ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 2009, p. 1242–1244 Vol. 53, No. 3
0066-4804/09/$08.00+0 doi:10.1128/AAC.01368-08

A 10-Year Survey of Antifungal Susceptibility of Candidemia Isolates from Intensive Care Unit Patients in Greece▽

George Dimopoulos,[1,2]† Aristea Velegraki,[3]†* and Matthew E. Falagas[2,4]

Department of Critical Care, University Hospital Attikon, Medical School, National and Kapodistrian Univeristy of Athens, Athens, Greece[1]; Alfa Institute of Biomedical Sciences, Athens, Greece[2]; Mycology Laboratory, Medical School, National and Kapodistrian University of Athens, Athens, Greece[3]; and Department of Medicine, Tufts University School of Medicine, Boston, Massachusetts[4]

Received 11 October 2008/Returned for modification 15 November 2008/Accepted 18 December 2008

This study retrospectively reviews the susceptibility of 135 baseline ICU candidemia isolates (from 1997 to 2007) to nine antifungals as determined by the AFST-EUCAST microdilution method and identifies the most frequent causative agents of confirmed point-source candidemia outbreaks in local intensive care units. A minority of common and rare *Candida* species displayed decreased susceptibility to all antifungals.

Candidemia occurs in up to 10% of the patients in intensive care units (ICU), leading to prolonged use of mechanical ventilation and duration of hospital stay (5, 7, 8, 14, 16). As the choice of antifungals for treating candidemia in critically ill patients depends on knowledge of the local epidemiology, this study reviews the susceptibility of ICU candidemia strains isolated from 1997 to 2007 and archived in the UOA/HCPF1 collection (World Data Centre of Microorganisms, WDCM929 [http://wdcm.nig.ac.jp/hpcc.html]).

A total of 135 baseline *Candida* species from patients with ICU-acquired candidemia were tested. Isolates were identified to the species level by morphological and biochemical analyses and, where necessary, by sequencing of the internal transcribed spacer region and/or the D1/D2 domain, as described previously (4, 11, 12). MICs of pure compounds were determined with the AFST-EUCAST reference procedure (22). We tested standard compounds of fluconazole (Pfizer, Sandwich, Kent, UK), amphotericin B (Sigma, St. Louis, MO), itraconazole (Janssen, Beerse, Belgium), and flucytosine (Sigma, St Louis, MO), voriconazole (Pfizer, Sandwich, Kent, United Kingdom), caspofungin (AS MedCare, Lexington, KY), and posaconazole (Schering Plough Research Institute, Kenilworth, NJ). Newer echinocandins, micafungin (Astellas Pharma Inc., Osaka, Japan) and anidulafungin (Pfizer, Groton, CT), were also tested. Quality control was performed for every testing occasion by using the designated quality control strains, *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019, which were within the MIC range given for the EUCAST-evaluated drugs (22). The quality control MIC range for anidulafungin was 0.25 to 0.5 μg/ml and 0.5 to 1 μg/ml for micafungin and caspofungin. For ATCC 22019, anidulafungin and micafungin MIC ranges were 0.5 to 2 μg/ml, and the MIC range for caspofungin was 2 to 4 μg/ml.

Inclusion of outbreak strains in this study may have constrained the accuracy of *Candida* species ranking order, but it revealed the predominant causes of point source ICU outbreaks of candidemia during the study period. In that respect, *C. lusitaniae* (1), *C. krusei*, and *C. parapsilosis* (data not shown) were causes of verified ICU outbreaks according to the related case definitions and descriptive and molecular epidemiology parameters. Therefore, a higher percentage (66.6%) of non-*C. albicans* isolates is reported than the 42% recorded for Europe in the SENTRY study (17). Despite *C. parapsilosis* being reported as the most frequent non-*C. albicans* ICU candidemia agent in Europe, geographical and possibly time-associated variations are highlighted in certain European studies in which *C. glabrata* is the second most frequently reported ICU candidemia agent (10, 13). Temporal variation was shown in a recent study in which, in 2002, *C. parapsilosis* reached 51% of the total isolation rates yet without reference to ICU outbreaks, while the absence of *C. tropicalis* was noted in the years 2000 to 2001 (3). Our study indicates that in the past 10 years, *C. glabrata* and *C. tropicalis* were always isolated at fluctuating isolation rates, whereas *C. dubliniensis*, known for its potential to acquire fluconazole resistance (2, 20), comprised 8.8% of the *C. albicans* isolates and was isolated only in the first 5 years of the study.

Our results show that drugs traditionally used in the ICU as standard treatments, such as fluconazole and amphotericin B, generally demonstrate low MICs, in agreement with previous observations (9, 19). However, high amphotericin B MICs were noted in two outbreak-unrelated *C. krusei* strains, the outbreak strain *C. lusitaniae* (MIC, 4 μg/ml) (Table 1), and 3/10 *C. tropicalis* strains (MIC, 2 μg/ml). Interestingly, high flucytosine MICs (32 μg/ml) were recorded for 6/45 *C. albicans* isolates (MIC, ≥64 μg/ml), compared with 3/19 *C. krusei* isolates, 2/18 *C. lusitaniae* isolates, 1/2 *C. rugosa* isolates, and a *C. intermedia* isolate. Besides *C. glabrata* and *C. krusei* resistance to fluconazole, only 4/18 outbreak-unrelated *C. parapsilosis* isolates demonstrated resistance (MIC, 32 μg/ml) (21). Nonetheless, fluconazole resistance associated with ICU fluconazole usage over the study period was not documented. Overall, posaconazole and voriconazole displayed potent antifungal activities against most ICU candidemia isolates. Yet, voriconazole resistance was identified in 8/19 *C. krusei* and 2/10 *C. tropicalis* isolates in accordance with their increased itracon-

* Corresponding author. Mailing address: Mikras Asias 75, Goudi, Athens 115 27-GR. Phone: 30-210-746 2146. Fax: 30-210-746 2147. E-mail: aveleg@med.uoa.gr.
† These authors contributed equally and share first authorship.
▽ Published ahead of print on 29 December 2008.

azole MICs. Elevated posaconazole MICs were recorded for the same *C. albicans*, *C. krusei*, *C. glabrata*, and *C. tropicalis* strains, which displayed high itraconazole MICs and resistance to fluconazole and voriconazole, indicating azole cross-resistance.

Elevated echinocandin MICs were recorded for some *C. parapsilosis*, *C. guilliermondii*, and *C. rugosa* isolates, for a single *C. kefyr* isolate, and for some *C. tropicalis* isolates. The overall MIC_{90} for anidulafungin and caspofungin against the six most frequently found *Candida* isolates was 0.5 μg/ml and for micafungin was 0.03 μg/ml, whereas 92% of the 121 isolates frequently found (Table 1) were inhibited at anidulafungin and caspofungin MICs of ≤2 μg/ml, as recorded previously (6, 19). Overall, the MIC_{90} of echinocandins against *C. parapsilosis* (2 μg/ml) was conspicuously higher (18) than those recorded for the common *Candida* species, such as *C. albicans* (MIC range, 0.06 to 0.25 μg/ml), *C. glabrata* (MIC range, 0.12 to 1 μg/ml), and *C. tropicalis* (MIC range, 0.12 to 0.25 μg/ml).

The majority of *Candida* species demonstrated limited species-related and echinocandin-specific trailing and paradoxical (14) (Eagle) phenomena. With anidulafungin, *C. albicans* trailing was limited to 7/45 isolates. Paradoxical phenomena occurred in only 10/45 *C. albicans* isolates, 1/18 *C. lusitaniae* isolates, and 1/4 *C. dubliniensis* isolates, while these phenomena occurred with micafungin (15) in only 4/45 *C. albicans* and only 1/4 *C. dubliniensis* isolates.

Data for the relative isolation rates and the recognition of outbreak-associated *Candida* species, including isolate susceptibility to nine established and recently licensed antifungal agents from Greek ICU, are reviewed for the first time. Indigenous ICU isolate susceptibility trends show that decreased susceptibility to all antifungals has a sporadic distribution among all isolates.

TABLE 1. Antifungal agent MIC ranges against 135 baseline ICU candidemia isolates and median MIC_{90}s of 121 *Candida* species isolated at high frequency

Drug[a]	EUCAST microdilution MIC (μg/ml) for:																							
	C. albicans ($n = 45$)		*C. krusei* ($n = 19$)[b]		*C. parapsilosis* (sensu lato) ($n = 18$)[c]		*C. lusitaniae* ($n = 18$)[d]		*C. glabrata* (sensu lato) ($n = 11$)[e]		*C. tropicalis* ($n = 10$)		*C. dubliniensis* ($n = 4$)	*C. guilliermondii* ($n = 3$)		*C. rugosa* ($n = 2$)	*C. pelliculosa* ($n = 1$)	*C. kefyr* ($n = 1$)	*C. lipolytica* ($n = 1$)	*C. pulcherrima* ($n = 1$)	*C. intermedia* ($n = 1$)
	Range	MIC_{90}	Range	MIC_{90}	Range	MIC_{90}	Range	MIC_{90}	Range	MIC_{90}	Range	MIC_{90}	Range	Range	MIC_{90}	Range	MIC_{90}					
ANID	0.03–0.5	0.12	0.03–0.5	0.06	0.5–4	2	0.03–0.5	1	0.03–0.12	0.12	0.03–8	0.25	0.03–1	0.5–2	0.5	0.12–2	1.0	16	0.03	0.03	0.03
MICA	0.03–0.5	0.06	0.03–8	0.12	0.5–4	2	0.03–2	0.12	0.03–0.12	0.25	0.03–8	0.12	0.03–05	0.25–2	0.5	0.03–8	0.5	4	0.03	0.03	0.03
CASP	0.12–1	0.12	0.5–8	0.12	0.5–8	2	0.25–8	1	0.25–4	1	0.12–4	0.25	0.25–2	1–16	0.25–8	0.25–8	0.03	8	0.12	0.03	0.03
FC	0.12–32	2	0.12–64	2	0.12–0.5	0.5	0.12–64	2	0.12–0.5	0.25	0.12–0.5	0.5	0.12–1	0.12–0.25	0.12	0.5–64	0.12	0.12	0.12	0.12	64
POS	0.06–4	0.12	0.06–2	0.5	0.03–2	0.12	0.03–0.5	0.03	0.12–4	0.25	0.03–8	0.12	0.01–2	0.12–0.5	0.03	0.03–0.25	0.03	0.25	0.03	0.03	0.25
VO	0.01–2	0.03	0.12–16	1	0.06–2	0.06	0.12–4	0.5	0.25–8	0.5	0.06–16	0.12	0.03–4	0.06–0.5	0.01–0.5	0.12	4	0.03	0.03	0.25	0.06
IT	0.03–16	0.5	0.12–8	0.12	0.03–8	0.12	0.12–4	0.25	0.5–8	0.5	0.12–16	0.12	0.06–8	1–4	0.03–1	0.25	0.25	0.25	0.12	0.12	0.12
FL	0.12–32	0.5	32–64	32	1.0–32	4	0.12–4	4	8.0–64	16	0.5–4	2	0.12–0.25	0.25–4	2.0–16	2.0	0.12	1	0.25	0.25	1
AB	0.12–4	0.12	0.25–4	0.25	0.12–4	0.25	0.12–4	4	0.5–4	0.5	0.25–2	0.25	0.06–1	0.5–2	0.12–1	0.25	0.5	1	0.5	0.12	0.5

[a] ANID, anidulafungin; MICA, micafungin; CASP, caspofungin; FC, flucytosine; POS, posaconazole; VO, voriconazole; IT, itraconazole; FL, fluconazole; AB, amphotericin B.
[b] Includes tests for 1/10 outbreak strains.
[c] Differential MICs for *C. orthopsilosis* and *C. metapsilosis* were incorporated; includes tests for 1/13 outbreak strains.
[d] Includes tests for 1/8 outbreak strains.
[e] Differential MICs for *C. bracarensis* and *C. nivariensis* within the *C. glabrata* clade were incorporated.

This study was supported partly by an unrestricted grant from Gilead Sciences, Greece, and partly by the University of Athens SARG Kapodistrias K.A 70/4/5905 and the mycology laboratory K.A 70/3/6915.

G.D. received speaker honoraria from Gilead, Wyeth, Pfizer, and Sanofi-Aventis. M.E.F. received speaker honoraria from Merck, Wyeth, AstraZeneca, Cipla, and Grunenthal.

REFERENCES

1. **Arabatzis, M., K. Kollia, P. Menounos, M. Logotheti, and A. Velegraki.** 2004. Delineation of *Clavispora lusitaniae* clinical isolates by PCR-SSCP analysis of the ITS1 region, a retrospective study comparing five typing methods. Med. Mycol. **42**:27–34.
2. **Bassetti, M., E. Righi, M. Tumbarello, A. Di Biagio, R. Rosso, and C. Viscoli.** 2006. *Candida* infections in the intensive care unit: epidemiology, risk factors and therapeutic strategies. Expert Rev. Anti. Infect. Ther. **4**:875–885.
3. **Bassetti, M., E. Righi, A. Costa, R. Fasce, M. P. Molinari, R. Rosso, F. B. Pallavicini, and C. Viscoli.** 2006. Epidemiological trends in nosocomial candidemia in intensive care. BMC Infect. Dis. **10**:6–21.
4. **Belazi, M., A. Velegraki, A. Fleva, I. Gidarakou, L. Papanaum, D. Baka, N. Daniilidou, and D. Karamitsos.** 2005. Candidal overgrowth in diabetic patients: potential predisposing factors. Mycoses **48**:192–196.
5. **Blumberg, H. M., W. R. Jarvis, J. M. Soucie, J. E. Edwards, J. E. Patterson, M. A. Pfaller, M. S. Rangel-Frausto, M. G. Rinaldi, L. Saiman, R. T. Wiblin, and R. P. Wenzel for the National Epidemiology of Mycoses Survey (NEMIS) Study Group.** 2001. Risk factors for candidal bloodstream infections in surgical intensive care unit patients: the NEMIS prospective multicenter study. Clin. Infect. Dis. **33**:177–186.
6. **Chryssanthou, E., and M. Cuenca-Estrella.** 2002. Comparison of the antifungal susceptibility testing Subcommittee of the European Committee on Antibiotic susceptibility testing proposed standard and the E-test with the NCCLS broth microdilution method for voriconazole and caspofungin susceptibility testing of yeast species. J. Clin. Microbiol. **40:**3841–3844.
7. **Dimopoulos, G., F. Ntziora, G. Rachiotis, A. Armaganidis, and M. E. Fal-**

agas. 2008. *Candida albicans* versus non-*albicans* bloodstream infection in critically ill patients: differences in risk factors and outcomes. Anesth. Analg. **106**:523–529.
8. **Falagas, M. E., K. E. Apostolou, and V. D. Pappas.** 2006. Attributable mortality of candidemia: a systematic review of matched cohort and case-control studies. Eur. J. Clin. Microbiol. Infect. Dis. **25**:419–425.
9. **Lass-Flörl, C., A. Mayr, S. Perkhofer, G. Hinterberger, J. Hausdorfer, C. Speth, and M. Fille.** 2008. Activities of antifungal agents against yeasts and filamentous fungi: assessment according to the methodology of the European Committee on Antimicrobial Susceptibility Testing. Antimicrob. Agents. Chemother. **52**:3637–3641.
10. **León, C., F. Alvarez-Lerma, S. Ruiz-Santana, M. A. León, J. Nolla, R. Jordá, P. Saavedra, and M. Palomar for the EPCAN Study Group.** 2008. Fungal colonization and/or infection in non-neutropenic critically ill patients: results of the EPCAN observational study. Eur. J. Clin. Microbiol. Infect. Dis. [Epub ahead of print.] doi:10.1007/s.10096-008-0618-z.
11. **Linton, C. J., A. M. Borman, G. Cheung, A. D. Holmes, A. Szekely, M. D. Palmer, P. D. Bridge, C. K. Campbell, and E. M. Johnson.** 2007. Molecular identification of unusual pathogenic yeast isolates by large ribosomal subunit gene sequencing: 2 years of experience at the United kingdom mycology reference laboratory. J. Clin. Microbiol. **45**:1152–1158.
12. **Lolis, N., D. Veldekis, H. Moraitou, S. Kanavaki, A. Velegraki, C. Triandafyllidis, C. Tasioudis, A. Pefanis, and I. Pneumatikos.** 2008. *Saccharomyces boulardii* fungaemia in an intensive care unit patient treated with caspofungin. Crit. Care **12**:414.
13. **Magill, S. S., S. M. Swoboda, E. A. Johnson, W. G. Merz, R. K. Pelz, P. A. Lipsett, and C. W. Hendrix.** 2006. The association between anatomic site of *Candida* colonization, invasive candidiasis, and mortality in critically ill surgical patients. Diagn. Microbiol. Infect. Dis. **55**:293–301.
14. **Ostrosky-Zeichner, L., J. H. Rex, P. G. Pappas, R. J. Hamill, R. A. Larsen, H. W. Horowitz, W. G. Powderly, N. Hyslop, C. A. Kauffman, J. Cleary, J. E. Mangino, and J. Lee.** 2003. Antifungal susceptibility survey of 2,000 bloodstream *Candida* isolates in the United States. Antimicrob. Agents Chemother. **47**:3149–3154.
15. **Pai, M. P., A. L. Jones, and C. K. Mullen.** 2007. Micafungin activity against *Candida* bloodstream isolates: effect of growth medium and susceptibility testing method. Diagn. Microbiol. Infect. Dis. **58**:129–132.
16. **Peres-Bota, D., H. Rodriguez-Villalobos, G. Dimopoulos, C. Melot, and J. L. Vincent.** 2004. Infections with *Candida* spp. in critically ill patients are primarily related to the length of stay in the intensive care unit. Clin. Microbiol. Infect. **10**:550–555.
17. **Pfaller, M. A., D. J. Diekema, R. N. Jones, H. S. Sader, A. C. Fluit, R. J. Hollis, and S. A. Messer for the SENTRY Participant Group.** 2001. International surveillance of bloodstream infections due to *Candida* species: frequency of occurrence and in vitro susceptibilities to fluconazole, ravuconazole, and voriconazole of isolates collected from 1997 through 1999 in the SENTRY antimicrobial surveillance program. J. Clin. Microbiol. **39**:3254–3259.
18. **Pfaller, M. A., D. J. Diekema, L. Ostrosky-Zeichner, J. H. Rex, B. D. Alexander, D. J. Andes, S. D. Brown, V. Chaturvedi, M. A. Ghannoum, C. C. Knapp, D. J. Sheehan, and T. J. Walsh.** 2008. Correlation of MIC with outcome for *Candida* species tested against caspofungin, anidulafungin, and micafungin: analysis and proposal for interpretive MIC breakpoints. Antimicrob. Agents Chemother. **46:**2620–2629.
19. **Richards, M. J., J. R. Edwards, D. H. Culver, and R. P. Gaynes.** 2000. Nosocomial infections in combined medical-surgical intensive care units in the United States. Infect. Control Hosp. Epidemiol. **21**:510–515.
20. **Rodriguez-Tudela, J. L., J. P. Donnelly, M. A. Pfaller, E. Chryssantou, P. Warn, D. W. Denning, A. Espinel-Ingroff, F. Barchiesi, and M. Cuenca-Estrella.** 2007. Statistical analyses of correlation between fluconazole MICs for *Candida* spp. assessed by standard methods set forth by the European Committee on Antimicrobial Susceptibility Testing (E.Dis. 7.1) and CLSI (M27-A2). J. Clin. Microbiol. **45**:109–111.
21. **Schelenz, S.** 2008. Management of candidiasis in the intensive care unit. J. Antimicrob. Chemother. **61**(Suppl. I)**:**i31–i34.
22. **Subcommittee on Antifungal Susceptibility Testing (AFST) of the ESCMID European Committee for Antimicrobial Susceptibility Testing (EUCAST).** 2008. EUCAST definitive document EDef 7.1: method for the determination of broth dilution MICs of antifungal agents for fermentative yeasts. Clin. Microbiol. Infect. **14**:398–405.