Antifungal Susceptibility Testing

P9-001. *In vitro* susceptibility of *Candida* isolates to amphotericin B, Abelcet and Ambisome

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The MICs of Amphotericin B (AMB), and their lipid formulations Abelcet and Ambisome have been determined for 138 isolates of clinical specimens of eleven species of *Candida*. The microbroth method following the M27-A document was used with RPMI 1640 plus glucose as culture medium. The MIC ranges were 0.01-1.0 µg/ml for AMB, 0.01-8.0 µg/ml for Ambisome, and 0,01 - 2.0 µg/ml for Abelcet. Only 4 isolates (2,8%) presented MICs≥ 1.0 µg/ml forAbelcet as opposed to 8 isolates for AMB (5,7%) and in 38 isolates (27%) for Ambisome.
MICs 50% and MICs 90% were determined for the six more numerous species.
Using the NCCLS standardised method, the lowest MIC values were found for Abelcet, ($p<0.001$). Only three isolates of *C. krusei* and one of *C. albicans* presented values ≥1.0 µg/ml considered as resistance. On the contrary 27% of the isolates showed MICs ≥1 µg/ml for Ambisome.)

	Amphotericin B		Abelcet		Ambisome	
Species	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
C. albicans	0.12	0.25	0.03	0.12	0.12	1.0
C. glabrata	0.25	0.25	0.03	0.06	0.06	0.5
C. tropicallis	0.12	0.25	0.03	0.12	0.12	1.0
C. krusei	0.25	0.5	0.12	0.5	2.0	2.0
C. dubliniensis	0.06	0.12	0.01	0.01	0.03	0.06
C. parapsilosis	0.12	0.25	0.03	0.03	0.25	0.25

In vitro Abelcet is also more active than conventional Amphotericin B against most of *Candida* species. MICs were noticably lower for the new species *C. dubliniensis* and for the emergent yeast *C. parapsilosis.*

P9-002. *In vitro* activity of voriconazole, a new antifungal agent, against *Candida* spp

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Aim: To compare the *in vitro* activity voriconazole (VOZ) to that of amphotericin B (AMB), fluconazole (FLZ) and itraconazole (ITZ) against 250 clinical yeast isolates, including 171 *C. albicans*, 45 *C. glabrata*, 15 *C. parapsilosis*, 12 *C. tropicalis* and 7 other especies *(2 C. guillermondii, 1 C. kefyr, 1 C. krusei, 1 C. lusitaniae, 1 C. rugosa and 1 S. cerevisiae).*
Methods: Susceptibility testing was performed using the NCCLS Standard Reference Method (M27-A, macrobroth dilution) The newly recommended quality control strains *C. parapsilosis* ATCC 2209 and *C. krusei* ATCC 6258 were included in each run.
Results: With a MIC 90 of 1 mg/L, AMB remained the most active drug, only one strain having a MIC of 2 mg/L. Likewise VOZ showed excellent activity with also a MIC 90 of < 1 mg/L, 15 strains (11 *C. albicans* and 4 *C. tropicalis*) having a MIC > 16 mg/L. The MIC 90s for itraconazole and fluconazole were 2 mg/L and 64 mg/L respectively. Only 65% of the strains could be considered fully susceptible to ITZ against 80 % to FLZ (NCCLS susceptibility breakpoints of ≤ 0.125 mg/L and ≤ 8 mg/L respectively). Another 15% and 10% of the strains were categorized as Susceptible-Dose Dependent for ITZ and for FLZ respectively (NCCLS S-DD breakpoints of 0.25-0.5 mg/L and 16-32 mg/L respectively). Twenty percent and 10% of the strains were considered resistant to ITZ and PLZ respectively (NCCLS breakpoints for resistance of ≥ 1 mg/L and ≥ 64 mg/L respectively)
Conclusions: These data suggest that VOZ may have potential efficacy in the therapy of *Candida* infections and merits further *in vitro* and *in vivo* studies.

P9-003. Unchanged antifungal susceptibility patterns of Swedish *Candida* sp. Blood isolates between 1994 and 1998

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Background: Swedish Reference Group for Antimycotic - Methodology inaugurated the first nationwide retrospective survery of antifungal susceptibility patterns of *Candida* sp. in 1998.
Methods: Candida sp. isolates cultured from blood 1994-1998 were requested from microbiological laboratories in 15 Swedish regional hospitals. A total of 232 *Candida* sp. isolates were included and they comprised *C. albicans* (n= 123), *C. glabrata* (n=51), *C. parapsilosis* (n=33), *C. tropicalis* (n=11), *C. krusei* (n=9) and *C. lusitanie* (n=5).
Antifungal susceptibility testing was performed by broth macrodilution utilising the National Committee for Clinical Laboratory Standards (NCCLS) M27-A methodology.
Results and conclusions: Unchanged antifungal susceptibility patterns of *Candida albicans* and non- *albicans Candida* sp. was demontrated within the 5 year period. MIC for 50% of the isolates for each year were as follows:
fluconazole 0.25 mg/L for *C. albicans* and 16-32 mg/L for non-*albicans*, itracazole 0.063 mg/L for *C. albicans* and 0.5-1 mg/L for non-albicans, amphotericin B 0.5-1 mg/L and flucytosine 0.125 mg/L for both *C albicans* and non-albicans *Candida* sp. Most of the *C. glabrata* and *krusei* isolates showed reduced susceptibility against both fluconazole and itraconazole. Antifungal resistance was uncommon in the other *Candida* sp.; only 5 *of C parapsilosis* isolates were resistant against fluconazole and 6 of the isolates (5 *C. parapsilosis*, 1 *C. albicans)* against flucytosine.

P9-004. Comparison of two commercial antifungal susceptibility tests in candidemias

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Background: With the emergence of fluconazole (FLU) resistant yeast strains, a rapid and effective susceptibility test has become necessary for the optimal management of candidemias. The NCCLS method is the reference for fungal susceptibility testing, although fastidious to perform routinely. Recently more commercial tests are being developed and present a rapid and easier alternative for daily practice. We compared two commercial tests with the standard NCCLS antifungal susceptibility testing.
Method: The susceptibility results of NCCLS method, Fungitest® (Sanofi Pasteur) and Sensititre® yeast one (Trek Diagnostic System Limited) were compared for amphotericin B (AMB) and FLU in 20 yeast strains isolated from blood cultures of patients with fungemia, and 4 ATCC yeast strains. Yeast isolates were identified by API-ID32C. The MIC's were determined following NCCLS recommendations (M27-A) adapted to microdilution broth technique. Plates were read with a spectrophotometer. Fungitest® and Sensititre® were performed according to manufacturer's recommendations. The MIC's determined by NCCLS method and Sensititre® were expressed in "SIR" in order to compare them with the Fungitest® results.
Results: No discordant results were obtained for AMB (all strains were "S" by the three methods). By NCCLS 1 *C. albicans* (16%) and 3 *C. krusei* (100%) were R to FLU (MIC>32) and 2 *C. glabrata* (40%) were I (MIC 16 and 32) to FLU. Two major discordances were shown (1 *C. glabrata* "R" for Fungitest® and 1 *C. tropicalis* "S" for Sensititre®) compared to NCCLS (S/R respectively). A minor discordance was obtained for FLU with 2 *C. krusei* for Fungitest® and Sensititre®, and 1 *C. glabrata* for Fungitest® compared to NCCLS determinations.
Conclusions: Both commercial methods are similar in term of results. However the reading and interpretation of results was easier and more rapid with Sensititre® than Fungitest® (some results needed up to 48 hours), and allow moreover a MIC determination.

P9-005. Comparison of *in vitro* antifungal susceptibilities of conidia and hyphae of *Aspergillus* spp

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The minumum inhibitory concentrations (MICs) and minimum fungicidal concentrations (MFCs) ofamphotericin B, lipid based amphotericin B formulations, itraconazole and voriconazole againts 37 isolates of *Aspergillus* spp. comprising 15 isolates of *Aspergillus fumigatus*, 12 of *Aspergillus flavus* and five each *of Aspergillus niger* and *Aspergillus terreus* were tested using a broth microdilution method according to the guidlines of the National Committee for Clinical Laboratory Standards (NCCLS M 38-P). In addition, we applied a broth microdilution assay for hyphae of *Aspergillus* spp. since the invasice form is manifested by the appearance of hyphal structures.
MICs and MFCs of hyphae were significantly higher ($p<0.001$) than those of conidia for almost all isolates of *Aspergillus* spp. and various antifungal agents. In contrast, the *in vitro* efficacy of the antimycotics did not differ significantly within hyphal and conidial inocula (MIC, $p=0.047$; MFC, $p=0.060$).
The MICs (MIC ranges, MIC50, MIC90) and MFCs (MFC ranges; MFC50, MFC90) within conidia and hyphae against amphotericin B and lipid-based amphotericin B formulations showed narrow ranges with minor variations. Similar data were observed for the azoles, itraconazole and variconazole.
This study demonstrates the importance of the type of inoculum used to test antfungal susceptibilities of *Aspergillus* spp. The significance of these results for *in vivo* outcome needs to be determined.

P9-006. Discrimination between susceptible and resistant strains of *Candida* spp. to amphotericin B

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Aim: To evaluate the effect of medium and pH and the ability to distnguish between strains that are susceptible and resistance to amphotericin B.
Methods: A total of 14 *Candida* species were evaluated, 6 resistant to AB (4 *C. lusitaneae*, 1 *C. albicans* and 1 *C. tropicalis*) and 8 susceptible to amphotericin B (AB) (4 *C. albicans*, 3 *C. parapsilosis* and 1 *C. lusitaneae*). *Candida krusei* ATCC 6258 and *parapsilosis* ATCC 22019 were used as control strains. The following media were used: RPMI and AM3 buffered with MOPS at pH 5 and 7; AM3 buffered with acetate at pH 5 and buffered with phosphate at pH 7. Glucose was added to each media to achieve a final concentration of 2%. AB concentration ranged between to 16-0,016 µg/ml. Inocula were preparated spectrophotometrically by adjusting the transmitance to 75-77% in order to obtain 1-5 x 10^6 CFU/ml. The microtitration plates were inoculated with a final inoculum of 1-5 x 10^3 CFU/ml and inculated at 35ºC for 24 and 48 h. The MIC was defined as the lowest concentration of drug that inhibited all visible growth. The pH of the drug-free well was measured after 24 and 48h. Minimal fungicidal concentration (MFC) was also determined by subculturing 0.1 ml from each well with no visible growth onto drug-free Sabouraud agar plates. All data were analyzed by Mann Whitney test.
Results: The differences in geometric means between the MICs of susceptible and resistant strains were always higher when AM3 used than RPMI 1640. For AM3 after 24h the differences were larger at pH 5 than pH 7 with the largest difference axhibited in AM3 buffered with MOPS (3 times, p=0.004). After 48 h the discrimination between susceptible and resistant strains was better at pH 7 with the largest difference exhibited in AM3 buffered with phosphate (3.19 times, p=0.0013) although the AM3 buffered with MOPS showed similar results (2.96 times, p=0.0007). When RPMI 1640 beffered at pH 7 with MOPS used, the difference were larger after 24h (1.33 times, p=0.0027) than after 48h but always lower compared with AM3. The discrimination in MFC better in AM3 compared with RPMI especially when it was buffered with phosphate at pH 7 (2.38 times, p=0.02). Concerning of the measurement of pH, pH 7 was more stable than pH 5. At pH 5 the MIC´s were higher than those at pH 7.

P9-007. *Ex vivo* evaluation of keratinophilic fungi susceptibility to antimycotics

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The susceptibility of fungi to antimycotic agents commonly used in the treatment of nail mycoses was evaluated using a previously worked out method based on *ex vivo* infecting distal nail plate fragments.
Three fungal strains were used: two *Trichophyton mentagrophytes*, and one *Scopulariopsis brevicaulis*. The strains originated from clinical materials. The following antimycotics were tested: systemic: ketoconazole, itraconazole, terbinafine; topical: bifonazole, naftifine, amorolfine, cyclopirox.
Two *ex vivo* techniques were used: in one of them the nail plates were pretreated with the drugs under study to incorporate the drug into them, and then they were infected with the fungi; in the second one, the nail plates previously *ex vivo* infected with the fungi, were then treated, using various drug concentrations and treatment time periods.
Terbinafine turned out to be most effective drug against both of the dermatophyte strains, even though the effects of amorolfine and naftifine were also satisfactory. However, amorolfine was the most potent drug against *S. brevicaulis.*

P9-008. Influence of media on the susceptibility of *Candida* species to fluconazole tested in a microdilution assay according to DIN 58940

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The influence of medium on the MIC results to fluconazole (FCA) was evaluated by testing 762 clinical yeast strains in a multicenter study (16 centres). All media, RPMI1640 (Sigma #7755), HR medium (Oxoid CM 845), and HR with 0.25 mg/L methylene blue (HRM), had a final concentration of 2% glucose. The MICs were determined by microdilution assays using ready-to-use panels (Merlin Diagnostics, FRG), according to the proposed DIN 58940. The MIC distributions and the susceptibility classification to susceptible (S; MIC ≤8 mg/L); susceptibility dose dependent (SD; MIC >8 mg/L - ≤32 mg/L) and resistant (R; MIC >32 mg/L) were compared statistically.
The total proportion of resistant strains after 18 hours/48 hours incubation time differed medium dependent as follows: RPMI 7.1%/20.4%, HR 8.0%/23.5% and HRM 5.2%/10.7%. No significant differences could be found between the distribution of susceptibility classes for all isolates comparing HR and RPMI after 18 hour, but between HRM and RPMI. The differences were time and species dependent.

P9-009. Azole and echinocandin antifungal agents against *Candida* species

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Introduction: The increasing incidence of candidiasis and other fungal infections has resulted in the need for an expanded arsenal of antifungal agents. Agents at different stages of development include new generation azole derivatives and echinocandins. Azoles are fungistatic drugs that inhibit ergosterol biosynthesis, whereas echinocandins target glucan synthesis and are fungicidal.
Methods: A total of 90 *Candida* isolates, including *C albicans* (n=28), *C. dubliniensis* (n=19), *C. glabrata* (n=21) and *C. krusei* (n=22) were recovered from different anatomical sites from patients in several geographic areas of Spain. NCCLS techniques using a broth microdilution method and reading of endpoints at 24 and 48 h were used to assess their *in vitro* susceptibility against fluconazole, itraconazole, voriconazole, posaconazole (SCH-56592), ravuconazole (BMS-207147) and Cancidas -Caspofungin- (MK-0991). For Cancidas, both RPMI 1640 and Antibiotic Medium 3 were used as test media.
Results: In general all investigational agents were active against most isolates tested. Cross-resistance between the different azole drugs was observed for several isolates, although in most instances these were isolates for which trailing endpoinds were found. Cancidas demonstrated high efficacy against all isolates tested, including those that showed decreased susceptibility to all azole derivatives. Differences in Cancidas MIC values were also found according to test medium.
Conclusions: These new antifungal agents display potent *in vitro* activity against *Candida*. These agents should constitute effective therapeutic options for the treatment of fungal infections, including those caused by organisms clinically refractory to currently used antifungal therapies.

P9-010. Comparison of the Sensititre YeastOne colorimetric Antifungal Panel with NCCLS broth microdilution method for susceptibility testing of *Candida* isolates against fluconazole and itraconzole

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Introduction: The Sensititre YeastOne Antifungal Panel (Accumed International, USA) which incorporates Alamar Blue as a colorimetric indicator may provide an alternative to more laborious NCCLS methods for determination of antifungal susceptibility. *Methods:* A total of 90 *Candida* isolates, including *C. albicans* (n = 28), *C. dublinensis* (n = 19), *C. glabrata* (n =21) and *C. krusei* (n = 22) were tested against fluconazole and itraconazole using both the Sensititre and the NCCLS broth microdilution methods, with reading of endpoints at 24 and 48 h. Three comparisons of MIC pairs were evaluated to obtain percentages of agreement: 24- and 48-h MICs and 24-h Sensititre readings versus 48-h NCCLS MICs.
*Results:*For both azole derivatives and all species tested the best correlations were observed with 24-h readings. MICs for *C. krusei* isolates displayed the best percentage of agreement in all comparisons whereas MIC values for *C. glabrata* showed the least agreement. In the case of *C. albicans*, lack of correltion at 48-h for individual isolates was mainly due to trailing endpoints observed using the colorimetric method.
Conclusion: The Sensititre YeastOne Antifungal Panel constitutes an alternative to NCCLS methodology for use in the clinical laboratory for the antifungal susceptibility testing of most *Candida* species, and its colorimetric reaction facilitates the reading of MICs.

P9-011. Saturated saccharose solution (SSS): its *in vitro* and *in vivo* action on *Paecilomyces lilacinus*

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Paecilomyces lilacinus (P1) , is an ubiquitous oftenly associated with human infections. For this work we employed a *Pl* strain isolated from cutaneous lesion of a immunologically normal patient. Mycological diagnosis was performed by direct analysis with OHK and subsequent growth in Sabouraud and pathogens selective agar.
The *in vitro* SSS activity was assayed by mixing a *Pl* suspension with saturated saccharose solution, eugenol, polyetienglycol 400 and recording the fungal survival time.
For *in vivo* analysis, the patient gave her written informed consent and a topical treatment was followed. *In vitro* results showed, after 50 expe-

riences, that *P1* is able to survive 2 minutes in contact with SSS, and *in vivo* treatment demonstrated that after only 2 months of topical SSS application a visible remission could be observed, with total cure at 6 months.
Due to the natural resistence to usual fungicidal therapy of this specie and the non-toxic nature of SSS, it is viable to consider this solution as an alternative therapeutic approach for treatment of *Pl* cutaneous infections.

P9-012. Comparision of *in vitro* activity of amphotericin B and nystatin against zygomycetes
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Objetives: To compare the *in vitro* activity of amphotericin B (AMB), amphotericin B deoxycholate (D-AMB), nystatin (NYS) and liposomal nystatin (L-NYS) against zygomycetes. The influence of mild heating of AMB (hAMB) and D-AMB (hD-AMB) on their *in vitro* activity was also tested.
Methods: MICs were determined for 39 zygomycetes isolates comprising 16 *Rhizopus* spp., 13 *Mucor* spp., 4 *Absidia* spp., 3 *Rhizomucor* spp., 2 *Cunninghamella* spp., and 1 *Apophysomyces elegans*. Susceptibility testing was performed using an NCCLS-based microdilution technique (M38-P). Spore suspensions were counted in an haemocytometer and adjusted to obtain a final concentration of 1 x 10^4 CFU/ml. MICs were read visually after incubation at 37ºC for 24H. AMB and D-AMB were tested directly or after heating at 70ºC for 20 min. All isolates were tested in duplicate with similar results.
Results:

		GM* MIC (µg/ml)					
Strains	n	AMB	hAMB	D-AMB	hD.AMB	NYS	L-NYS
Rhizopus spp.	16	0.50	0.54	0.19	0.18	3.83	1.61
Mucor spp.	13	0.12	0.12	0.08	0.07	0.62	0.62
Absidia spp.	4	0.18	0.15	0.09	0.09	0.71	0.84

*Geometric mean

Conclusion: (1) Heating of AMB and D-AMB did no significantly reduce their *in vitro* activity. (2) NYS was less active than AMB or D-AMB. (3) L-NYS appeared to be as active as free NYS. (4) The clinical sifnificance of these *in vitro* results remains to be determined *in vivo*.

P9-013. Sensitivity testing of filamentous fungi with two different methods and correlation with the clinical outcome
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Aim of study: Sensitivity determination of clinical strains of filamentous fungi and correlation to the clinical outcome of the patients.
Material and Methods: Twenty four cases of systemic mycoses due to filamentous fungi were studied from November 1998 to May 2000 in an Athens hospital. Sensitivity testing of the isolated strains was performed with the broth microdilution method and with Etest according to the available guidelines. The antifungal agents amphotericin B, flucytosine, fluconazole, ketoconazole and itraconazole were used.
Results: The patients, 4 women and 20 men, aged 19 to 70 years old, suffered mostly from hematologic malignancies and over 50% were febrile. The isolates were collected from the respiratory tract (13): 6 *Aspergillus fumigatus*, 6 *Aspergillus flavus*, 1 *Paecilomyces* sp., from the paranasal sinuses (7): 4 *Rhizopus* sp., 3 *Asp. flavus* and from soft tissue (4): 1 *Alternaria alternata*, 1 *Asp. flavus*, 1 *Fusarium solani*, 1 *Rhizopus* sp.
The obtained MICs were well comparable between the two methods (broth microdilution and Etest) regarding ketoconazole (MIC50=1 vs. 2µg/ml), flucytosine (MIC50=>32vs.>32µg/ml) and fluconazole (MIC50=>256vs.>256µg/ml), and less well comparable regarding amphotericin B with MIC50=0,5 vs. 2µg/ml and itraconazole with MIC50=0,5 vs 2µg/ml and itraconazole with MIC50=0,125 *vs.* 1µg/ml.
Most patients were treated with amphotericin B or its liposomal analoges and some also with itraconazole, with succesful outcome in 33% and partial response in 29%. The morality of 37% (=9 patients) was not only due to the mycosis but also to the underlying diseases, and sometimes therapy failure was observed although *in vitro* MIC-results regarded to be sensitive.
Conclusions: A deviation of the MIC50 for amphotericin B remains up to date first choice in therapy of infections due to filamentous fungi with satisfactory results even if *in vitro* results resistance. No statistically significant correlation between *in vitro* sensitivity and clinical outcome of therapy could be found.

P9-014. *In vitro* activity of UR-9825, a novel triazole agent, compared with that of ten other antifungal drugs against 506 strains of dermatophytes
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Data related to susceptibility testing of dermatophytes are scarce. The *in vitro* activity of UR-9825, a novel triazole agent (J. Uriach & Cia, S.A, Barcelona) was compared with that of itraconazole (I), fluconazole (F), amphotericin B (A), voriconazole (V), terbinafine (T), sertaconazole (S), miconazole (M), clotimazole (C), ketoconazole (K) and G-1 (furvina from Centro de Biactivos Químicos, Cuba) against 506 strains of dermatophytes. We tested 22 strains of *Epidermophyton floccosum*, 158 of *Microsporum* ssp and 320 of *Trichophyton* spp. Minimum inhibitory concentrations (MICs) were determined according to the NCCLS microdilution method for molds (M38-p) with some modifications. We used the RPMI 1640 medium, a final inoculum of 103-104 CFU/ml (65-70% T at 530 nm) and4-10 d of incubation at 28ºC . MICs for A, G-1 and T were defined as 100% of growth inhibition and for the azoles as 50% inhibition. UR-9825, V, T and C demonstrated excellent activity against the majority of the isolates. Their activity expressed as a geometric mean MICs (GMIC) and MIC90 (µg/ml) were: UR-9825 0.14, 0.25; V 0.14, 0.25; T 0.21, 0.06 and C 0.17, 0.25, respectively. *Microsporum racemosum* and *M. cookei* showed the highest GMICs (16 µg/ml) of T and C. M, I, K, S and A displayed very similar MIC90 (0.5-2 µg/ml). G-1 and F were those that had the lowest activity (GMICs of 16 and 32 µg/ml, respectively). Our results demostrated a high effectiveness of UR-9825 and V against dermatophytes, but clinical and bioavailability studies are needed to determine whether these *in vitro* data can predict *in vivo* outcome.

P9-015. *In vitro* antifungal susceptibility of filamentous fungi using agar diffusion methods
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Recently, it has been proposed a reference method (NCCLS M38-P document) for filamentous fungi which describes both micro- and macrodilution tests form the more common pathogenics moulds. Nevertheless, the development of this reference procedure does not eliminate the need for simpler methods of testing that can be performed in non-specialist laboratories. The in vitro antifungal susceptibility of 50 strains of filamentous fungi against amphotericin B, ketoconazole, itraconazole, fluconazole and flucytosine was evaluated using two commercially available agar diffusion tests: E-test (AB biodisk) and Neo-Sensitabs (Rosco). The filamentous fungi tested were mainly isolated from clinical specimens: *Scedosporium apiospermum* (7 isolates), *S. prolificans* (3), *Aspergillus fumigatus* (10), *A. flavus* (5), *A. niger* (5), *A. terreus* (4), *Fusarium solani* (5), *F. moniliforme* (5) y *F. oxysporum* (5). *Paecilomyces variotii* ATCC 22319 was included as a quality control strain. Inoculum was adjusted to 0.5 McFarland (Densimat bioMerieux) for *Aspergillus* spp, to 0.7 for *Scedosporium* spp and to 0.8 for *Fusarium* spp. MIC ranges obtained by E-test were in most cases similar to the available MIC data reported for these fungal species obtained by dilution techniques. A high correlation between both methods was obtained taking into account the break-points reported in the literature to define resistance.

P9-016. Activity of new and conventional antifungal drugs against *Exophiala spinifera*

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Aim: To evaluate the *in vitro* activity of polyenes, azoles, allylamines and others: amphotericin B (AB), itraconazole (ITZ), terbinafine(TF), 5-fluocytosine (5FC), fluconazole (FCZ),voriconazole (VCZ), BMS-207147 (BMS) against 15 strains of *Exophiala spinifera.*
Methods: A broth microdillution method was performed according to the NCCLS guideline uising RPMI 1640 medium with glutamine and without sodium bicarbonate buffered with MOPSat pH 7.
An inoculum if 0.4-5.0 x 10^6 CFU/ml was obtained by spectrophotometric procedure. The microtitration plates were incubated at 35ºC and the MIC was read at 48, 72 and 96 h. The MIC for AB was the lowest concentration of the the drug which inhibited all visible growth and for the remaining drugs, the MIC was defined as the lowest concentration of the drug which inhibited 50% of the growth (MIC-2) compared to the growth control. Minimal fungicidal concentration (MFC) were also determined by subculting 0.1 ml from each well with no visible growth onto drug-free Sabouraud dextrose agar plates.

Results:

The geometric means and ranges of MIC´s and MFC after 72 h of incubation were:

Drugs	Gm MIC´s (µg/mL)	MIC´s range (µg/mL)	Gm MFC (µg/mL)	MFC range (µg/mL)
AB	0.79	0.25-4	5.99	2-16
FCZ	24.25	8-64	>128	64->128
VCZ	0.16	0.063-0.5	2.38	1-8
TB	0.24	0.063-0.5	5.04	1-16
5-FC	1.49	0.125-16	>64	>64
BMS	0.30	0.125-2	3.56	1-8
ITZ	0.05	0.031-0.125	0.71	0.125-4

Gm: geometric mean.

Conclusion: The new azoles VCZ and BMS showed *in vitro* activity against *Exophiala spinifera* althought ITZ was more active. FCZ showed no activity. The lowest MFC were for ITZ, followed by VCZ and BMS.

P9-017. *In vitro* antifungal activities of porfirins against clinically important fungi

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Objectives: Candida albicans is the organism often associated with serious fungal infections, nevertheless other *Candida* species and genres have emerged as clinically important pathogens associated with opportunistic infections.
The limited ranged drugs, associated with drug toxicity and the appearance of resistant strains, leed us to study the *in vitro* activity of diferent porfirins (PFs), and compare this activities with those of two currently available azole drugs, used in systemic fungal infections: fluconazole (FLC) and itraconazole (ITC).
Material and Methods: In vitro antifungal activity of porfirins were determined against yeast isolates recovered from immuno-compromised individuals. ITC (Janssen Cilag-Farmaceutica, Portugal) and porfirins were solubilized in dimethyl sulfoxide, and FLC (Pfizer Roerig, Portugal) in sterile water. The inoculum was prepared in Sabouraud to obtain a final concentration of 1 x 10^3 CFU/ml. Minimal inhibitory concentrations (MICs) were determined according National Committee for Clinical Laboratory Standards (NCCLS). Cytotoxic effect was evaluated by Finter's method.
Results: For all of the compounds tested, PF1 showed antifungal activity. MICs for *Candida* isolates are ranged between 4-64 mg/L., and for the *Cryptococcus neoformans* 0.03-0.125 mg/L. The MICs for ITC and FLC against *C. neoformans* are ranged between 0.03-0.25 mg/L and 0.5-8 mg/L, respectively.
Conclusions: The MICs values obtained *in vitro* for PF1 showed good antifungal activity against all yeast isolates tested, however for C. neoformans the same compound revealed lower MIC values than comercial drugs (ITC, FLC). In this case , the MIC90 was 0.06 *g/ml, a value much lower than citotoxic concentrations.

*J.P. Frade, acknowledges fellowship of PRAXIS XXI-BD 19900/99

P9-018. Evaluation of different conditions for the antifungal susceptibility test for *Exophiala spinifera*

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Aim: to investigate the *in vitro* activity of antifungal drugs in order to find an approach of the better conditions for susceptibility test for the group *Exophiala spinifera.* For this purpose different temperatures, times and endpoints were evaluated.
Methods: the study were determined by a broth microdilution method recomended by the National Committee for Clinical Laboratory Standars in RPMI 1640 medium with glutamine and without sodium bicarbonate buffered with MOPS at pH 7. Inocula were performed by spectrophotometric procedure and the turbididities of conidial suspensions were measured at 530 nm adjusted to different percentages of transmitance and also the number of conidia were done microscopically to obtain a concentration of 0,4-0,5 x 10^6 CFU/ml.
Also the inoculum quantitation was performed by plating 0,01 ml of 1:100 dilutions of the adjusted inoculum on Sabouraud agar to determine the viable number of CFU per mililiter, incubated al 28 to 30º C and observed daily for the the presence of fungal colonies.
The microtitration plates were incubated at 28,35 and 37ºC and the MIC was reading ar 48, 72 and 96 hs. The MIC for amphotericin B, fluconazole, voriconazole, terbinafine, BMS-207147, 5 fluocytosine and itraconazole were evaluated at three endpoints: (MIC 0) as the lowest concentration of the drug that showed 100% of inhibition of all visible growth, (MIC 2) as the lowest concentration of the drug that showed 50% of inhibition of the growth and (MIC 1) as the lowest concentration of the drug that showed 75% of inhibition of the growth, all respect to the growth control. Minimal fungicidal concentration (MFC) were also determined by subculturing 0.1 ml from each well with no visible growth onto drug-free Saboureaud plates. All data were analyzed by ANOVA (Dunn´s Multiple comparision test).
Results: We found that after 48 hs the MIC's at 37º C were lower than the MIC's at 28 and 35º C and the growth were not enough at this time for all temperatures. Bases on the endpoints analized, the differences among the MIC's 1 at different temperatures were not statiscal significance (p>0,14), compared with the MIC's 0 and MIC's 2. Respect the period of incubation the statical analize showed differences at 48 and 96 hs. For the 93,8 of the range was 1,5-6 x 10^6 CFU/ml.
Conclusion: This preliminary study suggest that for this fungi the better conditions for the susceptibility test could be: determine the MIC's at 72 hs, % of transmitance between 77-82, endpoint as 75% of inhibition of growth (MIC-1).
More investigation of all these conditions are necessary to confirm this data in order to find a standarization method for dematiaceous fungi.

P9-019. A new methodological approach for the antifungal susceptibility testing of filamentous fungi

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Background: All methodologies for the MIC determination of antifungal drugs against filamentous fungi are based on visual, colorimetric or spectrophotometric assessment of fungal growth after a certain time of incubation. In this study a new approach for the antifungal susceptibility testing of filamentous fungi was developed based on real time growth curves of fungi exposed to antifungal agents.
Methods: 3 different species of filamentous fungi (*Aspergillus fumigatus*, *Scedosporium prolificans* and *Rhizopus oryzae*) were tested against 2-fold dilution series of itraconazole (ICZ), amphotericin B (AB) and terbinafine (TB). Two inocula, 10^6 and 10^4 conidia per ml, were prepared spectrophotometrically. After the inoculation, the microtitration plates were incubated at 37ºC and a growth curve was generated by measuring the optical density (OD) of each well every 15 min for 24 h. For each concentration of antifungal drug the following kinetic parameters (KP) were calculated: the first significant increase in OD, the average of OD changes per second, the slope of the growth curves, the area under the kinetic curve and the maximal sloe. After 24 h and 48 h of incubation, the MIC of the antifungal drugs for each species were determinated visually and compared with the KP. The correlation coefficients between the visual MIC and the KP were calculated.
Results: High levels of corrrelation were obtained when the MIC derived by visual reading and KP. The mean correlation coefficient between KP and the MICs ranged from 0.695 to 0.944 for all species tested. The correlation was slightly better for *A. funigatus* (r=0.895) and TB showed the highest (r=0.925) for all KP. Among KP, the best correlation was found with the average of OD changes per min (r=0.944) and the slopes (r=0.923).
Conclusions: A new methodology of antifungal susceptibility testing of filamentous fungi was described based on the estimation of K.P. This methodology can be optimised by establisching endpoints based on KP for better correlation with the conventional methods. Furthermore, the MICs of antifungal drugs can be determined earlier since visible growth is not required.

P9-020. The effect of two antifungal drugs on different stages of the growth of *Aspergillus fumigatus* in different nutrient media

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Background: The methods for antifungal susceptibility testing of filametous fungi are based on conidia and not on hyphae which cause invasice disease. In this study, the susceptibility of *Aspergillus fumigatus* conidia, germinated conidia and hyphae of different ages were investigated against amphotericin B (AB) and itraconazole (ICZ) in 4 nutrient media. A kinetic model was developed in order to monitor real time changes in the growth curves of *A. Fumigatus* before and after exposure to the drugs.
Methods: Conidia suspensions of 10 4 cfu/ml were inoculated in 100µl of antibiotic medium 3 (AM3), yeast nitrogen base medium (YNB), liquid Sabouraud (SAB) and RPMI-1640. All media were 2x concentrated and buffered with MOPS at pH 7.0. The conidia were incubated in a 96-flatbottom microtiltration plate for 96h at 37º C and the optical density of each well was measured every 15min. After 0,20 h, 40 h, 60 h and 80 h of incubation, 22µl of drug solutions were added into each well, respectively in order to obtain final concentrations of 10 x MIC (10 mg/l). The growth curves were segmented and each part was analysed separately. The slopes of the growth curves before and after the administration of the drugs were estimated by regression analysis and were compared with each other for each medium and time point. Large changes in slopes were correlated with higher susceptibility of the fungal structures.
Results: The growth of fungus was completely inhibited by AB independent of the time of exposure. Interestingly, the inhibition of the growth of hyphae older tha 40 h was reversible in SAP and AM3. A fumigatus conidia were completely inhibited by ICZ. However, the inhibition of hyphae was dependent on the age of hyphae and the nutrient medium (p=0.036). The divergence of the growth curves after the drug administration was less enhanced in RPMI (35%) compared with YNB (90%) and AM3 (70%).
Conclusions: The susceptibility of fungi was dependent on the stage growth and the nutrient medium. The effect of ICZ was less pronounced in RPMI, which may indicate failure to distinguish small differences in susceptibilities.

P9-021. Susceptibility testing of *Trichosporon* isolates to five antifungal drugs

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The genus *Trichosporon* is best known as the causative agent of white piedra an innocuous superficial hair infection. Since 1970 deep infection cases have been reported.
Six different species are generally involved in human diseases : *T. ashaii, T. asteroides, T. cutaneum, T. inkin, T. mucoides, T. asteroides, T. ovoides.*
Conventional antifungal treatments for *trichosporon* isolates: *T. ashaii* (7)*, T. coreniforme* (2), *T. cutaneum* (5), *T. inkin* (3), *T. mucoides* (1), *T. ovoides* (1) from environmental and human origin. The antifungal activity of amphotericinB (AMB), 5-flucytosine (5FC), itraconazole (ITR), ketoconazole (KET), fluconazole (FLU) against these isolates using strips of Etest(AB_BIODISK) with two different media Sabouraud dextrose agar (DIFCO) and Yeast Nitrogen Base (DIFCO) was performed in accordance with the manufacture´s instructions.
Saboraud Dextrose Agar medium showed a better performance for the development of *Trichosporon* isolates and for the reading of the Etest results.
For its practicity the Etest method has potentially utility as an alternative method to the NCCLS. Notwithstanding the heavy growth of microcolonies inside almost all of the most difficult reading pattern.
Modifications in the Etest method to produce sharper reading end points might further improve the performance of the test.

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P9-022. Effect of pyrimethamine, trimethoprim and sulfonamides against *Aspergillus* spp

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Aim: To evaluate the *in vitro* activity of: trimethoprim (TMP), sulphamethoxazole (SMX), sulphadiazine (SDZ), sulfamethoxypyridazyne (SMP), pyrimethamine (PMT), clotrimoxazole (CTX), sulfisoxazole (SSX), dapsone (DDS) against *Aspergillus spp.*
Matherials and methods: Among a total of 60 *Aspergillus* strains, were tested: 20 *Aspergillus fumigatus*, 10 each of *flavus, niger, ustus* and *terreus*. The final concentrations of the drugs were ranged from : 400 to 0.3 µg/ml for SDZ and SMP, 16 to 0.01 µg/ml for TMP, 320 to 0.3 µg/ml for SMX, 2 to 0.002 µg/ml for PMT, 8 to 0.008 µg/ml for DAP and 16-320 to 0.01-0.3 µg/ml for CTX (TMP plus SMX dil. 1:20)
A broth microdilution method was used according to the guidelines NCCLS using RPMI 1640 medium with glutamine and without sodium bicarbonate buffered with MOPS at pH 7.Inocula were performed by spectrophotometric procedure and the turbidities of conidial suspensions were measured at 530 nm adjusted yo 80-82 percentages of transmitance to obtain a concentration of 0,4-5,0 x 10 6 CFU/ml. The microtitration plates were incubated at 35ºC and the Mic´s were reading at 24 and 48 h. The MIC for this drugs was defined as the lowest concentration of the drug wich inhibited 50% of the growth respect to the growth control (MIC´s 2)
Results: The geometric means (GM) and the ranges of MIC´s (µg/mL) after 48 h were :

	TMP		SMX		CTX		PMT		DDS		SSX		SMP		SDZ	
Strain	GM	MIC*	GM	MIC	GM	MIC	GM	MIC	GM	MIC	GM	MIC	GM	MIC	GM	MIC
A. fumigatus	>16	>16	54.6	40 160	56.6	40.8	2.46	1->2	>8	>8	758	125-500	137	50 400	100	25->400
A. flavus	>16	>16	197	80-320	184	160-320	2.3	1->2	>8	>8	>500	>500	303	200-400	>400	>400
A. niger	>16	>16	49.2	40-80	98.5	40-160	1.32	0.13-2.0	>8	>8	933	500->500	81.2	50-200	100	12.5 800
A. ustus	>16	>16	367	80-320	394	80-320	3.25	2->2	>8	>8	>500	>500	348	100-400	>400	>400
A. terreus	>16	>16	139	80-160	149	80-320	2.83	0.5->2	>8	>8	>500	>500	400	50-400	>400	>400

GM : Geometric means; MIC*: MIC's range

Conclusion: These preliminar study shows that TMP, DDS and SSX were not active *in vitro* against *Aspergillus* species.
PMT was active against some isolates of *niger*, SDZ and SMP were active against *fumigatus* and *niger*.
SMX and CTX were active against all of the trains except *ustus.*

P9-023. *In vitro* activity of pentamidine against *Aspergillus* species

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Pentamidine (PTM) is an aromatic diamidine structururally similar to other antiprotozoal agents that and is known far its activity against *Pneumocystis carinii*. The activity of PTM aganinst molds is unknown.
Aim to evaluate the *in vitro* activity of PTM against *Aspergillus* species.
Matherial and methods: A total of 70 *Aspergillus species,* were tested : 20 *Aspergillus fumigatus*, 10 each of *flavus, niger, ustus,* and *nidulans.*
The final concentrations of the drug was ranged from: 128 to 0.12µg/ml.
The *in vitro* activity as determined by the broth microdilution method recommended by the NCCLS using RPMI 1640 medium with glutamine and without sodium bicarbonate buffered with MOPS at pH 7. Inocula were prepared and adjusted to a concentration of 0,4-5,0 x 10^6 CFU/ml by spectrophotometric procedure.
The microtitration plates were incubated at 35ºC ant the MIC´s were read after 24 and 48 h. The MIC for this drugs was defined as the lowest concentration by subculturing 0.1 ml from each well no visible growth onto drug-free Sabouraud dextrose agar plates.
Results: The geometric means (GM) and the ranges of MIC´s (µg/mL) after 48 h were :

	Pentamidine			
Strain	Gm MIC´s (µg/mL)	MIC´s range (µg/mL)	Gm MFC (µg/mL)	MFC range (µg/mL)
A. fumigatus (20)	>128	>128	>128	>128
A. flavus (10)	>128	>128	>128	>128
A. niger (10)	3.2	1-16	48.5	1->128
A. ustus (10)	9.8	0.25-32	103.9	64->128
A. nidulans (10)	2.1	0.25->128	5.65	0.5->128
A. terreus (10)	8.0	1-16	>128	>128

Gm : Geometric mean

Conclusion: Pentamidine seems to be active *in vitro Aspergillus niger, A. ustus, A. nidulans* and *A. terreus*, but not activity was observed to *Aspergillus fumigatus* and *A. flavus*. According with the MFC result, this drug could present fungistatic action.

P9-024. Comparision of the *in vitro* antifungal activities of sertaconazole and other vaginal antifungal drugs using microdilution and casitone agar dilution methods against clinical isolates of *Candida* spp

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Sertaconazole is an antifungal agent with a wide-spectrum activity. It has shown high efficacy and good tolerance in the treatment of topical and vaginal infections.
RPMI 1640-2% glucose supplemented-microdilution technique (M27-T / NCCLS) and Casitone agar dilution method were permormed to fluconazole (FLZ), clotrimazole (CLZ), itraconazole (IZ) and Ketoconazole (KZ), against 79 clinical isolates of *Candida* spp. (27 *C. albicans*, 16 *C. tropicalis*, 13 *C. parapsilosis*, 13 *C. krusei* and 10 *C. glabrata*) in order to find the overall agreement rates among the two methods.
The results obtained were analysed to find the statistically significant differences for SZ MICs in comparision with the other antifungal agents MICs, for *Candida* spp. strains and against the species tested for each method (Wilcoxon tests, $p<0.05$)
When the microdilution method was performed , our findings showed that : SZ was significantly more potent than FLZ for all the yeasts species tested. SZ was significantly more potent than IZ (or there were not found statistically significant differences) for all the species tested. SZ was significantly more potent than KZ (or no significant differences) for *C. glabrata, C. albicans* and *C. krusei*. And finally SZ was significantly more potent than CLZ for *C. glabrata* and *C. albicans*. When the Casitone agar dilution method was used, our findings showed that: SZ was significantly more potent than FLZ for all the yeasts species tested. Sz was significantly more potent than IZ (or no significant differences) for all the yeasts tested except *C. Albicans*. Sz was significantly more potent than KZ (or no significant differences) for *C. glabrata* and *C. tropicalis*. And finally, there were not found statistically significantly differences between SZ and CLZ for *C. glabrata.*
In this paper, the agreement (%), the kappa index and the intraclass correlation coefficient were used to evaluate the correlation of statistically significant differences, between the MICs of sertaconazole and the MICs of the other antifungal agents, comparatively to the methods used. It can be concluded that a good agreement between two methods was demonstrated for sertaconzole MICs vs. Clotrimazole MICs and sertaconazole MICs vs. itraconazole MICs. Nevertheless, the results also pointed out a lower agreement when sertaconazole MICs vs. ketoconazole MICs were compared among the two methods.

P9-025. Antifungal activity of voriconazole against candidal isolates obtained from Ugandan HIV-infected females

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Objective: In Uganda, HIV infection predisposes women to candidal infection. Though the predominant species from vaginitis has been shown to be *Candida albicans*, the prevalence of other yeast isolates has not been determined. Previous studies have also found unusual phenotypic and genotypic variations among African *C. albicans* isolates.1 These isolates were able to form germ tubes but did not form chlamydospores. Determining whether these variants may also exhibit antifungal resistance is an important tool in management of therapy.
Methods: Three hundred vaginal yeast isolates from AIDS patients seen at the STD Clinic, Mulago Hospital, Kampala, Uganda were tested against clotrimazole (CLO), fluconazole (FLU), miconazole (MIC), nystatin (NYS), and voriconazole (VOR) using a microdilution broth method.
Results: All *Candida albicans* isolates (249 or 83%) were susceptible to the five antifungals, with VOR achieving the lowest MIC range (<.06 – 1.0 µg/ml). Other isolates, including *C. glabrata* (7%), *C. tropicalis* (3%), *C. krusei* (1%), and *C. lipolytica* (.6%), showed varying susceptibilities to the antifungal panel, though none exhibited high MICs to VOR. The MIC_{90} of *Candida albicans*, defined as the concentration (in µg/ml) at which 90% of isolates were inhibited, was 0.25 for CLO, 1.0 for FLU, 0.125 for MIC, 2.0 for NYS, and .125 for VOR.
Conclusion: Voriconazole was shown to be a potent antifungal agent against all vaginal isolates from the population of AIDS patients in Africa.

P9-026. Susceptibility to antifungal agents of *Candida* spp bloodstream isolates. Results from a four years study (1996-1999)

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Aim: To determine the species frequency of *Candida* spp blood isolates and their susceptibility to seven antifungal agents.
Methods: All strains isolated since January 1996 to December 1999 were included in the study. The strains were identified according to the colony color on CHROMagar *Candida* medium and conventional biochemical methods. The *in vitro* activity of fluconazole (FL), itraconazole (IT), voriconazole (VO) ketoconazole (KT), amphotericin B (AB), 5-Fluorocitosine (5F) and LY303366 (LY) was determined using the broth microdilution version of the NCCLS reference method described in the M27-A document.
Results: A total of 129 isolates and five species were identified. The order of frequency of these species was as follows: *C. albicans* (47/36%), *C. parapsilosis* (32/24.8%), *C. tropicalis* (23/17.8%), *C. glabrata* (19/14.7%) and *C. krusei* (8/6.2%). Among azole compounds, VO was the most active drug overall with a MIC90 of 0.25 mg/L. For these drugs, the rank order of activity (based on MIC90 results) was VO > KT (MIC90, 0.5 mg/L) > IT (MIC90, 1 mg/L) > FL (MIC90, 32 mg/L). Against *C. albicans*, *C. parapsilosis* and *C. tropicalis* isolates, VO (MIC90, 0.03-0.25 mg/L) was four-fold and 16 to 32-fold more active than IT (MIC90, 0.12-1 mg/L) and FL (MIC90, 0.5-8 mg/L), respectively. VO and IT showed similar *in vitro* activity against *C. glabrata* (MIC90, 1 mg/L) and both were 32-fold more active than FL. LY was quite active against all *Candida* spp isolates (MIC90, ≤0.5 mg/L), except against *C. parapsilosis* (MIC90, 4 mg/L). AB MICs ranged from 0.12 to 1 mg/L.
Conclusions: C. albicans and *C. parapsilosis* were the most frequently isolated species. VO showed a potent *in vitro* activity against *Candida* spp bloodstream isolates and is a promising antifungal agent.

P9-027. Susceptibility of *Candida glabrata* to fluconazole and the correlations to different phenotypes and genotypes

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The susceptibility of the emerging pathogen *Candida* (*Torulopsis*) *glabrata* to fluconazole (FCA) differs in a wide range, partly strain dependent. In this study, the susceptibility of 62 *C. glabrata* isolates of 31 patients from 5 centres were evaluated in microdilution assay in HR-medium with 2% glucose according to the NCCLS M27 A and the DIN 58940-84. The results were correlated with those of phenotype analysis by Fourier-Transform Infrared Spectroscopy (FTIR) and genotyping by arbitrarily primed (AP) PCR. The susceptibility distributions classified to susceptible (S; MIC ≤8 mg/L), susceptibility dose dependent (SD; MIC >8 mg/L -≤32 mg/L) and resistant (R; MIC >32 mg/L) were as follows after 24 hours/48 hours: S 61.3%/22.6%; SD 35.5%/33.2% and R 3.2%/45.2%.
FTIR phenotyping showed three main classes (P1 41.9%; P2 33.9%; P3 24.2%) as well as AP-PCR genotyping (A 17.7%; B 62.9%; C 19.4%). All strains susceptible after an incubation time of 48 hours belonged to genotype B. All strains of genotype C displayed phenotype P1 and were resistant after 48 hours incubation time. Further validation of these preliminary data should be performed.

P9-028. Sterol and fatty acid compositions of *C. lusitaniae* isolates resistant to amphotericin B

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C. lusitaniae is an emerging opportunistic pathogen now recognised as an important nosocomial agent. Acquired resistance to amphotericin B and, less frequently, primary resistance are known to occur in this species. Resistance to amphotericin B is rare and most studies of its mechanism come from laboratory-derived mutants or clinical isolates cross-resistant to fluconazole. Usually, this resistance is associated with quantitative and/or qualitative modifications in the lipid content of the cell, especially sterols. To investigate the molecular basis of amphotericin B resistance in *C. lusitaniae*, we selected six clinical isolates known to be resistant or susceptible on the basis of *in vitro* and/or *in vivo* data. Antifungal susceptibility patterns were assessed by Etest. One isolate, recovered in a patient during amphotericin B prophylaxis, was highly resistant to amphotericin B (MIC D 16 µg/ml). Fatty acid analysis and sterol analysis were performed by gas chromatography after saponification of the cells. All the resistant isolates showed a greatly reduced ergosterol content and changes in sterol composition, both consistent with a defect in δ8→7 isomerase. Sterol composition did not correlate with degree of resistance. The susceptible isolates also showed reduced ergosterol content. All isolates showed similar fatty acid profiles. These results show that factors other than changes in lipid composition are involved in resistance of *C. lusitaniae* isolates to amphotericin B. Modifications of cell wall composition may play a role in this resistance. The low level of ergosterol in susceptible isolates may explain the unusual ability of this species to develop amphotericin B resistance during treatment.

P9-029. ECMM working party on cryptococcosis – UK isolates susceptibility results

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Data on the incidence of cryptococcal meningitis in the UK have been compiled from the Mycology Reference Laboratories in Bristol and Leeds and the PHLS Communicable Disease Surveillance Centre in London for 1997, 1998 and 1999.
Diagnosis was by detection of cryptococcal antigen and/or isolation in culture of blood or CSF. Isolates, identified as *Cryptococcus neoformans* var *neoformans*, were available in 90 of 147 cases, in the remaining 57 cases the diagnosis was based on serology.
Regimens including the combination of amphotericin B and flucytosine or fluconazole alone at 400 mg/d were commonly employed as initial therapy. Susceptibility patterns of isolates were assessed with an adaptation of the NCCLS M-27A method in YNBG medium. Analysis of results suggests no change in susceptibility over this time period. Only one of 45 isolates tested demonstrated antifungal drug resistance *in vitro*. This isolate was resistant to flucytosine (>64 mg/L), susceptible to amphotericin B (1.0 mg/L) and voriconazole (0.25 mg/L) and gave a profile that was susceptible-dependent-upon-dose with fluconazole (32 mg/L) and itraconazole (0.5 mg/L). A further 19 isolates produced MICs that were susceptible-dependent-upon-dose with itraconazole (0.25-0.5 mg/L) but were susceptible to the other antifungal agents. Minimum lethal concentrations (MLCs) were also assessed. For all drugs except flucytosine MLC results were generally a doubling dilution or two higher than the MIC results. In contrast flucytosine was often not fungicidal at 64 mg/L the highest concentration tested.
It is apparent that cryptococcal meningitis remains a disease predominantly of HIV-infected individuals and is regularly recorded as an AIDS-defining illness: 24 cases in 1997, 19 cases in 1998 and 24 cases in 1999. Despite the widespread use of fluconazole for treatment and as life-long maintenance therapy in HIV-infected patients resistance to this agent has not emerged as a significant clinical problem.

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P9-030. Phenotyping and antifungal drugs susceptibility study of *Candida albicans* isolated from Mexico City patients

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Nosocomial yeast infections have became a major cause of morbimortality in immunocompromised patients. In the last years the resistance development is a great trouble in treatment of mycoses. The aim of this study was determine the morphotype and serotype in 126 *Candida albicans* strains from healthy individual´s mouth (22) and vagina (20); AIDS patients mouth (20) and esophagus (19); and from vaginal candidiasis (45). All colonies were morphologically homogeneous and identified as *C. albicans* by germ tube production and chlamidoconidia formation in cornmeal and by their assimilation pattern of carbohydrates. Morphotype was identified by Phongpaichit et al technique. Serotype was determined by Hasenclever and Mitchel method. Antifungal susceptibility test was performed by ATB fungus kit ® and Fungitest ® for 50 strains (10 strains for each group).
The more frequent morphotype were 000 0 (23,8%) and 532 0 (11,1%), both absent in the healthy women of vaginal samples. In all study groups serotype B (81,7%) was more common than serotype A.
Concerning to different antifungal drugs, *C. albicans* strains exhibited more resistance to ketoconazole , itraconazole and fluconazole.

P9-031. *In vitro* activity of amphotericin B and itraconazole against *Aspergillus* spp. and the influence of incubation time

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Introduction: The majority od *Aspergillus* isolates are susceptible to both amphotericin B (AMB) and itraconazole (ITR). Recently, three *A. fumigatus* isolates from two patients with invasive aspergillosis have been documented to be resistant both *in vitro* and *in vivo* to ITR. This situation requires the performance of susceptibility testing.
Methods: The *in vitro* activity of AMB and ITR against 68 strains of *Aspergillus* (*30 A. glaucus*, 25 *A. fumigatus*, 9 *A. niger*, 2 *A. terreus*, 2 *A. flavus*) isolated from sputum and ear exudates was determined by the M38-P method. Inoculum suspensions were prepared from 7 day culture growth on PDA at 35ºC . Conidia were recovered and adjusted to 0.9-0.11 optical density. Final inoculu : $0.5x10^4$-$5x10^4$ CFU/ml. Readings were made after 48 and 72h of incubation.
Results: All the strains were inhibited with ≤1 µg/ml of AMB and ≤0.25 µg/ml of ITR. The MICs for AMB increased one dilution for 50% of strains when readings were performed at 72h, only 2 strains increased two dilutions. For ITR, the MICs for 94% of strains were the same at 48 and 72h. The geometric mean (GM) MICs of AMB at 48/72h for *A. glaucus* were 0.28/0.41, for *A. fumigatus* 0.36/0.58, and for *A. niger* 0.16/0.25. With regard to ITR, the GM MICs for *A. fumigatus* 0.06/0.06, and for *A. niger* 0.055/0.067. *A. fumigatus* were more resistant to AMB and ITR than other species