



Pilze: Resistenz und Empfindlichkeitsprüfung

Resistenz bei Pilzen

Wirkstoff	Testmethodik	Standard	Spezies / Bemerkung	Abstract
Echinocandine	beta-Glucan-Assay	-	<i>Candida parapsilosis</i>	M-1724
Fluconazol	MLST	-	<i>Candida tropicalis</i>	M-1536
Fluconazol	MBD - Resistenzinduktion	CLSI	<i>Candida parapsilosis</i>	M-2195
FLC; ITR; VOR; 5-FC	MBD	DIN	<i>C. glabrata; Candida spp.</i>	M-1537
FLC; VOR	MBD	k.A.	Echinocandin-Resistenzinduktion	M-2192
Voriconazol	CFU-Abnahme; Maus-Modell	-	<i>Aspergillus fumigatus</i>	M-1553
Voriconazol	Kombination mit Anidulafungin	CLSI	<i>A. fumigatus</i> ; FIC-Bestimmung	M-3764
Voriconazol	Resistenzinduktion durch Azole	CLSI	<i>Candida krusei</i>	M-2185

MBD = Mikrobouillondilution; AGF = Agardiffusion; ET = Etest; AGD = Agardilution; k.A. = keine Angabe;

FIC = Fractional Inhibitory Concentration

5-FC = Flucytosin; FLC = Fluconazol; ITR = Itraconazol; VOR = Voriconazol

Empfindlichkeitsprüfung: Pilze

Wirkstoff	Testmethodik	Standard	Spezies / Bemerkung	Abstract
Anidulafungin	AGD	CLSI	QC-Parameter (2 µg TBL-Breakpoints)	M-1541
TRB, CIC, VOR	MBD vs. XTT-Assay	CLSI	Methodenvergleich mit Dermatophyten	M-1539
Diverse	MBD	CLSI; EUCAST	<i>Candida braccarensis</i>	M-711 ; M-720
Diverse	Vitek-2	-	<i>Candida fermentati</i>	M-1528
Diverse	MBD	CLSI; EUCAST	<i>Candida nivariensis</i>	M-711 ; M-720
Diverse	Vitek-2; ET; MBD	EUCAST	Methodenvergleich mit <i>Candida</i> spp. und <i>Cr. neoformans</i>	M-1543
Diverse	M.I.C.E. (MIC-Evaluator Strips)	ISO	Oxoid „MICE“ Streifen (Konkurrenzprodukt zu Etest) Evaluierung	D-2243

MBD = Mikrobouillondilution; AGF = Agardiffusion; ET = Etest; AGD = Agardilution;

TRB = Terbinafin; CIC = Ciclopiroxolamin; VOR = Voriconazol

XTT = 2,3-bis (2-methoxy-4-nitro-5-[(sulphenylamino)carbonyl]-2H-tetrazolium hydroxid

D-2243

Validation of the MIC Results of MIC Evaluator Strips Following ISO Guidelines

M. H. NABUURS-FRANSEN, M. BOHNE, A. V.MILL, J. W. MOUTON;
Canisius Wilhelmina Hosp., Nijmegen, Netherlands.

Background: The M.I.C.Evaluator (M.I.C.E.) test strips (Oxoid Ltd) is a new product for determining the MIC. These strips provide a gradient of antibiotic stabilized on a polymer strip covering 15 doubling dilutions. The aim of the study was to evaluate the MIC values of the M.I.C.E. products vs the MIC reference method (ISO 20776-1) according to ISO guidelines (ISO 20776-2).

Methods: Following the ISO protocol, during daily clinical laboratory work 362 consecutive gram negative strains (fresh isolates) were collected and identified. From each strain, the MICs were determined following ISO guidelines (ISO 20776-1) and instructions of the M.I.C.E. manufacturer, respectively. The MIC values were read following instruction by two independent lab technicians. The following M.I.C.E. were evaluated: gentamicin 256-0.016 mg/L (CN 256), gentamicin 1024-0.064 mg/L (CN 1024), amoxicillin 256-0.016 mg/L (AML), amoxicillin-clavulanic acid 256-0.016 mg/L (AMC), cefotaxime 32-0.002 mg/L (CTX 32), cefotaxime 256-0.016 mg/L (CTX 256), levofloxacin 32-0.002 mg/L (LEV), imipenem 32-0.002 mg/L (IPM), ciprofloxacin 32-0.002 mg/L (CIP). Discrepancy analysis was performed following the ISO guideline.

Results: The isolates collected were: *Citrobacter* spp (n=20), *Enterobacter* spp (n=47), *Escherichia coli* (n=99), *Hafnia alveii* (n=1), *Klebsiella* spp (n=98), *Morganella morganii* (n=12), *Pantoea agglomerans* (n=1), *Proteus* spp (n=74), *Providencia rettgeri* (n=1), *Serratia marcescens* (n=9). Overall, 71% of the strains were susceptible for the different antimicrobial agents. The essential agreement after discrepancy analysis was: CN 256 97%, CN 1024 97%, AML 99%, AMC 100%, CTX 32 97%, CTX 256 97%, LEV 99%, IPM 92% and CIP 99%.

Conclusions: The M.I.C.Evaluator strips demonstrated an excellent performance, essential agreement 92 - 100%, to determine the MIC value for the antimicrobial agents tested and can be used during routine practice.

M-711

Candida nivariensis* and *bracarensis*: Rare Pathogens with Susceptibility Profiles Similar to *Candida glabrata

S. R. LOCKHART¹, S. A. MESSER ¹, M. GHERNA ², J. A. BISHOP ², W. G. MERZ ², M. A. PFALLER ¹, D. J. DIEKEMA

¹,
¹Univ. of Iowa, Iowa City, IA, ²Johns Hopkins Med. Inst., Baltimore, MD.

Background: Recent reports indicate that some clinical yeast isolates phenotypically identified as *C. glabrata* (CGLA) are the closely related species *C. nivariensis* (CNIV) and *C. bracarensis* (CBRA). Some of these CNIV and CBRA isolates have been resistant to 1 or more antifungals. We sought to determine the % of CGLA that are actually CNIV and CBRA, and to determine whether their susceptibility profile differed enough from CGLA to warrant routine identification of these isolates.

Methods: All isolates were collected as part of the ARTEMIS antifungal surveillance program and identified as CGLA using VITEK and conventional methods. Isolates were plated on BBL Chromagar to determine colony color and CGLA specific primers were used to amplify the ITS region of the rDNA. All isolates negative for CGLA specific amplification were subjected to peptide nucleic acid fluorescence *in-situ* hybridization (PNA-FISH) with probes specific for CGLA, CNIV, and CBRA. We determined the MICs (in ug/mL) for fluconazole, caspofungin, anidulafungin and micafungin by CLSI M27-A3 method. MICs for amphotericin B were determined by E-test.

Results: Of the 1603 presumed CGLA isolates, 1595 were positive by CGLA specific PCR. Of these, 1584 were mauve on Chromagar. All of the PCR negative isolates, and a subset of PCR positive isolates, were analyzed by PNA-FISH. All PCR positive isolates tested were confirmed positive with CGLA probe. Of the 8 remaining isolates, 1 was positive by CNIV probe, 2 were positive by CBRA probe and five could not be identified. The CNIV isolate originated in Australia and both of the CBRA isolates were from the US. None of the isolates had elevated MICs for fluconazole or the echinocandins. However, the amphotericin B MICs were 1, 1 and 8 for the CNIV and two CBRA isolates, respectively.

Conclusions: CNIV and CBRA currently comprise only a small fraction of CGLA isolates, and did not display fluconazole or echinocandin resistance.

M-720

Prevalence of *Candida nivariensis* (Cn) and *Candida bracarensis* (Cb) in Candidemia in Spain

A. GOMEZ-LOPEZ¹, I. CUESTA ¹, M. GUILLERMINA ISLA ¹, A. ALASTRUEY-IZQUIERDO ¹, D. RODRIGUEZ ², B. ALMIRANTE ², A. PAHISSA ², J. RODRIGUEZ-TUDELA ¹, M. CUENCA-ESTRELLA ¹;

¹CNM-ISCIIII, Majadahonda, Spain, ²Hosp. Vall d'Hebron, Barcelona, Spain.

Background: Recently two new *Candida* species have been described which are closely related to *C. glabrata* (Cg). These are named *C. nivariensis* (Cn) and *C. bracarensis* (Cb) and usually they are misidentified as *C. glabrata* using morphological or phenotypical methods. Antifungal susceptibility data revealed that these species are less susceptible than Cg to some antifungals. We describe the prevalence of the new discovered species in candidemia. Data from active population-based surveillance for candidemia in Barcelona were analyzed.

Methods: A total of 31 isolates firstly identified as Cg were included. Each clinical isolate represented a unique isolate from a patient. They accounted for 9% (31/345) of the cases of candidemia evaluated (between January 2002 to December 2003). The isolates were identified by sequencing of ribosomal DNA (ITS and D1D2 domains). The susceptibility profile was also analysed following EUCAST reference method . The antifungal agents used in the study were: Amphotericin B (AMB), fluconazole (FLC), itraconazole (ITC), voriconazole(VRC), ravuconazole (RVZ), posaconazole (POS) and caspofungin (CAS)

Results: All 31 isolates studied shared identical physiological characteristics. All of them were identified as Cg using ITS and D1D2 sequences by maximum parsimony clustering methodology. Cn nor Cb were found among the isolates analyzed. Antifungal susceptibility data were consistent with those of Cg reported in other studies: GeoMean and MIC₉₀ in µg/mL were as follow: AMB: 0.11/0.25, FLC: 6.04/16, ITC: 0.39/ 1, VRC: 0.25/0.5, RVZ: 0.27/1, POS: 0.42/1, CAS: 0.18/0.5

Conclusions: Although the prevalence of these new species remains unknown our data suggest a low persistence in candidemia in Spain. Further studies from multiple locations are needed to elucidate the epidemiology and antifungal susceptibility profile of these new species.

M-1528

Identification and Susceptibility Profile of *Candida fermentati* Isolates from a Worldwide Collection of *Candida guilliermondii* Clinical Isolates

S. R. LOCKHART, S. A. MESSER, M. A. PFALLER, D. J. DIEKEMA;
Univ. of Iowa, Iowa City, IA.

Background: The *Candida guilliermondii* (CGUI) complex comprises a number of species, most of which are rarely associated with human disease. However, the closely related species *Candida fermentati* (CFER) has been infrequently associated with human disease in the past. Because it cannot easily be distinguished from CGUI by phenotypic methods, we sought to determine what proportion of CGUI isolates were CFER and whether the CFER isolates had a susceptibility profile similar to that of CGUI.

Methods: We used a screening method outlined by Lan and Xu (Microbiology 152:1539) whereby CGUI and CFER were distinguished by unique restriction patterns of two separate enzymes on the PCR amplified ribosomal synthase gene fragment. Isolates collected between 2001 to 2007 as part of the ARTEMIS global surveillance study and identified as CGUI by VITEK were screened. MIC values were determined by CLSI M27-A3 method. Amphotericin B MICs were generated by E-test. Mean MICs of CGUI and CFER were compared using the Student's t-test. **Results:** One hundred forty-nine yeast isolates with a VITEK identification of CGUI were screened. CFER comprised 8.7% of the isolates. The CFER isolates had a global distribution pattern with isolates coming from North and South America, Europe, Australia and Asia. The majority of the isolates were from blood, but CFER was also recovered from joint fluid, peritoneal fluid and sputum. Mean MIC values (in micrograms/mL) by species are outlined in the table below.

Conclusions: CFER currently comprises a significant portion (almost 9%) of CGUI complex isolates and has a global distribution. CFER have lower echinocandin MIC values than do CGUI.

Species	Fluconazole	Amphotericin B	Caspofungin	Anidulafungin	Micafungin
<i>C. guilliermondii</i>	4.75	0.23	0.85	1.83	0.60
<i>C. fermentati</i>	8.46	0.36	0.43	1.58	0.38
P-value	0.079	0.088	0.056	0.362	0.0001

M-1536

Two Dominant Diploid Sequence Types of *Candida tropicalis* Isolates with Less Susceptibility to Fluconazole in Taiwan from 1999 to 2006

H. LO¹, S. LI², Y. YANG³, Y. LIN², H. KO³, A. WANG¹, K. CHEN², C. WANG¹;

¹Natl. Hlth.Res. Inst.s, Miaoli County, Taiwan, ²Ctr.s for Disease Control, Taipei, Taiwan, ³Natl. Chiao Tung Univ., Hsinchu, Taiwan.

Background: Among the 162, 244, and 246 *Candida tropicalis* isolates collected in Taiwan Surveillance of Antimicrobial Resistance of Yeasts (TSARY) in 1999, 2002, and 2006, 23 (14.2%), 0, and 132 (53.7%) isolates with minimum inhibitory concentrations (MICs) of fluconazole ³ 64 mg/ml, respectively.

Methods: Multilocus sequence typing (MLST) was used to characterize the genetic profiles of 102 *C. tropicalis* isolates collected from 19 hospitals in Taiwan.

Results: Among 61 differentiated DSTs, 11 had more than one isolates. Among 53 isolates with MICs ³ 64 mg/ml, 36 (69.7%) belonged to these 11 types whereas, only 16 (32.7%) ones with MICs \leq 16 mg/ml did ($p=0.005$). Furthermore, 24 (45.3%) with MICs ³ 64 mg/ml belonged to two closely related DSTs (140 and 98) whereas, only 4 (8.2%) ones with MICs \leq 16 mg/ml did ($p=0.00008$).

Conclusions: Thus, two related DSTs of *C. tropicalis* isolates with less susceptibility to fluconazole were dominant in Taiwan from 1999 to 2006.

M-1537

Candida glabrata: Parallel- and Cross-Resistance of Azoles (FLC,ITR,VOR), and of Flucytosine (FCY) in Clinical Isolates

A. SCHMALRECK¹, K. BECKER ², W. FEGELER ³;

¹MBS, Munich, Germany, ²I. Med. Micro., Univ., Münster, Germany, ³I. Med. Microb., Univ., Münster, Germany.

Background: *Candida glabrata* (Cg) is the second, resp. the third-most pathogen causing invasive candidiasis. To find probably new therapy options, the susceptibility test results of 1386 German Cg- isolates (18,3% of all *Candida* spp. isolated) were evaluated.

Methods: MIC determination was according to DIN 58940-84. MIC-reading was visually after 24 h at 36 °C with controls at 48 h. After the assignment of SIR-categories, comparison of all antifungal agents (AFA) could be performed for 1208 strains (87,2% of total Cg) by **S**usceptibility **P**attern (**SP**) **A**nalysis (**SPA**).

Results: AFA-susceptibility (S) /-resistance (R) percentage was as follows: FCY 97,6 / 1,9; FLC 84,6/4,9%; VOR 95,1 /2,8, and ITR 48,8 / 21,8. Except for the ITR-MIC₅₀ (0,25mg/l), all other MIC₅₀ were "S" (FCY: 0,125 mg/l; FLC 4 mg/l; VOR 0,125mg/l). However, AFA-MIC₉₀ and SIR categories were differing: (FCY 0,25mg/l: S; VOR 1mg/l: S; FLC 16 mg/l: I; ITR 2 mg/l: R).

30 Cg (2,5% of total Cg-isolates) showed parallel-resistance (PR) to all azoles, and most of them were susceptible against FCY (n=28; 93,3%). Whereas 80,3% of the ITR-resistant, and 30% of the FLC-resistant isolates were susceptible to VOR, all 35 VOR-resistant isolates were resistant to FLC and ITR. The isolates being resistant in parallel to only 2 azoles were: FLC/VOR 2,5%; ITR/VOR 2,9% and FLC/ITR 3,7%. Cross-resistance (CR) of the anti-metabolite FCY with all azoles was found in 0.2% (n=2). CR of the individual azoles with FCY was < 1% (FCY/FLC 0,3; FCY/VOR 0,2; FCY/ITR 0,6). More than 90% of the AZR showed susceptibility against FCY. Of the 23 FCY-resistant Cg, 13 strains (56,5%) showed S for FLC, 20 (87%) for VOR, and 10 (43,5%) for ITR.

Conclusions: Only little PR is found among the azoles. Therapeutic consequences which may result from the one-sided VOR-PR of FLC and ITR need further clarification. For severe Cg-infections, combination therapy with FCY could be considered increasingly, due to these in vitro results.

M-1539

Comparison Between Standard CLSI M38-A2 Method and A XTT-Based Method for Testing Antifungal Susceptibility of Dermatophytes

A. S. SHEHATA^{1,2}, P. K. MUKHERJEE¹, M. A. GHANNOUM¹;

¹Ctr. for Med. Mycology, Dept. of Dermatology, Univ. Hosp. of Cleveland, and Case Western Reserve Univ., Cleveland, OH, ²Microbiol. Dept., Faculty of Med., Suez Canal Univ., Ismailia, Egypt.

Background: The utility of 2,3-bis (2-methoxy-4-nitro-5-[(sulphenylamino)carbonyl]-2H-tetrazolium hydroxide (XTT)-based assay was tested in determining the antifungal susceptibility of dermatophytes to terbinafine (TRB), ciclopirox (CIC) and voriconazole (VOR).

Methods: Forty six dermatophyte isolates belonging to *Trichophyton rubrum* (TR, n=15), *Trichophyton mentagrophytes* (TM, n=7), *Trichophyton tonsurans* (TT, n=11) and *Epidermophyton floccosum* (EF, n=13) were tested by XTT-based method to determine MICs for the tested drugs. The obtained MIC results were compared with the results of Clinical and Laboratory Standards Institute (CLSI) M38-A2 method and the agreement between both methods was determined.

Results: With TR, XTT assay revealed MICs ranges (0.004->0.5 µg/ml, 0.125-0.25 µg/ml and 0.008-0.025 µg/ml for TRB, CIC and VOR, respectively. Similar MICs were obtained against TR using the CLSI M38-A2 methodology. Additionally, when tested with TM, TT and EF isolates, both XTT and CLSI methods resulted in comparable MICs ranges. The levels of agreement between both methods within 1 dilution were: 100% with TER, 97.8% with CIC and 89.1% with VOR. 100% agreement was obtained within two dilutions with all tested drugs.

Conclusions: This study revealed that XTT assay can be a useful tool for colorimetric antifungal susceptibility testing for dermatophytes and compares favorably with the CLSI methodology.

M-1541

Proposed Quality Control (QC) Parameters for Disk Diffusion (DD) Tests with Anidulafungin

J. E. ROSS¹, P. A. HOGAN², D. J. SHEEHAN², R. N. JONES¹;

¹JMI Lab., North Liberty, IA, ²Pfizer Inc, New York, NY.

Background: Anidulafungin (ANID) is a new echinocardin with high potency compared to other class agents (micafungin, caspofungin) via a β -glucan synthesis inhibition mechanism. An 8-laboratory study determined potential DD QC zone diameter ranges with four Clinical and Laboratory Standards Institute (CLSI)-recommended QC strains.

Methods: QC strains (*C. parapsilosis* [CPAR] ATCC 22019, *C. albicans* [CALB] ATCC 90028, *C. krusei* [CKRU] ATCC 6258, *C. tropicalis* [CTRO] ATCC 750) were tested in eight laboratories using 2 disk lots (MAST #216096 and DIFCO #8018129) and 3 Mueller-Hinton agar lots (Accumedia #08058A, Oxoid #08057O, DIFCO #08057D) each supplemented with 0.5 μ g/ml methylene blue and 2% glucose. Each site tested the QC strains by CLSI M44 method on 10 separate occasions generating 1,920 ANID results overall, along with 1,440 results for 2 control antifungals (fluconazole, voriconazole). Ranges were selected to contain 95% of results while minimizing the breadth to \leq 12 mm, where possible. 2- μ g ANID disks also contained 1% DMSO + 0.1% polysorbate-80 (Odabasi et al., 2003).

Results: Intra-laboratory zone range variations were 9-19, 10-18, 11-19 and 12-18 mm for the 4 QC strains. Also inter-laboratory media zone variations were extreme with results of 18-24, 26-38, 23-37 and 25-36 mm among QC organisms. Attempts to include 95% of results in range were achieved (Table), but proposed QC ranges were wide (13 to 18 mm). CPAR and possibly CKRU offer narrowest reproducible DD QC ranges.

Conclusions: ANID DD QC requires wide ranges (13-18 mm) to contain ³95% of reported results due to extensive intra- and inter-laboratory variation. ANID disk content (DMSO + P-80; used for CPAR testing) may require further refinement.

Proposed ranges in mm (% in range):

QC Organism	All laboratories	Seven laboratories
CPAR ATCC 22019	15-28 (95.9)	15-27 (95.5)
CALB ATCC 90028	22-41 (95.3)	24-39 (95.0)
CKRU ATCC 6258	20-38 (97.4)	20-35 (94.7)
CTRO ATCC 750	21-39 (96.4)	21-38 (94.6)

M-1543

Performance of the VITEK2 Yeast for Susceptibility Testing of Antifungal Agents against *Candida* spp. and *Cryptococcus neoformans*

F. BOTTEREL^{1,2}, C. CORBEL¹, F. FOULET¹, C. FARRUGIA^{1,2}, E. CAMBAU^{1,2}, C. CORDONNIER^{1,2}, S. BRETAGNE^{1,2};

¹APHP, Creteil, France, ²Université Paris 12, Creteil, France.

Background : VITEK2 yeast (bioMérieux) is designed for yeast identification and susceptibility testing of antifungal agents. We compared susceptibility testing results obtained by VITEK2 Yeast to those obtained using reference methods. This has never been done for *Cryptococcus neoformans*.

Methods : Were included in the analysis: 115 isolates of *Candida* spp from blood cultures (65 *C. albicans*, 22 *C. glabrata*, 10 *C. tropicalis*, 10 others) and 10 isolates of *C. neoformans*. The isolates were tested for amphotericin B (AMB), 5-flucytosine (5FC), fluconazole (FCZ) and voriconazole (VRZ) susceptibility using VITEK2, Etest and the EUCAST reference method. Essential Agreement (EA) was defined as MIC endpoints within +/- 2 dilutions. Categorical agreement (CA) was calculated based on previously published breakpoints.

Results: Excellent EA between VITEK2 and 24h-EUCAST was observed for AMB (97%), 5FC (98%), FCZ (89%) and VRZ (98%). With regard to species, the EA was > 95% for all species-drug combinations but *C. glabrata* (EA 70% for FCZ). CA between VITEK 2 and EUCAST ranged from 91% for FCZ to 98% for 5FC for *Candida* spp. For *C. neoformans*, EA ranged from 70% for FCZ to 100% for 5FC and CA ranged from 90% for 5FC to 100% for FCZ. Similar results were observed between VITEK2 and Etest with CA ranging from 93% for FCZ to 99% for VRZ. VITEK2 endpoints could be determined after 15.07 +/- 1.73 h of incubation for *Candida* spp. and 19.70 +/- 1.42 h for *C. neoformans*, whereas endpoints with Etest and reference method were 24 and 48 hours.

Conclusions: These results indicate that VITEK2 can accurately detect high-level resistance among *C. neoformans* and *C. albicans* isolates with excellent quantitative and qualitative agreements with the reference EUCAST method. VITEK2 can also provide faster results than Etest.

M-1553

In Vivo Efficacy of Posaconazole Against Laboratory-Selected Voriconazole-Resistant *Aspergillus fumigatus* in a Neutropenic Murine Pulmonary Aspergillosis Model

S. KRISHNAN-NATESAN^{1,2}, E. K. MANAVATHU ¹, J. L. CUTRIGHT ¹, G. J. ALANGADEN ¹, P. H. CHANDRASEKAR ¹;
¹Wayne State Univ., Detroit, MI, ²John D. Dingell VA Med Ctr, Detroit, MI.

Background: *A. fumigatus* VCZ-F33 is a laboratory-selected voriconazole-resistant (VCZ-R) isolate (derived from *A. fumigatus* F55064 with G448S mutation in the 14 α -lanosterol demethylase gene; ATCC 208995) with an MIC of 16 μ g/ml, but susceptible to posaconazole (POS) (MIC 0.25 μ g/ml). We investigated the *in vivo* efficacy of POS against VCZ- R isolates of *A. fumigatus* using a murine pulmonary aspergillosis model.

Methods: Six week old ICR female mice (n=100) were fed grape fruit juice daily and immunosuppressed by four injections of cyclophosphamide (300mg/kg) on days -4, -2, +1 and +4 (day 0 -day of infection). The mice (20 animals/group) were infected with either F55064 (VCZ-S) or VCZ-F33 (VCZ-R) *A. fumigatus* (1X10⁶ conidia/mouse). Treatment was initiated on day +1 with VCZ 25 or 50mg/kg; POS 25 or 50mg/kg and continued for 7 days. The efficacy of the antifungal treatment was evaluated by measurement of fungal burden in the lungs of infected mice.

Results: The fungal burden in the controls infected with F55064 was 12,043 CFU/lung whereas that in mice treated with either VCZ (25 or 50mg/kg) or POS (25 or 50mg/kg) was 280 CFU/lung and 469 CFU/lung respectively, resulting in ~ 2 log₁₀ reduction in both groups. However, the fungal burden in the lungs of mice infected with VCZ-F33 was 6,177 CFU/lung and that in mice treated with either VCZ (25 or 50 mg/kg) or POS (25 or 50 mg/kg) was 1010 CFU/lung (~0.75 log₁₀ reduction) and 14 CFU/lung (~3 log₁₀ reduction) respectively. While mortality rate was reduced in VCZ or POS treated mice infected with VCZ-S parent compared to controls (40% versus 70%), no survival advantage could be demonstrated with POS in mice infected with the VCZ-R *A. fumigatus*.

Conclusion: Lab-selected VCZ-R *A. fumigatus* had similar infectivity as the VCZ-S parent; POS had superior activity to that of VCZ in reducing fungal burden in mice infected with VCZ-R *A. fumigatus*.

M-1724

Novel Echinocandin Resistance Mechanisms in *Candida parapsilosis*

M. LI, J. D. SOBEL, R. A. AKINS;

Wayne State Univ. Sch. of Med., Detroit, MI.

Background: Echinocandins (Caspofungin, Micafungin, Anidulafungin) are relatively new antifungals to which acquired clinical resistance is still rare. In *Candida albicans*, resistance arises from point mutations in either of two “hot-spot” regions in the target gene, FKS1, encoding b-glucan synthetase. *C. parapsilosis* isolates typically are slightly less susceptible to these agents, and *in vitro*, readily mutate to resistance. We show here that these resistant mutants do not have mutations in the hot-spot regions of CpFKS1 and probably do not have hyperactive synthetase activity.

Methods: Spontaneous, independent mutants were selected from independent isolates of *C. parapsilosis* by single-step selection on each of the echinocandins. The CpFKS1 gene was amplified and sequenced with primers that immediately flank sequences hot-spot domains. b-glucan levels in culture supernatants of well defined cultures were determined with the Fungitell assay.

Results: Resistant mutants have MIC values >8 fold higher than their parent strains, yet had no mutations within the hot-spot domains 1 or 2. b-glucan levels in supernatants of parent and mutant cultures showed two phenotypes. Some mutants had wild type levels of glucan production/elaboration, and were inhibited by micafungin to the same extent as parents. Other mutants had reduced levels of glucan production/elaboration, and this level was not further altered by exposure to micafungin.

Conclusions: Assuming, for the moment, that supernatant levels of b-glucan are proportional to synthetase activity, these data indicate that echinocandin resistance in *C. parapsilosis* is likely not due to activating mutations in CpFKS1. We hypothesize that mutations which downregulate synthetase activity may be responsible for resistance in some mutants, by causing an upregulation of compensatory cell wall pathways, critically before exposure. In other mutants, the likely mechanism is direct activation of the compensatory pathways. More work is needed to substantiate these hypotheses.

M-2185

In Vitro Induction of Voriconazole Resistance in *Candida krusei* by Azole Exposure

F. GRENOUILLET, G. EGLIN, P. MURET, N. DEVILLARD, J. LEROY, L. MILLON;
CHU Jean Minjoz, BESANCON, France.

Background: *C. krusei* (*Ck*) is innately resistant to fluconazole (FLU), but susceptible to voriconazole (VOR). We identified a *Ck* strain from urinary tract of a kidney transplant patient, with in vivo acquisition of VOR resistance following VOR exposure. We hypothesized that exposure to suboptimal VOR concentrations could lead to VOR resistance. Thus, we investigated in vitro generation of VOR-resistant derivatives of *Ck* by VOR or FLU-exposure.

Methods: Nine *Ck* clinical strains, susceptible to VOR and with no previous voriconazole exposure, were used. Each strain was grown in brain-heart broth (BHB) containing 0.001 µg/mL of VOR, with daily subcultures in fresh BHB for 30 days. Then subcultures in BHB without VOR were made over 30 additional days. Each clinical strain was also submitted to 54 sequential subcultures in BHB containing FLU (10 µg/mL). MICs of VOR were assessed for sequential derivatives using CLSI M27-A2 method.

Results: Exposure to low VOR concentrations generated VOR-resistant derivatives of *Ck*. Geometric means of VOR MICs were respectively: 0.3 µg/mL before exposure, 5.4 µg/mL at day 6 and 11.8 µg/mL at day 30. Eight strains produced VOR-resistant derivatives by day 30. VOR-resistance was relatively stable after the 30 days without VOR exposure: decrease in mean of VOR-MIC (6.6 µg/mL) ; only 2 strains with at least a four-fold decrease of MICs. After 54 days of FLU-exposure, only one of the nine strains showed derivatives with significant increase in VOR-MIC (0.25 µg/mL at day 0 and 2 µg/mL at day 54).

Conclusions: 1) Our experimental study confirms the risk of occurrence of VOR-resistance in *Ck* following exposure to suboptimal concentrations of VOR, especially in patients presenting *Ck* colonization of body sites, i.e. urine, in which VOR concentrations are low. 2) FLU-exposure is not frequently associated with VOR-resistance in *Ck*, even if induction of VOR-resistance does seem to be possible. Further studies are needed to assess the impact of *in vivo* FLU-exposure on VOR susceptibility of *Ck* clinical strains.

M-2192

Development of Azole Resistance in *Candida glabrata* after Echinocandin Exposure

J. A. VAZQUEZ, M. WIERMAN, A. GOLEMBIESKI, D. VAGER, D. BAXA;
Henry Ford Hosp., Detroit, MI.

Background: Echinocandins (EC) are an important part of antifungal therapy. We describe a case of recalcitrant invasive *C. glabrata* (Cg) infection with persistent blood cultures for 4 months. Previously pt had received 2 wks of caspofungin (cfgn); on admission pt was placed on micafungin (mfgn) 150mg/d. Pt was never exposed to azoles. The goal of the study was to characterize the evolution of this unique Cg strain and evaluate the different plausible mechanisms of resistance.

Methods: In-vitro testing was done on 14 sequential isolates recovered over 16 wks. Genotyping was performed for strain relatedness. To evaluate the mechanisms of resistance the *FKS1*, *FKS2*, and *FKS3* genes were sequenced and compared. Gene expression analysis of *CDR1*, *CDR2*, *ERG11* and *SNQ2* were performed. The relative gene expression levels with respect to the 1st isolate and an ATCC Cg control were expressed as fold-increases in the amount of transcript detected.

Results: In-vitro assays revealed initial cfgn & mfgn MICs of 16µg/ml & an anidulafungin (afgn) MIC of 2µg/ml, while remaining susceptible to flz & vcz. After 7 days of mfgn the MIC to afgn increased to 4µg/ml and the MICs to flz & vcz increased to > 64µg/ml & 4µg/ml, respectively. Isolates were identical by CHEF. Comparison of the *FKS1*, *FKS2*, & *FKS3* sequences of the initial & sequential strains revealed a novel point mutation in *FKS1* at G1950A, a stop codon at amino acid 650. Gene expression analysis revealed a step-wise increase in *CDR1/2* & *ERG11* expression when compared to baseline isolates.

Conclusion: We report the development of a Cg with RES to all 3 ECs and the simultaneous development of flz & vcz resistance without azole exposure and with exposure only to ECs. Possibilities include the point mutation in *FKS1*, along with the overexpression of *CDR1/2* & *Erg11*. This study shows the possible and unknown interaction between the glucan synthase and ergosterol pathways that may be affected by ECs. The data demonstrate the complexity of antifungal resistance pathways and the importance of antifungal exposure history.

M-2195

In Vitro Induced Resistance to Fluconazole in *Candida parapsilosis* Does Not Revert Following Withdrawal of the Antifungal

A. P. SILVA^{1,2}, S. COSTA-DE-OLIVEIRA^{1,2}, A. SILVA-DIAS^{1,2}, A. G. RODRIGUES^{1,3,2}, C. PINA-VAZ^{1,2};

¹Lab. of Microbiol., Faculty of Med., Porto, Portugal, ²Cardiovascular Res. & Dev. Unit, Faculty of Med., Porto, Portugal,

³Burn Unit and Dept. of Plastic and Reconstructive Surgery, Hosp. S. João, Porto, Portugal.

Background: The widespread use of antifungals has selected *Candida* species that are intrinsically resistant or with easily inducible resistance. Our previous studies, described the rapid development of azole resistance by *C. parapsilosis* after exposure to fluconazole (FLU), voriconazole (VOR) and posaconazole (POS). In this study we evaluated the stability of the *in vitro* induced azole resistance in *C. parapsilosis*. Additionally, we studied a *C. parapsilosis* blood isolate from a patient admitted at a surgical intensive care unit treated with FLU for ten days, which changed from susceptible to susceptible-dose dependent (S-DD).

Methods: Three strains with azole resistant (R) phenotypes (which were *in vitro* induced by FLU, VOR and POS) and the FLU S-DD clinical strain were sub-cultured in drug-free medium during thirty days. Minimal inhibitory concentration (MIC) values were re-determined each five days according to CLSI M27-A3 protocol.

Results: Regarding the *in vitro* induced azole resistant organisms, MIC of FLU, of VOR, and of POS didn't significantly change after thirty days of incubation in drug-free medium, maintaining the R phenotypes to azoles. The S-DD clinical isolate kept its phenotype following incubation in the absence of drug.

Conclusions: The present data shows that, when R strains previously grown in FLU, VOR and POS were sub-cultured in antifungal-free medium, the MICs of azoles for such strains didn't revert, retaining R phenotypes. Similar findings occurred with a clinical isolate previously exposed to FLU *in vivo*. In summary, we demonstrated the irreversible development of azole resistance in *C. parapsilosis* attending to the fact that such strains kept its phenotype even following prolonged withdrawal of the antifungal.

M-3764

Laboratory-selected Voriconazole-resistant (VCZ-R) *Aspergillus fumigatus*: Synergistic In Vitro Activity of the Combination of Voriconazole and Anidulafungin (AFG)

S. KRISHNAN-NATESAN^{1,2}, E. K. MANAVATHU³, P. H. CHANDRASEKAR³;

¹Wayne State Univ. and John D Dingell VAMC, Detroit, MI, ²John D. Dingell VA Med Ctr, Detroit, MI, ³Wayne State Univ., Detroit, MI.

Background: Invasive aspergillosis (IA) is a major cause of mortality in immunocompromised patients. VCZ resistance has been reported in clinical isolates of *A. fumigatus*. A clinical trial comparing the efficacy of the combination of VCZ and AFG for treatment of IA is in progress. We therefore investigated the *in vitro* activity of VCZ in combination with AFG against laboratory-selected VCZ-R isolates of *A. fumigatus*.

Methods: Conidial suspensions (2×10^4 cfu/ml) were prepared from 7 laboratory selected VCZ-R isolates (with a known G448S mutation in the 14α -lanosterol demethylase gene /target gene) that were derived from 3 different VCZ-susceptible isolates (VCZ-S) of *A. fumigatus*. The MIC of VCZ (lowest concentration of the drug that showed no visible growth) for the VCZ-S and VCZ-R isolates were 0.25 µg/ml and 2-32 µg/ml respectively. MEC values (lowest concentration of the drug that produced microcolonies) were used for AFG (range: 0.0325-0.25 µg/ml) for all isolates tested. Fungal viability was further confirmed using the MTT assay (3-(4,5-Dimethylthiazolyl)-2,5-diphenyltetrazolium bromide). The *in vitro* susceptibility of *A. fumigatus* to the combination of VCZ and AFG was evaluated by the fractional inhibitory concentration index (FICI) method, using the CLSI broth microdilution technique. ($FICI = (A[c] / A[a]) + (B[c] / B[a])$ where A[c] and B[c] are the MIC of drugs A (VCZ) and B (AFG) in combination; A[a] and B[a] are the MIC of drugs A and B respectively (drug interaction is synergistic if $FICI \leq 0.5$)).

Results: The combination of VCZ with AFG showed synergistic interaction (FICI range was 0.12- 0.50) for all VCZ-R isolates tested. Similar results were obtained using the MTT assay.

Conclusion: Synergy was demonstrated with VCZ in combination with AFG suggesting that the addition of AFG to VCZ may provide enhanced activity in the treatment of IA due to VCZ-R *A. fumigatus*.