of BAL, sputum, cerebrospinal fluid and discharge from sinuses was positive in 28% of cases. *Aspergillus* spp. were isolated in 26% of cases. Level of GM index in samples differed: in serum was 0,5 - 2,7, CSF - 0,5 - 2,0, and in BAL - 1,0–7,5.

Sensitivity of GM test in BAL were higher, than in serum on 12,9% at similar specificity (83% vs. 86,0%). The positive results of GM test in BAL samples and serum samples correlated with positive results of microscopy and culture of BAL in 60% and 30%, respectively. In hematological patients with IA a higher level GM index (2, 0–7,5) was found in the BAL samples more frequently along with a positive microscopy than with positive mycological culture.

Conclusion This study indicates that GM test in serum, BAL and CSF has a significant impact on the diagnostics of IA in patients with hematologic malignancies. The sensitivity of "Platelia *Aspergillus* EIA"test was higher than that of classical mycological methods and depends on the type of biological specimen.

P184

Epidemiology and outcome of mucormycosis in hematopoietic stem cell transplant recipients

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Background In recent years mucormycosis (M) has become an important cause of morbidity and mortality in allogeneic hematopoietic stem cell transplant (allo-HSCT) recipients. This study focuses on etiology, clinical forms and outcome of M and factors which influences on overall survival (OS) in allo-HSCT recipients.

Methods 14 pts with M after allo-HSCT were included in 2009–2013 yy. Baseline patient characteristics and treatment are outlined in Table 1. EORTC/MSG 2008 diagnostic criteria of proven and probable invasive fungal disease (IFD) were used.

Results Incidence of M was 2,4% (14/582, allo-HSCT 2009–2012). There were 6 proven (43%) and 8 probable (57%) cases of M in 14 pts with predominantly acute leukemia (71%). Median date of M onset after allo-HSCT was D + 105 (14–449). One case of M was diagnosed post-mortally. Main clinical forms of M were pulmonary 79%, rhinocerebral 7%, subcutaneous/osteomyelitis 7%, and bowel 7%. In 79% pts bronchoscopy was performed, in 100% cases it was informative for the diagnosis. In 57% of cases diagnosis was confirmed by culture. Etiologic agents of M were *Rhizopus* spp. (75%), *Rhizomucor* spp. (12.5%), *Mucor* spp. (12.5%), and *L. corymbifera* (12.5%). In 50% cases M was diagnosed after or with invasive aspergillosis (IA). 12-week OS after diagnosis of M was 31%, 6-months OS in - 31%, and 1-year OS - 23%.

Table 1

Baseline patient characteristics with mucormycosis	(n = 14)
Variable	
Demographic characteristics	
Age, years – Median, range	21 (4-45)
Sex – Male/Female	10/4
Underlying disease	
Diagnosis	
Acute leukemia, Acute myeloid leukemia/Acute lymphoblastic leukemia	5/4
Others	4
Status at the moment of HSCT	
Remission/Relapse or without remission	5/9
Transplant characteristics	
Hematopoietic stem cells (HSC) source	
Bone marrow (BM)	5
Peripheral blood (PBSC)	9
Type of donor	
Unrelated matched/ Unrelated mismatched	7/1
Family matched/ Haploidentical	3/3
Conditioning regimen (CR)	
Myeloablative (MAC)	3
Reduced intensity (RIC)	11
Secondary alloHSCT	2
Antifungal prophylaxis	
Primary – fluconazole/voriconazole	7/2
Secondary – voriconazole/pozaconazole	3/2
Mucormycosis characteristics	
Clinical forms (isolated and/or in combination)	
Pulmonary	11
Rhinocerebral	1
Subcutaneous/osteomyelitis	1
Bowel	1
Treatment of mucormycosis	
Combination antifungals base on Amphotericin B/lipid forms	3/3
Caspofungin	2
Pozaconazole	4
Monotherapy	8
Pozaconazole	2
Lipid complex amphotericin B	1
Amphotericin B deoxycholate	2
Caspofungin	1
Voriconazole	2

Table 2

Factors which improved overall survival after diagnosis of Mucormycosis following allogeneic HSCT		
Factors associated with characteristics of …	factors	p Log-Rank
univariate	12 weeks OS	
Patient	childhood	0.04
	remission of underlying disease at the moment of HSCT	0.04
Transplant	haplo-HSCT	0.008
	busulfan base conditioning regimen	0.04
Therapy	ampho B in first line therapy	0.03
	combination antifungal therapy	0.008
	control of underlying disease	0.01
	6 months OS	
Transplant	haplo-HSCT	0.03
	busulfan base conditioning regimen	0.04
Therapy	combination antifungal therapy	0.009
	control of underlying disease	0.01
Cox regression multivariate	12 weeks OS	
	childhood	0.01
	haplo-HSCT	<0.001
	combination antifungal therapy	<0.001
	6 months OS	
	haplo-HSCT	<0.001
	combination antifungal therapy	<0.001

We established positive prognostic factors of 12-weeks, 6-months and 1-year OS after diagnosis of M (Table 2). Predictors of death ($p < 0.05$) were invasive fungal disease (IFD) before allo-HSCT and hemoptysis.

Conclusions We reveal mucormycosis in 2,4% alloHSCT recipients. *Rhizopus* spp. (75%) was main etiological agents. Main clinical form of M was pulmonary (79%). In 50% cases M were diagnosed with IA. In allo-HSCT recipients with M the 12-weeks and 6-months OS was 31%. Positive prognostic factors were age <18 y.o. and combination antifungal therapy, negative – IFD before the allo-HSCT and hemoptysis.

P185

Invasive candidiasis in hematopoietic stem cell transplant recipients in Saint Petersburg, Russia

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Background Invasive candidiasis (IC) has remained an important cause of morbidity and mortality in allogeneic (allo-HSCT) and autologous (auto-HSCT) hematopoietic stem cell transplant recipients. This study focuses on incidence, etiology and outcome of IC in HSCT recipients.

Methods 16 pts with IC after allo- and auto-HSCT were included in 2008–2012 yy (1056 HSCT). Baseline patient characteristics and treatment are outlined in Table 1. EORTC/MSG 2008 diagnostic criteria of proven invasive fungal disease (IFD) were used.

Results Incidence of IC in allo-HSCT was 1,6% (11/685): adult - 1,9% (8/423), children - 1,1% (3/262). Incidence of IC in auto-HSCT was 1,35% (5/371): adult - 0,3% (1/276), children - 4,2% (4/95). There were 26 samples of *Candida* spp. blood culture in 16 pts with predominantly acute leukemia in allo-HSCT group and solid tumor in auto-HSCT group. Median date of IC onset after allo-HSCT was

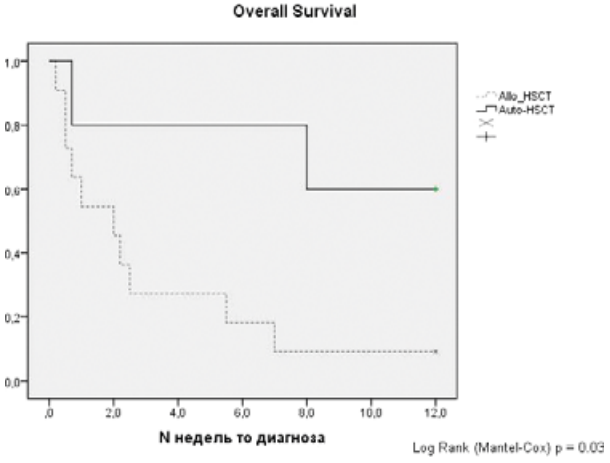

Figure 1

D + 114 (12–236). Median date of IC onset after auto-HSCT was D + 12 (8–33). In 100% of cases diagnosis was confirmed by blood or central venous catheter (CVC) culture. Blood cultures were received from following sites: blood from CVC 46%, blood 35%, removed CVC 19%. Etiologic agents of IC in allo-HSCT were *C.krusei* (28%), *C.parapsilosis* (18%), *C.albicans* (18%), and *C.intermedia* (9%), *C.glabrata* (9%), *C.rugosa* (9%), *C.sake* (9%). Etiologic agents of IC in auto-HSCT were *C.parapsilosis* (40%), *C.albicans* (40%), and *C.tropicalis* (20%). In 37,5% cases IC was diagnosed after or with invasive aspergillosis (IA). 30-days overall survival (OS) after diagnosis of IC was 50%, 12-week OS - 25%. Outcome of IC at 12-weeks in auto-HSCT group was better than in allo-HSCT group (60% vs 9%, Picture 1). Positive prognostic factors of 30-days and 12-weeks OS after diagnosis of IC were CVC removing (at 30-days: 78% vs 14%, at 12-weeks: 44,4% vs 0%) and engraftment (at 12-week: 36,4% vs 0%). Predictor of death was hepatic failure ($p = 0.03$).

Table 1

Baseline patient characteristics with invasive candidiasis (IC)	(n = 16)
Variable	
Demographic characteristics	
Age, years – Median, range	21,5 (1-59)
Sex – Male/Female	6/10
Underlying disease	
Diagnosis	
Acute leukemia	10
Lymphoma	3
Solid tumors	3
Status at the moment of HSCT	
Remission/Relapse or without remission	5/11
Transplant characteristics	
Hematopoietic stem cells (HSC) source	
Bone marrow (BM)	7
Peripheral blood (PBSC)	9
Autologous HSCT	5
Type of donor	
Unrelated matched/ Unrelated mismatched	6/0
Family matched/ Haploidentical	3/2
Conditioning regimen (CR)	
Myeloablative (MAC)	12
Reduced intensity (RIC)	4
Secondary alloHSCT	2
Antifungal prophylaxis	
Primary – fluconazole/voriconazole	7/4
Secondary – voriconazole/caspofungin	2/1
No prophylaxis	2
Treatment of IC	
Empiric / preemptive	13
Echinocandins	10
Amphotericin B, lipid forms	3
Target	16
Echinocandins	16
Removing central venous catheter	9

Conclusions We reveal invasive candidiasis in 4,2% auto-HSCT pediatric recipients, which significant higher than other HSCT groups. Median date of onset after auto-HSCT was significantly earlier than after allo-HSCT. Etiology of invasive candidiasis after allo-HSCT was wider and *C.krusei* was the main etiological agents. In 37,5% cases invasive candidiasis were diagnosed with or after invasive aspergillosis. At 30-days after diagnosis of invasive candidiasis OS was 50%, at 12-week OS - 25%. Outcome of invasive candidiasis in auto-HSCT group was better than in allo-HSCT. Predictor of death was hepatic failure. CVC removing significant improved OS in pts with invasive candidiasis after HSCT.

P186

Using of 'Pastorex Crypto Plus' test in complex diagnosis of cryptococcal meningitis in HIV-infected patients in Saint Petersburg

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Cryptococcosis is a severe fungal disease in HIV-infected patients involving brain and other organs. Laboratory diagnosis of cryptococcosis is based on detection of the etiologic agent at microscopy and culture from cerebrospinal fluid (CSF), blood and other clinical samples. Also detection of cryptococcal antigen in clinical materials by means of test "Pastorex *Crypto* Plus"is widely used.

Objectives The aim of the study: to evaluate efficacy of test "Pastorex *Crypto* Plus"in diagnosis of cryptococcosis in HIV-infected patients.

Methods In 2011–2012 yy. 84 samples (42 - CSF, 42 - serum) from 21 HIV-infected patients with cryptococcal meningitis hospitalized at 2 clinics in Saint Petersburg were studied. Control group included patients without cryptococcosis (n = 48). CSF samples were tested by "Pastorex *Crypto* Plus"and examined by microscopy and culture. Also *Cryptococcus* antigen was tested in serum. Results of "Pastorex *Crypto* Plus"test were considered as positive if antigen was detected in 2 consecutive samples from the same patient. Detection of cryptococci at microscopy was performed in Indian-ink preparations of CSF. Cultures were obtained on Sabouraud agar and identified by "Auxacolor2"test (BioRad). Sensitivity and specificity of tests was estimated.

Results *Cryptococcus* antigen was detected in 38 CSF samples and 34 serum samples of patients with cryptococcosis. Sensitivity of "Pastorex *Crypto* Plus"test was 90% for CSF and 80% for serum. Specificity in control group of patients was 95,8% and 97,5%, accordingly.

Mycological investigation of CSF was positive in 71,4% of patients. Positive results of "Pastorex *Crypto* Plus"test in CSF correlated with positive results of microscopy and culture in 60% and 40% of samples, correspondingly.

Conclusion

1 Determination of *Cryptococcus* antigen in CSF and sera is an obligatory method of cryptococcosis diagnosis in HIV-infected patients.
2 Sensitivity and specificity of "Pastorex *Crypto* Plus"test for diagnosis of cryptococcosis in HIV-infected patients varies according to the type of clinical sample (CSF, serum).
3 Laboratory diagnosis of cryptococcosis in HIV-infected patients should include complex of mycological and immunological methods of investigation.

P187

Adhesion and biofilm formation by *Candida albicans* isolates from vulvovaginal candidiasis in human immunodeficiency virus (HIV) positive women

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Objectives To asses the expression of two virulence factors, adhesion and biofilm formation, of *Candida albicans* isolates from HIV positive women in use of Highly active antiretroviral therapy (HAART) with different symptomatology.

Methods This study was conducted with 27 strains of *C. albicans*, one reference strain from the American Type Culture Collection (ATCC 90028) and 26 isolates obtained from HIV seropositive women with vulvovaginal candidiasis (VVC) in use of HAART, belonging to the archive collection from Medical Mycology Laboratory and Clinical Laboratory Citology of State University of Maringá, Maringá, Paraná, Brazil. The isolates were separated into groups according to the symptoms presented by the carrier, symptomatic or asymptomatic. For adhesion and biofilm were formed on 96 well plates immersed in Sabouraud Dextrose Broth. The quantification of adhesion and biofilm biomass were analyzed after 2 h and 24 h of incubation, respectively and the samples were quantified by crystal violet method (CV). All procedures were performed in triplicate and repeated in three separate assays. Statistical analysis was performed, involving mean and standard deviation (±SD). The Mann-Whitney test was used and $p \leq 0.05$ were considered statistically significant.

Results It is possible to observe that the yeast adhesion ability in symptomatic group was higher than asymptomatic group. Curiously, for biofilm formation and adhesion ability, between groups, was not found any statistical significance, p (>0.05).

Conclusion For the development of VVC predisposing factors related to the host are very important, especially immunosuppressive diseases such as HIV infection. In addition to host factors, virulence mechanisms may contribute to the transition from commensal *C. albicans* to infectious agent, confering ability to colonize host surfaces, to invade deeper host tissue or to evade host defenses. Adhesion to host surfaces and biofilm formation, are considered the first step to initiate *Candida* infection. Our findings showed that isolates from symptomatic groups developed greater adhesion capacity, however, others virulence factors have being studied to understand the infection process by *C. albicans*.

Table 1 Ability of adhesion and biofilm formation of Candida albicans isolates from seropositive patients with vulvovaginal candidiasis.

Adhesion		Biofilm	
Asymptomatic	Symptomatic	Asymptomatic	Symptomatic
0.46±0.18	0.53±0.21	0.44±0.24	0.42±0.16
p=0.75		p=0.29	

P188

Chronic headache in patients with AIDS, think meningitis by *Cryptocccus neformans*: a review from 2002 to 2005 in Western General Hospital

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Objectives Identify the cause of chronic headache in patients with AIDS in a public hospital in Guadalajara, Mexico. Specific objectives: relate the cause of chronic headache in patients with AIDS.
Methods Quantitative study, descriptive, observational, cross-sectional, retrospective.
Results We reviewed records of 18 patients diagnosed with AIDS, who entered the ER the Western General Hospital (HGO) from 2002 to 2005. The admission was a clinical suspicion of cryptococcal meningitis. Five female, thirteen male. The average age was 34 years. Fourteen of the patients who came to the ER, were receiving ART, and four were diagnosed with AIDS, to get to ER. Twelve patients reported moderate to severe headache, mild headache two patients and 4 patients reported no pain cephalic. Of the 14 patients who reported headache, 4 had: altered state of consciousness (somnolent, unconscious), 6 had confusion and the rest unchanged. For the duration of headaches are classified as chronic, subacute and acute Chronicle of 1 year acute evolution and 24 hours prior to admission. The location was: front in 4 patients, bilateral temporal 3; generalized five, one occipital, and parietal one.

The headaches are accompanied by vomiting in 6 patients, visual disturbances in 6 patients and seizures in 3. clinical findings: 38.8% presented: Babinski, Brudzinsky and / or stiff neck. The 88.8% of patients had no alterations fundus. Tendon reflexes normal in 77.7% of patients. At 100% of patients lumbar puncture was performed on admission to the emergency meeting the following: the appearance of CSF in 100% of patients were of normal appearance (clear / transparent). 83.3% had hypoglycorrhachia. India ink staining of CSF was positive in 17 (94.4%) patients. Cryptococcal antigen detection was performed in only three patients (16.6%) was found positive in 100% of cases.
Conclusions The clinical presentation of cryptococcal meningitis is an acute, sudden, accompanied by headache, vomiting, confusion, and other symptoms. however, in our sample, over 50% of patients reported headache have filed more than six months. In general, patients do not exhibit mental confusion, stupor or coma, although there were some exceptions. Amphotericin B treatment with a dose of 0.7 mg/kg/day for two weeks, and fluconazole 800 mg/day two weeks removed fungus. But as sequels three patients had seizures and three patients visual disturbances. We consider two important conclusions based on the immune status of the individual. On one hand, *Cryptococcus neoformans*, a capsule undeveloped due to lack of immune response, generates a bit severe inflammatory response, leading to headaches developed more than 6 months duration, the clinical symptom should meet in suspect of meningitis *C. neoformans*. And consequently, because the immune response is depressed, the meningeal inflammatory reaction can be poor and meningeal irritation manifests as chronic headache, which explains the styles oligosymptomatic.

P189

A. Fumigatus pulmonary aspergillosis in breeding donkeys

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Objectives In veterinary as in human medicine, *Aspergillus* spp. is often responsible for invasive fungal infections. In particular, *Aspergillus fumigatus* is the most common cause of pulmonary fungal infection (Hilton *et al*., 2009; Garcia-Vidal *et al*., 2013). Episodes of mortality caused by pulmonary aspergillosis are rarely observed in equine clinic (Carrasco *et al*., 1997; Johnson *et al*., 1999; Sweeney *et al*., 1999). Here we describe an outbreak of pulmonary aspergillosis by *A. fumigatus* in donkeys of an Umbrian farm (Central Italy).
Methods Five 21- to 45-day old Asinara donkeys, died without any apparent specific respiratory symptom. Depression was reported as the only clinical sign. Necropsy revealed the presence of whitish pulmonary nodules. Lung samples were therefore collected for bacteriological, virological, histological and mycological exams. For the mycological analysis, lung biopsies were seeded on Sabouraud Dextrose Agar (SDA) plates prior to a 25, 37 or50°C incubation. Colonies were macro- and microscopically identified and their susceptibility to antimycotics was tested.
Results Multifocal to coalescing whitish nodules (up to 3–4 cm in diameter) with a necrotic core and a peripheral hyperemic halo were observed in the basal lobes of both lungs. Lymph nodes were enlarged. Bacteriological and virological analysis results were negative. Histological observation revealed coagulative necrosis with a rim of degenerated neutrophils and multiple fungal hyphae embedded in the necrotic material. On SDA, velvety-powdery colonies, white at first then turning to greenish or grey developed after 4–5 days at 25°C or 48 hours at 37 and 50°C. The reverse side of the colonies was white to tan. Light microscopy examination revealed short (<300 μm) and smooth conidiophores harboring uniseriate phialides, often located only on the upper two-third of the vesicle, parallel to the conidiophore axis. These macro- and microscopic morphology features and the heat tolerance (growth at 50 °C) of the fungus allow us to identify it as *A. fumigatus* (Larone, 2002).

In vitro susceptibility to antimycotics showed a pattern identical to that of *A. fumigatus* of human origin, except for a higher sensitivity to itraconazole.
Conclusions *A. fumigatus* is today the most prevalent airborne fungal pathogen causing severe and usually fatal invasive infections in immunocompromised hosts. Despite the dramatic increase in the incidence of human invasive aspergillosis, the pathogenesis of *A. fumigatus* infection is still poorly understood (Latgè, 2001) and little is known on its pathogenic behavior in animals (Thierry *et al*., 2013). Furthermore the determinants for virulence identified so far in *A. fumigatus* are not unique to this species. In the scope of development of therapeutical strategies, it would be important to identify the pathological determinants and mechanisms specific of *A. fumigatus*, which make it a more opportunistic pathogen than other commonly encountered environmental molds.

P190

Triple mixed invasive candidiasis with fatal outcome – a case report

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Objectives Occurrence of mixed invasive fungal infections due to two different fungal species is relatively scarce, but not uncommonly described in the literature. A triple mixed invasive fungal infection has not been reported so far. Here we present such a case of triple mixed candidaemia at an 80 year old female patient with fatal outcome.

Case history A polymorbid 80 year female patient with history of cerebral apoplexia three years ago, metabolic syndrome and cardial insufficiency was admitted due to acute renal failure and circulation instability. Based on the significantly elevated liver parametes abdominal sonography was performed showing a liver tumor. Consecutively, abdominal CT-scan, PET-CT and endoscopic retrograde Cholangio-Pancreaticography (ERCP) were performed. Histology gained by ERCP revealed a low grade adenocarcinoma of the distal ductus choledochus (distal cholangio-carcinoma). For the treatment several central intravenous catheters were installed. Despite a broad-range antibiotic treatment the patient died due to a foudroyant septicaemia.

Results of further investigations Cultivation findings of bile secretions were two times negative for yeasts within four weeks, but revealed *Streptococcus salivarius*, *Corynebacterium spp.*, *Staphylococcus hominis* followed than by *Enterococcus faecium* - Vancomycin sensitive and *Staphylococcus haemolyticus* - Methicillin resistant. During the intravenous treatment with Meropenem 3 x 500 mg and Vancomycin 2 x 500 mg the patient developed *Clostridium difficile* associated diarrhea (CDAD) which was treated with Metronidazol 3x500 mg orally and Fidaxomicin 2 x 200 mg orally. Ten days later following this regimen the patient developed fever again. A peripheral gained blood culture revealed *Candida albicans*. A consecutive blood culture gained via a central venous catheter (Perm-cath-type) detected *C. albicans* again and additionally *C. glabrata* and *C. tropicalis* by one single blood culture.

All three isolates showed low MICs for the standard antifungals tested (Fluconazol, Voriconazol, Caspofungin, Amphotericn B, all below 1 mg/l) regarding to EUCAST criteria. Another two days later the patient died due to this multiple yeast sepsis. No antifungal treatment was initiated either as a preemptive - or as a targeted therapy.

Conclusions Mixed invasive fungal infections are rare clinical events fequently difficult to diagnose. We have detected and identified simultaneously even three different causing yeast pathogens in a single blood culture. Our case confirms that the presence of long term central venous catheter and prolonged antibiotic treatment represent a high risk to develop an invasive fungal disease in such critically ill patients. Furthermore the advanced age of this patient has also to be considered as an additional risk factor for invasive candidiasis. One should think about timely preemptive antifungal therapy with echinocandines according to the current guidelines for this patient group to overcome the potentially poor outcome of the patients.

P191

Invasive aspergillosis in patients with hematological malignancies in Slovakia: CELL fungal infection database (FIND) analysis, 2005–2011

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Objectives Evaluation of risk factors, diagnostic procedures and treatment outcomes of proven/probable episodes of invasive aspergillosis (IA) that occurred in patients with hematological malignancies.

Methods Retrospective analysis of data from 2005 to 2011 at 5 hematology centers in Slovakia.

Results There were 96 cases of IA identified. The majority of underlying hematological disease represented acute leukemia and myelodysplastic syndrome (44%), and induction or re-induction treatment (45%). The most frequent risk factor was neutropenia (57%). Chest HRCT performed in 85/96 patients at the time of diagnosis detected an abnormality in all episodes. Lungs were the most frequently affected site of infection (91%). The dominant findings were bilateral infiltrates (36%), signs more specific for IA were described less frequently.

Galactomannan (GM) detection in serum and bronchoalveolar lavage (BAL) were the major methods used in diagnosis assessment. Considering positive two consecutive serum samples with index of positivity >0.5, serum GM test was found positive in 73% of cases. GM test was positive in 31/96 (94%) of obtained BAL fluids (index of positivity >0.5).

Neutrophil count and antifungal prophylaxis did not affect the serum GM positivity rate, while the use of mold-active empirical antifungal therapy decreased the serum GM positivity rate. The BAL GM positivity rate was not influenced by prophylactic nor empirical antifungal treatment administration.

42% of the overall IA cases responded to initial antifungal therapy. There was only slight difference in response rate between voriconazole monotherapy and combination of voriconazole and echinocandin (47% vs 40%).

Conclusion Taking into account IA as an important cause of morbidity and mortality in hematological patients, the identification of diagnostic and treatment characteristics could improve the outcome in this group of patients.

P192

Disseminated histoplasmosis by *Histoplasma capsulatum* var *duboisii* in an HIV patient: first case reported in Spain

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Objective Hıstoplasmosıs ıs an endemıc mycose related to an ıncrease ın mortalıty ın HIV patıents, parttıcularly ın thoses lıvıng ın endemıc countrıes where dıagnostıc methods and antıretrovıral therapy are not avaılable. Nevertheless, reports of Afrıcan hıstoplasmosıs ın AIDS patıents are rare.

Results A 32 year-old woman from Equatorıal Guınea and resıdent ın Spaın sınce 2006 was admıtted to our hospıtal on 8th of March 2012 wıth fever of 40 C, chılls, dıarrhea and headache. Physıcal examınatıon only revealed a hard ınguınal lymphadenopathy of 3 cm ın her rıght sıde and smaller ones on the same sıde. Not toxıc habıts or any hıstory of ınterest except: cholecystectomy and appendectomy was referred. Sudy of stool (for parasıtes and enteropathogens), blood (for bacterıa, mycobacterıa, Plasmodıum and Leıshmanıa) and cerebrospınal fluıd (for bacteıa, mycobacterıa and Cryptococcus) were all negatıve.

Fıve days after the patıent worsened (pancytopenıa and ıncreased LDH). CT revealed multıple adenopathıes and a nodular dıffuse ınterstıtial lung pattern. HIV ınfectıon stadıum C3 and hepatıtıs A were confirmed on 9th March. On 15 th of March the patıent was submıtted to bronchoscopy. Bronchoalveolar aspırate, lavage and bronchıal bıopsy were used to bacterıa, mycobacterıa, vıruses and fungı cultures, Pneumocystıs and galactomannan detectıon. Cultures were only posıtıve for Hıstoplasma capsulatum. On 22th of March, bone marrow and the ınguınal adenopathy bıopsıes confirmed the presence of HC. Yeast wıth a sıze of 8-15 um ın dıameter compatıble wıth HC var dubosıı were observed ın both clınıcal samples.

The dıagnosıs of Hıstoplasma capsulatum var dubosıı was confirmed by a molecular approach. A RT PCR (Buıtrago et al., 2006) was performed ın a serum sample obtaınıng a posıtıve result. OLE gene amplıfıcatıon and the analysıs of the sequence, as descrıbed Kasuga et al (1999), revealed HC var dubosıı.It ıs noteworthy that ın the ınıcıal serum the galactomannan was posıtıve suggestıng false posıtive by dıssemınated hıstoplasmosıs and 1-3 BD glucan was negatıve. On 22th of March antıretrovıral treatment was stardted and on 27th antıfungal therapy (AmBısome and later swıtched to ıtraconazole)

Conclusıon Thıs ıs the fırst case reported ın Spaın of dısseminated hıstoplasmosıs by HC var dubosıı ın HIV patıent form afrıca. In nonendemıc areas, these mycoses are ıncreasıngly frequents. Clınıcal suspıcıon ın ımmunocompromısed patıents from these regıons could reduce the tıme to dıagnosıs and treatment, decreasıng the mortalıty associated to HC ınfectıons.

P193

Pneumocystis jirovecii pneumonia in solid organ transplant recipients in the Swiss Transplant Cohort Study

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Objectives To describe the epidemiologic characteristics of P. jirovecii (PCJ) infections in solid organ transplant (SOT) recipients in Switzerland.

Methods Retrospective observational study involving all patients included in the Swiss Transplant Cohort Study (STCS) from May 2008 to December 2011. Primary PCJ prophylaxis included trimethoprime-sulfamethoxazole, atovaquone, or aerosolized pentamidine. Infections risk factors for time to PCJ infection were analyzed with multi-state models with non-parametric hazards in the framework of the Cox model.

Results 20 patients among 1321 SOT recipients presented with PCJ infections in the following transplanted organs: 3/87 heart (3%), 3/246 (0.8%) liver, 13/806 kidney (1.6%), 1/60 (1.6%) pancreas. Deaths occurred in 3 patients, due to PCJ in 1. Microbiological diagnosis was obtained by bronchoalveolar lavage in 19 patients. PCJ was detected by fluorescence for 3 patients, immunofluorescence antibodies for 11 patients and PCR for 5 patients. 1 case was diagnosed by histopathology. 13 cases presented radiologically with ground glass opacities or lung infiltrates. All patients were receiving mycophenolate mofetil in association with either tacrolimus or cyclosporin. Median time from transplantation to infection was 290 days (range 67–664). 18 patients were not receiving anti-PCP prophylaxis at the time of infection. Twelve patients (60%) had cytomegalovirus (CMV) infections diagnosed before PCJ, 10 out of them in the 3 months before. Three patients presented CMV event at the time of PCJ infection. Rejection before PCJ occurred in 11 (55%) patients, in the 3 months before PCJ in 4 patients. Three patients presented with both, CMV event and rejection before PCJ infection. In multivariate analysis, risk factors for developing PCJ infection were diabetes, exposure to antibiotics, low creatinine clearance, prior occurrence of CMV event and prior occurrence of rejection.

Conclusion PCJ prophylaxis is highly protective and should be considered for SOT recipients in case of CMV infections or rejection episodes treated by increased immunosuppression.

P194

Voriconazole in the treatment of children with acute lymphoblastic leukemia and invasive aspergillosis: how long should we treat?

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Introduction Between Juli 2010 and December 2012, 8 pediatric patients with acute lymphoblastic leukemia (ALL) were diagnosed with invasive aspergillosis during induction therapy. Although the diagnostic criteria and treatment strategies for invasive aspergillosis are clear, data lack on how long the treatment should be continued or how the infection and response to therapy should be monitored.

Data 3/8 patients had proven, 4/8 probable and 1/8 possible disease. All patients were treated with Voriconazole, initially intravenously and afterwards orally. One patient underwent a lobectomy because a cavernous lesion.

We used imaging studies as the main tool to evaluate the response till disappearance of the lesions or in case of clinical signs. Voriconazol levels were measured weekly.

Findings Voriconazol dosage was aimed to obtain through levels between 2 an 6 mg/L but most patients had levels below the lower limit throughout the treatment, notwithstanding adjustment of the dose. The treatment was continued in all patients until the start of maintenance therapy. One patient suffered a relapse while treated with oral Voriconazole.

Conclusion We treated 8 pediatric patients with Voriconazole for invasive aspergillus. It was difficult to maintain good Voriconazole trough levels, but nevertheless only one patient suffered a relapse. No conclusions can be drawn concerning the duration of the treatment, but the fact that 1 patient relapsed indicates that the treatment should be continued at least for several months and careful monitoring should be continued, even after cessation of therapy.

P195

Probable/proven invasive aspergillosis in children with malignancy registered in database FIND, Study on behalf of the Czech Leukemia Study Group for life CELL

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Objectives Despite increasing spectrum of diagnostic tools and available antifungals invasive aspergillosis (IA) still remains serious life-threatening complication of intensive chemotherapy in children with malignancy. Here we present analysis of retrospectively obtained data in database FIND about diagnosis, therapy and outcome of children diagnosed with probable or proven IA.

Methods 32 children in the median age of 10 years (range 2–17) from four pediatric hemato/oncological centers with proven (12; 37.5%) or probable (20; 62.5%) IA were registered between 2002 and 2012. Underlying disease in 21 patients was acute leukemia (lymphoblastic in 18; 56%), 5 patients suffered from solid tumor and 6 from other hematological malignancy (MDS, NHL). 6 patients previously underwent allogeneic and 2 autologous stem cell transplantation. Diagnosis of proven/probable IA was defined according to EORTC/MSG criteria.

Results Pulmonary IA was the most frequent form (23; 72%) followed by disseminated form (4) or other (5). Among major risk factors for IA were: malignancy (100%), stem cell transplantation or other chemotherapy (100%), neutropenia (<0.5 x 10.9/l > 10 days) in 19 patients (59%), steroid therapy (>21 days) in 19 patients (59%) and graft versus host disease in 6 patients. Galactomannan was positive in blood in 8/27 (30%) and in BAL in 8/9 (89%) of patients tested. Cultures were positive for *Aspergillus* in 6/12 of patients with proven and 13/20 with probable IA with predominance of *A. fumigatus* (14/19; 74%) followed by *A. niger* (2), *A. terreus* (1) or other (3). Median time to diagnosis of IA from start of empirical therapy was 8 days. Empirical antifungal therapy (26/32; 81%) consisted of amphotericin B formulations in 43%, echinocandins in 23% and voriconazole in 15%. Targeted therapy was initiated in 30/32 patients (94%) and consisted of amphotericin B formulations (10 pts), voriconazole (5 pts), echinocandins (2 pts), echinocandins with voriconazole (5 pts) or other (8 pts). After 1st line therapy out of 30 treated patients 10 (33%) achieved complete/partial response,13 pts progressed, 4 remained stable, 3 cannot be assessed for response. At the end of entire treatment 22 patients (69%) achieved complete/partial response,7 pts progressed (22%) and 3 cannot be assessed for response (2/3 not treated). 14/32 patients died in median 1.1 months after the diagnosis of IA. Overall survival (OS) with median follow-up of 32 months in surviving patients is 54% with statistical difference (log rank test $p = 0.031$) between those with probable IA (OS 67%) or proven IA (OS 33%). 5/14 patients (36%) died in a direct consequence of IA, 4/14 patients died due to progression of malignancy, 4 died of other reasons but IA was considered as an important co-factor.

Conclusions IA is a life-threatening condition with high direct or indirect negative influence on survival of children treated for malignant disease. Only 33% of pediatric patients responded to first line therapy with 69% complete/partial final response. Proven IA significantly negatively influenced the overall survival.

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P196

Identification of *Candida fabianii* as the new emerging opportunistic pathogen in infants in Croatia

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Objectives Infections caused by rare low virulent *Candida* species have become increasingly common in high risk infants within hospital settings. The use of widespread commercially available biochemical systems for routine yeast identification, including API-32C, may lead to misidentification of uncommon *Candida* species, like *C. fabianii*. According to several published reports this pathogen can be *in vitro* resistant to azoles and/or amphotericin B. Therefore, the main objective of this study was to confirm species identification of 57 *Candida utilis* isolates (55 clinical and 2 environmental), collected over a five-year period from 9 children (8 newborns and 1 child) with candidaemia, urinary tract infections and/or colonization and from neonatal intensive care unit (NICU) during hospitalization of infected newborns. Additionally, we investigated the susceptibility profiles of these isolates to amphotericin B, flucytosine, fluconazole, itraconazole, and voriconazole.

Methods Samples were taken in accordance with clinical indication starting from January 2008 and lasting until December 2012. The isolates originated from various clinical specimens (19 from blood culture, 26 from urine samples, 5 from tracheal aspirates, 3 from stool samples, 1 from gastric lavage, and 1 from groin skin, respectively) and from air samples (2 isolates) obtained from NICU of the Children's Hospital Zagreb. The isolates were submitted to the Reference Centre for Systemic Mycoses (RCSM) at the Croatian Institute of Public Health in Zagreb for identification and *in vitro* antifungal susceptibility testing. All isolates were initially identified by API ID 32C kit (bioMérieux, Marcy l'Etoile, France) as *C. utilis*. *In vitro* antifungal susceptibility testing was performed by the ATB FUNGUS 3 (bioMérieux, Marcy l'Etoile, France) microdilution method and results were interpreted according to EUCAST recommendations.

All isolates stored at the Culture Collection of RCSM were retrospectively molecularly identified by the Department of Microbiology,

Faculty of Science and Informatics, University of Szeged by means of PCR-amplification (using universal primers ITS1 and ITS4), sequencing and sequence analysis of a fragment ribosomal DNA region.
Results Based on the sequence data, all isolates were found to belong to the species *Candida fabianii* (teleomorph: *Cyberlindnera fabianii*). The examined sequences of these isolates were shown to be identical, no variability was observed between them independently of their source and study period. No *in vitro* resistance to amphotericin B, flucytosine, fluconazole, itraconazole, and voriconazole was observed.
Conclusion Identification of rare *Candida* species presents a major challenge for mycology laboratories. Commercial biochemical method used in routine practice failed to provide the correct identification of *C. fabianii*, emphasizing the need for molecular-based assay for unequivocal species identification. In the light of these data, our results suggest that *C. fabianii* could be regarded as the new opportunistic neonatal pathogen in Croatia.

P197

Candida albicans in a chronic mucocutaneous candidiasis patient under continuous antimycotic treatment

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Objectives Chronic mucocutaneous candidiasis (CMC) is a recurrent or persistent infection of mucosal tissues, skin and nails, caused by *Candida albicans* and to a lesser extent non-albicans species of *Candida*. The disease affects usually people with primary immunodeficiencies. The most frequent treatment used is fluconazole, but clinical isolates of *C. albicans* are becoming increasingly resistant to this and other antifungals. We characterised *C. albicans* isolates from a child with CMC who is under continuous treatment with fluconazole by molecular, microbiological and immunological methods.
Methods Samples were taken sequentially at different time points from one patient. The interval between the swabs ranged from 1–3 months. The isolates were then characterised using three microsatellite markers (*CDC3*, *EF3*, *HIS3*) amplified by multiplex PCR. Fragment analysis of these three loci leads to a discriminatory power of 0.97. We also used a neutrophil killing assay to test the resistance of the different isolates to fungicidal activity of neutrophils. Finally, the susceptibility to fluconazole and anidulafungin were carried out to detect differences among the strains. The data were then compared with those obtained from isolates derived from other CMC patients and healthy volunteers.
Results The *C. albicans* populations harboured by the studied CMC patient are clonal. The *CDC3* locus, downstream of cell division cycle protein, showed in all isolates a 112:128 genotype. The *HIS3* locus, downstream of the coding sequence for the imidazole glycerolphosphate dehydratase, was also stable with a genotype of 146:158. In the second isolated population an allelic length difference in the *EF3* locus appeared. The 128:136 genotype changed to 128:128 and remained stable until now. The repeated units present in the alleles reduced from 32:34 to 32:32. Drug resistance and neutrophil killing data follow isolate-specific patterns.
Conclusion Microsatellite markers are a good tool to follow genetic modifications of clinical isolates over time. The *C. albicans* populations of the patient under investigation were clonal, but with a process of microevolution in the *EF3* locus. This microevolutionary event due to a single mutational step, and possibly related to the continuous fluconazole treatment, might provide a selective advantage to the pathogen. Differences in the survival rates in the neutrophil killing assay and in resistance to fluconazole and anidulafungin may provide evidence of the importance of the pathogen genotype in addition to the host's immunological status.

P198

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P199

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P200

Bloodstream infection by *Malassezia* and *Candida* species in critical care patients

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Objectives The diagnosis of bloodstream infections (BSI) by *Candida* spp. and *Malassezia* spp. (Miceli et al, Lancet Infect Dis 2011) increased also due to the growing number of immunocompromised patients. However, whilst fungemia by *Candida* species has been recognized as cause of morbidity and mortality in hospitalized patients worldwide (Pfaller et al., Diagn Microbiol Infect Dis 2012), the epidemiology of *Malassezia* spp. fungemia remains largely unknown. In this study we report the results of a one-year survey of BSI by *Malassezia* and *Candida* species in the Neonatal Intensive Care Unit (NICU) and in the surgical pediatric ward of a large hospital of the Apulia (Italy).
Methods From July 2011 to July 2012, 290 neonates or preterm infants and 17 pediatric patients with complicated post surgery features, were enrolled. After the onset of fungemia, clinical data (i.e., age, gender, underlying diseases, antifungal prophylaxis and therapy, day of catheter removal and fungemia duration) were collected in individual anamnestic files, along with skin swabs. Biological specimens and swabs were subjected to culture on Sabouraud Destrose Agar with 0.5% chloramphenicol and Dixon Agar and incubated at 37°C for 5 days and at 32°C for 10 days, respectively. Following preliminary yeast identification using the automated system Vitek2 (Biomerieux, France), *Malassezia* spp. was also characterized based on morphological, biochemical and molecular features (Cafarchia et al., Mol Cell Probes 2011)
Results A total of 12 fungemia episodes were registered: 10 in NICU, of which 6 (2.1%) caused by *M. furfur* and 4 by *Candida* spp. (1.4%; i.e., 2 *C. parapsilosis*, 1 *C. albicans*, and 1 *C. glabrata*) and 2 (11.8%) by *M. furfur* in pediatric ward. All patients received total lipid parenteral nutrition (mean = 38 days, from 24 to 60 days) via a central venous catheter (CVC). Seven patients were preterms (4 with *M. furfur* and 3 with *Candida* spp. fungemia) and five of them (3 with *M. furfur* and 2 with *Candida* spp. fungemia) received systemic antifungal prophylactic treatment with fluconazole (3 mg/Kg/72 h). Only patients with *M. furfur* BSI were yeasts skin colonized. Fungemia by *M. furfur* and *Candida* spp. occurred after an average of 26 days and 42 days of hospitalization, respectively. After the onset of fungemia all patients were treated with intravenous liposomal Amphotericin B at dosage 5 mg/Kg/day and resolved *Candida* spp. and *M. furfur* BSI after 6 and 16 days, respectively.
Conclusion Although neither clinical features nor predisposing factors (i.e., CVC, parenteral lipid nutrition, preterm birth, extended

length of hospitalization) allow to discriminate *M. furfur* from *Candida* spp. BSI, skin colonization seems to be a discriminating factor for *M. furfur* fungemia. Since in *Candida* and *Malassezia* fungemia the clinical features, strategies of patient management and outcomes do not differ, a more accurate etiological diagnosis is needed in high-risk patients by adding lipid supplemented culture media for *Malassezia* in the current mycological routine.

P210

Paracoccidioidomycotic granulomas developed in oral lesions of patients show similar patterns to those developed by experimentallly infected mice

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Introduction Severe clinical paracoccidioidomycosis (PCM) forms are characterized by the presence of numerous disseminated granulomas (Gr), in contrast with mild forms that have few compact Gr. Formation of G can be interpreted as a host defense mechanism to destroy or contain *P. brasiliensis* (Pb) by immune mechanisms. Our objectives were to characterize the oral lesions observed in PCM patients and compare these data with those previously described in the experimental murine model of PCM.
Material and methods For the studies using patients, the demographical data of patients were retrieved from anatomopathological files. Microscopical analysis was done in 38 cases of HE and Grocott stained slides. The architecture of the Gr, the presence of morphologically preserved or destroyed fungi, the composition of the cellular infiltrate and the pattern of deposition collagen fibers was compared to those previously observed in lesions of susceptible and resistant mice infected with Pb, after similar preparation of the material.
Results The best indicators of control of experimental PCM were the presence of compact Gr, containing few Pb with altered morphology, delimited by continuous deposit of collagen arranged in concentric orientation, and of multinucleated giant cells (MGC) containing Pb. In patients the most frequent sites of lesions were gengiva, hard and soft palate, and oral mucosa. Microscopy revealed predominance of intense, diffuse inflammatory infiltrate, mainly constituted by lymphocytes and plasmocytes. In most cases, no compact Gr were found, while MGC of the Langhans type were observed in 68% of cases. Pb were frequently found dispersed in connective tissue and inside MGC. A patient showed clinical symptoms suggestive of PCM, which was confirmed by microscopy following biopsy. An intense diffuse mononuclear infiltrate was observed at the lamina propria, with formation of compact Gr and presence of MGC containing Pb. The overall aspect of his lesions was similar to that observed in resistant mice, in contrast to the disseminated Gr of susceptible mice. He was treated for 6 months with 200 mg per day Itraconazol, resulting in complete cicatrization of the lesions and is nowadays completely asymptomatic.
Conclusion We can conclude that few Gr may indicate depression of cellular immunity in some patients and presence of compact Gr suggest control of the infection by local immune mechanisms in both, experimental animals and patients.

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P211

The immunomodulatory role of neutrophils and immune response to opportunistic fungi during neutropenia

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Objectives Invasive fungal infection (IFI) is a major cause of mortality and morbidity in immunocompromised patients, of which *Aspergillus* and *Candida* are the most common fungi. The recognition of neutropenia as a risk factor for development of IFI underlies the role which neutrophils play in mediating the host immune response to opportunistic pathogens. Acute leukemia patients undergoing induction chemotherapy are at one of the highest risks in developing IFI. To date, the immune status during febrile neutropenia remains not well characterized. We aim to study the mechanism through which neutrophils modulate response to fungal pathogens and the changes in the net immune response through the state of neutropenia.
Methods From healthy volunteers, peripheral blood mononuclear cells (PBMC) were separated by Ficoll density gradient centrifugation, after which polymorphonuclear neutrophils (PMN) were isolated from the erythrocyte fraction by hypotonic lysis. PBMC and PMN were plated in 24-well Transwell plates at final concentration of 1 x 10^6/ml or 2 x 10^6/ml respectively in total volume of 1 ml in RPMI culture medium. Stimulation was performed with *Candida albicans* 1x10^6/ml and *Aspergillus fumigatus* 1 x 10^6/ml. After 24 h and 48 h of incubation, supernatant were extracted from which individual cytokines were measured using ELISA.

Patients undergoing induction chemotherapy for acute myeloid leukemia were recruited for the study with informed consent. Serial blood specimens were obtained prior treatment, and at Weeks 1, 2, 3 and 4 post chemotherapy. Using whole blood, stimulations were performed using *A. fumigatus*, *C. albicans*, Toll like receptor (TLR) 4 ligand *Escherichia coli* lipopolysaccharide and TLR2 ligand Pam3Cys. At 24 h and 48 h, cytokine production was measured by ELISA.
Results Polymorphonuclear neutrophils by themselves did not induce much cytokine response to *Candida* or *Aspergillus*. When co-incubated with PBMC, PMN attenuated the production of tumour necrosis factor alpha (TNFa) and interleukin 1 beta (IL-1b) to the fungi. The use of the Transwell to effect separation between PBMC and PMN partly reversed the attenuation on cytokine response, suggesting that the phenomenon was dependent on secreted product as well as PMN-PBMC contact. The effect could be replicated through the addition of neutrophil myeloeroxidase to PBMC and *Candida*, but not with arginase-1.

In patients undergoing induction chemotherapy, an enhanced TNFa and IL-1b cytokine response to *Candida* and *Aspergillus* at Week 2 and Week 3 post commencement of chemotherapy. This coincided with the periods of neutropenia.
Conclusions Neutrophils play an important role in attenuating the immune response to fungal pathogens, mediated partly through secreted myeloperoxidase and cellular contact. During febrile neutropenia, the absence of neutrophils results in an accentuated cytokine milieu which may be implicated in the development of IFI.

P212

The effects of *Candida Albicans* cell wall protein fraction on dendritic cell maturation

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Objective *Candida albicans* is a member of the normal human microflora. *C. Albicans* cell wall is composed of several protein and carbohydrate components which have been shown to play a crucial role

in *C. albicans* interaction with the host immune system. Major components of *C. albican* cell wall are carbohydrates such as mannans, β glucans and chitins, and proteins that partially modulate the host immune responses. Dendritic cells (DC), as the most important antigen-presenting cells of the immune system, play a critical role in inducing immune responses against different pathogens. We investigated the effect of the cell wall protein fraction (CPF) of *C. Albicans* on DC maturation.

Methods The CPF of *C. albicans* cells was extracted by a lysis buffer containing sodium dodecyl sulphate, 2-mercaptoethanol and phosphate-buffered saline. The extract was dialyzed and its protein pattern was evaluated by electrophoresis. Dendritic cells were purified from Balb/c mice spleens through a three-step method including mononuclear cell separation, as well as 2-h and overnight cultures. The purified CPF was added at different concentrations to DC. The purity and maturation status of DC were determined by flow cytometry using monoclonal antibodies against CD11c, MHC-II, CD40 and CD86.

Results Treatment of DC with 10 μg/ml of CPF increased the expression of maturation markers including MHC-II, CD86 and CD40 on DC compared to the control group.

Conclusion In this study we used *C. albicans* CPF with the molecular weight of 40–45 kDa for pulsing and maturation of dendritic cells. Since according to our results CPF significantly increased the expression of maturation markers on DC, we suggest that CPF may act as an efficient immunomodulator, or may be used as a potential adjuvant to boost the host immune system against infections.

P213

The Candida albicans quorum sensing molecule is a modulator of innate immune cell function

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Objectives Farnesol is the first quorum sensing molecule discovered in an eukaryote and produced by the polymorphic fungus *Candida albicans*. The ability of the fungus to cause disease is linked to the morphological switch from yeast to filamentous form. Farnesol as a blocking molecule of this transition is well studied but its role in interaction with cells of the innate immune system has been only partially elucidated. The purpose of this study is to determine if innate immune cells are affected by farnesol.

Methods We used human primary monocytes isolated from buffy coats of healthy donors. Next to primary monocytes we examined monocyte-derived immature dendritic cells (DCs) generated over 6 days in the presence of GM-CSF and IL-4. Final maturation was induced by stimulation with LPS for 24 h. With an *in vitro* apoptosis assay using Annexin and PI staining we determined the cytotoxicity of farnesol on primary monocytes and DCs. The immunophenotype of monocytes and dendritic cells was defined by differential FACS analyses. Cell spreading was performed on fibronectin coated plates and analysed via microscopy. To determine the APC function of farnesol treated mature DCs we measured CFSE fluorescence in proliferating T cells by flow cytometry.

Results For physiological concentrations up to 100 μM, farnesol had no effect on viability of either primary monocytes or dendritic cells. Analysis of the surface phenotype of farnesol-treated primary monocytes showed an enhanced expression of the activation markers HLA-DR and CD86 whereas immature dendritic cells showed no significant change in surface marker expressions after farnesol treatment. Instead, the immunophenotype during the maturation process from monocytes to DCs is significantly affected by farnesol treatment. In presence of farnesol, CD1a, a marker for immature and mature DCs, is not expressed on the cell surface during maturation. Furthermore the maturation marker CD83 and the costimulatory molecule CD80 were reduced. Accordingly, farnesol impairs the ability of dendritic cells to act as a professional antigen-presenting cell. Farnesol treated mature DCs showed an inhibition in stimulating T cell-proliferation. In addition we could observe that these cells had an attenuated cell spreading on fibronectin coated plates.

Conclusions So far the experimental results suggest that farnesol modulates monocytes and dendritic cells as a part of the innate immune system. The most prominent effect was seen on the maturation process from monocytes to DCs. In further experiments we try to elucidate how farnesol impairs the ability of dendritic cells to act as a professional antigen-presenting cell.

P214

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P215

A virtual infection model enables quantification of innate effector mechanisms and fungal immune escape in human blood

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Objectives *Candida albicans* is the most important fungal pathogen in nosocomial bloodstream infections. So far little is known about the interplay of different cellular and non-cellular immune mechanisms for mounting a protective response against *C. albicans* in blood. Whereas many parameters are accessible to direct experimental quantification in whole blood infection models, others are not due to experimental limitations. To overcome these limitations we generated a virtual infection model that allows detailed predictions on the dynamics of host-pathogen interaction in human blood.

Methods Fungal cells were added to lepirudin-anticoagulated human whole blood to analyze the innate immune response against *C. albicans* in a situation similar to *in vivo*. Initial analyses included phagocytosis of *C. albicans*, fungal killing as well as activation patterns on immune cell populations and humoral marker quantification. To simulate the obtained time-resolved data we used a state-based modeling approach to set up a virtual infection model that determined the *a priori* unknown transition rates using the Monte Carlo method of simulated annealing.

Results Experimental results showed a predominant role of neutrophils in the early immune response against *C. albicans*. These phagocytes effectively take up viable *Candida* cells to kill them intracellularly and release antifungal effector molecules upon activation that may result in extracellular killing of the fungus. Quantification of respective transition rates in the model revealed that both mechanisms together account for 97.5% of *C. albicans* killing, underlying the central importance of neutrophils. In addition, we used the model to analyze the average contribution of single PMN to elimination of *C. albicans* and found that PMN contained predominantly one *C. albicans* cell (82.9% after 180 min). This prediction could be experimentally verified by manually counting *C. albicans* cells per PMN in blood smears with quantitatively comparable results. However, a fraction of *C. albicans* cells escaped phagocytosis and remained extracellular. This immune escape was independent of filamentation and fungal viability as we detected identical amounts of extracellular fungal cells for a non-filamentous *Δcph1/Δefg1* mutant or thimerosal killed *C. albicans* cells. Furthermore, immune escape was not linked to exhaustion or inactivation of innate immune cells.

Conclusion The human whole blood infection model allows the time-resolved analysis of innate immune activation after inoculation of *C. albicans*. Simulation of experimental data by a state-based modeling approach enabled us to make detailed predictions on the time-dependent distribution of *C. albicans* in immune cells and to quantify

with high accuracy transition rates that are experimentally not directly accessible.

P220

Clinical spectrum of *Exophiala* infections and a novel *Exophiala* species, *Exophiala hongkongensis*

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Background *Exophiala* species are saprophytic fungi which have been isolated from environments rich in hydrocarbons or from hot, humid, and oligotrophic environments. These fungi are considered as dematiaceous moulds; and due to their phenotypic characteristics at the beginning of colony formation, they are also often referred to as 'black yeasts', a misnomer which sometimes may mislead the choice of antifungal agents. When the cultures mature, brown hyphae are formed bearing conidiogenous cells referred to as annellides, a typical characteristic of this fungal genus. Although *Exophiala* species are environmental fungi, they should not be disregarded as contaminants when they are isolated from clinical specimens. These fungi are causative agents of skin and subcutaneous tissue infections and of systemic infections, such as prosthetic valve endocarditis, dialysis-associated peritonitis, and disseminated infections, especially in immunocompromised patients. Unfortunately, *Exophiala* species can often only be identified to the genus level by phenotypic characterisation.
Objectives The aims of this study were to study the clinical spectrum of *Exophiala* infections in Queen Mary Hospital, Hong Kong by a polyphasic approach, and to characterise a potentially novel *Exophiala* species, *Exophiala hongkongensis* (ex-type strain HKU32^T).
Methods All *Exophiala* strains characterised in this study were isolated from patients during a 15-year period (1998–2012) and were retrieved from the collection in the clinical microbiology laboratory at Queen Mary Hospital, Hong Kong. The strains were characterised phenotypically by microscopic examination of fungal structure using the agar block smear preparation method and phylogenetically using the internal transcribed spacer (ITS) region and *Rpb1* gene. In addition, a unique strain, HKU32^T, was further characterised phenotypically by scanning electron microscopy, enzyme activity test using the API-ZYM system, and growth tests on different temperatures and culture media. HKU32^T was also further phylogenetically characterised using β-tubulin and β-actin genes. All the phylogenetic analyses were performed by the maximum likelihood method using MEGA 5.0.5.
Results Microscopic examination of the young cultures of all the 12 strains showed subspherical, budding, yeast-like cells. Sequencing of the ITS region and partial *Rpb1* gene showed 11 of the 12 strains were known *Exophiala* species, including *E. oligosperma* [*n* = 3], *E. jeanselmei* [*n* = 2], *E. lecanii-corni* [*n* = 2], *E. bergeri* [*n* = 1], *E. cancerae* [*n* = 1], *E. dermatitidis* [*n* = 1], and *E. xenobiotica* [*n* = 1]). As for HKU32^T, it displayed unique morphological features and was positive for eight enzymes in the API-ZYM test. Optimal growth was observed at 30°C on potato dextrose agar or at 24°C on cornmeal agar. HKU32^T also occupied unique phylogenetic positions in all the phylogenetic analyses, with *Exophiala nishimurae* being the most closely related species. Clinical spectrum of *Exophiala* infections in Hong Kong included chronic skin infection, colonisation of gastrointestinal tract, continuous ambulatory peritoneal dialysis (CAPD) peritonitis, onychomycosis, pneumonia, tinea pedis, and wrist or finger nodule.
Conclusion *Exophiala* species could cause a wide range of infections and the most frequent species isolated from patients in Hong Kong was *Exophiala oligosperma*. *Exophiala hongkongensis* sp. nov. is proposed to describe the unique strain HKU32^T.

P221

Genome and transcriptome profiling of serial genetically-matched susceptible and FKS-resistant *C. glabrata* clinical isolates

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Background and Objectives *Candida glabrata* is an important opportunistic pathogen and is the second most common cause of candidiasis after *C. albicans* in North America. Echinocandin antifungal drugs are first-line therapy for *C. glabrata* infections but drug resistance is a clinical confounding factor that is on the rise. Reduced susceptibility to echinocandins has been associated with amino acid substitutions in highly conserved hot-spot regions of the Fksp subunits of glucan synthase. To better understand underlying cellular factors contributing to the emergence of echinocandin resistance in *C. glabrata,* we applied high-throughput sequencing technologies to sequence the genome and transcriptome of an echinocandin resistant clinical isolate harboring a point amino acid substitution in the *FKS2* gene (S663P) and its isogenic drug sensitive counterpart.
Methods *C. glabrata* genomic DNA was extracted from yeast cells of serial genetically-matched susceptible and *FKS*-resistant clinical isolates grown overnight in YPD broth with a Qbiogene FastDNA kit. Total RNA of exponentially growing cells was extracted using the RNeasy kit (Qiagen), with DNAse treatment. Whole fungal genome re-sequencing and RNA sequencing (RNA-seq) were carried out using the high-throughput Illumina HiSeq2000 sequencing technology by BGI Americas. Reference sequence *C_glabrata_CBS138* was used for bioinformatics analysis.
Results and Conclusions RNA-seq preliminary data revealed differential expression of 234 genes (175 genes were up-regulated and 59 down-regulated) including genes previously identified as involved in drug tolerance, genes not previously associated to a resistant phenotype, and novel transcripts whose function as a gene is uncharacterized. Preliminary studies indicate that genes for DNA repair are down-regulated, which may contribute to enhanced selection for resistance. These results provide new perspectives for our understanding of the genetic mechanisms that lead to the acquisition of drug resistance in *C. glabrata*, with potential for future improvements of therapeutic strategies.

P222

Screening of cryptic species among clinical *Aspergillus* isolates collected during one year period in a Portuguese reference laboratory

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Objectives Correct identification of *Aspergillus* species is important given that sibling species may show variable susceptibilities to multiple antifungal drugs and also because sharper definition of species may facilitate epidemiological studies. Thus, we screened *Aspergillus* clinical isolates from Portuguese hospitals to determine which, if any, of the cryptic species of *Aspergillus* were involved in patient infections.
Methods Over a one year period, *Aspergillus* isolates from Portuguese health institutions were collected. These isolates were identified on the basis of microscopic morphology and through the use of molecular tools. Genomic DNA was prepared from each isolate and the sequencing of the Internal Transcribed Spacers (ITS) regions, specifically the ITS1 and ITS2 non-coding regions flanking the 5.8S rDNA was used to determine the species complex, whereas β-tubulin

and calmodulin sequencing was done to achieve the correct species identification.
Results Over the study period, 57 *Aspergillus* isolates from clinical samples were collected from 10 Portuguese health institutions. According to the morphological observations, 29 isolates were identified as *Aspergillus fumigatus*, 11 *A. flavus*, 8 *A. niger*, 3 *A. nidulans*, 2 *A. terreus*, 2 *A. candidus* and 2 *Aspergillus* sp. Among those isolates, six species-complexes were detected by ITS sequencing, and were distributed as follows: *fumigati* (50.1%), *flavi* (21.0%), *nigri* (15.8%), *terrei* (5.3%), *nidulantes* (3.6%) and *versicolores* (3.6%). β-tubulin and calmodulin sequencing resulted in ten (17.5%) cryptic species being identified among the 57 isolates. Six of those isolates belonged to the *nigri* complex (*A. brasiliensis*, *A. awamorii* and *A. tubigensis*), two to the *versicolores* complex (*A. sidowii* and *A. fructus)*, one to the *fumigati* complex (*A. lentulus*) and one to the *nidulantes* complex (*Emmericella echinulata)*.
Conclusion With rigorous application of molecular tools, cryptic species of *Aspergillus* are not uncommon in the clinic. The identification of cryptic species among the collected clinical isolates of *Aspergillus* alerts the clinician to isolates with reduced susceptibilities to antifungal drugs and emphasizes a correct identification to species level.

P223

Identification of dermatophytes from the clinical samples of the skin clinics of the Tonekabon City through the real time ITS – PCR sequencing method

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Objectives Gaining access to a rapid and accurate laboratory technique to identify the dermatophytes in the level of genus and species is very important. Direct microscopic identification and culture of the components of the fungus from the clinical samples is a rapid identification method but lacks the specificity and sensitivity. In this research, we study the direct and rapid diagnosis of these fungi using the seminested Real-Time PCR Method in skin sample of the patient.
Methods In this study, 30 samples of crust from patients were collected and for identifications of Dermatophytes, seminested PCR was done. DNA extraction by phenol-chloroform method was done and for first step PCR, ITS 1-4 universal primer was used. First step PCR products for Realtime-PCR by ITS 86-4 universal primer were used. PCR products for identification of dermatophytes were sequenced.
Results The results for the 30 samples PCR-Sequensing fungus, *Trichophyton rubrum* and 8 as the dermatophyte and non-dermatophyte molds were identified as one *candida bombi*. The six colonies of dermatophytes: *Trichophyton rubrum*, *Trichophyton mentagrophytes* and *trichophyton verrucosum* was isolated by culture.
Conclusion This method be can an useful method in the quick identification of the pathogenic samples and this case is for the purpose of the fast treatment of the patients whit the proper drug and the accurate dose of drug and the appropriate period of the drug consumption.

P224

DNA-based quantification of mycotoxin producing fungi in French dwellings

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Introduction The toxic effects of the ingestion of mycotoxins are quite well known. What is less clear is the role of the inhalation of mycotoxins in water-damaged homes and their effects on human health. Because there are potentially hundreds of indoor mycotoxins, it is not practical to measure all of them and some selection process is needed.
Methods We utilized a DNA-based approach, quantitative PCR, to measure 36 common indoor fungi, including *Stachybotrys chartarum*, *Aspergillus ochraceus* and *A. versicolor*, in 40 homes in Brittany, France. Analysis of these 36 fungi resulted in the description of the fungal contamination by a metric called the Environmental Relative Moldiness Index (ERMI). The ERMI value for a home is derived from the mathematical treatment of the concentration of each of the 36 fungi to arrive at single number, the ERMI value.
Results The ERMI values in these homes ranged from -2.7 to 28.8 but there was a clear separation of the low-fungal contamination homes with ERMI values (<6) (n = 20) from the high ERMI value homes (>8) (n = 20). By comparing the populations of each mold in high versus low ERMI homes, we found, for example, that high ERMI homes had 79 cells of *S. chartarum* per mg of dust compared to 1 cell per mg dust for low ERMI value homes. *Aspergillus versicolor* occurred at 321 cells for every cell in low ERMI homes and *A. ochraceus* was found at a ratio of 6 to 1, high ERMI versus low ERMI homes.
Discussion A metric like the ERMI should provide a practical approach to describing the fungal contamination in epidemiological studies of possible mycotoxic effects from inhalation exposures. In addition, the QPCR analysis of the mycotoxin producing fungi is highly sensitive and specific and can help targeting the mycotoxins that might be quantified in epidemiological studies.

P225

Candida nivariensis – possible new pathogen, study on Clinical Hospital yeasts collection

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This work was supported by the National Science Centre (Grant no N N401 646140)
Objectives Two genetically new and closely related species, a potential source of opportunistic infections - *C. nivariensis* and *C. bracarensis* were identified within the *C. glabrata* complex. "Phenotypic switching"- a phenotypic dimorphism within the same strain discovered by Lachke et al - occurred in *Candida glabrata* similarly as in other yeast-like fungi. In *C. glabrata*, it manifests as the ability to produce varicoloured colonies on chromogenic agar media. Current studies on new species suggest that white colony phenotype is specific for *C. nivariensis*. The best known *C. glabrata* virulence factors are *YPS* family genes encoding aspartyl proteases. The purpose of the study was to establish the prevalence of new *Candida glabrata*-complex species: *Candida nivariensis* and *Candida bracarensis* isolated from clinical material, evaluate their phenotypes and the prevalence of

gene family encoding extracellular glycosylphosphatidylinositol-linked aspartyl proteases (YPS), crucial for *Candida glabrata* virulence.
Methods Study material included 224 *Candida glabrata* clinical strains. *Candida glabrata* phenotypes were identified using CHROMagar *Candida* medium. Afterwards strains were analyzed by using *Candida glabrata*-specific PCR with the CGL1 / CGL2 primers for the internal transcribed spacer (ITS) region to confirmed the identification. To identify *Candida nivariensis* and *Candida bracarensis* strains, the D1/D2 region of 26S rRNA was sequenced. The prevalence of YPS-family proteases genes were detected using standard PCR method.
Results *Candida nivariensis* amounted about 6% among the total number of *Candida glabrata* strains. No *Candida bracarensis* strain was identified. *Candida nivariensis* strains had a white phenotype (W) on chromogenic agar media and assimilated two sugars - trehalose and glucose in the ID 32C test. Among the 13 *Candida nivariensis* strains, 10 did not present any YPS-family protease genes. Coexistence of all detected YPS-family protease genes was specific for *Candida glabrata* species.
Conclusion
1 *C. nivariensis* strains were identified, but not *C. bracarensis.*
2 The analysed *C. nivariensis* strains presented only white phenotype.
3 Most of the analysed *C. nivariensis* strains did not possess any of the tested genes of the yapsin-family protease. This could indicate a lower virulence potential than in *C. glabrata.*

P226

A comparative study of DNA extraction methods in onychomycoses

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Objectives Due to the demanding and time consuming methodology for the diagnosis of onychomycoses, there is an attempt in the recent literature to provide clinical diagnosis and identification by the use of modern molecular techniques. The aim of the study was to compare three different protocols of DNA extraction in nail mycoses.
Methods Nail specimens were collected from 45 subjects, characterized as positive (31) or negative (14), according to the result of the nail culture. DNA extraction was performed on weighed nail clippings of equal size (approximately 4 mm) and further chopped, using three different protocols:

Protocol 1 (Pr1): DNA was extracted according to the nail-specific protocol of Tahir and Watson (1995), with a purification step by the Macherey-Nagel NucleoSpin® Blood Quick Pure extraction kit, after an enzymatic digestion with Proteinase K (Qiagen®).

Protocol 2 (Pr2): DNA was extracted according to the Denmark patent WO/2006/133701, proposed by the Statens Serum Institute, Copenhagen.

Protocol 3 (Pr3): DNA extraction was performed using the DNA IQ™ System, DC6701 (Promega), protocol for liquid samples. A paramagnetic resin was used to capture a consistent amount of DNA. The sample was homogenized by the addition of proteinase K (Qiagen®), acid-washed glass beads, 710–1,180 μm (Sigma-Aldrich) and ultra pure, nuclease-free water, strong vortexing (15 min) on a TurboMix™, Vortex-Genie® disrupting system, and incubation overnight at 56°C.

The evaluation parameters included: 1) DNA purity (ratio of OD260/OD280), 2) DNA concentration (μg/mL), 3) the ratio of total DNA yield/g of tissue, 4) method cost and 5) protocol duration. Data was expressed as mean±SD and analyzed using Kruskal-Wallis and Mann-Whitney tests.

Results Higher ratio was obtained by Pr3 (1.92 ± 0.49), followed by Pr1 (1.28 ± 0.21) and Pr2 (1.03 ± 0.21), $p < 0.001$.

In Pr2, DNA concentration (367.00 ± 211.07) was significantly higher, compared to Pr1 and Pr2 ($p < 0.001$), and the same was between Pr1 (110.83 ± 56.10) and Pr3 (70.57 ± 18.96) ($p < 0.001$).

DNA yield/g of tissue was also significantly higher in Pr2 (4040.58 ± 9087.12) compared to Pr1 (670.63 ± 1279.04) and Pr3 (145.68 ± 158.01) ($p < 0.001$). No significant difference was observed between Pr1 and Pr3 ($p = 0.065$).

Pr2 was the least expensive (0.10€/sample) or time consuming (1 h). For Pr1 and Pr3, both duration and cost were higher, 23 h and 7.54€ respectively for Pr1, 20 h&30 min and 13.57€ for Pr3.

Statistical results were the same, in general, when the comparisons were performed separately for positive or negative specimens.

In an effort to evaluate the total performance of the three methods, we calculated for each parameter (concentration, yield/g of tissue, ratio) the median, separately for each protocol in the general population. Subsequently, the value was expressed in multiples of the mean of medians for each parameter. The three values (one for each parameter) were added for each protocol and found that: Pr1 = 1.94 Pr2 = 5.34 Pr3 = 1.89, Pr2 > Pr1 > Pr3.
Conclusions Pr2 protocol showed high concentration and DNA yield/g of tissue. The duration and cost of this method were very advantageous too. However, DNA purity was low. Therefore, Pr3 and Pr1 (cheaper compared to Pr3) would be more appropriate for specific molecular techniques which demand higher DNA purity.

P227

Rapid identification of phenotypic similar *Candida* species from culture using high resolution melting analysis

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Objectives *Candida albicans* remains worldwide the most common fungal isolate from blood, but many studies have described an increasing trend in non-*albicans* infections. Correct identification of *Candida* species is crucial for providing adequate antifungal therapy and for epidemiological reasons. The aim of this study was to identify three groups of *Candida* sp., which are hardly distinguishable by phenotypic methods.
Methods We have developed real-time PCR followed by high resolution melting analysis (HRMA) for differentiation of phenotypic similar *Candida* species. The assay was performed with the following reference strains confirmed by sequencing: *C. albicans* (CCM8320) and *C. dubliniensis* (CCY29-177-1) (Group 1), *C. fabianii* (CCY38-20-1), *C. pelliculosa* (CCY29-6-7) and *C. utilis* (CCY29-38-74) (Group 2), *C. parapsilosis* (CCM8260), *C. orthopsilosis* (MUCL49939) and *C. metapsilosis* (MUCL46179) (Group 3). In addition, 73 clinical isolates at least 10 *Candida* isolates of each "*Candida* group"cultured from different clinical specimens were included in the study. Their HRMA outcomes were compared with biochemical results. Sequencing of clinical strains was done only in cases of discrepancy in species determination between biochemical tests and HRMA.
Results Real-time PCR with HRMA was performed with *Candida* reference strains in duplicates and revealed characteristic melting peaks for each species. Species could be identified by the shape of the melting curves and values of melting temperatures (Tm) and comparing them with reference list. Only Tm values for *C. orthopsilosis* and *C. metapsilosis* occurred repeatedly in the narrow range, therefore these 2 species could not be reliably distinguished. Some intra-species differences on HRMA were observed in clinical isolates of *C. albicans* and *C. metapsilosis* and were confirmed by sequencing, whereas other species revealed unified melting curves according to the reference panel.

Conclusion Real-time PCR followed by HRMA is a simple, rapid and inexpensive tool to identify quoted *Candida* species. It seems to be a suitable complement to current clinical diagnostic approach based on usage of commercially available biochemical kits where *C. fabianii*, *C. orthopsilosis* and *C. metapsilosis* are not involved.

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P228

Molecular characterization and identification of *Aspergillus* isolates collected in Saint Petersburg, Russia

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Objectives *Aspergillus* species are associated with a variety of invasive and cutaneous infection diseases, from which infections of the bronchopulmonary system, ear canals and sinuses are the most common forms. Species identification of *Aspergillus* may be important due to differences in antifungal drug susceptibility. Phenotype-based identification of *Aspergillus* allows recognizing isolates to species complex level. DNA sequence-based identification can demarcate species within the complexes and reveal cryptic species, that are not possible to identify using classical methods and frequency of which is not known at present time.

The objectives of this study was to characterize spectrum of *Aspergillus* species in clinical isolates collected in Saint Petersburg area (Russia) using morphological criteria and sequence analysis of fragments of rDNA and β-tubulin genes.

Methods Seventy one *Aspergillus* isolates were obtained from the Russian Collection of Pathogenic Fungi at Kashkin Research Institute of Medical Mycology in Saint Petersburg (Russia). 50 isolates were from *different clinical* specimens. 21 isolates were obtained from All Russian Collection of Microorganisms in Moscow (VKM) and Komarov Botanical Institute in Saint Petersburg. Isolates were identified both morphologically and by two-loci sequencing (ITS1-5.8S-ITS2 and β-tubulin). Sequences were compared with GenBank database. The identification to species level was concluded at 99-100% sequence identity. Phylogenetic analysis was done by MEGA 5.03 software.

Results Morphological analysis identified 68 isolates to species complex level and three isolates to genus level. 46 (64%) isolates were unambiguously identified to species level by ITS sequencing (all were *A. fumigatus* and *A. terreus* isolates), whereas 65 (92%) isolates were unambiguously identified to species level by β-tubulin sequencing. Sixth (8%) ambiguously identified isolates belonged to *A. niger* and *A. versicolor* species complexes. For precise species identification of these isolates phylogenetic analysis of their β-tubulin sequences and β-tubulin sequences of the type strains available in GenBank was performed. Two ambiguous "versicolor"isolates grouped together with *A. jensenii* on the neijbour-joining tree. Phylogenetic analysis of "niger"group revealed four isolates with an intermediate genotype between *A. awamorii* and *A. niger*.

Thirty isolates were obtained from patients with invasive aspergillosis of lung. The species associated with this disease were *A. fumigatus* (n = 14), *A. flavus* (n = 5), *A. tubingensis* (n = 3), *niger* (n = 3), *A. sydowii* (n = 2), *A. terreus* (n = 1), *A. amstelodami* (n = 1) and *A. calidoustus* (n = 1). Then isolates were obtained from patients with otomycosis. The species associated with this disease were *A. niger* (n = 3), *A. awamori* (n = 3), *A. flavus* (n = 2), *A.terreus* (n = 1) and *A. sydowii* (n = 1). Three isolates were obtained from patients with fungal sinusitis. The species associated with it were *A. flavus* (n = 2) and *A.sclerotiorum* (n = 1).

Conclusion In most cases, β-tubulin locus sequencing can adequately discriminate *Aspergillus* species, including cryptic species. We have revealed four isolates that genetically correspond to an intermediate species form between *A. awamorii* and *A. niger*. The most common species associated with invasive aspergillosis of lung was *A. fumigatus*, whereas with otomycosis - *A. niger* and *A.awamori*, with nasal aspergillosis - *A. flavus*.

P229

Abstract withdrawn

P230

PCR-test for diagnosis of onychomycosis

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Onychomycosis is a mycotic lesion of nails generally caused by dermatomycetes of *Trichophyton* genus. However, opportunistic micromycetes such as yeasts (*Candida* spp.) and filamentous nondermatomycetes (*Aspegillus* spp., *Fusarium* spp. and others) can play an important role in the occurrence of this disorder. Commonly used cultural and histological methods are time-consuming and insufficiently sensitive for fungi detection.

Objectives The objective of our work was to develop and evaluate a multiplex PCR-test for detection and identification of pathogenic fungi that commonly occur in patients with onychomycosis in Saint-Petersburg region and to evaluate the usefulness of the test for monitoring of therapeutic efficacy.

Methods The PCR-test was developed to identify pathogenic fungi belonging to four genera - *Trichophyton* (a representative of dermatomycetes) and *Aspergillus*, *Fusarium* and *Candida* (representatives of non-dermatomycetes) in nail samples. The test is a multiplex PCR with electrophoretic detection of PCR products. Evaluation of the clinical sensitivity and specificity of the PCR-test was carried out with reference to KOH-microscopy (which is a gold standard for today's laboratory diagnostics of onychomycosis), to cultural method and to both of them together.

Results By now our system has been tested on 63 nail samples from two groups of patients: 56 patients with diagnosis "onychomycosis"and 7 patients with diagnosis "onychodystrophy". Nail specimens were collected from patients of the mycological clinic of Kashkin Research Institute of Medical Mycology, Saint-Petersburg during the period from May 2012 to May 2013. According to the preliminary assessment the sensitivity of the PCR-test is 77%, 86% and 78% and the specificity is 70%, 52% and 76% with reference to KOH-microscopy, culture and both of them, respectively. All specimens with onychodystrophy were negative by the PCR-test. For three patients, monitoring of the efficacy of therapy was conducted. In one case, the therapy was changed according to the PCR result that led to improvement of the patient condition. In another case we observed the disappearance of the specific PCR signal during the course of the therapy. In the third case that was onychodystrophy the absence of PCR signal was in accordance with the clinical follow-up data.

Conclusion We have developed a multiplex PCR-test for determination and identification pathogenic fungi of four genera - *Trichophyton*, *Aspergillus*, *Fusarium* and *Candida*. The test identifies pathogenic fungi in nail specimens to genus level. In contrast to cultural analysis, which takes 14–28 days to complete, the PCR-test is completed in one day. Rather low specificity of the PCR-test may be connected to the fact that the specificity was calculated with reference to KOH-microscopy and culture analysis, whose own sensitivity is a subject of scientific discussion today. To better assess sensitivity and specificity of the PCR-test a larger number of patients (with clinical follow-up) needs to be evaluated.

P231

Is the extraction by Whatman FTA filter matrix technology and sequencing of large ribosomal subunit D1/D2 region sufficient for identification of clinical fungi?

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Objectives Conventional identification of pathogenic fungi based on the combination of tests evaluating their morphologic and chemical characteristics in clinical laboratories, they are time consuming and labor-intensive. Also commercially available yeast identification systems are often insufficient in the identification of infrequent species or the differentiation of closely related species. A new molecular diagnostic approach is PCR amplification of target DNA and then sequencing of amplicons. For this purpose, the sequence analysis of D1/D2 or ITS regions in ribosomal RNA large subunit (rLSU) gene has been used in identification of various yeasts and molds. In our study, we evaluated the DNA extraction by Whatman FTA filter matrix technology and then identification by DNA sequencing of D1/D2 region in rLSU of clinical yeasts and molds.
Methods A total of 1323 clinical yeast and 160 mold isolates were included in this study. At the beginning, yeast identification was made by conventional (macroscopic and microscopic morphology, germ tube production) and commercial (API 20C, Biomerieux and CHROMagar *Candida*, BD Diagnostics) tests, molds were identified by macroscopic and microscopic characteristics. Fungal DNA extraction was made by using Whatman FTA microcards with indicator. The amplification of genomic DNA was carried out by nested PCR for yeasts and seminested PCR for molds. Amplification success was evaluated by%1.2 agarose jel electrophoresis. BigDye terminator cycle sequencing Ready Reaction kits (Applied Biosystems) were used for cycle sequencing reactions. Resulting products were then analyzed on an automated capillary DNA sequencer (ABI Prism 3130 Genetic Analyzer, Applied Biosystems). Isolates were formally identified by using BLAST (Basic Local Alignment Search Tool) searches against fungal sequences in existing DNA databases.
Results Unidentified 20 yeasts (*Candida* and *Geotrichum* species) and all of the molds isolates (124 *Aspergillus*, 19 *Fusarium*, 6 *Trichophyton*, 3 *Scedosporium*, 2 *Alternaria*, 1 *Rhizopus*, 1 *Mucor*, 1 *Paecilomyces*, 1 *Hortaea*, 1 *Acremonium*, 1 *Microsporum* identified by microscopically and macroscopically) were included to sequence analysis. Also 6 quality control isolates were used. Whatman FTA filter matrix technology provided an easy and extremely rapid method of preparing both yeast and mould genomic DNAs. Amplicon lengths from sequencing were 392–578 bp for yeasts, 404–628 bp for molds. Conventional identification results were compatible with molecular identification results in general. Yeasts were identified with 100% identity. The sequences of 35 isolates in *Aspergillus* genus were shown 100% identity both *A. flavus* and *A. oryzae*. Ten *Fusarium* isolates exhibited 100% identity with *F.subglutinans* and *F.proliferatum*. Though *Trichophyton* species could not be identified by sequencing, *Microsporum* isolates were shown 100% identity.
Conclusion Whatman FTA matrix technology is an extremely rapid, practical and successful method for fungal DNA extraction. Although conventional and commercial phenotypic methods are substantially sufficient for identification of clinical important fungi, DNA sequence analysis may be a reliable alternative for some special or uncommon fungi. Sequence analysis of rLSU rRNA gene D1/D2 region was found considerably successful in identification of many clinical fungi. However addition of ITS regions may require for identification of closely related fungi.

P232

Abstract withdrawn

P233

Real-time PCR-based detection of dermatophytes in a diagnostic laboratory

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Objectives Real-time PCR-based detection of dermatophytes has increasingly been introduced to microbiological diagnostic laboratories in order to improve specificity, sensitivity and time to diagnosis of dermatophytosis. In comparison to a turnaround time of 1–4 weeks for conventional identification by microscopy and culture, application of the molecular method gives a result within only 2–4 days. In our study, a real time PCR assay was evaluated by comparing it to conventional identification methods, using 231 clinical specimens received in our diagnostic mycological laboratory.
Methods The real-time multiplex PCR assay was developed by Wisselink *et al.* 2011 (J Microbiol Methods 85, p. 62–66) and enables identification of the most prevalent dermatophytes in nail, skin and hair, namely *Trichophyton rubrum*, *Trichophyton interdigitale/mentagrophytes*, *Trichophyton violaceum*, *Trichophyton tonsurans* and *Microsporum* spp. Furthermore the assay design includes a pan-dermatophyte probe for detection of dermatophytes as a group. Specificity of the real-time PCR assay was determined using eight dermatophyte and five non-dermatophyte strains. In the period from January to March 2013, a total of 231 specimens (nail = 178, skin = 50 and hair = 3) were received with enough material to perform culture, microscopy and real-time PCR in parallel. Discrepancies between results obtained with real-time PCR and microscopy were further investigated using DNA sequencing for detection of dermatophyte DNA and/or non-dermatophyte DNA.
Results Using real-time PCR, dermatophytes were identified in 101 specimens (43.7%) of which 61 were dermatophyte positive in culture and 99 were positive in microscopy, as indicated in Table 1. In total, 63 of the 231 specimens were positive for dermatophytes by culture (27.3%). The dermatophytes detected by real-time PCR were 78 *T. rubrum*, 19 *T. interdigitale/mentagrophytes* and one *T. violaceum*. Three samples were identified as dermatophyte positive without further molecular identification; one of these was identified as *Epidermophyton floccosum* by culture. Concordance between real-time PCR and culture was achieved to the species level in 95.1% (58/61). Positive microscopy results were obtained in 26 samples without real-time PCR-based detection of dermatophytes. Of these, two samples were

Table 1. Real-time PCR, culture and microscopy results for 231 clinical specimens.

	Real-time PCR positive	Real-time PCR negative
Culture positive (dermatophytes)	61	2
Culture negative (dermatophytes)	39	129
Microscopy positive	99	26
Microscopy negative	2	104

identified as dermatophytes by culture and/or DNA sequencing and 21 samples were identified as non-dermatophytes by culture or DNA sequencing. The high concordance of negative results obtained by real-time PCR and microscopy (104 versus 106) indicates that the increase in detection rate using real-time PCR instead of culture was not due to false positives or contamination. In the specificity testing of the real-time PCR assay, all eight dermatophyte strains were correctly identified by the pan-dermatophyte probe and their corresponding specific probes. None of the non-dermatophyte strains yielded positive signal in real-time PCR.

Conclusion The high concordance between real-time PCR, culture and microscopy, as well as the considerably increased detection rate of dermatophytes establish the assay as a reliable method for diagnosis of dermatophytosis. Furthermore the turnaround time was significantly reduced compared to conventional identification methods.

P234

Use of rolling circle amplification to rapidly identify species of *Cladophialophora* potentially causing human infection

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The genus *Cladophialophora* comprises etiologic agents of disease in immunocompetent patients, ranging from mild cutaneous colonization to cerebral encephalitis, in addition to saprobic species. Due to the high degree of phenotypic similarity between closely related species of the genus, identification problems are imminent. In the present study, we described rapid and sensitive rolling circle amplification (RCA) method based on species specific padlock probes targeted for the internal transcribed spacer regions of rDNA. ITS regions of 12 Cladophialophora species were sequenced, and subsequently, 10 specific padlock probes were designed for the detection of single nucleotide polymorphisms. The majority of circularizable padlock probes were designed based on single nucleotide polymorphisms (SNPs), while for *C. bantiana, C. immunda* and *C. devriesii* were characterized by two or more nucleotides. Individual species-specific probes correctly identified in all ten *Cladophialophora* species correctly by visualization on 1.2% agarose gels used to verify specificity of probe-template binding; no cross-reactivity was observed. Simplicity, sensitivity, robustness and low costs provide RCA a distinct place among isothermal techniques for DNA diagnostics. However, restriction and specificity and sensitivity should be lowered and increased, respectively, to be useful for a wide variety of clinical applications.

P235

Trichosporon mycotoxinivorans: antifungal susceptibility and identification by MALDI-TOF as compared to other *Trichosporon* species

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Objective *Trichosporon* species are associated with both localized and disseminated infections in both immunocompromised and immunocompetent patients. *Trichosporon mycotoxinivorans* is increasingly associated with pulmonary infections in cystic fibrosis patients, with some strains of this species exhibiting potential resistance to antifungals resulting in clinical failure. MALDI-TOF is a powerful tool for the rapid and accurate identification of micro-organisms. We evaluated the activity of various antifungals against *Trichosporon mycotoxinivorans* and other *Trichosporon* species as well as the performance of MALDI-TOF for species identification.

Table 1

Antifungal	Amphotericin B	Fluconazole	Itraconazole	Posaconazole	Voriconazole	Terbinafine
All *Trichosporon* spp. (n = 59)						
MIC Range	0.06 – >16	< 0.125 - >64	<0.03 – 1	<0.03 – 1	<0.03 - >16	< 0.03 - >2
MIC50	0.5	1	0.06	0.125	<0.03	1
MIC90	1	32	0.5	0.25	1	>2
GM MIC	0.575	1.70	0.091	0.100	0.084	0.799
Trichosporon mycotoxinivorans (n = 26)						
MIC Range	0.25 - >16	<0.125 - >64	<0.03 – 1	<0.03 – 0.5	<0.03 - >16	<0.03 - >2
MIC50	1	1	0.06	0.06	< 0.03	1
MIC90	2	>64	1	0.5	16	>2
GM MIC	0.923	1.62	0.067	0.082	0.095	1.05
Trichosporon asahii (n = 11)						
MIC Range	0.25 – 4	1 – 4	0.125 – 0.5	0.125 – 0.25	<0.03 – 0.125	0.125 - >2
MIC50	1	2	0.25	0.25	<0.03	1
MIC90	4	4	0.5	0.25	0.125	> 2
GM MIC	0.939	2.13	0.25	0.194	0.044	0.882
Other Trichosporon spp. (T. asteroids, T. domesticum, T. inkin, T. loubieri, T. mucoides) (n = 22)						
MIC Range	0.06 – 1	<0.125	<0.03 – 1	<0.03 – 1	<0.03 – 32	0.125 - >2
MIC50	0.25	1	0.06	0.06	0.06	0.5
MIC90	1	32	0.5	0.5	2	>2
GM MIC	0.257	1.60	0.078	0.092	0.101	0.550

Methods Sixty-one clinical isolates sent to the Fungus Testing Laboratory, a large mycology reference center, for antifungal susceptibility testing and/or species identification were evaluated. Antifungal MICs were measured for amphotericin B, fluconazole, itraconazole, posaconazole, voriconazole, and terbinafine against 59 isolates according to CLSI broth microdilution guidelines. Species were identified by API 20C panels, morphologic/physiologic characteristics, and/or molecular sequencing of established loci (ITS, D1/D2, IGS). MALDI-TOF was performed on all isolates (Vitek MS, bioMerieux). Dendrograms were generated and the threshold for species identification was set at 65%.

Results The extended spectrum triazoles itraconazole, posaconazole, and voriconazole were the most potent antifungals against *Trichosporon* species. The geometric mean MICs of these agents (0.091, 0.100, and 0.084, respectively) were significantly lower than those of amphotericin B, fluconazole, and terbinafine (0.575, 1.70, and 0.799, respectively; $p < 0.001$). The marked potency of itraconazole, posaconazole, and voriconazole were maintained regardless of the species including *T. mycotoxinivorans*. However, elevated MICs to these agents and the other antifungals tested were observed against some *T. mycotoxinivorans* strains. MALDI-TOF identification was in agreement at the genus level with 90.1% (55/61) isolates. Identification to the species level by MALDI-TOF was in 100% (10/10) agreement for isolates that were determined by sequence analysis, including 6 *T. mycotoxinivorans* strains. In contrast, species identities between MALDI-TOF and API 20C & morphologic/physiologic characteristics were in agreement for only 48.1% of isolates. This included 9 isolates identified as *T. mycotoxinivorans* by MALDI-TOF but as *T. loubieri* by API 20C and morphologic/physiologic characteristics.

Conclusions The extended spectrum triazoles itraconazole, posaconazole, and voriconazole had the most potent activity against each Trichosporon species while the lowest activity was observed with fluconazole. MALDI-TOF identification was in good agreement to the genus level with other tests used to identify these fungi

P250

Photodynamic treatment of onychomycosis by means of novel multifunctional photosensitizers

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Objectives To demonstrate the usefulness of photodynamic treatment (PDT) for dermatophytic onychomycosis by means of novel compounds that can act both as nail penetration enhancer and as antifungal agent. The concept of PDT (see figure 1) refers to a treatment that requires light-activated agents, named photosensitizers, in combination with light of a proper wavelength and, depending on the reaction

type, the presence of molecular oxygen. To overcome the problem of the nail plate barrier we designed two porphyrin multifunctional photosensitizers (MFPS, figure 2), 5,10,15-tris(4-N-methylpyridinium)-20-(4-phenylthio)-[21*H*,23*H*]-porphine trichloride (PORTH) and 5,10,15-tris(4-N-methylpyridinium)-20-(4-(butyramido-methylcysteinyl)-hydroxyphenyl)-[21*H*,23*H*]-porphine trichloride (PORTHE).

The possibility of these compounds to weaken the nail plate is based on their keratolytic capacity, viz. the possibility of SH-containing compounds to chemically reduce the covalent disulphide bonds as present in keratin protein fibres in human nail plates and responsible for the nails rigidity and hardness.

Methods The potential functions of these compounds were evaluated *in vitro* and in an *ex vivo* human nail model that mimics the clinical onychomycosis situation as much as possible.

Nail penetration enhancement was screened (pH 5 and 8) by measuring the water uptake in human nail compared to other chemical enhancers. *In vitro* MFPS PDT efficacy was tested (pH 5, in presence and absence of oxygen) with *Trichophyton mentagrophytus* microconida using 5 mW/cm^2 of a 532 nm continuous-wave laser (0.6 J/cm^2) and various MFPS concentrations.*Ex vivo* PDT efficacy of the novel compounds was tested (pH 5) 7 and 35 days after infection of human nail clippings with *T. mentagrophytus.* Until the PDT, with 30 mW/cm^2 of 532 nm laser light (27J/cm^2) and different concentrations of PORTH and PORTHE, infected nails were kept at 37°C and 6.4% CO_2. The effect of 24 h pre-treatment of the nails before PDT with 2-Methyl-2,4-pentanediol, urea and propaan-1,2-diol was investigated as well.

Results *In vitro* laser treatment of *T. mentagrophytus* microconidia resulted in 0.1 log kill while PDT under normal air conditions (2 μM MFPS) gave 4.6 log kill for PORTH and 3.2 for PORTHE (4.1 for 10 μM). Anaerobe circumstances decreased the PDT efficacy of PORTH but increased the efficacy of PORTHE (4.7 log kill at 1.5 μM). Under *ex vivo* conditions treatment efficacy of PORTHE (40 - 80 μM) compared to PORTH proved to be higher and fungicidal after 1 treatment while pre-treatment with a minimum of 7% (v/v) 2-Methyl-2,4-pentanediol increased the overall treatment efficacy. Similar penetration enhancement effects were observed for PORTH (pH 5 and 8) and PORTHE (pH 8).

Conclusions Under normal oxygen conditions PORTH (pH 5) gives the best *in vitro* PDT and penetration enhancement effect while PORTHE displays the best overall treatment effect *ex vivo* and works under anaerobic circumstances as well. This makes PORTHE a realistic candidate for PDT of onychomycosis using a formulation that includes at least 7% (v/v) 2-Methyl-2,4-pentanediol.

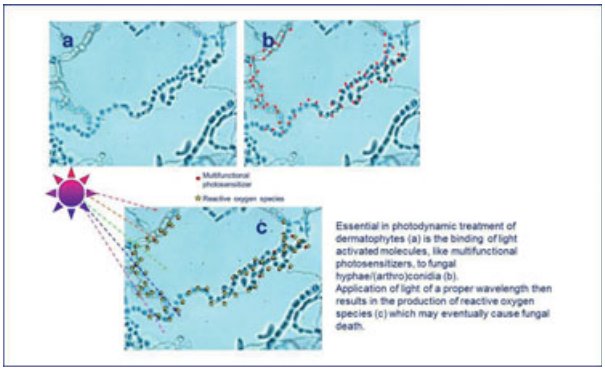

Figure 1 The concept of antifungal PDT.

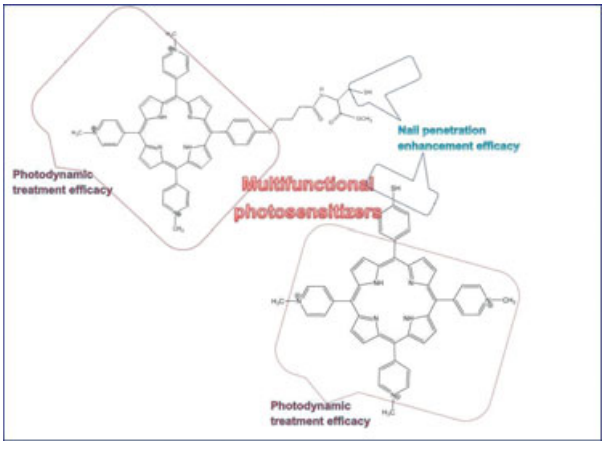

Figure 2 Multifunctional photosensitizers.

P251

Panning commercial compound libraries for antifungals: can a University research lab succeed in finding novel leads?

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The number of life-threatening, invasive fungal infections has risen dramatically over the last 20 years. Today, 4% of all patients dying in modern tertiary care hospitals have invasive aspergillosis, while 2% have invasive candidiasis. Existing treatments for invasive fungal infections remain unsatisfactory with an unacceptably high mortality rate in high-risk patients. Therefore, there is an urgent and unmet need to develop additional and novel antifungal drugs that inhibit essential fungal-specific cellular targets and pathways. We have screened a diverse commercial library of ~80,000 compounds to identify those interfering with virulence-essential pathways including cell wall stability, iron and zinc uptake, and synthesis of essential aromatic amino acids, metabolic precursors and vitamins. Our results suggest that it is possible to identify 'hit' compounds specifically affecting most of these pathways and inhibiting fungal growth at micromolar concentrations. However, most are active only under defined conditions in vitro. The implications of our findings will be discussed.

P252

Clinical isolates of *Candida glabrata* and *Candida tropicalis:* sensitivity to the essential oils of bergamot and lavender

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Introduction Yeasts of the genus *Candida* are present in the mucous membranes and on the skin of humans from birth. Any drop in immunosuppression favors infections, making this one of the most common fungi in nosocomial, dermatological and gynecological infections. Today there are difficulties in the therapy of fungal infections due to the high toxicity and high cost of some composites and resistance to their actions. The search for new molecules and the development of new products with antifungal activity is necessary, and thus medicinal plants and their compounds are an important source for research. *Lavandula officinalis* is a plant endemic to the Mediterranean region, Arabian Peninsula, Canary Islands, Spain and India. This plant is the source of essential oils responsible for

sedative, antiseptic, anti-inflammatory, antioxidant and anticholinesterase actions. *Citrus bergamia*, a plant typical of southern Italy, produces a fruit known as bergamot, from which an essential oil with antiseptic therapeutic properties is extracted from its structural components.
Aim The objective of this study was to evaluate the inhibitory effect of essential oils of bergamot and lavender on strains of the genus *Candida* and compare the inhibitory action of these substances to conventional drugs such as fluconazole and Amphotericin B.
Methods The antifungal activities of the essential oils of bergamot and lavender and the conventional drugs Fluconazole and Amphotericin B were evaluated on seven clinical and ATCC strains of *Candida tropicalis* and *Candida glabrata* using the disk diffusion method according to the M44-A2 Protocol (CLSI) and M27-A2 microdiffusion (CLSI) with modifications for the essential oils.
Results Both oils exhibited significant inhibitory action against all the strains tested. The average inhibition halos of *C. tropicalis* were: bergamot (29 mm), Lavender (21 mm), Fluconazole (31 mm) and Amphotericin B (17.5 mm). For *C. glabrata* the average inhibition halos were: bergamot (28 mm), Lavender (32 mm), fluconazole (20 mm) and Amphotericin B (13.5 mm). The essential oils of lavender and bergamot presented minimum inhibitory concentrations (MIC) of 17.5–4.3 mg/mL and 8.7–1.0 mg/mL, respectively. All the isolates were also sensitive to Amphotericin B with a MIC ranging from 0.1–0.03 μg/mL. With Fluconazole the *C. tropicalis* species were susceptible with a MIC of 0.125–0.03 μg/mL and against *C. glabrata*, as expected, dose dependent resistance was seen with Fluconazole with a MIC of 128–16 μg/mL.
Conclusion The results confirm the significant antifungal activity of the essential oils of bergamot and lavender for the clinical strains and ATCCs of *C. tropicalis* and *C. glabrata*. Given the intrinsic resistance to Fluconazole, for some species, these results support the notion that plant essential oils and extracts may have a role as pharmaceuticals.

P253

Screening selection of a new antifungal candidate by means of acute and chronic candidiasis models

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Objective In the frame of a screening program aimed at selecting a potential new antifungal candidate, 6 new molecules with proven *in vitro* antimycotic activity were tested in preclinical models of invasive candidiasis. For this purpose, acute and chronic murine models of systemic candidiasis were performed.
Method Female CD1 mice were infected intravenously (i.v.) with *Candida albicans*. Fungal inocula of $2x10^6$ for the acute and of $1x10^6$ for the chronic model were used. The test compounds, namely P-26233, P-26609, P-27471, P-27473, P-27530 and P-27758, were administered daily, by intraperitoneal route, from the day of infection until the death of the animal in the acute model or for 5 days in the chronic model. Doses were 3 and 30 mg/kg for the acute and 30 mg/kg for the chronic model. In the acute model, mice were monitored for survival. In the chronic model, mice were monitored for survival, fungal growth of kidney and brain and histopathology of the kidney. Control groups were dosed either with single dose of Caspofungin (2.5 mg/kg) or with the vehicle.
Results In the acute model, P-27530 at 30 mg/kg was the only compound that significantly increased the median survival time from 3 days of vehicle treated control to 10 days (Fig. 1).

In the chronic model, treatment with P-27530 exhibited efficacy leading to a significant reduction of the fungal burden in the kidneys and dissemination in the brain (Fig. 2). This result was confirmed by the histological analysis of the kidney, where P-27530 showed a marked reduced inflammation compared to untreated control.

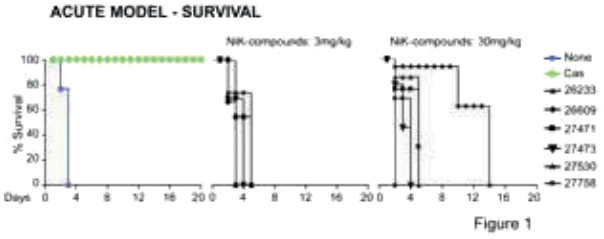

Figure 1

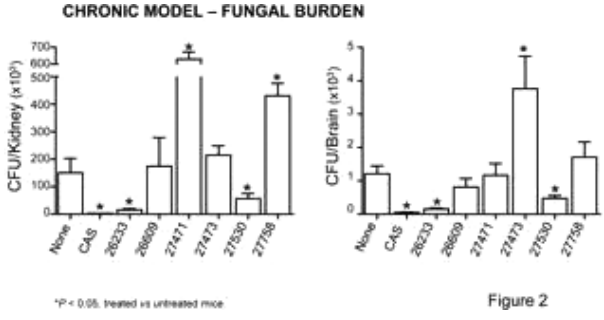

Figure 2

Conclusions The results obtained in the present study showed that P-27530 has potential antifungal activity as demonstrated by the increased survival in the acute model and the reduced fungal growth and inflammatory pathology in the chronic systemic candidiasis.

P254

A new anti-Aspergillosis inhibitor targeting N-myristoyltransferase in *Aspergillus fumigatus*

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Objectives MyristoylCoA:protein *N*-myristoyltransferase (NMT, EC: 2.1.3.97) is a highly conserved protein that catalyzes the covalent attachment of a 14-carbon saturated fatty acid myristate onto the N-terminal glycine residue of a variety of eukaryotic proteins. Many of the target proteins are crucial components of signaling pathways, and myristoylation typically promotes membrane binding that is essential for proper protein localization or biological function. NMT has been genetically validated as a therapeutic target in several human pathogens, including parasitic protozoa *Leishmania major*[1] and *Trypanosoma brucei*[2] as well as fungal species *Candida albicans*[3] and *Cryptococcus neoformans*[4]. A number of NMT inhibitors have been reported in those species. However, little in known about NMT in human opportunistic pathogen *A. fumigatus*. Our work focuses on 1) does NMT exist in *A. fumigatus?* 2) is it essential and 3) is it druggable in *A. fumigatus?*
Methods Single *nmt* gene was cloned from *A. fumigatus*. Recombinant *Af*NMT from *Escherichia coli* was characterized. No knockout strain of *Af*NMT was obtained after several trials. Therefore, a conditional mutant was constructed by replacing the native promoter of the *A.fumigatus nmt* gene with the *Aspergillus nidulans alcA* promoter. Phenotypic analyses, including growth rate, temperature

sensitivity, germination and cell wall structure, were performed for conditional mutant. A small library of previously described *Ca*NMT and *Tb*NMT inhibitors was tested on both *Af*NMT enzyme and *A. fumigatus*. Furthermore, crystal structure of *Af*NMT was determined. Promising inhibitors from the screening were co-crystallized with *Af*NMT.

Results *A. fumigatus* possesses an active NMT, which shows low *Km* for both myristyl-CoA and peptide substrates. Under strict repression conditions conditional NMT mutant cannot grow, demonstrating that *nmt* is essential for *A. fumigatus* survival. Under partial repression condition the mutant exhibits retarded hyphal growth, cell wall defect, delayed germination and abnormal polarity establishment and maintenance. Different from the NMT mutants in other fungal species, the condition mutant is not susceptible to high temperature and myristic supplementation doesn't restore its normal hyphal growth, suggesting NMT targets in *A. fumigatus* are different from other fungi. Inhibitors screening revealed that although several inhibitors are very potent with IC_{50} in the nM range against *Af*NMT, only a derivative of *Tb*NMT inhibitors showed inhibition on *A. fumigatus* in a *Af*NMT-dependent manner. Co-crystallized *Af*NMT-inhibitors complexes provided structure guides for more potent anti-aspergillosis inhibitors.

Conclusion *A. fumigatus* has an essential and active NMT that targets proteins different from other fungi. A promising *Af*NMT inhibitor showed potent inhibition both on this enzyme and the organism, representing a new anti-Aspergillosis inhibitor that would be exploitable in the future.

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P255

Evaluation of the efficacy of P-27530 in the oral and topical treatment of dermatophytosis in a guinea pig model

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Objectives Dermatophytosis causes significant discomfort and cosmetic problems. More effective therapies are needed. This study evaluated the efficacy of P-27530 following oral and topical treatment in a *Trichophyton mentagrophytes* -dermatophytosis guinea pig model.

Methods Male albino GPs 400 - 450 g were clipped, shaved and skin abraded. GPs were inoculated with *T. mentagrophytes* 10^7 conidia and randomized into 5 groups. Study designs were as follows: Oral Therapy: P-27530 10 mg/kg, P-27530 30 mg/kg, terbinafine 10 mg/kg, vehicle control and untreated control groups. All treatments were given orally daily, starting from the day of infection to day 9; Topical Therapy: 4% P-27530, 8% P-27530, 8% ciclopirox, vehicle control and untreated control groups. All treatments were given topically daily, starting from day 4 post-infection to day 10. P-27530 efficacy was assessed, on day 10 for the oral and on day 13 for the topical studies, by the assessment of mycological (hair route invasion test) and clinical (5-point scale skin evaluation) endpoints.

Results Following both oral (Table 1) and topical (Table 2) therapy, all groups, with the exception of the vehicle control, showed significant clinical and mycological efficacy when compared to untreated control ($P < 0.05$). The untreated control showed hair loss, ulcerated, scaly skin and the highest average fungal positive hair.

Conclusions The results obtained in this study demonstrated that P-27530 is efficacious in the treatment of *T. mentagrophytes* skin infection in the guinea pig model when administered either by oral or by topical route, supporting P-27530 as a potential treatment for dermatophytes infections.

Table 1 Summary of the clinical and mycological efficacy of oral treatment groups as compared to the untreated control.

Test Compounds	Dose (mg/kg)	Percent Efficacy	
		Clinical	Mycological
P-27350	10	30.2*	61.3*
	30	26.0*	90.3*
Terbinafine	10	70.8*	80.7*
Vehicle	N/A	2.1	26.9

* *P*-value of < 0.5 when compared to the untreated control

Table 2 Summary of the clinical and mycological efficacy of topical treatment groups as compared to the untreated control.

Test Compounds	Dose	Percent Efficacy	
		Clinical	Mycological
P-27350	4 %	35.7*	65.5*
	8 %	35.7*	64.4*
Ciclopirox	8 %	26.5*	56.9*
Vehicle	N/A	5.1	39.1*

* *P*-value of < 0.05 when compared to the untreated control

P256

Evaluation of the efficacy of two doses of P-27530 in the treatment of vaginitis caused by *Candida albicans* or *Candida glabrata* in a murine model

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Objectives Seventy five percent of women will experience the onset of vaginal candidiasis during their reproductive years. *Candida albicans* is the most common cause of vaginal candidiasis. *C. glabrata* is

Table 1 Vaginal tissue fungal burdens of treatment groups.

	Median Log CFU ± SD	
	C. albicans	*C. glabrata*
P-27530 1%	2.28 ± 0.92 [a,b]	0.74 ± 0.74 [a,b]
P-27530 2%	2.48 ± 0.57 [a]	0.70 ± 0.80 [a,b]
Fluconazole 2%	2.77 ± 0.65 [a]	0 ± 0 [a,b]
Vehicle Control	4.02 ± 1.01	1.96 ± 0.42
Untreated Control	4.52 ± 0.67	2.30 ± 0.63

[a] *P*-value of < 0.05 when compared to the untreated control.

[b] *P*-value of < 0.05 when compared to the vehicle control.

recognized as the second most common cause of *Candida* infections. Even though, they are usually treatable, incidences of resistance, particularly with fluconazole have been reported. Therefore, the development of new antifungal agents is needed. In two experiments, the animals were infected with either *C. albicans* or *C. glabrata*, then divided into groups (n = 10); P-27530 1%, P-27530 2%, fluconazole 2%, vehicle, and an untreated control. The mycological efficacies of the compounds were assessed.

Methods Female BALB/c mice with a body weight of ~ 20 g were used. Three days prior and four days post infection, mice were given 0.5 mg of estradiol valerate to induce and maintain pseudoestrus. Animals were anesthetized and challenged with 2.5×10^8 blastospores intra-vaginally. The efficacy of the treatment was assessed using the vaginal fungal burden.

Results Table 1 shows the median log CFU ± the standard deviation of the treatment groups against infection with *C. albicans* and *C. glabrata*. **1)** *C. albicans* The untreated and vehicle controls showed the highest fungal burdens with median log CFU ± SD of 4.52 ± 0.67 and 4.02 ± 1.01, respectively. Groups treated with 1% P-27530, 2% P-27530 and 2% fluconazole demonstrated median log CFU ± SD of: 2.28 ± 0.92, 2.48 ± 0.57 and 2.77 ± 0.65. The fungal burden in the 1% P-27530, 2% P-27530 and 2% fluconazole -treated groups were significantly lower compared to the untreated control group (*P*-values = 0.002, <0.001, and <0.001, respectively). Two percent P-27530 also showed significant efficacy when compared to the vehicle ($P = 0.019$). **2)** *C. glabrata* The untreated and vehicle treated controls showed the highest fungal burdens, 2.30 ± 0.63 and 1.96 ± 0.42, respectively. Groups treated with 1% P-27530, 2% P-27530 and 2% fluconazole demonstrated CFU counts 0.74 ± 0.74, 0.70 ± 0.80 and 0 ± 0. Fungal burdens in the 1% P-27530, 2% P-27530 and 2% fluconazole -treated groups were significantly lower compared to the untreated ($P < 0.001$, =0.001, and <0.001, respectively). P-27530 1% and 2% showed significant efficacy when compared to the vehicle ($P = 0.001$). However, the 2% fluconazole-treated group demonstrated significant efficacy when compared to the P-27530 1% and 2%-treated groups ($P = 0.023$ and 0.007, respectively).

Conclusions Both 1% and 2% P-27530 showed significant efficacy in reducing fungal burdens in these murine models of vaginal *C. albicans* and *C. glabrata* infection. There was no significant difference between the fungal burden of the P-27530 and the fluconazole-treated groups indicating that P-27530 is a potential alternative to fluconazole.

P257

The effect of 17-(allylamino)-17-demethoxygeldanamycin in combination with caspofungin therapy in an invasive pulmonary Aspergillosis rat model

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Introduction Invasive pulmonary aspergillosis (IPA) is a life-threatening fungal infection in immunocompromised patients, mostly caused by *Aspergillus fumigatus*. Despite new treatment options for IPA, therapy continues to be a major problem due to toxicity and emerging resistance against the first choice antifungal drug voriconazole. The echinocandin caspofungin (CAS) is used as salvage therapy. This drug has a different mode of action compared to the azoles and inhibits the biosynthesis of 1,3 β-glucan. The synthesis of 1,3 β-glucan is also influenced by the stress response of the fungus. One of the key players in this stress response is heat shock protein 90 (Hsp90) which is able to upregulate 1,3 β-glucan synthesis. 17-(allylamino)-17-demethoxygeldanamycin (17-AAG) is an Hsp90 inhibitor and enhances the therapeutic efficacy of CAS in an *in vivo* systemic aspergillosis model in the moth *Galleria mellonella*. However, no data are available for mammal models of IPA. To determine if combination therapy also shows a promising beneficial effect in IPA in mammals, we investigated if 17-AAG could improve the *in vivo* efficacy of CAS in IPA in transiently neutropenic rats.

Methods For 10 *A. fumigatus* strains, an XTT-based checkerboard titration was performed to assess *in vitro* synergy between CAS and 17-AAG. Subsequently, the therapeutic efficacy of 17-AAG in combination with CAS was studied in transiently neutropenic female RP rats with IPA. A left-sided pulmonary infection was established via intubation and administration of *A. fumigatus* conidia into the left lung lobe. Subgroups of rats were treated intraperitoneally according to the following 3 regimens: 17-AAG 1 mg/kg/day, 17-AAG 5 mg/kg/day or 17-AAG 20 mg/kg/day with or without the CAS dosage of 0.75 mg/kg/day which has suboptimal efficacy when administered as mono-therapy. Treatment duration was 10 days and started 16 h after infection. One group of control animals received CAS monotherapy and the other group control animals received vehicle.

Results *In vitro* activity, 17-AAG did not show an antifungal effect (MIC>128 μg/ml), but a synergistic interaction was observed when combining 17-AAG with CAS (FICI = 0.5).

In vivo efficacy, treatment with 1 mg/kg 17-AAG as mono-therapy was well tolerated, however did not show any efficacy in rats with IPA. Addition of 17-AAG to sub-optimal CAS therapy did not result in significantly increased therapeutic efficacy, 33% rat survival was obtained which was also observed after CAS mono-therapy. Increased dosages of 17-AAG at 5 mg/kg or 20 mg/kg in combination with CAS were not tolerated and resulted in renal and hepatic dysfunctions.

Conclusion Whereas synergistic activity between 17-AAG and CAS towards *A. fumigatus in vitro* is demonstrated, a synergistic effect of 17-AAG at the maximum tolerated dosage and CAS in IPA in neutropenic rats is not observed. Although combining 17-AAG with CAS proved to be beneficial in a moth model with systemic aspergillosis, this combination of drugs is not successful in a clinically-relevant animal model of IPA in immunocompromised rats.

P258

Herbal products: analysis of microbial action against *Candida albicans* and *Candida parapsilosis* on removable acrylic resin orthodontic appliances

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Objective To evaluate the antifungal action on the biofilm morphology of some herbal disinfectant solutions [*Cymbopogon (L.) Rendle, Casearia sylvestris Swartz, Hydrastis canadenses L.*] against *Candida albicans* and *Candida parapsilosis* on acrylic resin used in the manufacture of removable orthodontic appliances.

Methods Forty acrylic resin test pieces were made using metal molds. The test pieces were divided into two groups that were infected with either *C. albicans* or *C. parapsilosis* and incubated for 48 hours. Subsequently, subgroups of five test pieces of each of the two groups were immersed for ten minutes in the following solutions: distilled water, *Cymbopogon (L.) Rendle, Casearia sylvestris Swartz, Hydrastis canadenses L.* The surfaces of all the test pieces were scanned by electron microscopy.

Results *Cymbopogon nardus (L.) Rendle* was visually more effective against these two yeasts than the other two herbal solutions. However, on comparing with the control group (distilled water), all herbal solutions had disinfectant effects.

Conclusion *Cymbopogon nardus (L.) Rendle* was visually more effective against *C. albicans* and *C. parapsilosis* than *Casearia sylvestris Swartz* and *Hydrastis canadenses L.* however all the solutions had antifungal actions on the biofilm morphology on the acrilic resin surface used to mafufecture removable orthodontic appliances.

P259

Antifungal activity of the essential oil of angelica major

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Objectives In the last years, there has been an increase in the incidence of fungal diseases, particularly among patients with impaired immune systems. Also, the reduced number of available drugs and the increasing resistance to classical antifungal compounds justifies the research for efficient and economic therapeutic alternatives with low side effects. Aromatic plants and their essential oils (EO) have been traditionally used as antifungal agents. In the present work we report the antifungal activity on a range of representative human pathogenic yeast species of the EO of *Angelica major* from Portugal.

Methods The EO of *Angelica major* obtained by hydrodistillation was analysed by gas-chromatography and gas-chromatography/mass spectroscopy. The antifungal activity was evaluated against yeasts: eight strains from six species of *Candida* (*C. albicans* ATCC, M1 and D5; *C. glabrata*; *C. parapsilosis*; *C. krusei*; *C. tropicalis* and *C. dubliniensis*) and *Cryptococcus neoformans* using the reference CLSI broth macrodilution protocol M27-A3. Minimum fungicidal concentrations (MFCs) were the lowest concentrations showing no growth after incubation of 20 mL samples from clear tubes in the macrodilution test in Sabouraud agar at 37°C. The influence of sub inhibitory concentrations of the EO on the dimorphic transition in *C. albicans* was then studied.

Results The oil showed high percentages of α-pinene (21.8%) and *cis*-*β*-ocymene (30.4%). Minimum inhibitory concentrations (MICs) of the EO were dependent from the specie and the strain tested. MIC values ranging from 2.5 to higher or equal to 10 $\mu L.mL^{-1}$ for *Candida* strains and was 0.16 $\mu L.mL^{-1}$ for *C. neoformans*. However, its major components, α-pinene and *cis*-*β*-ocymene, showed MIC values lower, ranging from 0.08 to 1.25 $\mu L.mL^{-1}$ and from 0.16 to 1.25 $\mu L.mL^{-1}$, respectively. Furthermore, the α-pinene and *cis*-*β*-ocymene displayed a clear fungicidal activity, with MFCs equal to or just one dilution above the respective MICs, including against isolates with decreased susceptibility to commercial antimycotic drugs.

The EO also produces almost complete inhibition of filamentation in *C. albicans* at concentrations as low as MIC/32. The major constituents were also assayed and the activity of the EO is presumably due to both the contribution of α-pinene and *cis*-*β*-ocymene, considering that concentrations of 0.04–0.16 $\mu L.mL^{-1}$ inhibited almost completely the filamentation process.

Conclusions In addition to the antifungal activity of EO of *Angelica major* against *Candida* spp., this work revealed an important inhibition of filamentation in *C. albicans* strains. This is interesting, since filamentation has long been shown to be essential for virulence in *C. albicans* and its inhibition alone appears to be sufficient to treat candidosis. This data supporting the potential of the EO of *Angelica major* for the clinical management of mucocutaneous candidiasis.

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P260

Aspergillus fumigatus GNA1: fragment screening gets groovy

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Background & objective Diseases due to *Aspergillus fumigatus* present a spectrum of clinical, diagnostic and therapeutic challenges. A new generation of antifungal agents is required to address the toxicity and emerging reports of resistance in existing therapies. The cell wall of *A. fumigatus* (*Af*) represents a drug target. This dynamic interlaced polysaccharide structure is essential for survival of the fungus. Chitin is an integral component of the cell wall consisting of linear β (1–4) linked *N*-acetyl-D-glucosamine (GlcNAc). The Hexosamine biosynthetic pathway provides the sole source of the sugar nucleotide precursor UDP-GlcNAc.

A new potential antifungal target is glucosamine-6-phosphate *N*-acetyltransferase (*Af*GNA1). This enzyme *N*-acetylates glucosamine-6-phosphate using acetyl-CoA to *N*-acetyl-glucosamine-6-phosphate as an intermediary step in UDP-GlcNAc biosynthesis. Fragment-based lead drug discovery provides a complementary and contrasting approach to traditional high-throughput methods through the elaboration of weakly binding small molecules. Here we assess the "druggability"of *Af*GNA1 using a fragment screen.

Methods and results Purified *Af*GNA1 was chemically biotinylated and a fragment screen based on bio-layer interferometry (Octet Red, Forte Bio) performed to assess the binding of fragments to the target protein. Screening the Dundee Drug Discovery Unit fragment library gave a preliminary hit rate of 5.7% (37/652 with a response rate > 0.02 nm). A subset of seven fragments demonstrated stoichiometric binding with equilibrium dissociation constants in the micromolar range. X-ray crystallography was used to (a) confirm the hits and (b) determine the binding mode. Structural analysis of *Af*GNA1 in complex with fragment (A) illustrated the fragment binds in a groove behind the sugar substrate. This combined with initial kinetic data suggests fragment (A) may elicit a conformational change in the active site resulting in partial inhibition of *Af*GNA1 activity.

Conclusion Fragment screening explores a diverse range of chemical space in assessing the binding capabilities of a target. This work suggests *Af*GNA1 may be a "druggable"antifungal target with fragment screening identifying a series of ligand efficient molecules binding in a groove adjacent to the active site. Iterative cycles of medicinal chemistry and structural biology are required to optimise this chemical 'anchor' by extending into the active site to generate highly potent drug-like lead molecules.

P261

Fungicidal activity of new asymmetrically substituted porphyrins and their zinc complexes

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Nowadays the antibiotic resistance of main fungal pathogens has been increased. This situation is a concern and stimulates the search of new alternatives for therapy of mycoses caused especially by *Candida albicans*. A large number of natural and synthetic porphyrins of diverse chemical compositions already used in the photodynamic therapy of cancer (Dougherty T.J., 2002). Antimicrobial and antiviral activities of porphyrins are based on their ability to absorb photons and generate reactive oxygen species and partition into lipids of bacterial membranes (Wainwright M., 2000). Light-dependent, photodynamic activity of natural and synthetic porphyrins and their metal complexes against Gram-positive and Gram-negative bacteria has been well demonstrated (Bertoloni G. et al., 1992).

The aim of this work was to determine the mechanism of *Candida albicans* cell death in the presence of new asymmetrically substituted porphyrins and their zinc complexes after irradiation.

In this study the culture *C. albicans* ATCC 18804 was used. Establishing a form of cell death was carried out under the influence of photosensitizers by flow cytometer Facscan (Becton Dickinson, CA, USA) using the AV-FITC and propidium iodide (Coyle B. et al., 2004). During activation of studied substances intensity of light exposure was 20 J/cm^2 at the sample.

All investigated compounds were more effective photosensitizers compared with the Photofrin® medicine that used in photodynamic therapy. One of the processes that occur as a result of their exposure

is apoptosis, the maximum value of which was characterized for 5,10,15-tri-(4-N-methyl-pyridyl)-20-n-hexadecylporphyrin zinc. Zinc-containing porphyrins had higher photosensitizing antifungal activity values to compare with their free bases.

Thus, the characteristics of the interaction of microorganisms with photosensitizers indicates that the culture of *C. albicans* is formed by yeast cells with the ability to directly reaction to the porphyrin compound influence, as well as those whose response develops over time.

P262

Photodynamic inactivation in *Candida spp* isolated from oral cavity lesions of kidney transplant patients sensitized by cationic porphyrin 5,10,15,20-Tetrakis[2,3,5,6-tetrafluoro-4-(N-methylpyridinium-4-ylsulfanyl)phenyl] porphyrin tetra-iodide

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Objectives The *Candida* species colonizes oral cavity and can be related with opportunistic infections such as oral candidiasis. The therapeutic options are limited and the microorganism resistant have frequently been isolated. So, the search for alternatives therapies have become increasingly relevant. Photodynamic therapy has been an interesting option of alternative therapy due to efficiency, low cost and tolerability. However, little is known about the behavior of yeast exposed to photodynamic inactivation process (PDI). Thus, the aim of this study was evaluate the behavior of *C. albicans* and *C. glabrata* isolated from oral cavity lesions of the kidney transplant patients exposed to photodynamic inactivation process PDI, residing in Maringá, PR, Brazil.

Methods The photosensitizer used in this study was cationic porphyrin, 5,10,15,20-Tetrakis[2,3,5,6-tetrafluoro-4-(*N*-methylpyridinium-4-ylsulfanyl)phenyl] porphyrin tetra-iodide ceded by the Drug Synthesis Laboratory of Universidade Federal do Paraná. Light emitting LumaCareLC122 was used to the PDI assays (100mW/cm^2 for 20 minutes. Assays were conducted using yeast suspension (10^8 CFU/mL) with the cationic porphyrin at concentrations of 5, 2.5, 1 and 0.5 μM. Dark Control (DC) was performed at highest concentration of porphyrin (5 μM) protected from light, and a Light Control (LC) under the same test conditions, but without porphyrin. Controls and irradiated cells suspensions were plated on Sabouraud agar, incubation at 37 °C. After 48 h the number of colonies was determined. Each experiment was performed in duplicate and in three independent assays.

Results It was possible to observe the absence of growth starting from 1 μM to *C. glabrata* and 2.5 μM to *C. albicans*. However it is important to highlight that in 0.5 μM concentration was observed a decrease of four and three logarithmic scales, for *C. glabrata* and *C. albicans*, respectively.

Conclusions These results indicate that photodynamic inactivation seems to be effective in the treatment of oral candidiasis, with better yield on *C. glabrata* species, currently referred as an emergent species and naturally resistant to antifungal agents used in clinical practice.

P263

Antifungal activity evaluation of the cymbopogon nardus extract on dermatophyte isolated from animals and domestic environment in Maringá, Paraná, Brazil

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Objectives Dermatophytosis is an extremely contagious disease with high zoonotic potential and the most common fungal disease in dogs and cats in the Western world. The strategic treatment for disease must include the environmental descontamination to prevent the spread of infection. Products available to sanitizing for environmental or domestic use, present several implications as odor, difficulty in handling, toxicity, cost, potential for persistence and accumulation in the environment. The aim of present study was to evaluate *in vitro* activity of citronella extract (*Cymbopogon nardus*) on *Microsporum canis* isolated from domestic animals and environment in order to find a sanitizing alternative for the prophylaxis of dermatophytosis.

Methods The susceptibility test was performed by broth macrodilution technique in RPMI 1640 (Sigma) supplemented with 2% glucose, according to Clinical and Laboratory Standards Institute document (M38-A2, 2008) with the modifications for natural products. After seven days of incubation at 35°C, the visual reading was done. The Minimum Inhibitory Concentration (MIC) was considered the lowest concentration in which any fungal growth was observed. In this study 31 samples of *M. canis* isolates from animals and domestic environment were tested.

Results The citronella extract was able to inhibit *M. canis* isolates with MICs ranging from 9.75 mg/ml to 625 mg/ml. Correlating the values found in the literature with these results, the citronella extract showed a strong antifungal activity (<75 ug / ml) on 80.65% of the samples.

Conclusions *In vitro* results with citronella extract showed a potent antifungal effect against *Microsporum canis*. However, further researches are necessary, mainly, in the use of this extract as sanitizer prophylactic to dermatophytosis propagated by pets.

P264

Antifungal activity of ajoene on *Fusarium spp* onychomycosis isolates

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Background *Fusarium* is an opportunistic fungal pathogen with high mortality among immunocompromised patients. This genus is related as agent of infection of nails (onychomycosis) in immunocompetent individuals. These onychomycosis, are an important public health concern due to its persistent nature and high recurrence rates. Moreover, the *Fusarium* is resistant to antifungal agents and its low therapeutic response. Therefore, it becomes important to discover new antifungal agents for this resistant microorganism, and natural products could be an interesting alternative. The ajoene (4,5,9-trithiadodeca-1,6,11-triene 9-oxide) is an organosulphur derived from garlic extract which has shown an important antimicrobial agent. Thereby, the aim of this study was to evaluate the *Fusarium* spp. susceptibility to ajoene compared with terbinafine.

Methods The samples were obtained between January and December 2003 from patients attending in the Laboratory of Teaching and Research in Clinical Analysis of the State University of Maringá, Paraná, Brazil. The minimum inhibitory concentration (MIC) for

terbinafine was determined according to Clinical and Laboratory Standards Institute (M38-A2, 2008) method of microdilution in broth. For the ajoene MIC, this methodology was modified to the study of natural products.
Results In period of this study, 360 nail samples were confirmed positive for onychomycosis, these, 7.22% (26) were caused by *Fusarium* spp., and *F. oxysporum* and *F. solani* were the most frequently species (8 isolates for each specie). all *Fusarium* strains showed resistant to terbinafine. However, regarding to extract ajoene, the results were satisfactory, exhibiting sensibility, with MIC ranging from 3.65 μg/mL to 29.25 μg/mL.
Conclusions According our results the ajoene demonstrated seem be an excellent alternative for the treatment of onychomycosis caused by *Fusarium* spp., since most of antifungals available are not very effective against *Fusarium* spp., making treatment difficult.

P265

In vitro antifungal and antibacterial activity of Turkish propolis extract

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Propolis (bee glue) is a resinous substance extracted by bees from plants. Propolis has been effectively used in treatment of dermatological, laryngological, and gynecological problems, neurodegenerative diseases, in wound healing, and in treatment of burns and ulcers. Several studies in which crude solutions of propolis were tested against a wide range of bacteria were carried out by different researchers. However, the number of studies on antifungal activity of propolis is limited. The aim of the presented study was to examine the antimicrobial activity of ethanol extract of Turkish propolis against *Candida albicans*, *Candida tropicalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* strains *in vitro*.

Manually collected propolis was extracted in 95% v/v ethyl alcohol, in a hermetically closed glass vessel for 7 days at 24°C. The ethanolic extract was then filtered through a Whatman filter paper No 4 and evaporated on a rotary evaporator, under reduced pressure at 50°C. Minimal inhibitory concentrations of propolis extract were determined by the broth microdilution method. MICs was observed in the concentration of 0.12 mg mL^{-1} to the *C. albicans*, 0.25 mg mL^{-1} to the *C. tropicalis*, 0.06 mg mL^{-1} to the *S. aureus*, 0.12 mg mL^{-1} to the *P. aeruginosa*. The beneficial effects of the propolis have been mentioned since ages ago. According to our results, the analyzed fungal and bacterial strains were shown to be sensitive to the propolis in almost all the evaluated concentrations. Some of the bacterial and fungal infections have been characterized by the development of resistance among many strains after antifungal and antibacterial therapies. So, alternative therapies can be an option since more 'in vitro' studies may be done and related to some established parameters of the 'in vivo' efficacy.

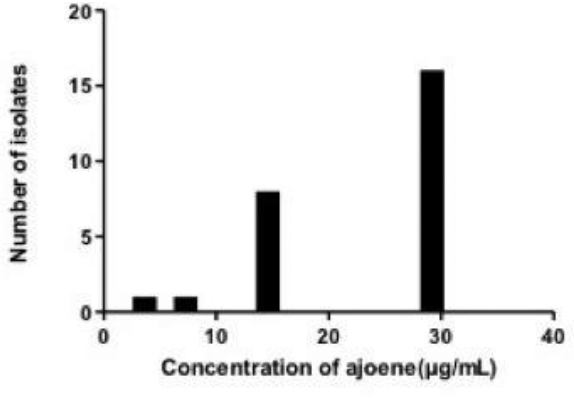

Figure 1: Minimum inhibitory concentrations (μg/mL) of the ajoene for 100% inhibition of growth, in 26 *Fusarium* spp. onychomycoses isolates

P280

MP65 gene is essential for drug resistance and virulence in *Candida albicans*

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Objectives The *MP65* gene of *Candida albicans* (orf 19.1779) encodes a cell wall mannoprotein of 65 kDa (Mp65p), with putative β-glucanase activity, which plays a main role in host-fungus interaction, morphogenesis, cell wall integrity, adherence and biofilm formation. In this study, we performed an extensive analysis of the *mp65Δ* mutant to assess the role of this gene in drug resistance and virulence.
Methods
***MP65* gene and drug sensitivity** To determine the effects of deletion of the *MP65* gene on drug sensitivity, we tested the *mp65*Δ mutant with different antimycotic agents [as Fluconazole (Flu), Voriconazole (Vor), Itraconazole (Itra) and Caspofungin (Cf)], by three assays: minimal inhibitory/fungicidal concentration (MIC/MFC), spotting in solid medium and time-kill curve.
***MP65* gene and drug resistance mechanisms** In order to understand which is or are the mechanism/s responsible for the increased sensibility of mp65Δ mutant to Flu and Cf, we analyzed the expression by real-time reverse transcription (RT)-PCR in wt and mp65Δ mutant strains of the drug targets Erg11 and Fks1, of the multidrug transporters Cdr1, Cdr2, Mdr1, Flu1 and Hst6 and of two key regulators of cellular stress responses, namely Mkc1 and Cna1. Moreover, to investigate the relationship between signaling pathways involved in Flu/Cf drug resistance and *MP65* gene, we studied by real-time reverse transcription (RT)-PCR the *MP65* expression in *erg3Δ*, *bck1Δ*, *cna1Δ* and *pkc1Δ* mutant strains.
***MP65* gene and clinical isolates** We studied the expression of the Mp65p in clinical strains by western-blot analysis.
***MP65* gene and virulence** To test whether genetic compromise of *MP65* activity holds therapeutic potential, we performed *in vivo* studies in BALB/c mice treated with Flu and Cf after intravaginal challenge with wt, *mp65*Δ mutant and revertant strains and we compared clearance acceleration by CFU count.
Results The *MP65* gene disruption conferred hypersensitivity to multiple antifungal drugs, including Flu and Cf, turning the fungistatic Flu to fungicidal, and affecting significantly transcriptional expression of different drug transporters (as Cdr1, Cdr2, Mdr1, Flu1, Hst6) and of two key regulators of cellular stress responses (namely Mkc1 and Cna1).

Moreover, *MP65* transcription decreased in *pkc1Δ* and increased in *bck1Δ* mutant strains, suggesting that *MP65* expression is regulated by *PKC* pathway. Also, Mp65p expression was higher in azole-sensible (s) than in azole-resistant (r) clinical strains. Finally, *in vivo* studies showed that Flu and Cf treatments were significantly more effective when *MP65* activity was genetically compromised.
Conclusion This work establishes that *MP65* gene is involved in two important biological aspects of pathogenicity: drug resistence and virulence, making it an attractive drug target for therapy of *Candida* infections.

P281

Opsonization of different Mucormycetes (Zygomycetes) species by complement modulates their recognition by phagocytes

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Objectives Mucormycoses are invasive fungal infections caused by filamentous fungi belonging to the class of Mucormycetes. Angioinvasion and frequent thrombosis are characteristic features of these diseases. In the bloodstream, the fungi have to cope with the complement system, which is a central component of the soluble innate immunity. Complement exerts multiple antimicrobial effector functions, including direct fungal damage, opsonization of the pathogen and hence support of phagocytosis, as well as attraction and activation of immune cells. On the other hand, exaggerated activity of this complex immune weapon might also induce strong inflammation and tissue damage and thus contribute to the high mortality of mucormycoses. We therefore aim to study in detail the interactions between Mucormycetes and the complement system.
Methods The capacity of various pathogenic species (*Lichtheimia corymbifera, L. ramosa, Rhizopus microsporus, R. oryzae, Rhizomucor pusillus,* and *Mucor* spp) of Mucormycetes to activate the complement system was tested *in vitro*; the opsonization of the fungal surface by various complement factors was examined. Furthermore, potential intra-species variations were assessed by testing up to three different clinical isolates from each species. Fungal spores were opsonized in human serum; the deposition of complement factors on the surface was detected by flow cytometry. Neutrophil granulocytes and platelets were coincubated with native and opsonized spores; interactions were examined microscopically.
Results The complement system was activated by the sporangiospores of all examined Mucormycetes species, which resulted in the deposition of the complement factors C1q, C3, and the Terminal Complement Complex (TCC) on the spore surface. Considerable variations in the degree of opsonization were detected between the different species as well as between isolates of the same species. *Rhizopus microsporus*, *Rhizopus oryzae*, *Rhizomucor pusillus* and *Mucor* spp showed substantial opsonization intensity on their spore surface, while the complement deposition on the two tested *Lichtheimia* species *L. corymbifera* and *L. ramosa* was weaker than on the other species.

Deposition of complement proteins on the Mucormycetes spores markedly influences the interaction with neutrophil granulocytes and platelets. Opsonization of the spores was shown to enhance the adherence to or even phagocytosis by neutrophils. Coincubation of opsonized spores with neutrophils and platelets resulted in the formation of large aggregates.
Conclusion These preliminary findings indicate that complement may cause both beneficial and detrimental effects in the pathogenesis of Mucormycoses. Deposition of complement factors on the fungi might facilitate their clearance by phagocytes. The formation of aggregates might on the one hand trap the pathogens and thereby support the host defence, but on the other hand also be a cause of massive thrombosis.

P282

New insights into the pathogenesis of invasive pulmonary aspergillosis: real time imaging of hypoxia and inflammation

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Objectives *Aspergillus fumigatus*, is responsible for relevant diseases especially in immunocompromised individuals. Among these, life-threatening invasive pulmonary aspergillosis (IPA) results in mortality rates ranging from 30–90%. While investigating the role of immune effector cells in host defense against *A. fumigatus* using a bioluminescent *A. fumigatus* reporter strain, we recently showed that one day post-infection lungs from cortisone acetate-treated mice displayed severe tissue necrosis and hypoxia (Brock et al 2008, Ibrahim-Granet O et al 2010, Galiger et al 2013). Development of hypoxia was confirmed by Grahl et al in 2011 using the hypoxia marker pimonidazole hydrochloride on lungs sections from euthanized mice with IPA.

In this study we focused on the *in vivo* real-time monitoring of hypoxia and inflammation within the lungs of mice from three immunologically distinct murine models of invasive pulmonary aspergillosis, i. e. cortisone acetate *versus* cyclophospahmide treatment *versus* CXCR2 knock- out mice (Ibrahim-Granet et al BMC 2010).
Methods To monitor disease progression, hypoxia and inflammation we used bioluminescence imaging and fluorescence molecular tomography systems (Perkin Elmer) on mice infected with the luminescent *A. fumigatus* strain. Here, (i) fungal growth was quantified by bioluminescence, (ii) hypoxia was quantified by the cell surface expression of carbonic anhydrase (CAIX) and (iii) inflammation was determined through the pan cathepsin proteases known to be produced by inflammatory cells. In addition, inflammation was investigated from lung tissues homogenates by studying the inflammatory cytokine patterns by ELISA.
Results Three days following infection hypoxia was detected under all infectious conditions regardless the immune status of mice. Even immunocompetent mice developed hypoxia levels five times higher than mock-infected (control) animals although no invasive aspergillosis developed. However, hypoxia was most pronounced in susceptible CXCR2 KO mice (28 times higher than control)

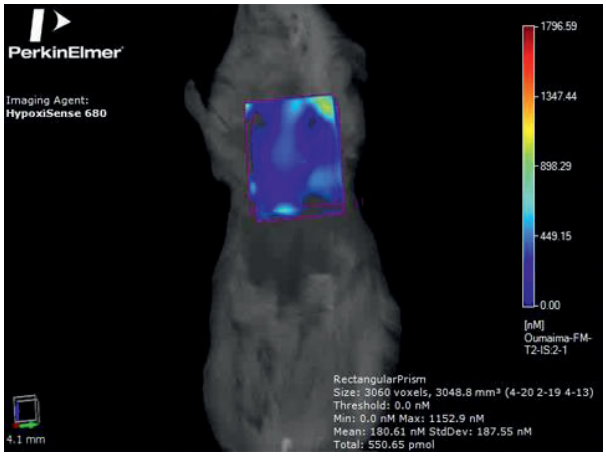

Figure 1 Real time acquisition of hypoxia in the chest of an immnosuppresed mouse D3 post inoculation with A. fumigatus conidia.

followed by corticosteroid and cyclophosphamide treated animals. In the CXCR2 KO mice, the high level of hypoxia is associated with an overwhelming inflammatory cytokines levels in the lungs homogenate. Regarding the cathepsin level, in immunocompetent animals, cathepsin was hardly detectable within the lungs of the mice. However, in corticosteroid animals the presence of cathepsin was more pronounced (11 times compared to control). Interestingly, in CXCR2 KO, showing the highest level of hypoxia, the presence of cathepsin was only moderate (3 times compared to control). This indicates that the pan cathepsin proteases activity is not necessarily linked to the severity of the infection and the role of these proteases in the clearance of the infection needs further investigations.
Conclusion This is the first study that correlates hypoxia with fungal growth in real-time from *in vivo* models of murine IPA. Importantly our data brings new insights on the contribution of hypoxia to lung inflammation during fungal infection. Impact of hypoxia on the innate immune system during invasive pulmonary aspergillosis has to be investigated.

P283

Interaction of airway epithelium cell lines (A459 and RPMI 2650) with fungi

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Objective The objective of this study was to determine the mechanism of interaction of fungi with airway epithelial cell lines (A549 & RPMI2650) *in vitro* and to determine the role of Toll Like Receptor (TLR), Protease activated receptor (PAR), Dectin and transcription factor (NF-kB & MAPK) in this process.
Methods The Cell lines were exposed to fungal culture filtrate (*Aspergillus flavus*). The inducible expression of Patterns Recognition Receptors (PRR) and transcription factors (NF-kβ & MAPK) involved in above interaction were analysed by RT PCR & real time PCR.
Result The fungi enhanced the expression of transcription factor NF-kβ50, NF-kβ52, NF-kβ65, ERk & p38 MAPK but downregulates the expression of TLR1, TLR2, TLR3, TLR4, TLR4, TLR5, TLR6, TLR7, TLR8, TLR8, TLR10, PAR1, PAR2, PAR3, and PAR4.
Conclusion Fungi released some inhibitory substance which downregulates the expression of PRR (TLR, PAR) and some other unknown receptor may be responsible for the activation of NF-KB & MAPK during above interaction.

P284

Evaluation of candidal colonization and specific humoral responses against *Candida albicans* in patients with psoriasis

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Objectives Psoriasis is an inflammatory skin disease that can considerably affect on patients' quality of life. Environmental and genetic factors, as well as microflora such as *Candida* species may play a role in exacerbation and persistence of psoriasis. In the present study we evaluated candidal colonization and specific humoral response against *C. albicans* in patients with psoriasis.
Methods One hundred patients with psoriasis vulgaris and 50 healthy individuals were enrolled in the study. Skin and oral specimens from all participants were cultured on CHOROMagar*Candida*medium. Isolated yeast like fungi were identified by using the sequence of the D1/D2 domain of the 26S rRNA gene. The enzyme-linked immunosorbent assay (GENESIS-Diagnostic, England) was used for detection of IgM, IgA and IgG antibodies against *C. albicans* in sera of patients and healthy individuals.
Results *Candida* species were isolated from the skin of 15% of the patients and 4% of the controls and from oral specimens of 60% of the patients and 20% of the controls. There was a significant difference between *candidal* colonization in patients and controls ($P < 0.05$).Serum IgM, IgA and IgG levels against *C. albicans* were significantly lower in patients with psoriasis than in controls ($P < 0.05$). There was no significant difference between the serum levels of specific antibodies against *C. albicans* and frequency of candidal colonization and clinical severity of the disease ($P > 0.05$).
Conclusion However, the results of our study showed a higher rate of candidal colonization in patients with psoriasis in comparison with controls but there was a reduction in humoral immune responses in patients groups.

P285

Potential pathogenicity of sexual *Aspergillus fumigatus* spores in embryonated chicken eggs

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Objectives *Aspergillus fumigatus* is an ubiquitous saprotrophic fungus available in the atmosphere, which is known to reproduce through both asexual and sexual means. The experimental sexual crossing studies showed that the mutated antifungal resistance genes will be subsequently transferred to its offspring.Gaining insights into poential virulence of sexual crossing and its subsequent progeny may help to further explore more in details the required approaches responible for the antifungal reistance and the natural peristence of *A.fumigatus*. In the present study, we therefore evaluated the virulence potential of *A.fumigatus* ascospores comparing to the asexual spores in an embryonated chicken eggs, as an alternative infection model.
Methods Two *A.fumigatus* strains (V 108-20 and V 108-21) were used for this study. The mating type of each strain was first determined and then crossed to eachother on the oatmeal agar plates. The crosses were assessed weekly until cleistothecia were developed. The ascospores then were harvested from surface of plates and heated for one hour at 70°C, in order to prevent the germination of asexual spores. The aliquots of the asexual and corresponding sexual spores were then used to determine their potential of pathogenicity in an embryonated chicken egg model. The fertilized chicken eggs were incubated at 37°C and 50 to 60% humidity in a specialized incubator. On day 10 of incubation, the eggs were infected through injection of a 0.1 ml inoculum to chorioallantoic membrane (CAM) using a sterile 1 ml syringe. The holes were sealed with paraffin and vitality (survival) was monitored daily by candling for up to 7 days.Each experiment was performed in two separate replicates.
Results All embryos infected with 10^3 to 10^6 asexual spores died within 3 to 7 days post challenge. However, the infection with heat-killed asexual spores was resulted to 100% survival. Chicken embryos infected with the heated ascospores suspensions, were killed

in a dose dependent manner, in which all the embryos infected with 10^6 ascospores died within 7 days post infection, but Killing was delayed at lower dose (10^3 ascospores) showing the mortality up to 50%. The morphological and histopathological examinations of CAM in dead embryos were revealed fungal growth and destruction of blood vessels.
Conclusion In the present study, the mortality of the chicken embryos was dependent on the viability and the heat-killed asexual *A.fumigatus* spores were inactivated for reproduction and virulence. Notably, our results revealed a link between sexual reproduction and virulence potential of *A.fumigatus*, indicating that adaptations to pathogenicity may occures in both sexual and asexual reporoduction means. This may suggests a real concern for the disease management of *A. fumigatus* due to the genetic variation,since sexual crossing might produce offspring with increased virulence or resistant to antifungal agents. Further molecular investigations are warranted to explore the putative virulence genes of ascospore and its posible roles related to antifungal resistance or persitence of *A.fumigatus* in the environement.

P286

Iron overload is a dominant risk factor for the manifestation of invasive zygomycosis

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Objectives To investigate the existence of predisposing factors and analyze the relative significance of each of them, for the development of invasive zygomycosis in a cohort of 15 patients, diagnosed in a tertiary hospital during a 15-year period.
Methods Clinical manifestations, laboratory features and course of all patients, diagnosed with invasive zygomycosis in the University Hospital of Patras, Greece, was analyzed and the preexistence of the known predisposing factors was investigated. The course and evolution of these patients was then compared, in relation to the presence of these factors.
Results Patients were 9 males and 6 females, with a median age at diagnosis of zygomycosis of 53 years (range 21–85 years). Twelve patients had an underlying hematological malignancy (ALL in 4, of whom 1 allotransplanted, with grade-1 acute GVHD, AML 1, myelodysplastic syndrome 3, multiple myeloma 2, non-Hodgkin's Lymphoma 2). Disease status was active/refractory in 7 and remission in 5. The remaining 3 patients had non-malignant diseases (β-thalassemia major 1, non-hematological disease 2). Ten patients were severely neutropenic (absolute neutrophil count <0.2/μl), 11 severely lymphopenic (absolute lymphocyte count <0.5/μl, and 10 were diabetics, of whom 3 poorly controlled. Eleven patients had previously received various immunosuppressive treatments (methotrexate 5, cyclosporine-A 3, cyclophosphamide 2, lenalidomide 2, ATG/Campath 2, fludarabin 1, other various 4). No patient was HIV positive or received parenteral nutrition. Two patients had been previously colonized with Candida species, 11 were receiving broad spectrum antibiotics, and 7 were also receiving antifungal prophylaxis or preemptive treatment with imidazole derivatives (6) or caspofungin (1), representing cases of breakthrough mycosis. At diagnosis of zygomycosis 13 patients (87%) exhibited severe iron overload, as defined by transferrin saturation >50% and/or by serum ferritin >1000 ng/ml, attributed to previous transfusions in 8. Median serum ferritin was 2450 ng/ml (range 346–8014 ng/ml) and median transferrin saturation 69.2% (range 33.2–91.4%), rendering iron overload the dominant feature for this group of patients. Two patients (before the year 2000) were treated with Amphotericin-B deoxycholate and the remaining 13 with high-dose Liposomal Amphotericin-B, 5–10 mg/kg/d for a median of 15 days (range 5–57 days). The course was fatal in 10 cases and curative in 5, but in one patient zygomycosis relapsed 6 weeks later, and he finally succumbed. The 4 patients, who survived, underwent repeated surgical debridement, and 3 of them also received adjuvant deferasirox treatment. These 3 patients finally died for reasons related to their underlying hematological malignancy, without recurrence of zygomycosis. Survivors had lower mean transferrin saturation and serum ferritin values, compared to patients died from zygomycosis (59.1 ± 20.1% versus 71.6 ± 11.6% and 1800 ± 864 versus 3369 ± 2054 ng/ml, respectively), yet these differences were not statistically significant ($p = 0.186$ and $p = 0.197$, respectively).
Conclusion Although already recognized, iron overload, either attributed to previous red-blood cell transfusions or not, appears to be a dominant risk factor for the development of invasive zygomycosis, in patients, either with an underlying hematological malignancy or not. Iron status should be examined in all cases, and if possibly, iron overload should be reversed with the appropriate iron-chelating treatment, preferably with deferasirox.

P300

Validation of a bioassay for the measurement of posaconazole levels in serum

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Objectives Posaconazole is a potent triazole with broad spectrum antifungal activity. It is used as a prophylactic or therapeutic antifungal agent in immunocompromised patients. In order to prove a sufficient and stable bioavailability and ascertain the efficacy and safety of treatment, the drug levels in blood should be determined. Microbiological (bioassays) and analytical assays are in use for antifungal therapeutic drug monitoring (TDM). The first are simplest and less expensive, while analytical assays are more sensitive and able to determine the possible metabolites. Concerning posaconazole, there are not active or clinically significant metabolites. Aim of this study was the development and validation of a bioassay for the determination of posaconazole levels in blood serum.
Method Following the validation and use of a bioassay for voriconazole, the method was further tested and validated for posaconazole measurements. The method is performed on 24 x 24 cm agar plates by observing the inhibition of fungal (*Candida kefyr*) growth, measuring the inhibition zones and forming a calibration curve according to which the unknown samples are quantified. The agar plates are supplemented with yeast nitrogen base, glucose, citrate solution. The pH is optimally adjusted to 7.0. All standards, controls and patients' samples are inoculated in duplicate or triplicate, randomly on the agar in order to avoid interference by the plate preparation. The validation was assessed by running seven core assays. The stock solution (1 mg/mL) was prepared by dissolving the posaconazole powder in DMSO/methanol (1:5 solution), while the working solution (10 μg/mL) was made by further dilution in methanol. The concentrations of the calibration curve (0.2, 0.5, 1.0, 4.0, 6.0 and 8.0 μg/mL) and the quality controls (0.5, 1.0 and 4.0 μg/mL) were prepared in pooled human serum. Three pairs of patients' samples were tested for the measurement of trough and 2 h post-dose levels respectively, in order to further validate the method.
Results The method was linear (mean $r^2 = 0.996$) for the specific range of standards concentrations (when the y axis of concentrations was transformed to the logarithmic scale). The precision, as inter run %CV, ranged from 9.4% to 11.1% for the standard curve, 2.6% to 12.1% for the quality controls and 2.6% to 10.9% for the patients' samples, while as mean intra run%CV from 8.4% to 12.4%, 2.6% to 11.4% and 2.1% to 11.2% respectively. The inter run accuracy (%

DIFF) ranged from -22.5% to 2.5% for the control samples. The stability of posaconazole was examined with repeated freeze-thaw cycles (5–8 times in four months) and found that the drug presented a very good stability (%CV: 7.8% to 18.5%). The recovery of posaconazole was 92.50 ± 10.35% (mean±SD). As preliminary measurements, in three adult haematological patients receiving 200 mg x 3/24 h of posaconazole, the trough levels were found 0.98 ± 0.23 μg/mL and the two hours post-dose 1.20 ± 0.14 μg/mL.

Conclusions The method is time consuming but simple, not expensive, and most important precise and accurate. It has to be further evaluated and compared to the analytical methods, but it is a very good alternative for posaconazole TDM.

P301

High-throughput equilibrium dialysis to determine protein binding characteristics of voriconazole

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Objectives Plasma protein binding (PPB) is an important aspect of a drug's pharmacokinetics. Equilibrium dialysis (ED) is the analytical method of choice (Wan & Rehngren, 2006, J Chromatograph). Voriconazole, a triazole with unpredictable pharmacokinetics, binds to plasma proteins for 58%, determined with ED in the premarketing studies, using a Dianorm dialyzer for 2 hours, with human plasma spiked with 1 mg/L voriconazole against a 0.1M phosphate buffer pH7,4 (Roffey *et al*, 2003, Drug Metab & Dispos). Nowadays, a newer high-throughput ED assay (HT-ED) is available (HTDialysis LLC; 37 Drive Gales Ferry, CT 06335, USA; Banker *et al*, 2003, J Pharm Sci). We have tested its applicability for voriconazole.

Methods In a 96-well plate (model HTD96b, HTDialysis), a semi-permeable membrane (12–14K) separates the sample solution of blank healthy human plasma spiked with clinically relevant voriconazole concentrations (0.7–11.2 mg/L), from PBS buffer pH 7.4 (Sigma-Aldrich LLC). During shaker incubation at 37°C, equilibrium is reached by diffusion of unbound drug. Tests were performed in triplicate or fivefold. Additionally, the influence of temperature during HT-ED, frozen storage of plasma, freeze-thaw cycles and pH fluctuations during HT-ED and frozen storage on PPB of voriconazole was investigated. Voriconazole concentrations were determined using LC-MSMS (Pauwels *et al*, 2012, Clin Chim Acta). Statistical analyses were performed using SPSS 20.0 for Windows (SPSS Inc. 2011, Chicago, IL, USA) and $p < 0.05$ was considered significant.

Results Mass balance indicated negligible drug loss through adsorption to the device. Volume shift is neglectable during ED of less than 4,5 h (Lagrange *et al*, 2000, J Pharmaceut & Biomed). After 4 hours of dialysis, equilibrium was reached for both 1,7 and 3,5 mg/L. The median (IQR) PPB of all experiments (N = 235) was 47,6% (45.3–50%). The percentage of PPB did not differ at concentrations ranging from 0,7 to 11,2 mg/L ($p = 0.653$).

Advantages of the assay are the possibilities to perform at 37°C or 25°C ($p = 0.713$) or in batch: PPB did not change during 1-3-5 freeze-thaw cycles ($p = 0.108$) and during frozen storage (-20°C) up to 9 months (Table 1). Alkaline pH during ED (range 7.4–7.7) and frozen storage (range 7,4 to 7,8) did not influence the PPB (Table 1). Remarkably, PPB was lower than 58%, possibly due to methodological differences. Roffey *et al.* only tested one voriconazole concentration (1 mg/L, which is clinically a low concentration according to the reference range of 1 or 2 up to 5,5 mg/L), in triplicate, after 2 hours of equilibrium, which could be suboptimal. Also differences in used volume (1 mL vs. 150 μL) and buffer composition (phosphate 0,012M vs. 0,1M) could contribute. In our opinion, HT-ED is preferred to determine clinically achieved unbound concentrations. It can be performed regardless from temperature, batching, freezing and thawing.

Conclusion HT-ED is a robust assay for determination of voriconazole PPB and can easily be used to determine unbound concentrations in daily practice.

Table 1 Median protein binding values of voriconazole tested at different periods of storage at -20°C and at different voriconazole concentrations.

Voriconazole concentration (mg/L)	Period of frozen storage (number of days)									
	0	1	14	28	56	84	112	140	168	252
0,7	51,11	50,00	49,00	50,00	47,73	47,62	53,49	48,89	47,62	53,49
1,4	48,91	48,44	45,45	48,31	49,43	47,73	51,14	50,00	47,78	51,19
2,8	49,17	48,59	50,00	46,11	52,88	46,29	51,67	48,15	46,59	50,56
5,6	48,46	48,39	47,88	45,94	53,80	47,53	52,34	45,88	47,47	51,18
11,2	44,90	48,55	46,53	45,29	53,51	43,29	51,05	46,12	46,43	48,95

Test for each voriconazole concentration and each period of frozen storage.
Healthy human plasma was spiked with different concentrations of voriconazole and frozen during different storage periods.

P302

Impact of hypoalbuminemia on plasma protein binding characteristics of voriconazole

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Objectives Since the launch of voriconazole, variable voriconazole plasma levels (VPLs) are a challenge. Little is known about the plasma protein binding (PPB). PPB is 58% according to in vitro data from the premarketing studies (Roffey *et al*, 2003, Drug Metab & Dispos). In our study, PPB and the impact of hypoproteinemia were evaluated in clinically relevant patient populations.

Methods Pediatric patients (>2 years) and adult hematology and ICU patients, treated with voriconazole and with a steady state VPL>0,4 mg/L were included. Unbound fractions were separated using high-throughput equilibrium dialysis (HT-ED) during 4 hours (HTDialysis LLC, USA), followed by LC-MSMS for voriconazole analysis (Pauwels *et al*, 2012, Clin Chim Acta). α-1-acid-glycoprotein (AAG) was determined using an IMMAGE-kit (Beckman Coulter, Ireland). Serum albumin concentrations were collected from the patient record. Statistical analysis was performed using SPSS 20.0 for Windows (SPSS Inc. 2011, Chicago, IL, USA), $p < 0,05$ was considered significant. Correlations were defined based on R^2 values.

Results 46 samples were collected from 34 patients during a 7-month study period (Table 1).

Median PPB did not differ between patient groups ($p = 0.130$). The overall median PPB (51,9%) deviates from the previously published 58%, which was based on equilibrium dialysis (ED) (Dianorm Dialyzer) with human plasma spiked with 1 mg/L voriconazole after 2 hours of equilibrium (Roffey *et al*, 2003, Drug Metab Disp), versus our method, carried out during 4 hours to reach equilibrium for all clinically relevant VPL. Next to technical reasons, pathophysiological factors and co-medication might contribute, as our study was conducted in patients.

Table 1 Demographics, clinical and biochemical characteristics.

Characteristics	Total	ICU	Hematology	Pediatrics
Number of patients	32	13	14	5
Age (years) - Median (IQR)	58,5 [47,365]	62 [57,5-71,5]	60,5 [49,865]	11 [5,5-15,5]
Actual Weight (kg) - Mean ± SD	65,4 ± 16,8	71,6 ± 14,5	68,4 ± 9,6	40,7 ± 18,7
Actual BMI (m²/kg) - Mean ± SD	23,2 ± 4,2 (N=29)	25,2 ± 4,2	22,7 ± 3,6 (N=11)	18,8 ± 1,3
Male/Female - N (%)	20 (62,5%)/ 32 (37,5%)	6 (46,2%)/ 7 (53,5%)	11 (78,6%)/ 3 (21,4%)	3 (60%)/ 2 (40%)
In-hospital mortality – N (%)	10 (31,3%)	6 (46,2%)	4 (28,6%)	0
28 day mortality – N (%)	10 (31,3%)	6 (46,2%)	4 (28,6%)	0
Number of samples	46	20	19	7
VRC IV/PO (N-%)	28 (60,9%)/ 18 (39,1%)	20 (100%)/ 0	8 (42,1%)/ 11 (57,9%)	0/ 7 (100%)
Albumin (g/L) - Median (IQR)	31,1 [27,8-33,3]	31,1 [28,4-33,2]	28,7 [26,3-32,1]	34,7 [33,3-37,1]
AAG (g/l) - Median (IQR)	1,8 [1,2-2,3]	1,8 [1,5-2,2]	1,94 [1,18-2,47]	0,93 [0,73-1,42]
Total protein (g/L) - Mean ± SD	56,8 ± 7,5 (N=44)	56,6 ± 7,3 (N=18)	56,5 ± 8,7	57,7 ± 4,9
Total bilirubin (mg/dL) - Median (IQR)	0,36 [0,260,75] (N=25)	0,41 [0,28-1,69] (N=12)	0,35 [0,25-0,50] (N=11)	0,25 [0,19-0,31] (N=2)
Dose (mg/day) - Median (IQR)*	560 [400-700]	580 [465-800]	400 [400-700]	490 [320-600]
Dose (mg/kg/day) - Median (IQR)*	8 [7,1-11,1]	8 [7,5-8,3]	6,8 [5,7-11]	14,6 [10,8-19,1]
VRC treatment (days) - Median (IQR)**	6 [4-14]	6 [4-13]	5 [4-11]	14 [5-44]
Total VRC level (mg/L) - Median (IQR)	2,9 [1,7-4,9]	2,4 [1,5-4,5]	3,5 [2,5-5,8]	2,6 [0,8-5,2]
Unbound VRC level (mg/L) - Median (IQR)	0,94 [0,59-1,43]	0,71 [0,51-1,46]	1,10 [0,65-1,37]	0,70 [0,26-1,98]
Bound VRC level (mg/L) - Median (IQR)	1,95 [1,16-2,69]	1,52 [1,07-2,57]	2,18 [1,33-2,94]	1,56 [0,56-3,72]
Protein binding (%) – Median (IQR)	51,2 [46,7-55,6]	49,6 [42,5-52,5]	53,9 [49,3-55,9]	50,1 [50,0-56,0]

VRC = voriconazole
AAG = alpha-1-acid-glycoprotein
* on day of sampling
** Days of VRC treatment before day of sampling

Table 2 Correlation coefficients (R^2) with plasma protein binding of voriconazole.

Parameter	Correlation Coefficient (R^2)			
	Total population	ICU	Hematology	Pediatrics
Correlation with plasma protein binding (%)				
Albumine level (g/L)	0,069	0,317	0,013	0,320
AAG level (mg/L)	0,019	0,000	0,181	0,194
Total plasma protein (g/L)	0,028	0,266	0,020	0,222
Voriconazole daily dose (mg/kg)	0,008	0,028	0,043	0,161

Neither albumin nor AAG did correlate with PPB (Table 2) in the total population. This was confirmed in vitro with phosphate buffered saline spiked with 3,5 mg/L voriconazole and clinically relevant concentrations of albumin, AAG or both. PPB was respectively 25,5% [21.4–29.8%], 4.5% [3.6–5.9%] and 27% [25.5–28.7%]. Voriconazole could bind to other blood constituents, as lipoproteins or globulins. However, in ICU patients, PPB decreased with decreasing albumin and total protein. This is unexpected, as voriconazole has only moderate PPB, and VPLs are far below the usual albumin levels, probably resulting in non-saturated PPB. It should be further explored whether, in the ICU population, other components or drugs saturate albumin binding. Based on the correlation between lower PPB and hypoalbuminemia/hypoproteinemia, one could expect, due to saturated adult metabolism, a higher free fraction within the same total VPL, potentially causing more toxicity. Future studies should investigate whether, in patients with very low albumin levels and VPLs at the higher end of the reference interval, doses should be decreased. Despite the limited pediatric sample size, the same correlation was seen between PPB and albumin/total protein levels. However, in this population lower total VPLs might be expected with hypoalbuminemia/hypoproteinemia, as a consequence of the rapid non-linear PK.

Conclusion PPB is lower than 58% in all studied populations. Plasma components other than albumin and AAG could contribute to the overall PPB. The correlation between PPB and albumin/protein levels should be confirmed in a larger sample size, with ideally, a broad scale of albumin levels and VPLs tested.

P303

Clinically achievable caspofungin, micafungin, and anidulafungin concentrations within the bloodstream: experience from a mycology reference laboratory

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Objective Therapeutic drug monitoring of antimicrobials is an accepted practice with the goals of improving clinical outcomes and avoiding drug toxicities in patients. In some institutions, therapeutic drug monitoring is routinely done for the azoles voriconazole, posaconazole, and itraconazole, and numerous studies have reported the utility of this practice with these antifungals. However, few clinical

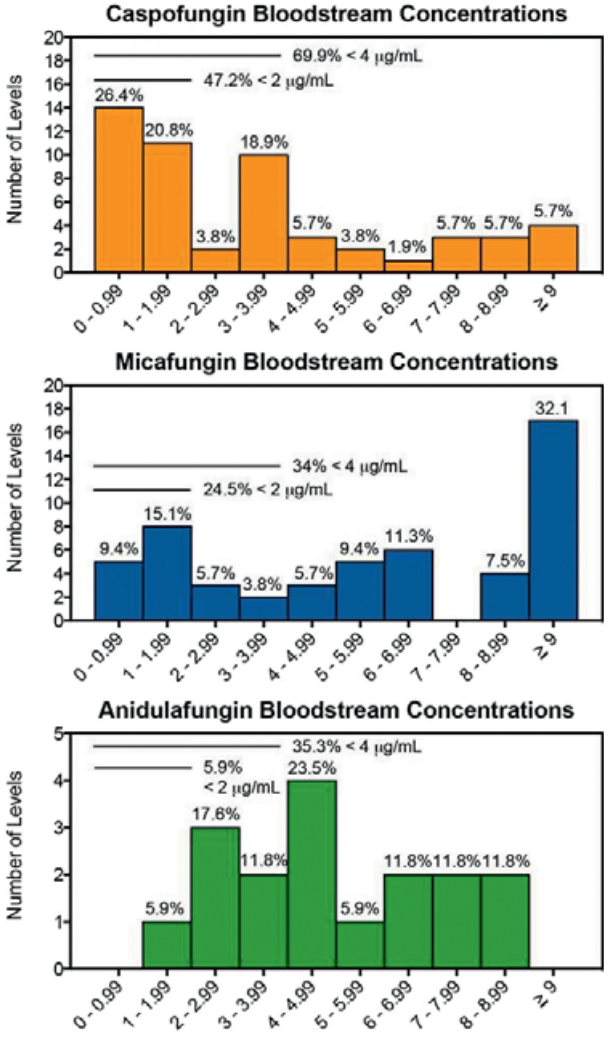

Figure 1

data are available regarding the therapeutic drug monitoring of the echinocandins. We reviewed a large dataset of antifungal drug concentrations with the goal of better understanding clinically achievable echinocandin concentrations.
Methods The antifungal drug level database in the Fungus Testing Laboratory at the UT Health Science Center San Antonio was used. This database is populated with antifungal drug concentrations from human and animal samples sent to this reference laboratory for measurement. All samples were appropriately processed, and the concentrations of caspofungin, micafungin, and anidulafungin were measured by validated HPLC assays with fluorescence detection. For this study, only confirmed bloodstream (serum/plasma) levels were analyzed.
Results In comparison to azoles concentrations, a limited number of echinocandin bloodstream concentrations were available. For caspofungin and micafungin, there were each 53 bloodstream levels measured in humans, and for anidulafungin 17 human bloodstream levels were available. Median detectable concentrations for caspofungin, micafungin, and anidulafungin were 3.28, 6.40, and 4.62 μg/mL, respectively. There were marked differences between the echinocandins in the distribution of concentrations. Caspofungin concentrations were skewed to the right with significantly more levels of this echinocandin below 1 μg/mL, the current CLSI clinical breakpoint for resistance against many *Candida* species (26.4%), compared to micafungin (9.4%; $p = 0.041$) and anidulafungin (0%; $p = 0.016$). Similarly, significantly more caspofungin concentrations were below 4 μg/mL (69.9%) compared to micafungin (34%; $p < 0.001$) and anidulafungin (35.3%; $p = 0.02$). Conversely, micafungin concentrations were skewed towards the left with significantly more levels >9 μg/mL (32.1%) compared to caspofungin (7.5%; $p = 0.003$) and anidulafungin (0%; $p = 0.007$).
Conclusions This dataset demonstrates disparity amongst the clinically available echinocandins in terms of achievable bloodstream concentrations. Caspofungin had the greatest number of levels distributed towards the lower end of the range, while micafungin had the highest number of elevated concentrations. However, for each member of this class a wide range of bloodstream concentrations was observed. These results suggest that consistency in bloodstream concentrations for the echinocandins may be lacking, thus making it difficult to predict achievable levels. Further studies are needed to determine the utility of therapeutic drug monitoring for this class of antifungals.

P304

Population pharmacokinetics of caspofungin in a phase II dose escalation study

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Objectives Caspofungin (CAS) is used for management of proven or probable invasive fungal infections. In a multicenter phase II dose escalation study, dose-dependent pharmacokinetics of CAS was studied.
Methods CAS was administered as 2 h infusion at doses from 70 to 200 mg QD. CAS pharmacokinetic sampling (n = 468 samples) was performed on day 1 and at peak and trough time points at days 4, 7, 14, and 28 (70 mg: 9 (96), 100 mg: 8 (80), 50 mg: 9 (94), 200 mg: 20 (198) patients (plasma samples)). Trough levels were analysed descriptively. Population pharmacokinetic analysis was performed using NONMEM and Pirana. For model evaluation, Bootstrap analysis, prediction corrected (pcVPC) as well as standardized (SVPC) visual predictive check were performed.Results: There was no difference in log-transformed dose-normalised trough levels of CAS (ANOVA). CASconcentration data fitted best to a two-compartment model with proportional error model, interindividualvariability (IIV) on clearance (CL), central (V1) and peripheral (V2) volume of distribution, covariance on CL and V1, interoccasion variability (IOV) on CL and body weight as covariate on CL and V1 (CL 0.411 L/h ± 29%, IOV on CL: 16%, V1: 5.785 L ± 29%, Q: 0.843 L/h, V2: 6.53 L ± 67%). No one of the other covariates (dose level, sex, body weight, age, serum bilirubin, creatinine clearance) further improved the model. Bootstrap results show robustness of the final PopPK model, VPC and SVPC confirm its predictability. Based on the final model, simulated peak plasma concentrations at steady state ranged from 13.8 to 39.4 mg/L (31%), trough concentrations from 4.2 to 12.0 mg/L (49%), and area under the concentration-time curve from 170 to 487 mg/L*h (34%) for the dosage range of 70 to 200 mg QD (geometric mean, geometric coefficient of variation).
Conclusion CAS showed linear pharmacokinetics across the investigated dosage range of 70 to 200 mg QD.Following administration of 100 mg QD, drug exposure in the study patients were slightly higher (7%) relative to results found in healthy volunteers.

P305

Trends of antifungal agents' use in a tertiary care hospital

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Objectives During the last decade significant changes have been made in antifungal therapy including wide use of new agents (AF) such as azoles, lipid amphotericin B formulations and echinocandins. In addition, evidence-based therapeutic guidelines have been recently published. The objective of this study was to investigate trends of AF use in different hospital departments during a 9-year period.
Methods The study was conducted in a tertiary care general hospital. Eleven departments were included: intensive care unit (ICU), solid organ transplant unit (SOT), 5 internal medicine departments (IM) and 4 surgical departments (S). Annual use rates of intravenously administered AF were measured using defined daily doses per 100 bed-days (DDD/100BD).
Results The median annual AF use rates in ICU, SOT, IM and S departments were 48, 13, 7 and 3 DDD/100BD, respectively. In ICU, an increase of total AF use was documented (from 26 to 98 DDD/100BD, $p < 0.01$). Specifically, azole (AZL) use (mainly fluconazole) was increased from 6 to 40 DDD/100BD ($p = 0.01$). While deoxycholate amphotericin B dramatically decreased, lipid formulations (LFAMB) exhibited a constant increase during the last 3 years ($p = 0.02$). Caspofungin (CAS), the only echinocandin (ECH) available until 2008, was the 3rd most commonly used agent in ICU showing a substantial increase since its first use in 2003 from 2 to 22 DDD/100BD ($p = 0.002$). In SOT, AZL use was decreased from 14 to 2 DDD/100BD ($p = 0.02$) with a parallel increase of ECH use from 0.9 to 6 ($p < 0.0001$). Use of LFAMB had a md of 2.5 DDD/100BD (mainly liposomal AMB). In IM departments, LFAMB were the predominant agents with a md annual use of 8.6 DDD/100BD followed by AZL (2 DDD/100BD). ECH use was low ranging from 0.3 to 1.7. In S departments, AZL were the most commonly used agents with a md of 2 DDD/100BD followed by LFAMB (0.08 DDD/100BD) and ECH (0.03 DDD/100BD).
Conclusion Pattern and trends of AF use significantly vary among hospital departments of a general tertiary care hospital. Substantial increase of total AF consumption in critically ill patients requires continuous monitoring and implementation of antifungal stewardship program.

P306

Synergistic interaction between voriconazole and amphotericin B against *Aspergillus fumigatus* in a pharmacokinetic/pharmacodynamic (PK/PD) model simulating free drug concentrations achieved in human serum

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Objectives Voriconazole is often combined with amphotericin B for the management of refractory invasive aspergillosis. The potential treatment benefit of polyene-triazole combination therapy is controversial because of conflicting in vitro and in vivo studies. However, in these studies pharmacodynamic effects were not assessed at clinically relevant concentrations since in vitro and in vivo studied did not simulated human serum concentration time profiles. We therefore investigated the efficacy of voriconazole in combination with amphotericin B against *A. fumigatus* simulating free drug concentrations achieved in human serum with a new *in vitro* PK/PD model.
Methods The *A. fumigtaus* isolate ATCC MYA-3626 with voriconazole and amphotericin B CLSI MICs of 0.5 and 1 mg/L, respectively, was used. Voriconazole and conventional amphotericin B human standard dosages of 4 mg/kg bid and 1 mg/kg od, respectively were simulated in a newly developed *in vitro* PK simulation model. The *in vitro* model consists of an internal compartment (IC, a 10 mL dialysis tube made out of semi-permeable cellulose membrane allowing the free diffusion of molecules smaller than 20kD) placed inside an external compartment (EC, a 700 mL glass beaker) whose content is diluted with a peristaltic pump at the same rate as the clearance of the drug in human plasma. The IC was inoculated with a conidial suspension (10^3 CFU/mL) and incubated at 37°C for 72 hours, while voriconazole and amphotericin B were injected alone and in combination in both compartments every 12 h and 24 h, respectively at maximum concentrations corresponding to the maximum concentrations of unbound drugs in human serum (fCmax) of 1.7 mg/l and 0.1 mg/l, respectively. Voriconazole and amphotericin B fCmax were determined based on the 58% and 95% protein binding and the mean maximum concentrations of total drugs of 4 mg/l and 2 mg/l achieved in human serum, respectively (Purkins et al AAC 2002, Ayastraran et al AAC 1996). Drug levels were determined by microbiological afar diffusion assays and fungal growth by measuring the galactomannan production in the IC using a commercially available sandwich-ELISA. The% of fungal growth was calculated based on the area under the galactomannan-time curve. The interactions were analyzed based on the Bliss independence model where the observed growth E_{OBS} was compared the theoretical E_{THE} if the two drugs were acting independently as $E_{VOR}xE_{AMB}$ where E_{VOR} and E_{AMB} are the% of voriconazole and amphotericin B alone, respectively. Synergy was concluded when $E_{OBS} < E_{THE}$ (t test $p < 0.05$). All experiments were repeated two times.
Results The model simulated well voriconazole and amphotericin B human pharmacokinetics as drug concentration-time profiles corresponded to those observed in human serum. Both monotherapies of amphotericin B and voriconazole resulted in $86 \pm 8\%$ and $40 \pm 3.6\%$fungal growth indicating that drug concentrations were suboptimal. The two-drug combination resulted in $8.6 \pm 1\%$ growth with Bliss interaction analysis showing a synergistic effect of $25.8 \pm 2.5\%$ ($p < 0.05$).
Conclusion The double combination of voriconazole plus amphotericin B may be synergistic against *A. fumigatus* at clinically achievable serum concentrations, enhancing the overall efficacy of polyene+azole combination therapy particularly in patients with suboptimal drug concentrations.

P307

Higher than predicted posaconazole penetration at the infection site in a murine model of invasive pulmonary aspergillosis

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Objectives Invasive aspergillosis (IA) is an important opportunistic fungal infection in immunocompromised patients with the overall mortality ranging between 30 to 88%. Inhalation of *Aspergillus fumigatus* conidia is the most common route of infection in IA, therefore adequate drug penetration to the infection site is crucial for optimal efficacy. We aimed to determine posaconazole (POS) concentrations in pulmonary epithelial lining fluid (ELF) and compare to the blood plasma levels.
Methods A total of 96 outbred CD-1 immunosuppressed mice were used. Animals received 0, 4, 8, 16 or 32 mg/kg of body weight POS once daily at days -2, -1, and 0 by oral gavage. On day zero mice were infected with the *A.fumigatus* isolate (MIC POS 0.5 mg/l) through instillation of conidial suspension in the nares. Blood and BAL samples were drawn at 8 predefined time points postchallenge (0, 0.5, 1, 2, 4, 8, 12 and 24 h, 3 mice per each time-point). POS concentration in blood and BAL were assayed by a validated HPLC method. The concentration of POS in ELF was calculated by determination of blood and BAL urea concentration utilizing QuantiChrom™ Urea Assay Kit (DIUR-500)(BioAssay Systems).
Results All 96 mice were alive at the time of the sample collection from 0 to 24 h post challenge and in total 288 samples were obtained. The maximum total drug concentrations (C_{max}) of POS in plasma were 5.51, 8.70, 10.92 and 15.04 mg/L, while lower amounts were obtained in ELF (1.58, 2.83, 3.62 and 5.32 mg/L) for dosages of 4, 8, 16 and 32 mg/kg, respectively. The area under the concentration-time curve from 0 to 24 h ($AUC_{0\text{-}24}$) for plasma was correlated significantly with the dose in a linear fashion from 4–32 mg/kg ($r^2 = 0.94$). The $\log_{10}$ $AUC_{0\text{-}24}$ for plasma were 1.87, 2.18, 2.30 and 2.47(hr.mg/L) and for ELF were 1.18, 1.63, 1.80, 1.61(hr.mg/L), resp. A significant correlation between POS concentration in blood and ELF was noted by linear regression analysis ($r^2 = 0.75$, $P < 0.0001$). Penetration of POS in ELF was calculated with respect to plasma results in a ratio (ELF: plasma $AUC_{0\text{-}24}$ ratio) that was between 0.14 and 0.31.
Conclusion Although posaconazole is highly protein bound, ELF concentrations were relatively high. The high intrapulmonary penetration of POS may be an advantage for protection against diseases caused by both azole susceptible and azole resistant *A.fumigatus* with MIC higher than susceptible breakpoints to POS.

P320

Embryonated eggs as an alternative infection model to investigate *Rhizopus microsporus* virulence

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Objective Infection models are essential tools for studying microbial pathogenesis. Murine models are considered the "gold standard"for studying in vivo infections. Cell culture or invertebrates are widely used for screening as well. To reduce the number of rodents in animal experiments and to bridge the gap between invertebrate models and mice, we have developed an alternative, low-cost, and easy-to-use infection model for *Rhizopus* species based on embryonated eggs.

The genus *Rhizopus* was first described in 1821 by Ehrenberg and belongs to the order Mucorales in the Zygomycotina. Since ancient time this group of fungi has been used for production of fermented food, especially in Far East Asian countries. *Rhizopus arrhizus* (*R. oryzae*) and *R. microsporus* are widely used for production of oriental fermented foods, but these species are also causative agents for severe human infections known as mucormycosis; at present this is the third most frequent, potentially fatal opportunistic infection in human. This group of fungi shows a ruderal strategy and can cause spoilage in agricultural products and diseases in plants.

Method Virulence potential of *R. microsporus* species was investigated using 20 strains from different sources including clinical as well as non-clinical samples. The strains were truly identified by phylogenetical approaches in advance and belonged to different geographical distinct. 10^6 spores / egg dilution from 2 days old culture were prepared and inoculated in chrioallantoic membrane. Incubation was performed at 37.6°C and 50 to 60% relative humidity in a specialized incubator (BSS 300; Grumbach, Germany) and post infection viability was monitored once a day for 7 days by candling method. PBS was used as a negative control and survival data were plotted as Kaplan-Meyer curves and were analyzed statistically by a log rank test using Graph Pad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA).

Results The outcome of infections in the egg model is highly reproducible. All strains showed high level of mortality between 60–100% regardless of source of isolation and behaved similar in terms of mortality pattern and speed. There was no side effect observed due to manipulations based on the negative control ones.

Conclusion Our results approved the opportunistic feature of these fungi and the fact that if they find a susceptible host they can be highly mortal even those strains that were isolated from food stuffs. As there was no mortality observed in negative controls, the method could be standardized easily and looking to the literatures it can be a good alternative in animal model tests.

[Correction added on 7 November 2013, after print publication and first online publication: The first author's name was changed to S. Dolatabadi and the affiliation for both authors were changed to *CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands* in Abstract P320.]

P321

Posaconazole prophylaxis in acute myeloid leukemia. Real life experience in four hematological centers

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Introduction Acute Myeloid Leukemia (AML) patients (pts) are at high risk of Invasive Fungal Diseases (IFD). Posaconazole (POS), an oral azole with a broader spectrum, was approved for the prophylaxis of IFD in high risk hematologic pts. We report a multicenter real-life experience with POS prophylaxis in AML. We also compare the performance of POS with an historical, well matched, control group of AML pts who received prophylaxis with Fluconazole (FLUCO) or Itraconazole (ITRA).

Patients and Results 120 unselected and consecutive AML pts (96 with active disease and 24 in CR) received POS prophylaxis (600 mg daily), between Jan 2009 and Dec 2012, in 4 Hematological Centers. Median age was 52 yrs (range 18–69). All cases were given intensive chemotherapy. The POS was started when neutrophil (PMN) count was less than 1000 mL and was stopped at PMN recovery. The median duration of severe neutropenia (PMN lower than 500 mL) was 16 days (range 7–71); 27/120 (23%) of cases had an oral mucositis grade II-III CTC and 74% of these pts received a proton pump inhibitor. An active diagnostic work up was made in all cases with GM assay, standard chest X-ray and thoracic CT scan in case of FUO lasting over 48 hours. The median duration of POS was 16 days (range 7–41). Only 17/120 (14%) of pts required parenteral empiric or pre-emptive antimycotic therapy and only 6/120 (5%) experienced a probable or proven IFD. Mortality IFD related was 0%. POS was well tolerated and only 8% of pts experienced mild drug related side effects. No cases of POS discontinuation were reported. When we compare the 120 pts who received POS with an historical control group of 120 AML pts who received FLUCO or ITRA prophylaxis, no significant differences were observed for underling disease status, age, IFD risk factors, days of severe neutropenia and days of prophylaxis. Instead, there were significant differences in number of cases who required parenteral antymicotic therapy (14% in POS group vs 25% in the control group; $P = 0.01$), and in days of parenteral antymicotic therapy (151 vs 374, with a saving of 223 days).

Conclusions This real-life experience confirms that POS prophylaxis is safe, well tolerated and effective in unselected AML pts. Only 14% of these high risk pts required parenteral antimycotic therapy and only 6/120(5%) experienced probable (5/120) or proven (1/120) IFD. We also confirm that POS, in real life setting, is more effective than FLUCO/ITRA as antifungal prophylaxis in AML pts.

P322

Different doses of micafungin for prophylaxis of invasive fungal diseases: A web-based non-interventional trial in four large university hospitals in Germany

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Objectives Treatment indications of new antifungals in clinical practice often deviate from the strict criteria used in controlled clinical trials. Under routine clinical conditions, beneficial and adverse effect not previously described in clinical trials may be observed. The aim of this study was to describe customary prescription and treatment strategies of micafungin (MIC).

Methods A registry was set up on www.ClinicalSurveys.net and physicians from German tertiary care centers were invited to provide retrospective information on cases they had treated with MIC. Documentation comprised demographic information, underlying disease, efficacy, safety, and tolerability of MIC. Types of invasive fungal disease (IFD) were defined by the EORTC/MSG criteria.

Results A total of 125 episodes of patients hospitalized between 10/2009–01/2012 were documented, of which seven episodes had to be excluded due to incomplete documentation. The most common underlying disease of patients receiving MIC was hematological malignancy (116, 98.3%), followed by stem cell transplantation (104, 88.1%). Risk factors for contracting IFD were antibiotic treatment > 3 days (115, 97.5%), central venous catheterization (112, 94.9%), parenteral nutrition (50, 42.4%), and other (66, 55.9%). Micafungin was administered as prophylaxis (PPX) in 106 (89.9%) and for treatment of possible, probable or proven IFD in 12 (10.1%) patients, respectively. In the group of antifungal PPX, mean duration of MIC treatment was 22.8 days (95% CI: 20.4 - 25.3); 53 of the patients (50%) received a dosage of 50 mg/day, while the other 53 (50%) received 100 mg/day. For the different doses, prophylactic outcome was rated as success in 42 (79.2%) vs. 52 patients (98.1%; $p = 0.002$). Fifty-five patients (51.9%) were treated with posaconazole before initiation of MIC, 30 (28.3%) also received amphotericin b inhalation during PPX with MIC. Four patients (3.8%) developed a proven IFD while being treated with 50 mg/day MIC, compared to no patients treated with 100 mg/day. At the end of MIC PPX, 24 (22.6%) patients were switched to fluconazole and 61 (66.1%) patients to posaconazole. Six patients (5.7%) died due to unknown or other reasons than an IFD.

Conclusion Our study demonstrates clinical effectiveness of MIC PPX in patients at high risk of contracting IFD. In most cases, MIC was part of a multi-modal antifungal PPX strategy. Investigators reported better outcomes in patients receiving therapeutic doses of MIC for PPX.

P323

Antifungal prophylaxis with posaconazole and micafungin bridging in allogeneic SCT recipients

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Introduction Invasive fungal diseases (IFDs) are an important cause of morbidity and mortality in patients undergoing allogeneic stem cell transplantation (SCT).
Methods To compare the clinical efficacy of two prophylactic antifungal regimens used as standard of care (SOC) in the setting of SCT during the periods of May 2006–September 2009 (oral posaconazole, POS) and October 2009–July 2011, (oral posaconazole with intravenous micafungin bridging, POS-MIC), data from the Cologne Cohort of Neutropenic Patients (CoCoNut) study were analyzed. The primary endpoint was the occurrence of breakthrough probable/proven IFD under prophylaxis. Among secondary endpoints were incidence and duration of persistent febrile neutropenia, incidence of unspecific pneumonic infiltrates, possible IFD, positive galactomannan tests, as well as fungal-free and overall survival.
Results Of 274 SCT observed during the study period 234 were included into the analysis. Patients receiving POS-MIC were less likely to present with an unspecific pneumonic infiltrate (36.3% and 23%; P = 0.025) or an infiltrate typical of IFD (15.9% and 5.2%; P = 0.006). They also experienced significantly less febrile days (6.15 and 4.43; P = 0.018) and improved fungal-free survival at day 100 (p = 0.031). No significant differences were observed for the incidence of probable or proven IFD, positive galactomannan tests, persistent febrile neutropenia, duration of hospitalization and overall mortality. There was no grade III or IV CTCAE toxicity related to antifungal prophylaxis.
Conclusion Our results suggest that both prophylactic regimens, POS and POS-MIC are feasible, safe and effective, whereas the POS-MIC group additionally profited from a significant reduction in infection-related endpoints.

P324

Azole-based chemoprophylaxis of invasive fungal infections in pediatric patients with acute leukemia: an internal audit

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Background Incidence rates of invasive fungal infections (IFI) of >10% and case fatality rates in the order of 50% justify systemic antifungal prophylaxis (SAP) in children and adolescents with acute myeloid leukemia (AML) and recurrent acute leukemia (RAL).
Methods Oral azole-based SAP has been implemented in 2006 as standard of care (SOP) in pts. with AML and RALs admitted to our department. Pts. ≥ 13 yrs receive posaconazole (200 mg TID), and pts. ≤ 12 to 2 yrs voriconazole (200 mg BID) starting upon completion of chemotherapy until hematopoietic recovery. Pts. with inability to take oral medication/ contraindications are placed on IV voriconazole, liposomal amphotericin B or micafungin as appropriate. Algorithms for neutropenic fever or signs and symptoms of infection include monitoring with blood cultures, high resolution computed tomography (HR-CT), and, in patients with lung infiltrates, serial serum galactomannan, invasive diagnostics and preemptive therapy with change in class.
Results From 2006–2010, 40 new pts. (median: 9 yrs; r, 0.8–17; 21 m, 19f) with AML (31) or recurrent leukemia (9) were admitted. 36 received a total of 149 courses of chemotherapy (reasons for not receiving prophylaxis: Contraindications/early death ≤ 3 days, resp.). The target attainment of episodes with azole prophylaxis was 87.2% (n = 130/149). Preemptive therapy for pulmonary infiltrates was initiated in 5/36 (13.8%) patients or 6/130 (4.6%) episodes for a mean duration of 12 days (range, 3–22). No proven or probable IFIs occurred. Antifungal prophylaxis was tolerated without grade III/IV toxicities. In simultaneously admitted new pts. with acute lymphatic leukemia (n = 101) and pediatric lymphomas (n = 29) not receiving SAP per SOP, proven/probable IFIs occurred in 4 pts. with ALL (3.9%), and 5/130 pts. (3.8%) received preemptive therapy.
Conclusions Azole-based SAP in high risk pts. with AML/RALs was satisfactorily implemented, well tolerated and effective with no breakthrough infections and few and short courses of preemptive treatment. The low rate of IFIs in patients with ALL and lymphomas confirm the lack of indication for general SAP in these populations at our center.

P325

Monitoring plasma levels of prophylactic posaconazole: is it worthwhile?

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Objectives Posaconazole has secured a place in the therapeutic and prophylactic approach of invasive fungal infections. Increasing evidence supports an exposure-response relationship for plasma posaconazole concentrations for prophylaxis and treatment. In view of the high mortality and cost associated with the treatment of invasive fungal infections, the poor and variable bioavailability of posaconazole and the common finding of insufficient plasma concentrations, therapeutic drug monitoring (TDM) has been suggested to be of clinical use. No guidelines are available to deal with posaconazole TDM in clinical practice. Due to saturable absorption of posaconazole, dose modifications have no effect. Actions to increase plasma levels are mainly based on modifying external factors that might improve bioavailability. In this observational study we wanted to investigate if such an approach, i.e. focusing on intake advice and drug interactions, helps to obtain prophylactic plasma levels.
Methods AZ Sint-Lucas is an 800-bed secondary care hospital with a hematology-oncology ward of 30 beds. In the period of Sept 2011 until Jan 2013, 12 hematologic patients were included in the study. All patients received posaconazole prophylaxis (200 mg three times a day) during the treatment of their acute myeloid/lymphoid leukemia. Clinical pharmacist, microbiologist, hematologist and dietician took part of a team to optimize posaconazole prophylaxis. Based on posaconazole levels, a protocol was set up to organize the collaboration between the different disciplines (Figure 1). First plasma posaconazole levels were obtained between day 7 and day 11 of dosing. Thereafter, samples were analyzed once-weekly using an UPLC-based method for the quantification of posaconazole. The prophylactic cut-off was set at 0.7 mg/l.
Results In total 68 samples from 12 patients were analyzed with a mean of 6 plasma samples per patient (Figure 2). Only 4 patients (33%) had a first posaconazole level above the prophylactic target. Eight patients had a first sub-prophylactic level, of whom 2 patients, after counseling, obtained higher posaconazole levels. Despite multiple interventions, 6 patients never obtained more than one posaconazole level above 0.7 mg/l. The serial measurements of low posaconazole levels resulted in multiple consultations, without direct effect on the posaconazole level. A majority (n = 4) of these 6 patients had problems that could not be readily adjusted, like

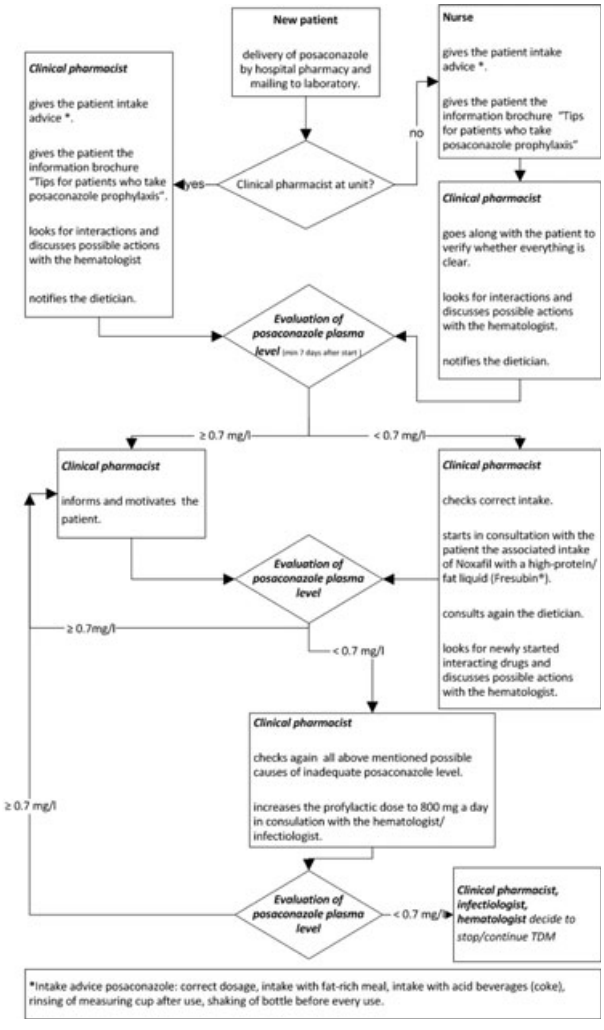

Figure 1 Flowchart posaconazole protocol based on therapeutic drug monitoring levels.

Table 1 Patient characteristics and posaconazole plasma levels.

				Posaconazole level (mg/l)											
patient	age (years)	AML/ALL	gender	first	second	third	last	number of samples	lowest posaconazole level	highest posaconazole level	effect of multiple dosing approach?	cause for low level	fungal infection during prophylaxis?	last posaconazole level before start antifungal therapy?	Number of days between last posaconazole level and diagnosis?
1	54	AML	M	<0,2	0,2	<0,2	<0,2	3	<0,2	0,2	no	compliance	-	-	-
2	61	ALL	M	2,7	1,3	2,2	2,6	13	1,3	1,3	no	-	-	-	-
3	59	AML	F	<0,2	<0,2	<0,2	<0,2	3	<0,2	<0,2	no	diarrhea	-	-	-
4	34	ALL	M	0,8	1,4	1,7	2,1	11	0,73	2,2	yes	-	-	-	-
5	70	AML	M	0,4	0,7	1,4	0,7	8	0,4	1,4	yes	diarrhea	-	-	-
6	71	AML	M	0,3	0,6	<0,3	0,4	4	0,3	0,6	no	mucositis	-	-	-
7	66	ALL	M	0,6	1,5	2,1	2,1	3	0,6	2,1	yes	-	probable IA	2,1	13
8	70	AML	M	0,6	0,5	0,9	0,3	5	0,3	0,9	no	mucositis	-	-	-
9	68	AML	M	0,7	1,2	1,0	1,0	3	0,7	1,0	no	-	possible IA	1,0	11
10	79	AML	F	0,6	0,6	<0,2	<0,2	4	<0,2	0,6	no	no	-	-	-
11	50	AML	F	0,2	0,3	0,2	0,3	8	0,2	0,6	no	no	-	-	-
12	79	AML	F	2,1	1,5	2,8	2,8	3	1,5	2,8	no	-	-	-	-

AML (acute myeloid leukemia), ALL (acute lymphoid leukemia), IA (invasive aspergillosis)

diarrhea or mucositis. For the other patients no clear explanation for the persistent low posaconazole levels was found.

Conclusion Despite multiple actions to optimize bioavailability of posaconazole, only 50% of the patients achieved stable prophylactic plasma levels. TDM may give an indication of the efficiency, but in clinical practice, only a limited number of interventions can be undertaken to increase the level successfully. In patients not attaining prophylactic levels, a lot of time and cost is invested in TDM and coupled actions, with only minimal effect. Moreover, there is not enough evidence to stop posaconazole prophylaxis based on low plasma levels. Currently we do not consider TDM to be an added value. However, future studies are needed to decide if monitoring of posaconazole in prophylactic setting is useful, and how we have to deal with low posaconazole levels.

P340

Retrospective study of oropharyngeal candidiasis in patients receiving treatment in Minsk public health centers (Belarus)

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Purpose of the study To analyze the results of laboratory tests used to diagnose Candida infection from oral and pharyngeal mucosa from patients receiving treatment in public health centers in Minsk.

Materials and methods An analysis of the results of bacteriological tests oral and pharyngeal mucosa received from scrapings was conducted in order to determine Candida species and other yeast-like fungi and their resistance to antimicrobial drugs during the years 2005–2011. The test results were provided by the microbiological laboratories which conduct research on biomaterial collected from 86 health centers in the city of Minsk. Automated biochemical tests and disk-diffusion methods were used to identify the species and determine antibiotic susceptibility. Statistical analysis was conducted with the use of BioStat 2009 5.8.4.

Results A 7-year period studied revealed a total of 6786 positive results for yeasts. The most frequently identified pathogen was C.albicans – 73.02 ± 0.54% of all cases, whereas non-albicans spp. (C.tropicalis, C.pseudotropicalis, C.guilliermondii, C.crusei) were identified in 25.29% ± 0.57% of cases, and other yeast-like fungi (Cryptococcus laurentii, Saccharomyces cerevisiae) appeared in only 1.69% ± 0.09% of cases. Because not all microbiological laboratories register negative results in their databases it is difficult to determine the percentage of cases in which yeasts are detected when confirming preliminary clinical diagnosis in laboratory. According to the City Skin and Venereal diseases Center, where 14.7% (n = 998) of all positive results in our study were obtained, the yeasts were verified microbiologically in 41.93% of patients who received a preliminary diagnosis of oropharyngeal candidiasis.

The results of the yeasts sensitivity to antibiotics were hard to compare because of different methods and various antifungal drugs used across laboratories. The results of susceptibility testing of C. albicans as the most common pathogen were analyzed. It was determined that approximately 90% of the isolates were sensitive to polyene antifungal drugs (96.9% of the isolates were sensitive to amphotericin B, and 91.4% – to nystatin). The strains studied were more resistant to azole antifungals, and the highest resistance observed was to fluconazole (62% of strains), followed by ketoconazole (45.2%), clotrimazole (33.3%), and finally, itraconazole (12.5%).

Conclusions The most frequently identified type of yeasts, obtained from the oral cavity and pharynx, is C.albicans (73.02%). However, the absence of Candida, primarily C.albicans, in the results of these laboratory investigations should not exclude the presence of other pathogens, which can cause oropharyngeal yeast infections. Therefore, a detailed study on various types of yeasts is required when conducting their identification. The fact of using different laboratory methods doesn't always make it possible to analyze total epidemiological accuracy. While conducting laboratory confirmation of the diagnosis of oropharyngeal candidiasis it is necessary to study the sensitivity of yeasts to antimycotic drugs in order to provide information for therapy and avoid failure in the subsequent treatment.

P341

Clinical features of onychomycosis due to *Trichophyton rubrum* infection in patients with noninsulin-dependent diabetes mellitus

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Dermatophytosis due to *Trichophyton rubrum* is the most common mycosis in the world. Onychomycosis and tinea pedis are significant predictors in the development of foot ulcers at patients with noninsulin-dependent diabetes mellitus (DM).Therefore, early diagnosis of onychomycosis is important to prevent complications.

Objectives It was examined 54 patients with diabetes and Onychomycosis due to *Trichophyton rubrum* infection (13 men and 41 women) aged 42–75 years with duration of mycotic nail infection 3 to 45 years (group I). The diagnosis was confirmed by laboratory methods (microscopy and culture). Age of noninsulin-dependent diabetes mellitus (DM) was 2 to 17 years.

Results The group II consisted of 26 patients with Onychomycosis due to *T. rubrum* infection with duration of disease 6 months–18 years (mean 7.5 ± 0.9 years) who did not have DM. A comparative analysis of two groups of patients showed the following clinical features of Onychomycosis in DM.

All patients with DM (group I) had foot deformities as aflattened foot, hallux valgus.Onychomycosis was more severe and was more prevalent on the foot, which was greater deformations. Patients ofthe group II did not have foot deformations.

Distal lateral subungual onychomycosis (DLSO) had 57.1% patients of group I and 78% patients of group II. Onychomycosis in patients with DM started with DLSO of one toe and quickly spreaded to all the nails of the foot. If onychomycosis preceded DM this trend was not found. Total dystrophic onychomycosis (TDO) was diagnosed at 42.9% patients of group I and 12% patients of group II. TDO 10 toes had more than half of the patients of group I.

Conclusion Thus, Distal lateral subungual onychomycosis was frequently diagnosed in both groups. Total dystrophic onychomycosis occurred in patients with diabetes in 3.5 times frequently than in the control group ($p < 0.05$). A characteristic feature was microtraumas of proximalis and lateralis nail folds caused by sloppy pedicure by the patient. These microtraumas could trigger deterioration in patient quality of life due to the complications induced by secondary bacterial infections.

P342

Tinea pedis in adult serbian patients: epidemiological and etological study

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Introduction Tinea pedis is a fungal infection of the foot caused by dermatophytes in the genera Trichophyton and Epidermophyton. There has been a significant increase in the incidence of tinea pedis infection in Europe, and if is not diagnosed and treated properly, it is prevalence might reach epidemic proportions in the near future.

Aim The aim of this study is to determine prevalence, epidemiology and etiology of tinea pedis in our patients.

Material and methodes This study was conducted in the Clinic of Dermatovenerology, Clincal Center of Serbia during period of one year (2009 - 2010), and it included adult patients with tinea infections. Samples were examined by microscopy using potassium hydroxide and were cultured on mycological media. The dermatophytes were identified by their macroscopic and microscopic characteristics.

Results Out of 123 patients with dermatophyte infections, we identified 40 (25.49%) patients with tinea pedis. Out of 40 patients, 23 were female (55.26%) and 17 male patients (43.59%). The highest frequency of disease was identified in the age 51–60, 12 (30%) and the lowest in the age 31–40, 3 patients (7.5%). Trichophyton mentagrophytes downy type (37.50%) was predominant pathogen isolated, followed by Trichophyton rubrum (25.00%), Trichophyton mentagrophytes granulosum (17.50%), Trichophyton mentagrophytes spp.(10.00%), Trichophyton spp. (7.5%) and Trichophyton terrestre (2.50%).

Conclusion Tinea pedis was the second most common dermatohytoses, and it more often infects middle age women. The principal fungus responsible for tinea pedis was Trichophyton mentagrophytes downy type and Trichophyton rubrum. All patients were infected by dermatophytes in genera Trichophyton. In the etiology of tinea pedis participated antropophyllic and zoophyllic dermatophytes. According to the literature, Epidermophyton floccosum is one the most frequet dermatophyte causing tinea pedis, but in our study Epidermophyton floccosum was not isoleted.

P343

Cutaneous eumycetoma caused by *Phialemonium curvatum* in a woman successfully treated with surgery and oral itraconazole

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A 76-year-old woman had medical histories of acute appendicitis and cecal necrosis after surgery, and vaginal and pulmonary tuberculosis had treated with Rifater and ethambutol for six months. She suffered from a progressive skin lesion on her hand for three months. It developed after a trauma during her agriculture activity. Upon physical examination, the lesion consisted with a firm, tender, movable subcutaneous nodule with overlying erythema and erosion on patient's right dorsal hand. Histopathology of skin biopsy showed a dense diffuse mixed-cell inflammatory infiltration with microabscesses in the dermis. There were sulfur granules composed of PAS-positive fungal hyphae in the center of microabscesses. A fungus grew from the culture of the skin specimen. The colony had a fluffy white surface and grey reverse. It was identified as *Phialemonium curvatum* by its microscopic characters and sequencing of internal transcribed spacers of ribosomal DNA. The subsequent pus cultures from the wound all grew the same fungus. The patient was treated with itraconazole 200 mg per day for ten weeks. The erythema subsided gradually, but the subcutaneous nodule persisted. The lesion was totally excised and sent for pathologic examination again. One piece of plant debris and a few small granules were seen in the microabscess surrounded by dermal fibrosis. One week of itraconazole of same dosage was given after surgery. No recurrence was noted at the last follow-up two months later.

P344

Superficial mold infections; a patient's ordeal

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Molds are considered opportunistic pathogens. When we speak about mold infections, we refer mainly to *Fusarium* and *Aspergilli* infections in immunocompromised hosts. Yet they account for the greatest rate of morbidity in healthy individuals. The cases of immunocompromised patients constitute only the tip of the iceberg among more general cases of infection. The prevalent mold pathology in immunocompetent individuals affects the nail unit.

Skin lesions of healthy patients present features similar to the ones of the immunocompromised. The only difference consists in their intensity and extension. A thorough knowledge of lesions in healthy patients is crucial both for preventing further development of chronic morbidity and for making possible an early diagnosis in immunocompromised patients.

Objectives Stressing the high rates of mold superficial infections and their oddity,and complexity concerning their multiple manifestations.

Pointing out our high cure rates – actually almost 100% of recovery, when practicing social medicine (that is personalized medicine sustained by a more active role on the part of the physician).

Methodology Our research is based on 26 years of mycological experience in dermatology ward. We followed the patient from the diagnosis towards complete recovery.

All the mycological positive results were confirmed by at least another exam and checked for clinical features stringency.

Results The extreme variety of the pathology's clinical features was observed in healthy hosts (some are still scarcely known and other are not yet reported).

Neuropsychotic Syndrome
*Fusarium*stomatitis
Gastrointestinal pain
Latent infections
Relapses
Site specific infections
Isolated paronychia
Paronychia without paronychia
Segmental infections
Family infections
In pregnancy
In teenagers
On angiomatous tissue
Locally very aggressive
Minimal infections (highly misleading)
Sovrainfected melanoma
Mixed infections
Abscesses
Rare molds: *Eutypella scoparia, Fusarium guttiforme/ananatum, Gymnoascus dankaliensis*

Conclusions Superficial mold infections greatly impact patients' wellness.

Despite their diagnosis being challenging (even for skilled physicians), nonetheless, in our area, podiatrists, cosmetologists, pharmacists and, in the best case scenario, general practitioners often find themselves dealing with such clinical challenge. Unfortunately patients often seek for the professional help of dermatologists, as their very last resource.

The required mycological exams are seldom carried out and often wrongly interpreted, because they are administered and evaluated mainly by biologists and microbiologists.

The knowledge on the part of physicians of the wide clinical spectrum of mold superficial infections remains unsatisfactory.

These difficulties, and, mostly, the fact that the professional expertise of dermatologists is rarely sought for, produce an inadequate management of this pathology and, ultimately, a clinical ordeal for most that many patients affected by this pathology.

We would like to call the clinicians' and health managers' attention to this pathology, in order that they might organize the most effective medical response to this issue. Above all, we want to stress the importance of social medicine for the management of this pathology. Only this approach can guarantee high figures of complete recovery. It is paramount to regain the trust of the public and redirect it towards specialized clinicians.

P345

The survey of presence *Candida* Spp in dividuals skin with clinical manifestation of acne referred to dermatology clinic of Sina hospital in Sari

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Objectives Candida is one of the etiologic agent of skin disease like Cutaneous Candidiosis and its also opportunistic pathogen. The microbial colonization and inflammatory response due to extra cellular exudes and superficial antigens are one of the main etiologic of acnetic lesions. In the other hand it was proven that candida is capable of producing skins disease and by considering that North of Iran condition is suitable for skin lesions and candida growth and also lack of any investigation in this field, this study was made to evaluated presence of candida in patients with acne and identify candida species in inside and surface of acne.

Methods 125patients(70female and 55male)enrolled in this study. The sample were collected from inside and surface of acne lesions and mounted by KOH to direct microscopic examination .Samples were also cultured on sabouraud dextrose agar with Chloramphenicol (Sc) .The isolated species were identified by morphologic and physiologic examination include:culturing and Scc media and chrom agar candida,germ tube test, chlamydospoe forming on cornmeal agar,growth in 45ċ and identification kit Hicandida.

Results Our finding showed that 57case were positive for yeast cells in direct microscopic examination .In total,45samples were positive in cultures that 11cases isolated from inside acne, include:*Candida parapsilosis,C.krusei, C.glaberata,C.dublininsis,C.kefy* and 34casesisolated from surface acne include:*Candida krusei,C.parapsilosis,C.guillermondii, C.dublininsis, C.albicans,C.glaberata,C.kefyr,C.zeylanoidea* .In 5cases candida were detected in both inside and surface of acne lesionsthat in one case the species were identify.

Conclusion In this study varied candida species isolated from skin acnetic patients but by considering that samples in this study were limit which can effect on result too our knowledge and there is no study in this field so it is recommended to design some more specific studies in different areas in the future.

P346

A case dermatophytosis: relapse or reinfection?

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Case report We describe a case of a 62-years-old immunocompetent patient with a 2-year history of relapsed dermatophytosis. In December 2009, the patient was referred to the Mycology Laboratory of the Dermatological Clinic of University of Naples "Federico II"with a one-year history of itching erythematous-desquamative lesions located mainly in buttocks and groin area. When the patient came to our attention, an accurate physical examination showed the presence of lesions even on toenails and interdigital spaces of the feet. The anamnesis did not reveal other pathologies and patient denied to have case in family of dermatophytosis. The mycroscopic examinations revealed the presence of fungal hyphae, whereas the cultural exam highlights the develop of colonies typical of *Trichophyton mentagrophytes*. Diagnosis of tinea corporis, tinea cruris, tinea pedis and tinea unguium was made. Griseofulvin 500 mg/die for 4 weeks, topical ciclopirox and nail polish amorolfine on nails for 3 months were prescribed.

In September 2010 symptoms reappeared and mycroscopical examinations were positive again. Before starting therapy, liver function tests

were asked and resulting in the normal ranges. Griseofulvin 1 g/die for 6 weeks and tintura rubra in the groin area for 2 weeks, and toenail polish amorolfine on nails for 3 months were prescribed. After 2 months the patient came for a control; mycroscopic examinations of buttocks, groin area, interdigital spaces and toenail were performed resulting negative except the toenails one. Itraconazole 400 mg/die for one week a month for 3 months and toenail polish amorolfine for 3 months were prescribed. Moreover a chemical onycholysis was made.

Lesions and symptoms reappeared in June 2011 again. Patient was submitted to the Department of Immunology ofUniversityofNaples"Federico II"to better understand the therapy resistance with specific tests. No alterations of humoral and/or cell mediated immunity were found.

In November 2011, the lesions reappeared and the mycological examinations were still positive too. We started to hypothesize the possibility of a series of reinfections; so we decided to investigate all the possible familiar source of infection for a better understanding of this atypical case of resistance in an immunocompetent patient. The physical examination of her husband showed suspected lesions on feet and toenails; hence mycroscopic examinations were done: the cultural exam highlights the develop of colonies of *Trichophyton mentagrophytes.*

A systemic and topical antimycotic treatment was prescribed for both the patient and her husband.

At the follow-up of February 2012 lesions disappeared and mycological examinations were negative.

Conclusions The present case highlights the importance of a careful anamnesis of the patient and of the other members of his/her family, especially of partners. We conclude that in all immunocompetent patients unresponsive to antimycotic treatment is important to evaluate the possibility of a reinfection for the presence of a source of infection among other family members, rather than a relapse.

[Correction added on 7 November 2013, after print publication and first online publication: The names of the second, third and fourth authors have been changed to M. Cantelli, A. Patalano and E. Fiammenghi respectively, in Abstract P346.]

P347

Many faces of pityriasis versicolor

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Introduction Pityriasis versicolor is a superficial mycoses that affects nearly 1% of the general population and has an incidence of up to 50% in some tropical climates. In temperate climates, eruptions occur more commonly in the summer than in the winter months.

Pityriasis versicolor is caused by dimorphic, lipophilic yeast, *Pityrosporum orbiculare*, which are part of the normal skin flora. Clinical disease occurs when these organisms convert from their saprophytic yeast form to their pathogenic mycelial or hyphal form.

Clinically, pityriasis versicolor presents as multiple well-demarcated, scaly, oval-to-round hypo- or hyperpigmented macules that frequently coalesce into larger patches. The scale on the patches is usually subtle and is often best visualized by gently scraping the skin with a scalpel blade, the edge of a glass slide, or a fingernail. As the term *versicolor* implies, the lesions can be of varying colors, such as white, pink, tan, light brown, and dark brown.

The lesions are typically distributed on the upper trunk, upper arms, and neck; however, lesions in unusual locations (face and legs) may present some diagnostic difficulties.

Less typical cases of pityriasis versicolor may present lesions that are *guttata* or *perifollicular* (1–2 mm in diameter), *nummular* (not confluent coin shaped and size patches), "*erythrasmoid*" (localized in body folds), "*circinate of Jadassohn*" (patches with well-defined slightly raised edges with scaling and erythema and pale centers), "*nevoid* " (a single brownish with clearly defined edges and desquamation that is not even noticeable after being starched), *papular* (with flat papular elements) and *atrophic*, sometimes secondary to steroid treatment.

Objectives The purpose of this study was to evaluate the incidence of atypical forms of pityriasis versicolor observed from 2003 to 2012.

Methods The retrospective study was conducted on a total of 480 patients suspected to have a pityriasis versicolor over a 10-year period (2003–2012), in order to evaluate atypical forms of the disease.

The patients referred to the Mycology Laboratory of Dermatological Clinic of University of Naples "Federico II", Campania, Italy; they were subjected to a screening by a Wood's Lamp examination and clinical diagnoses was confirmed by direct microscopic examination of scales after maceration in 20% KOH and by cultures on Sabouraud -dextrose-agar with olive oil.

Results Of the 480 patients examined, 365 (76%) were affected by pityriasis versicolor. 160 patients (44%) presented atypical form of pityriasis versicolor.

We underline in the last 5 years a 20% increase in the incidence of the disease.

Conclusion The diagnosis of pityriasis versicolor is mainly clinical.However, there are atypical forms in which mycological examinations are essential for the diagnosis. The sealing of the climate in the Mediterranean area resulted in an increased incidence of pityriasis versicolor, especially of atypical forms.

[Correction added on 7 November 2013, after print publication and first online publication: The names of the second and third authors have been changed to A. Patalano and E. Fiammenghi respectively, in Abstract P347.]

P348

Change of etiology of recurrent vulvovaginal candidiasis in women in Saint Petersburg, Russia

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Objectives Recurrent vulvovaginal candidiasis (RVVC) is a major clinical problem. Results of treatment of RVVC not always are satisfactory. According to some data failures of therapy of RVVC can be associated with an increasing role of fluconazole-resistant *Candida* spp. Aim of the study – to examine etiology of RVVC and in vitro fluconazole susceptibility of pathogens.

Methods RVVC was defined as four or more episodes of vulvovaginal candidiasis during 12-month period. Diagnosis of RVVC was based on association of clinical symptoms (vulval pruritus and burning, "cottage-cheese" vaginal discharge, vulval oedema, vulval and vaginal erythema) and standard laboratorial tests (exposure of budding yeasts cells, mycelium / pseudo mycelium by microscopy of vaginal smears, growth *Candida* spp. on Sabouraud dextrose agar). Isolates were identified by standard morphological and biochemical methods. In vitro susceptibilities to fluconazole of vaginal isolates were determined by disk-diffusion test according to the CLSI M44-A protocol. Statistical data manipulation was carried out by of methods of parametric and nonparametric statistics.

Vaginal *Candida* spp. isolates (n = 904) were obtained during prospective comparative clinical study (2007–2013) of 904 immunocompetent HIV-negative women with RVVC in age from 16 to 65 years (mean age – 29.3 ± 6.5 years). 251 immunocompetent HIV-negative women with RVVC in age from 15 to 70 years (mean age – 30.5 ± 8.7 years) were the group of "historical"control (2003–2006). Duration of disease in the main group (n = 904) was at the mean 5.8 ± 4.7 years (vs.3.8 ± 3.3 years in the group of "historical"control, $p < 0.05$).

Results *C. albicans* was the main etiologic agent of RVVC in both groups (90.4% in the main group vs. 83.3% in the control,group, $p < 0.05$). In the main group other pathogens were *C. glabrata* (3.2%), *C. parapsilosis* (1.5%), *C. krusei* (1.1%), *C. guilliermondii* (1.1%), *C. tropicalis* (1.0%), *C.dubliniensis* (1.0%), *C. kefyr* (0.7%), In the control group other pathogens were *C. glabrata* (5.2%), *C. krusei* (4.8%), *C. parapsilosis* (1.6%), *C. kefyr* (1.2%), *C. guilliermondii* (1.2%), *C. zeylanoides* (0.8%), *C. tropicalis* (0.4%), *C. lipolytica* (0.4%), *C. norvegensis* (0.4%), *C. famata* (0.4)%, *C.rugosa* (0.4%). Most of *Candida*

spp. isolates were susceptible to fluconazole *in vitro* (95% in the main group vs. 88% in the control group, $p < 0.05$). Among 817 *C.albicans* isolates in the main group and 209 *C.albicans* isolates in the control group, fluconazole susceptibility was revealed in 98.9% and 98.5%, and fluconazole-resistant *C. albicans* were isolated in 0.4% and 1% respectively. Among "non-*albicans*"species fluconazole susceptibility was more often in the intervention group compared with the control group (60% and 38% respectively, $p < 0.05$). Opposite, fluconazole resistance was more marked in the control group (41% vs. 18% in the main group, $p < 0.05$).
Conclusions *Candida albicans* are the cause of RVVC in 90% cases in SaintPetersburg. Fluconazole resistance decreased two times among *Candida* "non-*albicans*" - from 41% to 18%. Fluconazole resistance is not more than 1% among *Candida albicans.*

P349

Five years of onychomycosis in university hospitals in Mato Grosso state - Brazil

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Objective Onychomycosis are cosmopolitan infections caused by dermatophytes, yeasts and non-dermatophyte molds. This research aimed to verify the occurrence of onychomycosis, determining patient age, sex, anatomical site of the lesion and etiology in Mato Grosso, Brazil and comparison with onychomycosis surveys in the published literature.
Methods Five hundred and eighty-six patients with suspected onychomycosis who sought medical care were submitted for laboratorial exams in two university hospitals in Cuiabá, MT, Brazil, from January 2007 to December 2011. This observational study was achieved by analyzing data from patient medical and laboratory records.
Results During this study, a positive results for fungi were verified in cultures from 294 patients (50.2%). From these individuals, 331 clinical specimens were collected, 77.6% determined as yeasts (74.9% *Candida* spp and 2.7% *Trichosporon* spp), 12.7% as dermatophytes (8.8% *T. rubrum*) and 9.7% as non-dermatophyte fungi (8.5% *Fusarium* spp).
Conclusion The most affected age group was patients between 46–60 years-old (35.1%) and the least affected those under 15 years-old. The fingernails were the most frequently affected anatomical site in both sexes. In summary, among the population studied, yeasts occupied a predominant position in the etiological profile of onychomycosis, followed by dermatophytes and non-dermatophyte molds. According to the clinical criteria for onychomycosis, only half the cases were confirmed by laboratorial exams (positive culture).

P350

Fixed orthodontic appliances, clinical aspects of gingival tissue and enzymatic activity of *Candida* spp in the central-western region of Brazil

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Objective The purpose of this study was to evaluate orthodontic appliances, the clinical appearance of gingival tissue and virulence of yeasts isolated in 80 patients. Of these, 40 belonged to the control group and 40 used orthodontic appliances.
Methods Yeasts were identified by both classic and automated methods (VITEK 2) to determine enzymatic activity (proteinase and phospholipase). Among the 80 patients, *Candida* spp was isolated in 27 (64.3%) among those who used orthodontic appliances and 15 (35.7%) among non-users.
Results A statistically significant correlation was determined between the two groups (isolation of yeast in relation to the use of an appliance) ($p < 0.05$ and OR = 3.4). *Candida albicans* was the most frequent isolate (31 isolates), 17 (42.5%) in cases from the orthodontic appliances group and 14 (35.0%) in the control group. A statistically significant association was determined between the clinical appearance of the patients' gingival tissue and the presence of orthodontic appliances ($p < 0.05$).
Conclusion Control group patients were more likely to present clinically healthy gingiva (OR = 0.2). Proteins were present in 100% of the strains from both groups, while for phospholipases, positivity was 22.5% for patients using an appliance and 15.0% for the control group. The use of orthodontic appliances may predispose patients to alterations in the oral microbiota, resulting in greater probability of clinically unhealthy gingiva.

P351

Yeast and mold fungi isolated from superficial lesions of outpatients attending Cerrahpasa Medical Faculty Hospital, in Istanbul, Turkey (01 April 2010–01 April 2013)

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Background Superficial fungal infections are among the world's most common diseases and the distribution of their etiological agents varies in different countries and geographic areas and depends on several factors, such as life style, type of population, socioeconomical conditions, personal hygiene and climate.
Objectives The aim of this study was to determine the frequency of aetiological agents of superficial mycoses encountered in skin, nail and hair samples from outpatients attended to Dermatology Department of Cerrahpasa Medical Faculty, İstanbul.
Methods Clinical samples were collected from 1681 patients suspected to have superficial mycoses over a period of 2 years and were examined by direct microscopy and culture. Yeast-like and mold fungi grown were identified using conventional techniques based on morphologial and biochemical criteria.
Results Pathogen fungi (n = 538) were detected in 523 of the patients. Of the isolates were 169 (31.4%) *Candida* spp, 276 (51.3%) dermatophytes, (93%) other keratinophylic fungi, namely 15 (2.8%) *Fusarium* and 78 (14.5%) *Trichosporon* spp. *T. rubrum* was the most frequent isolate (134, 24.9%) and toenail onychomycosis was the most common type of infection (1080, 58.0%). Two different significant fungi were cultured as aetiological agents from samples of 15 (5.4%) patients.
Conclusions The data showed that the most common agents isolated were *Trichophyton* speciess, being *Candida* spp the second prevalent. Several studies were reported from different regions of Turkey, including two from Istanbul, with different spectrum and rank of orders of aetiologic agents indicating probable regional specificities. Epidemiological surveys will be a usefull tool for the awareness of emerging species and infection control.

P352

Association of *Malassezia* species in pityriasis versicolor

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Objectives Pityriasis versicolor (PV) is a most common chronic superficial infection of the stratum corneum, reported in 40–60% of the tropical population. The aim of our study was to determine the species of *Malassezia* implicated with PV and correlate its clinical presentation.
Methods The subjects include 50 PV patients, who attended dermatology OPD of our hospital and 50 healthy individuals. Same size area of the skin was sampled from lesional and non lesional sites in the patient group, and from forehead, cheek and chest of healthy individuals. *Malassezia* spp. isolated were identified by conventional method and confirmed by ITS2 PCR-RFLP and sequencing of D1/D2 region of 26S rDNA.
Results Majority of cases (94%) came to hospital during summer ($p < 0.05$). Eighty percent of patients presented with hypopigmented lesions and 20% with hyperpigmented lesions. From PV lesions, the most frequently isolated species was *M. furfur* (50%), followed by *M. globosa* (27.3%), mixture of *M. furfur* and *M. globosa* (15.9%), *M. sympodialis* (4.5%) and *M. slooffiae* (2.3%). From skin of healthy individuals *M. furfur*, mixture of *M. furfur* and *M. globosa* and *M. sympodialis* was isolated in 73.3%, 16.7% and 10% of cases.The average numbers of colonies from lesional site (neck, back, arms, axilla and shoulder) was 18.6 and 8 confluent, whereas from non-lesional site was 7.9 and 1 confluent. The average numbers of colonies from forehead, cheek and chest of normal healthy individuals were 10.1, 9.8and 12.4 respectively.
Discussion and conclusion Higher *Malassezia* density was found in lesional area compared to non-lesional area of PV patients and in healthy individuals ($p < 0.05$). As the coexistences of *Malassezia* spp. was observed on the skin, it is important to incubate the culture for longer period. Though *M. furfur* was the most prevalent species isolated from both patients and control, significantly higher isolation of *M. globosa* from the lesional area (27.3%) compared to non-lesional area (5%) indicates the possible role of *M. globosa* in causing PV ($p < 0.05$).

P353

Analysis of a three year study of superficial mycoses in Northern Greece

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Objectives To record and analyse the data concerning the superficial mycoses in Northern Greece (mainly the region of Central Macedonia), after a study lasting three years, between January 2010 and January 2013.
Methods There were studied specimens from 360 patients (110 M/250 F), aged 2–85 years old. 414 samples were collected from skin (64), hair (13) and nails (hands 62, feet 275) lesions. For each patient a form was filled with information concerning job, hobbies, pet possession, immunity situation (e.g. chemotherapy, corticotherapy, diabetes mellitus). When patients were under antifungal treatment (local or systemic), specimens were sampled 2–3 weeks after the discontinuation of therapy. Direct microscopy was performed under KOH 15%. Specimens were primarily inoculated on Sabouraud dextrose agar with chloramphenicol (0.05%) and Dermatophyte Test Medium. Cultures were incubated for 2–4 weeks at 30°C and checked at least twice a week for fungal growth. Several media for sub culturing, biochemical and physiological tests, assimilation tests and identification keys were used accordingly to the fungal isolate. Repetition of the examination was advised in some cases (negative culture and positive direct microscopy-8 cases, non-dermatopyhtes in culture, etc.).
Results Of a total of 151 positive cultures, 19 were considered as yielding clinically non-significant isolates (saprophytes). Among the rest, dermatophytes were the most prevalent isolates (73, 54.9%), followed by yeasts (42, 31.6%) and non-dermatophytes (18, 13.5%). *Trichophyton rubrum* (41, 56.2%), *Trichophyton mentagrophytes* (12, 16.4%) and *Microsporum canis* (11, 15.1%) were the most commonly isolates among dermatophytes, whereas *Candida parapsilosis* (21, 50%) and *Candida albicans* (7, 16.7%) were the most commonly isolates among yeasts. The most common among the non-dermatophytes were *Acremonium* and *Fusarium* species (5, 27.8% each). Concerning toe nails the commonest isolations were *T. rubrum* (31, 43.7%) and *T. mentagropyhtes* (8, 11.3%), non-dermatophytes (14, 19.7%) and *C. parapsilosis* (9, 12.7%) whereas concerning finger nails *C. parapsilosis* (11, 35.5%), *C. albicans* (6, 19.4.8%) and equally *C. guilliermondii* and *C. tropicalis* (4, 12.9% each). As for hair the commonest was *Microsporum canis* (5, 83.3%) and for skin *T. rubrum* (8, 32%) and *M. canis* (6, 24%). 115 of the 151 positive cultures had a positive direct microscopy (76.2%).
Conclusion Main pathogenic fungi were found to be the dermatophytes. Particularly *Trichophyton rubrum*, followed by *Trichophyton mentagrophytes var. interdigitale / mentagrophytes*, especially in toe nails lesions. *Microsporum canis* was the most frequently isolated fungus in lesions of the scalp. *Candida* species, mainly *Candida parapsilosis* and *Candida albicans* but also other *Candida* species were the main pathogenic fungi in finger nails onychomycoses. Concerning non-dermatophytic filamentous fungi, they are often considered contaminants, but in several cases could represent the cause of the infection and have to be studied cautiously.

P354

Distribution of *Malassezia* species isolated from the patients with pityriasis versicolor in Turkey

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Objectives Pityriasis versicolor is a common superficial mycotic disease of the skin which is caused by different *Malassezia* species. The lipophilic yeasts of the *Malassezia* genus are known to be normal inhabitant of the skin flora of human and warm-blooded animals. However, they are considered opportunistic yeasts of increasing clinical importance. The aim of this study was to determine the distribution of *Malassezia* species isolated from patients with pityriasis versicolor by using conventional culture methods.
Methods In total, 146 patients with pityriasis versicolor were included in this study. The samples from patients were obtained by scraping the patients' lesions with sterile scalpel and/or applying sterile sticky plaster. The specimens taken by scraping the lesions with sufficient scaling were microscopically examined by using potassium hydroxide (KOH) solution. Samples obtained by both methods were inoculated on modified Dixon's medium for culturing. The macroscopic features including shape, size, texture and color, and the microscopic appearance (i.e., cell morphology and budding location) of predominant colonies were examined. For species-level identification of suspicious *Malassezia* yeasts, conventional tests including catalase, esculin and Tween tests were performed. In addition, the assimilation of cremophor EL and the growth in Sabouraud glucose agar medium were also evaluated.
Results Demographic and clinical characteristics such as age, gender, location of lesions were determined. The highest prevalence of pityriasis versicolor in this study was observed in the 18- to 34-year-

Table 1. Species distribution of isolated *Malassezia* spp.

Species	Number	%
M. globosa	71	65.1
M. obtusa	19	17.4
M. japonica	8	7.4
M. nana	4	3.7
M. restricta	2	1.8
M. furfur	1	0.9
Malassezia spp.	4	3.7

old group and the trunk was the most effected anatomical site. Fungal elements were observed in 36% (40/110) of the samples by KOH examination. Of 146 clinical samples, 109 (75%) yielded a growth which were considered to be a *Malassezia* sp. in culture. Species distribution of the isolated *Malassezia* spp. was shown in Table 1. As shown, *Malassezia globosa* (65%) was the most commonly isolated species and it was followed by *Malassezia obtusa* (17%). However, four *Malassezia* spp. isolates could not been identified at species-level with conventional methods.

Conclusion We found a relatively low positivity rate (36%) with direct KOH examination. In culture, the positivity rate was 75% and Malassezia globosa (65%) was found to be the predominant species in patients with pityriasis versicolor

P355

Abstract withdrawn

P356

Dermatophyte detection and identification in nail specimens from patients with suspected onychomycosis using a simple multiplex PCR-based method

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Objectives Onychomycosis is a common nail disorder caused mostly by *Trichophyton rubrum* and *T. mentagrophytes var interdigitale*. Since other conditions may resemble onychomycosis the accurate detection and identification of the causal agent is mandatory. Conventional methods for detecting fungi in nail specimens are either nonspecific (microscopy) or insensitive and time consuming (culture), especially in cases of previous antifungal therapy. Recently, PCR-based assays have been introduced to improve diagnosis.

Methods A multiplex PCR-based method for detecting dermatophytes in nail specimens (Dermatophyte PCR kit, Statens Serum Institut, SSI Diagnostica, Denmark) was evaluated using 202 nail specimens from patients with clinically suspected onychomycosis. After a rapid two-step DNA extraction method, the multiplex PCR was performed using two sets of primers aimed at chitin synthase 1 for detecting dermatophytes generally and ITS2 (internal transcribed spacer) for detecting *T. rubrum*. Amplicon analysis was made using agarose gel electrophoresis. The test was completed within 5 h, including the extraction stage. PCR results were compared with those of the direct microscopy and culture of the nails.

Results Overall 68/202 (33.7%) of the samples were dermatophyte positive by PCR (59 for *T. rubrum* and 9 for dermatophytes), while 60/202 (39.7%) of them were positive by conventional methods (42 by microscopy and culture, 17 only by microscopic examination, and 1 only by culture). The number of positive samples was increased by 7.4% by the use of the PCR assay. Furthermore, the percentage of samples with a species identification was increased (68 out of 202 specimens were found to be *T. rubrum* or dermatophytes positive by the PCR, while only 43 out of 202 specimens were dermatophyte positive by culture; increase in species-specific identification by PCR: 11.4%). Among 42 microscopy and culture positive specimens, only 2 were not detected by PCR, possibly due to the small quantity of the examined material. Among 124 specimens negative by both conventional methods, 16 were PCR positive (15 *T. rubrum*), while the rest were confirmed by PCR as negative. Of the 17 microscopy-positive but culture-negative specimens, 9 were PCR-positive (5 for *T. rubrum* and 4 for dermatophytes). From 16 specimens diagnosed by conventional methods as non-dermatophyte or *Candida* species, one gave a positive PCR-result (*T. rubrum*). Finally, previous systemic or topical unsuccessful treatment did not affect PCR results - 6 specimens were PCR-positive: 5 for *T. rubrum*, one for dermatophytes.

Conclusion This easy and rapid multiplex-PCR method applied directly on nail specimens could be a promising diagnostic tool to conventional methods for the management of the patients with suspected onychomycosis.

P357

Prevalence of *Candida* spp in a population of denture-wearing immigrants

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Objectives Infection of the oral cavity and dentures by *Candida* species are frequent in denture wearers. *C. albicans* is the most common pathogen; however, other emerging *Candid*a species are also responsible for this condition. Minimal data are available about the occurrence of *Candida* species in the oral cavities of denture wearing migrants to Italy. In this study, we compare the *Candida* species found in the oral mucosa and dentures from a population of denture wearing migrants to Italy to a matched Italian group.

Materials and Methods Oral swabs were collected from dentures and the underlying mucosa of patients enrolled in the study and were then cultured to test for the presence of *Candida* species in each sample.

Results Out of 284 patients enrolled (113 Italians and 171 migrants), 79 Italians (69.8%) and 135 migrants (78.9%) tested positive for the presence of *Candida*. *Candida albicans* was the most frequently observed species overall; however, we found a higher occurrence of *C. glabrata* among migrants than among Italians. In addition, migrants displayed a higher incidence of *Candida* - associated stomatitis and a lower mean age than *Candida*-positive individuals from the Italian group.

Conclusion Migrants are more prone to longer colonization of the oral mucosa and dentures by *Candida*. In these patients, dentures must be checked periodically to prevent the presence of *Candida*. Because biofilm formation is a risk factor for *Candida* infection in denture wearers, it is advisable to periodically screen denture wearers for *Candida* presence. Additionally, screening denture wearers for predisposing factors, such as hygienic habits, could lead to the early diagnosis of *Candida* colonization, leading to prompt eradication treatment, even in patients with mild or no symptoms. Nevertheless, a prompt treatment is strictly required in patients with non-*albicans Candida*, as these are the yeast with the highest tendency to invade and destroy the underlying oral mucosa.

P358

Toenail onychomycosis by an unusual pathogen: *Phoma* spp

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Background Onychomycosis is caused mainly by dermatophytes but occasionally by nondermatophytic fungi.
Objectives We present an onychomycosis case occured by Phoma spp.
Patient/Methods The patient was a 37 yeas-old male teacher who dealed with gardening in summer times. He presented with a history of greenish-yellow discoloration and subungual hyperkeratosis on all the toenails. He was otherwise in good health and he denied nail trauma or dystrophic nail abnormalities prior to the onset of the present lesions. There was no history of other disease except for toenail dystrophy. Nail surfaces were disinfected by 70% ethanol and specimens were collected with the aid of a sterile scissors were placed in labelled sterile Petri dishes and processed freshly. Clinical samples were examined by direct microscopy and culture. Specimens were cultured on two Sabouraud dextrose agar (SDA) without cycloheximide and one with cycloheximide at 25º and 37°C. Fungus grown was identified by macroscopic and microscopic characteristics. Microscopic morphology of the isolate was examined by staining with lactophenol cotton blue and using unstained wet mounts with physiological saline. The patient was called two more times with two weeks intervals, to obtain fresh samples to confirm the pathogenic significance of the fungus by repeating cultures and to exclude contamination.
Results In mycological examination septate hyphae were observed in 30% KOH preparation from the toenail samples. Rapid growing green-gray colonies were occured on SDA. Microscopical preparation revealed hyaline to Brown septate hyphae, several picnidia with ostioles and unicellular conidia. The same fungus was isolated on a total of three consecutive cultures. Dermatophytes were absent. The isolated mold phenotypically identified as *Phoma* spp.
Discussion *Dermatophytes are the major causative agents and produce up to 90% of the toenail infections fallowed by Candida spp. Traditionally molds other than dermatophytes have been considered as contaminating fungi of the skin and nails. Phoma* is a genus of fungi, a picnidial coelomycetes with over 200 known species which was occasionally recovered in cases of human subcutaneous disease, endophthalmitis and deep tissue infection. In this case, *Phoma* spp was isolated from toenails, and clinically mimiced the signs and symptoms seen in dermatophyte infections. Careful diagnostic attention is required when identifying nondermatophytes as an etiologic agents.

P359

Presence of a clear bias towards high molecular weight alleles in the microsatellite containing ORF RLM1 in *Candida albicans* isolates from vulvovaginal infections

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Vulvovaginal Candidiasis (VVC) affects over 75% of women, being the second most common gynaecologic infection. The most frequent causative agent of VVC is the commensal yeast *Candida albicans*, which is responsible for 70–80% of all cases. Comparative genomic analyses suggest that *C. albicans* repeat-containing ORFs, such as *RLM1*, may be important as fitness determinants. CAI microsatellite confers great variability to the *C. albicans gene RLM1*. This gene encodes the transcription factor CaRlm1, from the MADS-box family which regulates the expression of genes involved in the cell-wall remodelling. Several works have correlated some specific CAI genotypes with Chinese isolates from VVC and balanitis. However, no such study has been conducted in other countries. In this way, the purpose of this study was to genotype *C. albicans* isolates from geographic regions and from different body locations and correlate the resulting CAI genotypes with the VVC isolates.

In order to achieve this, 383 *C. albicans* strains from Portugal, Greece, and Brazil were genotyped with CAI microsatellite. Furthermore, clustering and population differentiation tests were performed. Strains from vulvovaginal infections presented highly significant differences ($P < 0.0000$) from all other strains, in both genetic and genotypic distribution. Regarding the clustering of the strains, it was possible to group them into 3 groups (A, B and C), reflecting the molecular weight of the alleles. In group A, 67.9% of the isolates were from other sources rather than VVCs, while in group C, 81.6% derived from VVCs.

These results revealed a clear biased distribution towards CAI high molecular weight alleles in strains from vulvovaginal infections (group C) in comparison with strains from other sources. With this work we propose that the presence of high molecular weight alleles may confer higher fitness for these strains in the vaginal environment.

P360

Phenotypes of the chronic mucocutaneous candidiasis and immuno-genetic disorders

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Objectives Chronic mucocutaneous candidiasis (CMC) is associated with genetic defects causing innate or adaptive immune dysfunctions and T helper-17 (Th17) signaling disorders are frequent among its. However, the defect of these immune reactions may be caused by gene mutations. Therefore the assessment of an early inflammation with interleukine (IL)6/IL23/IL17 and IL12/interferon(IFN)γ Th1 cytokines activation of the immune response is interesting today. In this study the immuno-genetic features in Russian patient with several clinic variants of the CMC were evaluated.
Methods We studied immunological parameters in 6 patients with CMC more than 5 years. Subtypes of peripheral blood lymphocytes were determined with using of monoclonal antibodies (DAKO), levels of immunoglobulins (Ig) A, M, G in serum were estimated by the Orion Diagnostica Turbox assay. The supernatants of peripheral blood mononuclear cells from CMC patients after 24 h. incubation with phytohemagglutinin-P were tested by ELISAs specific for the cytokines IFN-α, IFN-γ, IL-17 and IL-10 ('Cytokine', 'Vektor-Best', Russia). Oxidative microbicidal activity of neutrophils in the test with nitrotetrazolium blue, their phagocyte and killing activity with use of Candida albicans were estimated.
Results In view of the family anamnesis, a clinic, immune disorders and genetic mutations we observed patients united in two groups. The first group included 3 patients with autosomal dominant Autoimmune Polyendocrinopathy Syndrome 1(APS1) (mutations in a gene regulator), the second – 2 patients with an autosomal dominant CMC with function disorders of a thyroid gland (mutations were found in a gene of Sygnal Transducer and Activator of Transcription 1 (STAT1) and 1 patient – with a family autosomal recessive CMC without endocrine disorders. There were not essential disorders in lymphocyte subtypes and Ig A, M, G levels. In the first group decrease of neutrophil killer activity was from 5 to 17% to compare reference values of

healthy people (25–45%). The productions of IFN-α were 5 to 20 pg/ml (vs 100–500 pg/ml). The IL-17 level was reduced in one patient (6 pg/ml). The IL-10 level didn't differ from healthy people (from 410 to 595 pg/ml). Decrease of IL-17 production (from 7 to 58 pg/ml) was accompanied by low IFN-γ (146–230 pg/ml) and IL-10 production (23–131 pg/ml) in the second group.
Conclusion The results show decrease of macrophage and T-cell activity cytokine production in CMC patients with different genetic mutations. However, clinical phenotypes of CMC (APECED, with or no other thyreoid gland disorders) dependent on genetic peculiarities.

P361

Two undescribed dermatophyte species, *Trichophyton* and *Microsporum*, isolated from Czech patients

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During two-year molecular-epidemiological study focused on the non-*Trichophyton rubrum* dermatophytes in the Czech Republic, two species with unusual morphology were isolated from Czech patients. These species showed unique fingerprinting pattern (M13-core primer) and sequence data (ITS rDNA, beta-tubulin and RPB2 gene). The combination of macro- and micromorphological, physiological and phylogenetic data collected in this study justified that both isolates represented undescribed species. The *Trichophyton* isolate was associated with a case of probable distal lateral subungual onychomycosis affecting the right great toenail of a 33-year-old man. However, the pathogenic role of this isolate was not confirmed by two consecutive nail samplings and also the direct microscopical examination of nail scrapings was negative. This species probably belonged to the geophilic dermatophytes based on phylogenetic analysis and was most closely related to the anamorphic *T. thuringiense*, homothallic *Arthroderma ciferrii* (anamorph *T. georgiae*) and heterothallic *A. melis*. The species is characterized by yellowish colonies, red reverse on several media, positive urease test, negative hair-perforation test, absence of growth at 34 °C, absence of macroconidia, formation of one-celled clavate microconidia, spiral hyphae and sterile gymnothecium-like structures covered by peridial hyphae. The *Microsporum* isolate was associated with a case of tinea corporis in a 45-year-old female. The skin lesion was located at the dorsal right wrist and at first consisted of three 6 mm wide lesions which later merged to form a single circular lesion having a diameter of 40 mm. Direct microscopic examination of epithelial scales revealed the presence of septate, branching hyphae suggestive of dermatophyte infection and the same species grew on all slants in pure culture. Based on phylogenetic analysis, the species was closest to geophilic *Microsporum* species such as *M. gypseum*, *M. fulvum*, *M. duboisii*, *M. praecox* and *M. persicolor*. The isolate grew as sterile mycelium and did not produce any conidia, making more detail morphological analysis impossible.

P370

Classification and phenotypic profile of clinical and environmental *Cryptococcus neoformans* complex strains maintened in stock culture

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Objectives Study the phenotypic profile of yeasts maintened in stock culture identified as *Cryptococcus neoforman.*
Methods Forty clinical and forty four environmental strains, were chosen ran domly. The strains had undergone biochemical typing for differentiation as *C. neoformans* and *C. gattii* in CGB medium. The strains that were positive in biochemical test for *C. gattii* underwent molecular typing (PCR-RFLP). All the strains were underwent in virulence test to production of phospholipase, protease, melanin and capsule.
Results Among the clinical strains 90% were typed as *C. neoformans* and 10% as *C. gattii*, already among the environmental strains were 95.5% *C. neoformans* and 4.5% *C. gattii*. The strains that were positive in biochemical test for *C. gattii* found that only four strain were actually *C. gattii* (VGII) and two other *C. neoformans* (VNI and VNII). When the factors related to virulence were studied, both the clinical and environmental strains were phospholipase positive but the clinical strains produced a greater amount of this enzyme. All strains were both clinical and environmental production of protease and also showed an intensity colony color (melanization). When the thickness of the capsule was evaluated, all of it showed a capsule, from the clinical strains 67% had an average capsule and from environmental strains 70% had an average capsule.
Conclusion The study of phenotypes (CGB medium), was determined six strains of *C. gattii*. These six were so passed through molecular typing by PCR-RFLP and only four were truly confirmed as *C. gattii*. Among the virulence factor studied, with the exception of phospholipase, there was no correlation between clinical and environmental strains of *C. gattii* and *C. neoformans*. We point out that phospholipase production would be used as marked to *C. neoformans* complex and a correct identification of strains maintained in stock culture is extremely necessary by the biochemical and molecular test.

P371

Aspergillus viridinutans complex: polyphasic taxonomy, mating behaviour and antifungal susceptibility testing

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The aim of this study was to determine the species boundaries within the *Aspergillus viridinutans* complex. The species belonging to this complex are predominantly soil-inhabiting organisms that are increasingly reported as opportunistic human and animal pathogens. The species show considerable phenotypic variability but typically share nodding heads (some vesicles borne at an angle to the stipe) and relatively poorly sporulating colonies with abundant aerial mycelium (in comparison with *A. fumigatus* s. str.). A total number

of 72 isolates from various substrates and countries was subjected to morphological, physiological and molecular analysis (RPB2, calmodulin, beta-tubulin and actin gene). In addition, the mating experiments were provided within and between isolates representing opposite mating types from all major clades (on different media and temperatures). The physiological data (maximum growth temperature, acid production on CREA and Ehrlich test) and morphology of the anamorphic state was taxonomically insignificant due to overlapping features. Phylogenetic analysis based on combined dataset and mating experiments data showed that *A. viridinutans* complex comprises at least five to six heterothallic species and two homothallic species. Fertile ascomata were observed in three heterothallic species. These species differed in morphology of ascospores (width of crests and convex surface ornamentation) and conditions suitable for teleomorph induction. Only *A. udagawae*, *A. felis* and one tentative new species represent clinically relevant species. Our four locus typing scheme was able to unambiguously distinguish almost all isolates tested (Simpson's index close to 1). The calmodulin gene had the highest discriminatory power. Antifungal susceptibility testing (YeastOne panels) showed that posaconazole was the most active antifungal agent across species. All species were resistant to 5-fluorocytosine and fluconazole, other antifungals showed species-specific susceptibility patterns. This work was supported by the project GAUK 607812 and by the Internal Grant Agency of the Palacký University Olomouc, Czech Republic (grant no. LF_2013_012).

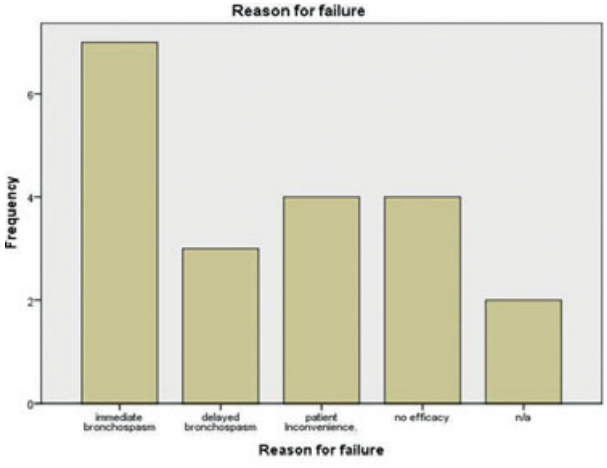

Fig 1: Reason for treatment failure in patients treated with nebulised (non-liposomal) Amphotericin B (Fungizone). Na= Not applicable (ie no failure observed).

P380

Nebulised amphotericin in allergic bronchopulmonary aspergillosis (ABPA) and severe asthma with fungal sensitization (SAFS)

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Background and rationale Allergic bronchopulmonary aspergillosis (ABPA) and severe asthma with fungal sensitization (SAFS) are debilitating lung diseases whose treatment is not yet fully established. Some published case reports/series suggest that nebulised amphotericin (liposomal) may have a role in the treatment of cystic fibrosis (CF) ABPA but little is known regarding its appropriateness in asthmatic ABPA and SAFS patients. We assessed the efficacy and safety of nebulised Amphotericin as second and third line therapy.
Methods 20 adult asthmatics with SAFS (n = 11) and ABPA (n = 9) were treated with nebulised amphotericin between January 2011 and May 2013. All patients had either failed itraconazole (n = 8), voriconazole preceded by itraconazole (n = 5) or developed adverse events (AEs) to either agent (n = 7). 10 mg of Nebulised amphotericin B (Fungizone) was administered using a Pari LC plus nebulizer twice/day, preceded by salbutamol, under direct physiotherapist observation. We audited clinical, radiological and immunological response, including change in the Asthma Quality of Life Questionnaire (AQLQ-J) scores .We also examined Asthma Control Questionnaire (ACQ) scores, change in lung function (FEV1), change in IgE (total and specific) and healthcare utilization. Patients were followed up for 12 months during which they were evaluated at 2, 4, 6, 9 and 12 months.
Results There were 20 patients analysed (SAFS, n = 11) and (ABPA n = 9), M: F = 8:12, median age 65.5 yrs (range = 24–78). The median duration of therapy was 30 days (IQR, 0.0–142). Clinical benefit was observed in 2 (10%) in which mean ACQ score improved from 6 to 2, overall mean AQLQ score improved by 0.95 and mean FEV1 improved by 1.2 L(63.1%). Seven (35%) failed the challenge due to adverse events (bronchospasm). 11 (55%) discontinued within 12 months of therapy due to delayed bronchospasm (n = 3, within 4 weeks), equipment problems/patient inconvenience (n = 4) and lack of clinical benefit (n = 4) (fig 1). There were no significant changes in immunological and radiological outcomes.
Conclusion Our data suggests that the overall efficacy of nebulised amphotericin in this group of patients may be poor and is associated with high frequency of adverse events. However, the responses were excellent in 2 (10%) patients. It is not clear which patients are likely to respond. Further studies need to be conducted to establish the optimal dose range (dose, frequency), nebulizer type, pressures and identification of patients who may respond.

P381

Efficacy and safety of amphotericin B Lipid Complex (ABLC) in adult oncohematological patients after failure of antifungal prophylaxis

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Objective To assess clinical response and safety of amphotericin B lipid complex (ABLC) administration in patients with hematologic malignancy and/or hematopoietic stem cell transplantation, with invasive fungal infections despite primary antifungal prophylaxis.
Methods Observational, mulicentric, non-interventional study from 3 centers in Slovakia during the years 2010–2012. Patients with breakthrough IFD while on antifungal prophylaxis who were treated with ABLC were enrolled. EORTC/MSG criteria 2008 were used for classification of IFD. Data regarding risk factors, prophylaxis, response to ABLC, toxicity and survival at the end of the study and at day + 30 after the last dose of ABLC was collected and analyzed.
Results 47 adult patients (26 males) with hematological malignancy, with average age 47 years, mainly with AML (60%) and ALL (17%) were enrolled. 5 patients underwent allogeneic HSCT. 36 patients were neutropenic for average 18 days (2–52 days). Prophylactic posaconazole was administred in 25, fluconazole in 13, vorikonazole in 8 patients and micafungin in 1 patient. Average duration of prophylaxis was 23 days (3–120 days). Proven/probable IFD was documented in 26 patients, possible IFD in 21 cases. The most common IFD reported was invasive aspergillosis (42 cases/89%). ABLC was administred in standard dose (3.0–5.2 mg/kg), in 42 patients as monotherapy, with average duration of 13 days (4–30 days). Complete and partial response (CR+PR) rate at the end of ABLC therapy

was 57% (14% + 43%). Survival at this time point was 85%. Survival at day 30 after the stop of ABLC therapy was 74%, majority of patients continued with other antifungal treatment (follow-on therapy or secondary prophylaxis in 80%). 14 patients (30%) developed toxicity (9 cases of nephrotoxicity). Average serum creatinine value was 71 and 97 (μmol/L) at the start and at the end of ABLC administration, respectively.

Conclusion Due to our observation, ABLC is reasonable choice for treatment of breakthrough IFD in high-risk hematological patients when azole prophylaxis failed. Clinical response and toxicity of ABLC were acceptable but follow-on antifungal therapy or secondary prophylaxis after polyene discontinuation is usually needed to maintain the response.

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P382

Comparison of efficacy of liposomal Amphotericin B (Fungisome™) and Amphotericin B deoxycholate in treating experimental fungal Endophthalmitis and Keratitis

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Objectives To develop an experimental model of fungal keratitis and fungal endophthalmitis in rabbits and to compare the efficacy of the indigenous liposomal Amphotericin B (Fungisome ™) and conventional Amphotericin B deoxycholate formulation in treating the above infection.

Methods The study was conducted using male and female adult New Zealand white rabbits (3-3.5 kg) as subjects and *A. fumigatus* (ATCC 13073) and *C. albicans* (ATCC 90028) as inoculum. Fungal Keratitis was developed either by using contact lens or intrastromal injection of the fungi and Fungal Endophthalmitis was developed by intravitreal inoculation of fungal inoculum. Fungisome ™ was used at a concentration of 0.05% and 0.1% while Amphotericin B deoxycholate was used at a concentration of 0.15% daily to treat these infections. The clinical outcome was graded daily using regular slit lamp examination and culture of vitreous tap. After eight days of therapy, the eyes were enucleated and histopathology and semiquantitative estimation of the fungal load were performed.

Results In fungal keratitis both drugs caused significant improvement of lesion compared to the control animals but there was no difference in the efficacy between these drugs at any concentration. In fungal endophthalmitis though complete recovery could not be achieved with either drug, significant reduction of fungal load and inflammation were observed by both drugs compared to the control animals. However, greater reduction of fungal load was noted in the Fungisome ™ (5 μg) treated eyes compared to the conventional Amphotericin B deoxycholate treated eyes.

Conclusion Both Fungisome™ and Amphotericin B deoxycholate had comparable efficacy in treating fungal Keratitis and Fungisome™ had better efficacy in treating fungal Endophthalmitis.

P383

Efficacy comparison between Liposomal Amphotericin B (L-AmB) and Voriconazole (Vori) in a murine infection model of pulmonary *Aspergillus flavus*

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Objectives Although *Aspergillus fumigatus* causes 66% of aspergillosis infections, the second most common causative agent is *Aspergillus flavus* with an incidence of 14%. *A. flavus* infections are more difficult to treat and the present study was done to determine if the combination of Vori and L-AmB, would be more effective than either drug alone for treating a murine *A. flavus* pulmonary infection.

Methods Swiss Webster mice were immunosuppressed with triamcinolone d-3, d0 and d+2, intranasally challenged with 8 x 10ex6 *A. flavus* (ATCC #203403) and 2 hours later treated with 10 mg/kg L-AmB (IV, qd), 40 mg/kg Vori (PO, bid), 10 mg/kg L-AmB (IV qd) + 40 mg/kg Vori (PO,bid) or 5% dextrose (D5W) (n = 17/group). At 48 hours post-challenge, mice (n = 7/group) were euthanized and blood collected for BUN. At this same timepoint, BAL and lungs were harvested for fungal burden (Log_{10} CFU/g), drug concentration (amphotericin B bioassay), and cytokine levels (multiplex bead assay). Mice (n = 10/group) were monitored for survival, weight change and disease signs to d+21.

Results Long-term survival to d+21 was significantly better with L-AmB alone or L-AmB + Vori compared to Vori (100% vs 40%, $p = 0.01$) with disease signs and weight loss paralleling the survival data. Day +2 (24 h post-2nd L-AmB dose; 12 h post-4th Vori dose) the fungal burden in the BAL was significantly lower with L-AmB vs all other treatments ($p \leq 0.05$). In the lungs, the mean CFU/g was 3.5 L-AmB, 4.5 Vori, 5.0 L-AmB + Vori, 5.5 D5W, although there was no significant difference amongst the groups. At this same timepoint, the mean drug level in the lungs of mice given L-AmB was 6.9 μg/g with no detectable Vori in the lungs of Vori-treated mice. BAL cytokine levels of IL-1a, IL-1B and IL-22 were significantly lower for Vori alone vs D5W ($p \leq 0.04$) but similar for Vori and L-AmB. In the lungs, IL-22 levels in mice given Vori alone were significantly lower than either D5W or L-AmB ($p = 0.03$). All BUN samples were within normal limits.

Conclusions Based on the endpoints of early reduction of fungal burden and long-term survival, L-AmB was more effective than Vori or L-AmB + Vori for treating murine pulmonary *A. flavus* infection. Although all animals survived to d+21 following L-AmB or L-AmB + Vori treatment, there was some suggestion of initial antagonism between the two agents based on fungal burden.

P384

The clinical response to a short-term course of intravenous liposomal amphotericin B therapy in patients with chronic pulmonary aspergillosis

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Objective This audit was performed to assess whether patients with chronic pulmonary aspergillosis (CPA) experience a sustained clinical benefit from a short course (< 6 weeks) of intravenous liposomal amphotericin B therapy (LAmB).

Methods CPA patients who had received their first short-course of intravenous LAmB therapy (Gilead) at the National Aspergillosis Centre in Manchester were identified. A retrospective patient case-note review was performed using a standardised proforma. Data collected included patient demographics, indication for LAmB treatment, the dose and duration of therapy and the clinical response to treatment. Patients who received <3 doses of LAmB were not evaluated for response (n = 2).

Results 48 CPA patients (23 females, 25 males) were identified aged 28–86 years (median 64) when treated. The median duration of prior azole therapy was 11.5 months (range 0–51 months). The dose and duration of intravenous LAmB given ranged between 2.47–5 (mean 3.03) mg/Kg daily and 1–36 (mean 16.9) days respectively. 1 patient was unable to tolerate the test dose of LAmB and received no further treatment. The primary reason for therapy was either respiratory (48%) or both respiratory and constitutional symptoms (52%). The average number of respiratory and constitutional symptoms per patient was 4.5 (range 1–7) symptoms.

15 of 47 (32%) patients developed an acute kidney injury whilst on LAmB therapy resulting in the need for IV rehydration; 3 with other contributing factors to their renal impairment. 5 patients needed to stop their LAmB treatment early, two of whom had received a LAmB dose reduction. 4 other patients successfully completed their LAmB course following dose reduction.

30 of 46 (65%) patients experienced a clinical response to LAmB therapy. Improvements in respiratory and constitutional symptoms were seen in 27 (58.6%) and 19 patients (41.3%) respectively. 27 (90%) out of 30 patients who completed the St George's Respiratory Questionnaire (SGRQ) Quality of Life Score pre and post treatment noticed an improvement in a least one modality with 18 (60%), 16 (53.3%), 12 (40%) and 15 (50%) patients experiencing an improvement in their symptom, impact, activity and total scores respectively. Response was usually delayed and only apparent at follow up post-discharge.

Improvement in the immunological markers of *Aspergillus* infection were observed in 9 (26.4%) out of the 34 patients who had adequate immunological data available. 13 patients had *Aspergillus* PCR performed on their sputum prior to LAmB treatment and 10 had a positive PCR result. Following treatment the *Aspergillus* PCR in sputum became negative in 7 patients.

Conclusion 65% of CPA patients receiving their first course of LAmB treatment experienced a clinical response. Improvements in respiratory and constitutional symptoms were seen in 58.6% and 41.3% of patients respectively. An acute kidney injury was observed in 32.6% of patients receiving LAmB therapy with 5 (10.9%) patients needed to stop their treatment course early. Routine intravenous rehydration at initiation of LAmB therapy and avoidance of other factors that may contribute to an acute kidney injury should be considered in all patients receiving LAmB therapy.

P385

Invasive candidiasis caused by *Candida metapsilosis*, *Candida orthopsilosis* and *Candida parapsilosis* in a Caenorhabditis elegans model

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Objectives To investigate the virulence of *Candida metapsilosis, Candida orthopsilosis* and *Candida parapsilosis* in a model of invasive candidiasis in *Caenorhabditis elegans* and to evaluate the efficacy of current antifungal agents for the treatment of the infection caused by these species.

Methods Reference strains of *Candida metapsilosis* ATCC 96143, *Candida orthopsilosis* ATCC 96139 and *Candida parapsilosis* ATCC 22019 were used for inducing the infection. Approximately 100 synchronized *Caenorhabditis elegans* (AU37 strain) worms L4 coordinated stage were inoculated onto the centre of agar plates inoculated with *Candida* lawn and allowed to feed for 2 h at 25 °C. Afterwards, 20 worms were dispensed into each well of a microtitre plate that contained M9 buffer, 10 μg/ml cholesterol in ethanol, and 90 μg/ml kanamycin. For the evaluation of treatment efficacy, the following final concentrations of antifungal agents were added to the wells: amphotericin B (1 μg/ml and 2 μg/ml), anidulafungin (4 μg/ml), caspofungin (4 μg/ml), micafungin (4 μg/ml and 8 μg/ml), fluconazole (32 μg/ml, 64 μg/ml and 128 μg/ml), and posaconazole and voriconazole (1 μg/ml and 2 μg/ml). The plate was incubated at 25 °C overnight and a visually scoring of live and dead worms was done at 24 h, using a dissecting stereomicroscope (Nikon SMZ-745). *Caenorhabditis elegans* survival curves were plotted and differences (log rank and Wilcoxon tests) were analyzed by the Kaplan–Meier method using SPSS 15.0 software.

Results *Candida parapsilosis* complex infection reduced significantly the survival of *Caenorhabditis elegans* in comparison to non-infected nematodes. *Candida parapsilosis* was less virulent than the related species *Candida metapsilosis* and *Candida orthopsilosis* ($p < 0.05$), which did not differ from each other ($p = 0.209$). Significant survival rates ($p < 0.05$) were observed in nematodes treated with amphotericin B: 52% and 68% survivals at 120 h in infections by *Candida orthopsilosis* and *Candida parapsilosis*, respectively. Anidulafungin increased significantly the survival rates in *Candida metapsilosis* and *Candida parapsilosis* infections ($p < 0.05$). Although, both caspofungin and micafungin increased significantly the survival of *Caenorhabditis elegans* in all candidiasis, only micafungin protected more than 50% of nematode at 120 h. Azoles increased significantly the survival rates of infected worms, estimated median hours of life and percentage of *Caenorhabditis elegans* survivors, on all invasive candidiasis ($p < 0.05$).

Conclusion *Caenorhabditis elegans* offers a simple and feasible model to study *Candida parapsilosis* complex virulence and to assess antifungal efficacy during infection. All antifungal agents studied showed efficacy in the treatment of *Caenorhabditis elegans* invasive candidiasis caused by the *Candida parapsilosis* complex.

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P386

Establishment of a new animal model of *Candida albicans* osteomyelitis: preliminary results

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Objectives *Candida* osteomyelitis causes significant morbidity if not recognized early or treated effectively. As there are no previous well defined animal models or prospective studies of this infection, there are insufficient data on its pathogenesis, diagnosis, imaging, and treatment. Clinical trials for *Candida* osteomyelitis are difficult to perform, due to the low incidence of this disease; therefore, the optimal treatment is elusive. Thus, the aim of this study is to establish an animal model for the study of the pathophysiology, diagnostic modalities, and therapeutic interventions of *Candida* osteomyelitis. Specific objectives were to determine inoculum response effect, to characterize inflammatory response, to establish radiologic characteristics overtime, and to develop positron emission tomography / computed tomography (PET / CT) scanning as a novel diagnostic tool.

Methods We used a modified version of the Norden rabbit model of tibial osteomyelitis: a cortical defect was introduced in the tibial metaphysis with a 2-mm drill, the medullary cavity was inoculated with *C. albicans*, and finally the drilling hole was plugged with sterile bone wax under general anesthesia. The isolate was derived from a patient with established *Candida* osteomyelitis. On days 7, 14, and 21 after infection, the animals underwent bone radiographs, ^{18}F-FDG PET/CT scan and blood sampling for blood cultures, blood counts, determination of ESR, and mannan antigen serum levels. On day 21, all animals were sacrificed. Infected tibias were harvested and specimens of cortical/cancellous bone, bone marrow, and surrounding soft tissue were sampled under sterile conditions for quantitative culture and histology.

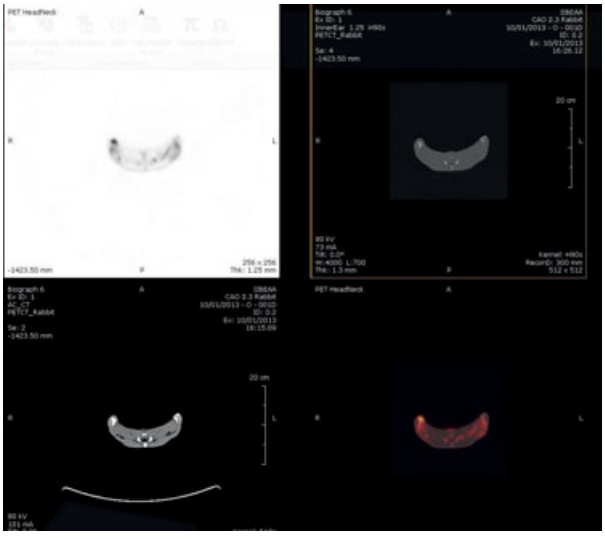

Figure 1 PET-CT scan showing increased uptake at the site of osteomyelitis.

Results Thus far, a total of seven animals have been infected with 10^6 CFU (4 animals); 10^7 CFU (2 animals); 10^9 CFU (1 animal), of the *Candida* isolate. The 10^9 inoculum was lethal as the animal died 6 days after inoculation. The rest 6 animals survived until day 21. In all animals, bone radiographs at days 7, 14, and 21 revealed radiological signs of progressive osteomyelitis. PET/CT scans on the same days showed for all animals time-dependent and inoculum-dependent increased ^{18}F-FDG uptake (range of SUVmax = 2.1–4.8) at the area of inoculation in the upper third of the tibia. All blood cultures, for all time points were negative. Leukocytosis (>10.000 cells/ml) was observed in 4/6 animals, while ESR was normal in all animals. Mannan serum levels were determined in 23 samples and were positive in 7, intermediate in 5, and negative in 11 samples. Five infected tibia were available for cultures; *Candida* growth was observed in all bone cultures (2731–21036 cfu/gr), in 3/5 bone marrow samples (68–6272 cfu/ml), and in 4/5 soft tissue samples (217–2598 cfu/gr). Histology of the infected bone revealed presence of neutrophils (5/5 samples), eosinophils (1/5 samples), giant cells (5/5 samples), bone necrosis (5/5 samples), fibrosis (2/5 samples), and budding yeasts (3/5 samples).
Conclusions We report the successful establishment of a new animal (rabbit) model of *Candida* osteomyelitis that may applicable in advancing our understanding of the pathophysiology, diagnostic modalities, and treatment of this debilitating infection.

P387

Medical management of prosthetic valve endocarditis due to Exophiala dermatitidis

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We report a case of Exophiala dermatitidis prosthetic valve endocarditis in a 75 year-old woman previously operated for culture negative prosthetic valve endocarditis. The blood cultures were initially reported to have grown a kind of yeast form fungus and she was treated with micafungin for 4 weeks, then switched to oral fluconazole. The result of blood cultures later revealed E. dermatitidis but the response to the treatment appeared well, resulting in the continuation of the same treatment. The oral therapy was discontinued after 7 weeks because of the suspected drug induced hepatotoxicity. She remained afebrile for about a week after the discontinuation of antifungals, but begun to have fever and another blood culture grew E. dermatitidis.The risk of preoperative mortality was estimated to be as high as 70%, and the decision was made to manage her medically again. She is under treatment with liposomal amphotericin B for 6 weeks and is planned to be switched to oral voriconazole for long term suppression therapy.

To our knowledge, this is the first case of prosthetic valve endocarditis due to E. dermatitidis and the first case of medically managed endocarditis due to E. dermatitidis.

P388

Interferon-gamma as adjunctive immunotherapy for invasive fungal infections

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Objectives The incidence of fungal infections is increasing at an alarming rate, despite development of new classes of antifungal agents. Invasive fungal infections remain associated with unacceptable high mortality rates and represent a major cause of death worldwide. The evolution of mechanisms for significant resistance to the current antifungal drugs further emphasizes the need for novel approaches to treat invasive fungal infections. As invasive fungal infections are most commonly observed in individuals with immune defects or dysfunction and the number of immunocompromised patients is steadily increasing. Adjunctive immunotherapy, to improve anti-fungal host defense, is therefore an attractive strategy to improve the outcome of patients with disseminated fungal infections.
Methods In a case series we included eight patients with severe invasive *Candida* and/or *Aspergillus* infections who were treated with recombinant IFNγ for 2 weeks. From these patients blood was drawn to measure the *ex-vivo* responsiveness of to fungal stimulation, HLA-DR expression and lymphocyte subsets.
Results Treatment with IFNγ significantly boosted *ex-vivo* IL-1β and TNFα response to *C. albicans* and LPS at the first days after initiation of treatment. Additionally, an increase in the capacity to induce T-cell cytokines IL-17 and IL-22 was observed after initiation of IFNγ therapy. Treatment with IFNγ also modulated the leucocyte subsets in the peripheral blood. We were unable to find significant changes in HLA-DR expression which might be explained by the fact that most of the patients were not immunoparalysed. However, patients that demonstrated decreased HLA-DR upon inclusion showed a trend towards increasing monocytes HLA-DR expression after IFNγ treatment.
Conclusion Collectively these data provide a first immunological proof-of-principle that adjunctive immunotherapy with IFNg might improve the outcome invasive fungal infections. Yet still, larger clinical trials are warranted to assess this possibility.

P389

Amphotericin B nanoemulsion: evaluation of *in vitro* and *in vivo* antifungal activity in chromoblastomycosis agents

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Objectives To evaluate the *in vitro* and *in vivo* susceptibility of chromoblastomycosis agents to amphotericin B nanoemulsion, for parenteral administration, produced by us, compared with a commercial nanostructured formulation (Abelcet ®), the conventional formulation (Fungizone®), and a free drug. In addition, to assess the toxicological parameters of nanoemulsion and the free drug.
Methods The amphotericin B nanoemulsion was produced by high-pressure homogenization method and characterized as to physicochemical parameters. The *in vitro* susceptibility was evaluated using microdilution methodology recommended by M38-A2 CLSI protocol. The animal model used to evaluate the *in vivo* antifungal activity was developed with *Fonsecaea pedrosoi* ATCC 46428. From this strain it was prepared a spore suspension containing 10^6 CFU/mL, which was inoculated in male Balb/c mice, intraperitoneally (100 μL), and in each hind paws (50 μl). After 15 days of inoculation, the treatment of the animals was initiated, until the 30th day. Daily administrations were performed, four of them being intralesional, anesthetizing the animals with isoflurane, intercalated with intraperitoneal administration, and intraperitoneal applications, only, the following days. The dose of amphotericin B for all treatments (amphotericin B free, Fungizone®, Amphotericin B nanoemulsion and Abelcet®) was 1 mg/kg/day. The effectiveness of the different treatments was evaluated counting the number of colonies in relation to the untreated controls, obtained by the inoculation of one of the paws macerated in Sabouraud dextrose agar plus chloramphenicol.Biochemical tests (creatinine, by Jaffé method without deproteinization; alanine aminotransferase, by kinetic UV method; and urea by UV enzymatic method), and histopathological analyzes of the kidneys, liver and paws were performed. The nanoemulsion was evaluated as to the potential to cause hemolysis *in vitro*, cytotoxicity (Clonogenic assay) and mutagenicity (*Salmonella*/Microsome assay).
Results The amphotericin B nanoemulsion for parenteral administration was satisfactorily produced. It was not possible determine the antifungal activity of both the amphotericin B nanoemulsion and the Abelcet ® in *in vitro* assay. In the *in vivo* assay, the nanoemulsion showed activity superior than free amphotericin B and Fungizone®, and reduced significantly the number of colonies compared to the control. In the determination of serum urea levels, it was observed a significant increase when free amphotericin B and Fungizone were administered, whereas the levels obtained with amphotericin B nanoemulsion and Abelcet® were similar to the control, suggesting a renal protection conferred with the latter treatments. With histopathological analysis, it was possible to verify that the nanoemulsion reduces the nephrotoxicity caused by amphotericin B, when compared with Abelcet®, Fungizone®, and free drug. There is a tendency the amphotericin B nanoemulsion and Abelcet® causes an hemolysis percentage lower than free amphotericin B and Fungizone®. Amphotericin B free was cytotoxic for V79 cells at concentrations from 4 μg/mL, while with nanoemulsion, no toxicity was observed in the same band concentrations applied to free amphotericin B. Both the amphotericin B nanoemulsion and free amphotericin B were not able to produce the revertant strains at concentrations evaluated.
Conclusions The produced Amphotericin B nanoemulsion, in the future, can be an alternative safe and effective treatment, for chromoblastomycosis.

P390

In vitro and *in vivo* susceptibility of chromoblastomycosis agents to itraconazole nanoemulsion

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Objectives An itraconazole nanoemulsion for parenteral administration was evaluated for *in vitro* and *in vivo* activity in chromoblastomycosis agents, as well as in relation to its toxicological potential.
Methods The nanoemulsion was produced by high-pressure homogenization technique and characterized as to the droplet size and polydispersion, zeta potential, viscosity, pH, association rate, drug concentration and the presence of instability phenomena. *In vitro* evaluation of the antifungal activity was carried out according to CLSI M38-A2 protocol. *In vivo*, a spore suspension of *Fonsecaea pedrosoi* ATCC 46428 strain, with a 10^6 CFU/mL concentration was inoculated into the hind paws and peritoneum of BALB/c mice. After 15 days of inoculation, the animals were treated until the 30th day. The dose of itraconazole (itraconazole nanoemulsion and itraconazole dissolved in DMSO) was 2.85 mg/kg/ day, being this dose proportional to the dose used in humans. Histopathological analyzes of kidneys, liver and paws were performed. The biochemical parameters (urea, creatinine and alanine transferase) were also analyzed. Regarding toxicological tests, the *Salmonella*/Microssoma and clonogenic tests were employed.
Results The produced nanoemulsion showed adequate for parenteral administration and antifungal activity *in vitro* similar to free drug. In the *in vivo* assay, animals treated with nanoemulsion showed a lower fungal colonies growth and reduced liver toxicity when compared to those treated with itraconazole. The reduction in toxicity was confirmed by alanine aminotransferase measurement. In toxicological tests, itraconazole was cytotoxic at a concentration of 0.41 μg/mL, while the nanoemulsion was not in a concentration of 4.915 μg/mL. The itraconazole was not able to produce the revertant in strains evaluated, until the concentration of 1 μg/plate without metabolization, while a volume of 5 μL of nanoemulsion (equivalent to 4.875 μg of itraconazole) caused no mutations both with and without metabolization.
Conclusion The itraconazole nanoemulsion was safe and effective against chromoblastomycosis agents.