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Echinocandin and Triazole Antifungal Susceptibility Profiles for Clinical Opportunistic Yeast and Mold Isolates Collected from 2010 to 2011: Application of New CLSI Clinical Breakpoints and Epidemiological Cutoff Values for Characterization of Geographic and Temporal Trends of Antifungal Resistance

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The SENTRY Antimicrobial Surveillance Program monitors global susceptibility and resistance rates of newer and established antifungal agents. We report the echinocandin and triazole antifungal susceptibility patterns for 3,418 contemporary clinical isolates of yeasts and molds. The isolates were obtained from 98 laboratories in 34 countries during 2010 and 2011. Yeasts not presumptively identified by CHROMagar, the trehalose test, or growth at 42°C and all molds were sequence identified using internal transcribed spacer (ITS) and 28S (yeasts) or ITS, translation elongation factor (TEF), and 28S (molds) genes. Susceptibility testing was performed against 7 antifungals (anidulafungin, caspofungin, micafungin, fluconazole, itraconazole, posaconazole, and voriconazole) using CLSI methods. Rates of resistance to all agents were determined using the new CLSI clinical breakpoints and epidemiological cutoff value criteria, as appropriate. Sequencing of *fks* hot spots was performed for echinocandin non-wild-type (WT) strains. Isolates included 3,107 from 21 *Candida* spp., 146 from 9 *Aspergillus* spp., 84 from *Cryptococcus neoformans*, 40 from 23 other mold species, and 41 from 9 other yeast species. Among *Candida* spp., resistance to the echinocandins was low (0.0 to 1.7%). *Candida albicans* and *Candida glabrata* that were resistant to anidulafungin, caspofungin, or micafungin were shown to have *fks* mutations. Resistance to fluconazole was low among the isolates of *C. albicans* (0.4%), *Candida tropicalis* (1.3%), and *Candida parapsilosis* (2.1%); however, 8.8% of *C. glabrata* isolates were resistant to fluconazole. Among echinocandin-resistant *C. glabrata* isolates from 2011, 38% were fluconazole resistant. Voriconazole was active against all *Candida* spp. except *C. glabrata* (10.5% non-WT), whereas posaconazole showed decreased activity against *C. albicans* (4.4%) and *Candida krusei* (15.2% non-WT). All agents except for the echinocandins were active against *C. neoformans*, and the triazoles were active against other yeasts (MIC_{90}, 2 μg/ml). The echinocandins and triazoles were active against *Aspergillus* spp. (MIC_{90}/minimum effective concentration [MEC_{90}] range, 0.015 to 2 μg/ml), but the echinocandins were not active against other molds (MEC_{90} range, 4 to >16 μg/ml). Overall, echinocandin and triazole resistance rates were low; however, the fluconazole and echinocandin coresistance among *C. glabrata* strains warrants continued close surveillance.

The spectrum of opportunistic yeasts and molds causing invasive fungal infections (IFIs) is clearly increasing in diversity throughout the world (1–3). Accurate identification (ID) and antifungal resistance testing of these organisms are important for guiding therapy and determining prognosis in these IFIs, as well as in epidemiologic surveys (2, 4–12). One of the most important aspects of global and regional surveillance programs is to use state-of-the-art methods for ID and antifungal resistance testing of the organisms that are implicated in IFIs (2, 6–8, 11, 13). Accurate ID of opportunistic fungi is important in identifying geographic trends in the resistance profiles of common species and in assessing the epidemiology, pathogenicity, and antifungal susceptibility profiles of less common or "cryptic" species (1, 6–8, 13, 14).

Antifungal testing has progressed from humble beginnings with limited available drugs to the current arsenal of agents exhibiting broad-spectrum activities against a wide variety of fungi (11, 15, 16). The introduction of these newer agents (triazoles and echinocandins) and the development of standardized testing methods by the Clinical and Laboratory Standards Institute (CLSI) Subcommittee on Antifungal Susceptibility Testing (17–19) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (20) have proven to be invaluable to the clinician (21–28), particularly with the introduction of clinical breakpoints (CBPs) (11, 29–31). The advent of global surveillance programs, backed by molecular ID and resistance detection, has resulted in a vast accumulation of data that has presented a clearer view of changing patterns in fungal species distributions and the emergence of less-susceptible and less-common species (2, 6–8, 12, 14, 32, 33). It also became clear that the previously established breakpoints for available antifungal agents needed refinement to more specifically address reduced reporting times and broad differences in susceptibilities among species to the different agents, as well as to specifically address those non-wild-type (non-WT)

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strains within a given species exhibiting reduced susceptibilities to the respective agents (11). Antifungal susceptibility testing has departed from a "one breakpoint fits all" approach (15, 34, 35) to using more-refined species-specific CBPs at the shorter 24-h reporting times (11, 12, 29). The recent surveillance focus has been on how best to detect the emergence of resistance within a population of a given fungal species (2, 7, 8, 12–14, 36). The development of epidemiological cutoff values (ECVs) has aided in the detection of non-WT strains in given populations, allowing a more focused approach to the molecular definition of strains presenting less-susceptible profiles (6, 8, 11, 13, 33, 37).

The SENTRY Antimicrobial Surveillance Program has been active since 1997 and has reported the frequency of pathogen occurrence and the susceptibilities to various antifungal agents on a global scale (12, 14, 38). In the present study, we summarize the results of the global SENTRY Program for 2010 and 2011, comparing the activities of 3 echinocandins and 4 triazoles tested against a collection of 3,418 clinical isolates of *Candida* (3,107 isolates, 21 species), non-*Candida* yeasts (125 isolates, 10 species), *Aspergillus* (146 isolates, 9 species), and non-*Aspergillus* molds (40 isolates, 23 species). This study is unique in that molecular methods were used to confirm the IDs of the less-common species of *Candida*, as well as those of the non-*Candida* yeasts and all of the filamentous fungi (8). Furthermore, we applied the newly revised CBPs for the echinocandins, fluconazole, and voriconazole to determine the resistance profiles of various *Candida* species (11, 29–31) and the ECVs for these agents, as well as itraconazole and posaconazole, to detect emerging resistance among less-common species of *Candida* (11) and among isolates of *Aspergillus fumigatus* (37) and *Cryptococcus neoformans* (39). Furthermore, we provide a molecular characterization of the mechanisms of echinocandin resistance among isolates of *Candida* spp. showing elevated echinocandin MIC results.

MATERIALS AND METHODS

Organisms. A total of 3,418 clinical isolates from patients with IFI were collected during 2010 and 2011 from 98 laboratories (34 countries) in North America (1,349 isolates), Europe (1,191 isolates), Latin America (492 isolates), and the Asia-Pacific region (384 isolates) as part of the SENTRY Program. In each case, collection was approved by the appropriate institutional review board. Each participating center recovered consecutive nonduplicated isolates from patients with bloodstream infections (2,227 isolates), normally sterile body fluid, abscess, and tissue samples (425 isolates), respiratory tract infections (352 isolates), and other or unknown sites (414 isolates). Isolates were identified at the participating institutions using methods routinely employed at the submitting laboratory, including Vitek, MicroScan, API, and AuxaColor, supplemented by classical methods for yeast and mold ID (40, 41). Isolates were submitted to JMI Laboratories (North Liberty, IA), where the ID was confirmed by morphological, biochemical, and molecular methods (6, 8, 42). Yeast isolates were subcultured and screened using CHROMagar *Candida* (Becton, Dickinson, Sparks, MD) to ensure purity and to differentiate *Candida albicans*/*Candida dubliniensis*, *Candida tropicalis*, and *Candida krusei*. Biochemical tests, including Vitek 2 (bioMérieux, Hazelwood, MO), trehalose assimilation (for *Candida glabrata*), or growth at 45°C (for *C. albicans*/*C. dubliniensis*), were additionally used to identify common *Candida* species. Molecular methods were performed on common species of *Candida* that could not be definitively identified using phenotypic methods or that presented unusual phenotypic or biochemical profiles, as well as for all uncommon species of *Candida*, non-*Candida* yeasts, and all molds. *Candida* spp. and other yeasts were identified using sequence-based methods for the internal transcribed spacer (ITS) region, 28S ribosomal subunit (D1/D2), and the intergenic spacer (IGS) (*Debaryomyces* spp.) and IGS1 (*Trichosporon* spp.) (6, 8). All mold isolates were subcultured and analyzed by ITS sequencing, followed by specific molecular species ID within genera: β-tubulin for *Aspergillus* spp., translation elongation factor (TEF) for *Fusarium* spp., and 28S for all other genera of molds (8). Nucleotide sequences were examined using Lasergene software (DNAStar, Madison, WI) and then compared to database sequences using BLAST (http://www.nbeti.nlmnih.gov.blast). *Fusarium* spp. isolates were analyzed for TEF sequence using the *Fusarium*-ID database (http://www.isolate.fusariumdb.org) and the *Fusarium* multilocus sequence typing (MLST) database (http://chs.knaw.nl/fusarium/) (8). Results were considered acceptable if homology was >99.5% with other entries in the databases used for comparison. Available sequences that were considerably different from the majority of entries for one species were considered outliers and were discarded in the analysis. Additionally, if no match was found in the database, the ID was based on species complex (SC), genus, family, or order, according to the most current classification systems.

Among the 3,107 isolates of *Candida*, there were 1,405 isolates of *C. albicans*, 571 of *C. glabrata*, 565 of *Candida parapsilosis*, 318 of *C. tropicalis*, 79 of *C. krusei*, and 169 of miscellaneous *Candida* spp. (55 *Candida lusitaniae*, 50 *Candida dubliniensis*, 19 *Candida guilliermondii*, 16 *Candida kefyr*, 8 *Candida fermentati*, 4 *Candida lipolytica*, 3 *Candida intermedia*/*Candida pseudointermedia*, 2 *Candida catenulata*, 2 *Candida haemulonii*, 2 *Candida inconspicua*, 2 *Candida nivariensis*, 2 *Candida pararugosa*, and 1 each of *Candida bracarensis*, *Candida fabianii*, *Candida norvegensis*, and *Candida pelliculosa*). The collection also included *C. neoformans* (84 isolates), *Trichosporon asahii* (9 isolates), *Saccharomyces cerevisiae* (6 isolates), *Rhodotorula mucilaginosa* (5 isolates), *Trichosporon* spp. (5 isolates), *Dipodascus capitatus* (3 isolates), *Lodderomyces elongisporus* (2 isolates), and 1 isolate each from *Aureobasidium pullulans*, *Debaryomyces fabryi*, and *Trichosporon mycotoxinivorans*. Molds included 97 isolates from *A. fumigatus* sensu stricto, 49 from *Aspergillus* spp. (19 *Aspergillus flavus* species complex [SC], 15 *Aspergillus niger* SC, 7 *Aspergillus terreus* SC, 2 *Aspergillus alabamensis*, 2 *Aspergillus nidulans*, 2 *Aspergillus sydowii*, and 1 each of *Aspergillus foetidus* and *Aspergillus ustus* SC), and 40 from other molds (6 *Gibberella fujikuroi* SC, 4 *Scedosporium apiospermum*, 3 *Fusarium solani* SC, 3 *Penicillium* subgenus *Aspergilloides*, 2 *Penicillium* subgenus *Terverticillata*, 2 *Alternaria* spp., 2 *Paecilomyces variotii*, 2 *Sarocladium* [*Acremonium*] *kiliense*, and 1 each of *Absidia* [*Lichtheimia*] *corymbifera*, *Acremonium strictum* genogroup III, *Coprinellus radians*, *Penicillium roqueforti* group, *Penicillium* subgenus *Fureatum*, *Penicillium variabile*, *Phialemonium curvatum*, *Rhizomucor pusillus*, *Rhizopus microsporus* group, *Rhizopus oryzae*, a *Curvularia* sp., a *Fusarium* sp., a *Penicillium* sp., a *Trichoderma* sp., and *Exophiala dermatitidis*).

In addition to these isolates, we included the echinocandin MIC distributions from an earlier (2003 to 2007) collection of 8,023 *Candida* spp. (36) in order to assess any temporal change in echinocandin resistance between 2003 to 2007 and 2010 to 2011. *P* values were calculated by the χ^2 test for trend.

Antifungal susceptibility testing. All yeast isolates were tested for *in vitro* susceptibility to the echinocandins (anidulafungin, caspofungin, and micafungin) and the triazoles (fluconazole, itraconazole, posaconazole, and voriconazole) using CLSI (17) broth microdilution (BMD) methods. The MIC results for all agents were read following 24 h of incubation when the agents were tested against *Candida* spp., whereas MIC endpoints for the triazoles were read after 48 h when the drugs were tested against non-*Candida* yeasts. In all instances, the MIC values were determined visually as the lowest concentration of drug that caused significant growth diminution levels (17, 18).

In vitro susceptibility testing of *Aspergillus* spp. and other molds against the echinocandins and triazoles (itraconazole, posaconazole, and voriconazole) was performed by BMD as described in CLSI document M38-A2 (19). The triazole MICs and echinocandin minimum effective concentrations (MEC) were determined as described in the CLSI reference method (19).

We used the recently revised CLSI CBP values to identify strains of the 5 most common species of *Candida* (*C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei*) that were resistant to the echinocandins, as well as those that were resistant to fluconazole and voriconazole (11): anidulafungin, caspofungin, and micafungin MIC values of >0.5 μg/ml were considered resistant for *C. albicans*, *C. tropicalis*, and *C. krusei*, and MIC results of >4 μg/ml were categorized as resistant for *C. parapsilosis*; anidulafungin and caspofungin MIC values of >0.25 μg/ml and micafungin MIC values of >0.12 μg/ml were considered resistant for *C. glabrata*; fluconazole MIC results of >4 μg/ml were defined as resistant for *C. albicans*, *C. parapsilosis*, and *C. tropicalis*, and MICs of >32 μg/ml were considered resistant for *C. glabrata*. All isolates of *C. krusei* were defined as resistant to fluconazole. The CLSI resistance breakpoint for voriconazole was >0.5 μg/ml for *C. albicans*, *C. tropicalis*, and *C. parapsilosis*, and MIC results of >1 μg/ml were categorized as resistant for *C. krusei*; CLSI has not assigned CBPs for voriconazole and *C. glabrata* and recommends the ECV of 0.5 μg/ml to be used to differentiate wild-type (WT) from non-WT strains of this species (11, 30).

CBPs have not been established for any antifungal agent and the less-common species of *Candida*, non-*Candida* yeasts, *Aspergillus* spp., or the non-*Aspergillus* molds; however, ECVs have been established for echinocandins and triazoles and 6 species of *Candida* that are encountered less frequently (*C. lusitaniae*, *C. guilliermondii*, *C. dubliniensis*, *C. kefyr*, *C. orthopsilosis*, and *C. pelliculosa*) (11). ECVs have been derived for *C. neoformans* and fluconazole (8 to 16 μg/ml), itraconazole (1 μg/ml), posaconazole (0.5 μg/ml), and voriconazole (0.25 μg/ml) (39). ECVs have also been developed for *A. fumigatus*, *A flavus*, *A. terreus*, and *A. niger* and itraconazole, posaconazole, and voriconazole (37): itraconazole and voriconazole MIC values of >1 μg/ml were considered non-WT for *A. fumigatus*, *A. flavus*, and *A. terreus*; itraconazole MIC values of >1 μg/ml and voriconazole MIC values of >2 μg/ml were considered non-WT for *A. nidulans*, whereas itraconazole and voriconazole MIC values of >2 μg/ml were non-WT for *A. niger*. Posaconazole MIC values of >0.5 μg/ml were considered non-WT for *A. fumigatus*, *A. terreus*, and *A. niger*, and MIC results of >0.25 μg/ml were non-WT for *A. flavus*; posaconazole MIC values of >1 μg/ml were non-WT for *A. nidulans*. Isolates of these *Aspergillus* spp. for which triazole MIC results exceed the ECV are considered to be non-WT and may harbor acquired mutations in the *cyp51A* gene (43, 44).

Quality control was performed as recommended in CLSI documents M27-A3 (17) and M38-A2 (19) using the strains *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019.

All isolates of *Candida* spp. that were resistant to ≥1 of the echinocandins were further characterized regarding the presence or absence of a mutation in the hot spot (HS) regions of *fks1* and *fks2* (*C. glabrata* only), as described previously (13, 33).

TABLE 1 Geographic distribution of organisms collected during 2010 to 2011 in medical centers participating in the SENTRY Antimicrobial Surveillance Program

Organism/organism group	No. of isolates in each indicated geographic region:				
	North America	Europe	Latin America	Asia-Pacific	Total
Total, yeasts and molds	1,349	1,191	492	384	3,418
Yeast					
All *Candida* spp.	1,211	1,097	433	366	3,107
C. albicans	503	552	186	164	1,405
C. glabrata	306	175	38	52	571
C. parapsilosis	173	195	104	93	565
C. tropicalis	109	89	76	44	318
C. krusei	40	28	8	3	79
Other *Candida* spp.[a]	80	58	21	10	169
Cryptococcus neoformans	36	9	29	10	84
Other yeasts[b]	8	21	10	2	41
Mold					
All *Aspergillus* spp.	74	51	15	6	146
Aspergillus fumigatus	45	34	13	4	97
Other *Aspergillus* spp.[c]	29	16	2	2	49
Other molds[d]	20	15	5		40

[a] Other *Candida* spp. include *C. bracarensis* (1 strain), *C. catenulata* (2 strains), *C. dubliniensis* (50 strains), *C. fabianii* (1 strain), *C. fermentati* (8 strains), *C. guilliermondii* (19 strains), *C. haemulonii* (2 strains), *C. inconspicua* (2 strains), *C. kefyr* (16 strains), *C. lipolytica* (4 strains), *C. lusitaniae* (55 strains), *C. nivariensis* (2 strains), *C. norvegensis* (1 strains), *C. pararugosa* (2 strains), *C. pelliculosa* (1 strain), and *C. intermedia*/*C. pseudointermedia* (3 strains).

[b] Other yeasts include *Aureobasidium pullulans* (1 strain), *Debaryomyces fabryi* (1 strain), *Dipodascus capitatus* (3 strains), *Lodderomyces elongisporus* (2 strains), *Rhodotorula mucilaginosa* (5 strains), *Saccharomyces cerevisiae* (6 strains), *Trichosporon asahii* (9 strains), *Trichosporon mycotoxinivorans* (1 strain), *Trichosporon* spp. (5 strains), and unidentified yeasts (6 strains).

[c] Other *Aspergillus* spp. include *A. alabamensis* (2 strains), *A. flavus* species complex (SC) (19 strains), *A. foetidus* (1 strain), *A. nidulans* (2 strains), *A. niger* SC (15 strains), *A. sydowii* (2 strains), *A. terreus* SC (7 strains), and *A. ustus* SC (1 strain).

[d] Other molds include *Absidia (Lichtheimia) corymbifera* (1 strain), *Acremonium strictum* genogroup III (1 strain), *Coprinellus radians* (1 strain), *Fusarium solani* SC (3 strains), *Gibberella fujikuroi* SC (6 strains), *Paecilomyces variotii* (2 strains), *Penicillium roqueforti* group (1 strain), *Penicillium* subgenus *Aspergilloides* (3 strains), *Penicillium* subgenus *Furcatum* (1 strain), *Penicillium* subgenus *Terverticillata* (2 strains), *Penicillium variabile* (1 strain), *Phialemonium curvatum* (1 strain), *Rhizomucor pusillus* (1 strain), *Rhizopus microsporus* group (1 strain), *Rhizopus oryzae* (1 strain), *Sarocladium* (*Acremonium*) *kiliense* (2 strains), *Scedosporium apiospermum* (4 strains), *Alternaria* spp. (2 strains), *Curvularia* spp. (1 strain), *Fusarium* spp. (1 strain), *Penicillium* spp. (1 strain), *Trichoderma* spp. (1 strain), and *Exophiala dermatitidis* (1 strain).

RESULTS AND DISCUSSION

Table 1 shows species distribution by geographic region of the fungi implicated in IFIs during 2010 and 2011. As expected, *Candida* spp. accounted for the vast majority of IFIs in all four regions (91.0%; range, 88.0% [Latin America] to 95.3% [Asia-Pacific]). *C. albicans* was most common in Europe (50.3%) and least common in North America (41.5%), whereas *C. glabrata* was most common in North America (25.3%) and least common in Latin America (8.8%). *C. parapsilosis* and *C. tropicalis* were most common in Latin America (24.0% and 17.6%, respectively) and the Asia-Pacific region (25.4% and 12.0%, respectively), and *C. krusei* was most common in North America (3.3%), as were other miscellaneous species of *Candida* (6.6%).

Non-*Candida* yeasts accounted for 3.7% of all IFI isolates, dominated by *C. neoformans* (2.5% of all IFI isolates [range, 0.8% to 5.9%] and 67.2% of all non-*Candida* yeasts).

Aspergillus spp. accounted for 4.3% of all IFI isolates, with *A. fumigatus* sensu stricto being the most common species of the genus in all regions (range, 60.8% of all *Aspergillus* spp. isolates [North America] to 86.7% [Latin America]). The other molds were largely reported from North America and Europe (Table 1).

Geographic trends in resistance to the echinocandins and azoles for the five most common species of *Candida* are shown in Table 2. Resistance to the echinocandins was distinctly uncommon (overall range, 0.0 to 1.2%) among *C. albicans* (0.0 to 0.6%), *C. tropicalis* (0.0%), *C. parapsilosis* (0.0 to 1.2%), and *C. krusei* (0.0%) isolates from all four geographic regions, using the new CLSI CBP values. Resistance to anidulafungin (3.8%), caspofungin (1.9%), micafungin (1.9%), and the triazoles (5.8% to 13.5%) was most prominent among isolates of *C. glabrata* from the Asia-Pacific region, whereas none of the *C. glabrata* isolates from Latin America were resistant to the echinocandins (Table 2). Fluconazole resistance was uncommon among isolates of *C. albicans* and

TABLE 2 Frequency of antifungal resistance among clinical isolates of *Candida* spp. by geographic region in the 2010-2011 SENTRY Surveillance Program

Candida species	Antifungal agent	No. of isolates (% resistant[a] to each antifungal agent) by region				
		North America	Europe	Latin America	Asia-Pacific	Total
C. albicans	Anidulafungin	503 (0.0)	552 (0.0)	186 (0.0)	164 (0.0)	1,405 (0.0)
	Caspofungin	503 (0.0)	552 (0.4)	186 (0.0)	164 (0.6)	1,405 (0.2)
	Micafungin	503 (0.0)	552 (0.4)	186 (0.0)	164 (0.0)	1,405 (0.1)
	Fluconazole	503 (0.6)	552 (0.2)	186 (0.0)	164 (0.6)	1,405 (0.4)
	Posaconazole[c]	503 (0.6)	552 (8.3)	186 (6.5)	164 (0.6)	1,405 (4.4)
	Voriconazole	503 (0.6)	552 (0.2)	186 (0.0)	164 (0.6)	1,405 (0.4)
C. glabrata	Anidulafungin	306 (1.6)	175 (1.7)	38 (0.0)	52 (3.8)	571 (1.8)
	Caspofungin	306 (1.6)	175 (1.7)	38 (0.0)	52 (1.9)	571 (1.6)
	Micafungin	306 (1.3)	175 (1.1)	38 (0.0)	52 (1.9)	571 (1.2)
	Fluconazole	306 (9.8)	175 (6.3)	38 (5.3)	52 (13.5)	571 (8.8)
	Posaconazole[c]	306 (3.3)	175 (2.9)	38 (5.3)	52 (5.8)	571 (3.5)
	Voriconazole[b]	306 (11.1)	175 (9.1)	38 (7.9)	52 (13.5)	571 (10.5)
C. parapsilosis	Anidulafungin	173 (1.2)	195 (0.0)	104 (1.0)	93 (0.0)	565 (0.5)
	Caspofungin	173 (0.0)	195 (0.0)	104 (0.0)	93 (0.0)	565 (0.0)
	Micafungin	173 (0.0)	195 (0.0)	104 (0.0)	93 (0.0)	565 (0.0)
	Fluconazole	173 (0.0)	195 (3.1)	104 (1.0)	93 (5.4)	565 (2.1)
	Posaconazole[c]	173 (1.2)	195 (0.5)	104 (3.9)	93 (6.5)	565 (2.3)
	Voriconazole	173 (0.0)	195 (0.0)	104 (0.0)	93 (1.1)	565 (0.2)
C. tropicalis	Anidulafungin	109 (0.0)	89 (0.0)	76 (0.0)	44 (0.0)	318 (0.0)
	Caspofungin	109 (0.0)	89 (0.0)	76 (0.0)	44 (0.0)	318 (0.0)
	Micafungin	109 (0.0)	89 (0.0)	76 (0.0)	44 (0.0)	318 (0.0)
	Fluconazole	109 (2.7)	89 (1.1)	76 (0.0)	44 (0.0)	318 (1.3)
	Posaconazole[c]	109 (7.3)	89 (7.9)	76 (2.6)	44 (0.0)	318 (5.3)
	Voriconazole	109 (0.9)	89 (0.0)	76 (0.0)	44 (0.0)	318 (0.3)
C. krusei	Anidulafungin	40 (0.0)	28 (0.0)	8 (0.0)	3 (0.0)	79 (0.0)
	Caspofungin	40 (0.0)	28 (0.0)	8 (0.0)	3 (0.0)	79 (0.0)
	Micafungin	40 (0.0)	28 (0.0)	8 (0.0)	3 (0.0)	79 (0.0)
	Posaconazole[c]	40 (17.5)	28 (7.1)	8 (25.0)	3 (33.3)	79 (15.2)
	Voriconazole	40 (2.5)	28 (0.0)	8 (0.0)	3 (0.0)	79 (1.3)

[a] Resistance is defined as an MIC of >0.5 μg/ml for anidulafungin, caspofungin, and micafungin against *C. albicans*, *C. tropicalis*, and *C. krusei*, an MIC of >4 μg/ml against *C. parapsilosis*, an MIC of >0.25 μg/ml for anidulafungin and caspofungin, and an MIC of >0.12 μg/ml for micafungin against *C. glabrata*; an MIC of >4 μg/ml for fluconazole against *C. albicans*, *C. tropicalis*, and *C. parapsilosis*, an MIC of >32 μg/ml against *C. glabrata*; and an MIC of >0.5 μg/ml for voriconazole against *C. albicans*, *C. tropicalis*, and *C. parapsilosis*.

[b] In lieu of clinical breakpoints for voriconazole against *C. glabrata*, the epidemiological cutoff value (ECV) of >0.5 μg/ml was used to identify non-wild-type (non-WT) isolates.

[c] Posaconazole ECVs were used to identify non-WT isolates of *C. albicans* (ECV > 0.06 μg/ml), *C. glabrata* (ECV > 2 μg/ml), *C. parapsilosis* (ECV > 0.25 μg/ml), *C. tropicalis* (ECV > 0.12 μg/ml), and *C. krusei* (ECV > 0.5 μg/ml).

C. tropicalis from all four regions and among *C. parapsilosis* isolates from North America, Europe, and Latin America. Similar to *C. glabrata*, the highest rate of fluconazole resistance among *C. parapsilosis* isolates was seen in the Asia-Pacific region. Aside from *C. glabrata*, resistance to voriconazole was minimal in all four geographic regions. Decreased susceptibility (MIC > ECV) to posaconazole was most prominent (>5%) among *C. albicans* isolates (ECV, 0.06 μg/ml) from Europe (8.3%) and Latin America (6.5%), *C. glabrata* isolates (ECV, 2 μg/ml) from Latin America (5.3%) and the Asia-Pacific region (5.8%), *C. parapsilosis* isolates (ECV, 0.25 μg/ml) from the Asia-Pacific region (6.5%), and *C. krusei* isolates (ECV, 0.5 μg/ml) from all regions (15.2% overall; range, 7.1% [Europe] to 33.3% [Asia-Pacific]). Among the 12 isolates of *C. krusei* characterized as being non-WT to posaconazole (ECV, 0.5 μg/ml), the MIC was 1 μg/ml for 10 isolates (83%) and 2 μg/ml for 2 isolates. Thus, the posaconazole MIC was ≤1 μg/ml for 77 of 79 isolates (97.5%) of *C. krusei*, comparable to the activity seen with voriconazole against this species. Isolates of *C. krusei* for which posaconazole MICs are 1 μg/ml (non-WT) yet which are classified as WT for voriconazole are very unusual and not explained by known mechanisms of resistance (45), suggesting that the ECV for posaconazole and *C. krusei* may be set too low or that technical factors may have led to falsely elevated MIC values in the present study. It should also be noted that the posaconazole ECVs for *C. krusei* and the other common species of *Candida* were derived from MIC distributions obtained from a single laboratory and may differ from those derived from a multicenter study. Furthermore, these ECVs are not meant to serve as a measure of susceptibility or resistance *in vivo* (11).

Table 3 provides a comparison of the MIC distributions for each of the echinocandins according to the species of *Candida* that were collected in the years 2003 to 2007 (36) and the present collection representing 2010 to 2011. Results from the two time periods were obtained with CLSI reference BMD methods in two

TABLE 3 Comparison of *in vitro* susceptibilities of three echinocandins versus isolates of *Candida* collected during two different time periods[a]

Candida species	Antifungal agent[b]	Years[c]	No. of isolates tested	% of isolates at an MIC (μg/ml) of:										
				≤0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	≥8
C. albicans	ANF	2003-2007	4,283	7.9	29.8	36.0	20.9	5.0	0.3		<0.1			
		2010-2011	1,405	9.3	22.6	35.4	24.7	7.0	0.5	0.4				
	CSF	2003-2007	4,283	2.1	27.6	47.6	21.0	1.6	0.1					
		2010-2011	1,405	0.7	16.2	45.7	25.9	10.0	0.9	0.4	0.1	0.1		
	MCF	2003-2007	4,283	14.2	68.9	14.6	2.1	0.1						
		2010-2011	1,405	4.2	40.2	47.6	6.3	1.1	0.2	0.3	0.1			
C. glabrata	ANF	2003-2007	1,236		0.6	13.0	57.8	25.9	2.1	0.2	0.2	0.2	0.1	
		2010-2011	571		0.7	15.2	52.0	25.7	4.6	0.4	1.1	0.2	0.2	
	CSF	2003-2007	1,236		10.7	59.1	26.6	2.1	0.6	0.6	0.1			0.2
		2010-2011	571		1.2	58.5	23.3	13.0	2.5	0.5	0.7		0.2	0.2
	MCF	2003-2007	1,236	16.8	75.6	5.7	1.0	0.3	0.2	0.1	0.2	0.1		
		2010-2011	571	9.8	68.5	17.5	2.1	0.9	0.2	0.7	0.2	0.2		
C. parapsilosis	ANF	2003-2007	1,238	0.1	0.2	0.1	0.1	1.1	4.0	25.8	61.8	6.9		0.1
		2010-2011	565		0.2		0.5	4.2	10.1	32.0	39.3	13.1	0.5	
	CSF	2003-2007	1,238	0.2	0.4	2.5	10.2	44.0	32.2	9.1	1.3	0.1		0.2
		2010-2011	565		0.4	1.4	10.4	52.4	26.2	8.8	0.4			
	MCF	2003-2007	1,238	0.2	0.2	0.1	0.8	5.3	21.1	54.6	17.8			0.2
		2010-2011	565				0.2	0.9	2.5	13.5	52.9	29.6	0.5	
C. tropicalis	ANF	2003-2007	996	4.1	25.5	49.5	17.4	2.4	0.7	0.1		0.3		
		2010-2011	318	11.9	37.1	38.7	10.7	1.3	0.3					
	CSF	2003-2007	996	1.7	31.9	48.4	16.2	1.2	0.4		0.1			0.1
		2010-2011	318	0.6	18.9	57.9	17.0	4.7	0.9					
	MCF	2003-2007	996	4.6	40.2	37.7	15.0	1.7	0.6	0.1	0.2			
		2010-2011	318	2.2	27.7	50.3	17.3	1.9	0.6					
C. krusei	ANF	2003-2007	270		1.5	58.9	33.7	5.2	0.4	0.4				
		2010-2011	79	1.3	7.6	31.6	45.6	12.7	1.3					
	CSF	2003-2007	270		0.4		51.9	29.3	14.8	3.0	0.7	0.4		
		2010-2011	79	1.3		2.5	30.4	21.5	39.2	12.9				
	MCF	2003-2007	270		1.5	10.4	78.1	7.8	2.2					
		2010-2011	79	1.3	1.3		27.8	68.4	1.3					

[a] All isolates were tested using CLSI broth microdilution methods (17).
[b] ANF, anidulafungin; MCF, micafungin; CSF, caspofungin.
[c] Data from the 2003-2007 period were compiled from the work of Pfaller et al. (36).

different laboratories (2003 to 2007 at the University of Iowa and 2010 to 2011 at JMI Laboratories); however, testing was performed by common personnel in both time periods, and quality control procedures were rigorously performed during both studies, suggesting that testing conditions did not influence the differences that we noted.

Among the 4,283 isolates of *C. albicans* from 2003 to 2007, 1 (0.02%) was resistant to anidulafungin and intermediate to both caspofungin and micafungin, whereas 3 isolates (0.2%) ($P < 0.001$) from 2010 to 2011 were resistant to caspofungin and 2 (0.1%) ($P < 0.001$) were resistant to micafungin (Table 3). The 3 caspofungin-resistant isolates in 2010 to 2011 were from Europe (2 isolates) and China (1 isolate), 2 of which were intermediate to anidulafungin (MIC, 0.5 μg/ml) and resistant to micafungin (MIC, 1 μg/ml), and 1 of which was susceptible to anidulafungin (MIC, 0.25 μg/ml) and intermediate to micafungin (MIC, 0.5 μg/ml). All 3 isolates were found to have a mutation in HS1 of *fks1* (Table 4).

Among the *C. glabrata* isolates from 2003 to 2007, 7 (0.6%) were resistant to anidulafungin (MIC, ≥0.5 μg/ml), 10 (0.8%) were resistant to caspofungin (MIC, ≥0.5 μg/ml), and 6 (0.5%) were resistant to micafungin (MIC, ≥0.25 μg/ml) (Table 3). In contrast, 10 isolates (1.8%) ($P < 0.005$) from 2010 to 2011 were resistant to anidulafungin, 9 isolates (1.6%) ($P = 0.0013$) were resistant to caspofungin, and 7 isolates (1.5%) ($P = 0.056$) were resistant to micafungin (Table 3). Taken together, the data indicate that a total of 12 isolates (2.1%) of *C. glabrata* from 2010 to 2011 were resistant to one or more of the echinocandins, 7 of which were from North America, 3 were from Europe, and 2 were from the Asia-Pacific region (Australia) (Table 4). Among these 12 isolates, 8 were found to contain mutations in HS1 of *fks2*, 2 were found to contain mutations in HS1 of *fks1*, and 2 did not have mutations in either *fks1* or *fks2* (Table 4). Of the latter 2 isolates, 1 was resistant to anidulafungin and caspofungin and susceptible to micafungin, and 1 was intermediate to anidulafungin and caspofungin and resistant to micafungin (Table 4). Although the clinical importance of such differential susceptibilities to the echinocandins in the isolates of *C. glabrata* is not clear, a recent study by Arendrup and colleagues (46) found such differences to be meaningful in an *in vivo* model of invasive candidiasis, irrespective of the presence

TABLE 4 Summary of *fks* alterations detected in echinocandin-resistant *Candida* sp. strains

Organism	State or country	Year	MIC (μg/ml) for each indicated antifungal:			1,3-β-D-Glucan synthase alterations in[a]:			
			Anidulafungin	Caspofungin	Micafungin	*fks1* HS1	*fks1* HS2	*fks2* HS1[b]	*fks2* HS2[b]
Candida glabrata	Indiana	2010	1	4	0.06	WT	WT	F641V	WT
C. glabrata	Belgium	2010	1	1	0.06	WT	WT	WT	WT
C. glabrata	Michigan	2010	0.25	0.5	0.03	WT	WT	D648E	WT
C. glabrata	Texas	2010	0.5	0.25	0.03	WT	WT	F641Y	WT
C. glabrata	Germany	2010	1	0.5	0.5	WT	WT	L644W	WT
C. glabrata	New York	2010	0.25	0.25	0.5	WT	WT	WT	WT
Candida parapsilosis	New York	2010	8	2	1	WT	WT	NT	NT
C. parapsilosis	New York	2010	8	1	1	WT	WT	NT	NT
C. parapsilosis	Argentina	2010	8	1	1	WT	WT	NT	NT
Candida albicans	Sweden	2011	0.5	1	1	S654P	WT	NT	NT
C. albicans	Scotland	2011	0.5	2	1	S629P	WT	NT	NT
C. albicans	China	2011	0.25	1	0.5	S645P	WT	NT	NT
C. glabrata	Australia	2011	0.5	0.25	0.12	F625S	WT	WT	WT
C. glabrata	Canada	2011	1	1	0.25	WT	WT	S659Y	WT
C. glabrata	Indiana	2011	1	0.5	0.5	WT	WT	S663Y	WT
C. glabrata	Australia	2011	1	1	0.5	WT	WT	S663P	WT
C. glabrata	Greece	2011	2	1	1	WT	WT	S663P	WT
C. glabrata	Louisiana	2011	4	16	2	S629P	WT	WT	WT

[a] WT, wild-type.
[b] NT, not tested.

or absence of *fks* mutations. Taken together, these results suggest that resistance to echinocandins may be increasing in *C. glabrata* isolates and that *in vitro* susceptibility test results are predictive of *fks* resistance mutations using the CLSI CBPs. It should also be noted that 38% of the echinocandin-resistant isolates of *C. glabrata* were also resistant to fluconazole.

Similar to that seen with *C. albicans*, echinocandin resistance was very uncommon among isolates of *C. parapsilosis*, *C. tropicalis*, and *C. krusei* from both time periods (Table 3). Three isolates of *C. parapsilosis* from 2010 to 2011 were resistant to anidulafungin (MIC, >4 μg/ml) and were susceptible (MIC, ≤2 μg/ml) to both caspofungin and micafungin, none of which contained an *fks* resistance mutation (Table 4).

Table 5 shows the frequency of decreased susceptibility of *A. fumigatus* to itraconazole, posaconazole, and voriconazole across the four geographic regions. Using the ECV of 1 μg/ml for both itraconazole and voriconazole, the only non-WT isolates in the present collection were from North America, for an overall frequency of non-WT strains of 1.0 to 2.0%. In contrast, 0.0% to 15.6% of isolates (9.4% overall) were classified as non-WT to posaconazole using the ECV of 0.5 μg/ml established by Espinel-Ingroff et al. (37). Among the 9 isolates characterized as non-WT to posaconazole, the MIC was 1 μg/ml for 8 isolates (89%) and 4 μg/ml for 1 isolate. The latter isolate was also non-WT to both itraconazole and voriconazole, suggesting a rare cross-resistance among the three triazoles. Isolates for which posaconazole MICs are 1 μg/ml (non-WT) yet which are classified as WT for both itraconazole and voriconazole do not conform to known mechanisms of resistance (44), suggesting that the ECV for posaconazole and *A. fumigatus* may be set too low, or that technical factors in testing, such as solubility of the drug, may have led to falsely elevated MIC values in the present study.

TABLE 5 Frequency of decreased susceptibility of *Aspergillus fumigatus* to azole antifungal agents by geographic region using CLSI epidemiological cutoff values

Antifungal agent	ECV[b] (μg/ml)	No. of isolates[a] (% non-wild-type to each antifungal agent) by region				
		North America	Europe	Latin America	Asia-Pacific	Total
Itraconazole	1	45 (4.4)	34 (0.0)	13 (0.0)	4 (0.0)	96 (2.0)
Posaconazole	0.5	45 (15.6)	34 (2.9)	13 (7.7)	4 (0.0)	96 (9.4)
Voriconazole	1	45 (2.2)	34 (0.0)	13 (0.0)	4 (0.0)	96 (1.0)

[a] All isolates tested using CLSI broth microdilution methods (19).
[b] ECVs as published by Espinel-Ingroff et al. (37).

It should be noted that whereas azole resistance in *A. fumigatus* is generally considered to be quite uncommon (47), reports from The Netherlands (48) and the United Kingdom (Manchester) (49) suggest that azole resistance may have increased in recent years. Recent estimates of the frequency of azole (itraconazole) resistance among European isolates of *A. fumigatus* range from 0.8% in France (50) to 5% in The Netherlands (48) and from 14 to 20% in a specialty referral laboratory in Manchester, United Kingdom (49). The lack of azole-resistant (non-WT) isolates in the present European collection may be explained in part by the fact that only 34 isolates were obtained from European study sites and that there were no isolates submitted from The Netherlands or from Manchester. The finding of 4% non-WT isolates of *A. fumigatus* in North America is similar to the findings of Baddley et al. (51), who reported 4% resistance to azoles in isolates of *Aspergillus* in the North American-based Transplant-Associated Infection Surveillance Network (TRANSNET).

The MIC distributions for the echinocandins and triazoles for the uncommon species of *Candida* and *Aspergillus*, the non-*Candida* yeasts, and non-*Aspergillus* molds for which molecular ID was confirmed are shown in Table 6 and in the supplemental material (species for which there were <5 isolates). Given that these species are uncommon in most regions of the world and that data concerning their *in vitro* susceptibility to most antifungals are lacking, we have elected to display the results as the number of isolates at each MIC value so that these results may eventually be

TABLE 6 MIC distributions for azole and echinocandin antifungal agents tested against isolates of uncommon species of *Candida* and *Aspergillus*, as well as non-*Candida* yeasts and non-*Aspergillus* molds identified by molecular methods (≥5 isolates per species) in SENTRY, 2010 to 2011

Species (no. tested)	Antifungal agent[a]	No. at an MIC/MEC (μg/ml) of:														
		0.007	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128
Candida species																
C. dubliniensis (50)	ANF	1	3	17	18	8	3									
	CSF	2	2	14	23	8	1									
	MCF	4	6	23	12	4	1									
	FLC				16	16	17									
	ITR	1	10	19	14	4		2								
	PSC	2	7	28	5	7		1								
	VRC	41	6	2		1										
C. fermentati (8)	ANF								4	4						
	CSF					4	2	2								
	MCF						1	6	1							
	FLC						1	1	1	3	1					1
	ITR						2	4	1			1				
	PSC				3	3	1					1				
	VRC			2	2	3						1				
C. guilliermondii (19)	ANF						1		5	8	4	1				
	CSF				3	3	3	4	4	2						
	MCF				1	1	5	4	5	2		1				
	FLC								1	7	4	3	2		1	1
	ITR						4	7	2	5		1				
	PSC					5	5	4	5							
	VRC			1	6	7	3		1	1						
C. kefyr (16)	ANF			8	4	2	2									
	CSF	1	13	1		1										
	MCF			6	7	2		1								
	FLC				1	5	8	2								
	ITR		1	3	7	3	2									
	PSC		1	3	7	3	2									
	VRC	13	3													
C. lusitaniae (55)	ANF			1	3	2	18	19	11	1						
	CSF		1		4	12	20	13	5							
	MCF		2	2	3	7	25	10	5	1						
	FLC				12	13	11	8	4	4	1			1	1	
	ITR	1		1	13	16	8	11	4	1						
	PSC	1	5	10	19	7	11	1	1							
	VRC	34	12	6	1		1	1								
Non-*Candida* yeasts																
Cryptococcus neoformans (84)	ANF												84			
	CSF											11	73			
	MCF												84			
	FLC							3	14	24	26	15	2			
	ITR			8	18	24	22	11	1							
	PSC		1	4	19	30	16	12	2							
	VRC	4	17	29	29	5										
Rhodotorula mucilaginosa (5)	ANF												5			
	CSF											2	3			
	MCF												5			
	FLC															5
	ITR								2	2		1				
	PSC								2	2	1					
	VRC							2			3					
Saccharomyces cerevisiae (6)	ANF				1	1	2	2								
	CSF					2	3	1								
	MCF					5	1									
	FLC								3	2	1					
	ITR						2	2	2							
	PSC						2	2	2							
	VRC			3	2	1										

(Continued on following page)

TABLE 6 (Continued)

Species (no. tested)	Antifungal agent[a]	No. at an MIC/MEC (μg/ml) of:														
		0.007	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128
Trichosporon asahii (9)	ANF												9			
	CSF										1	3	5			
	MCF												9			
	FLC							2		5	1					1
	ITR					1	1	5	2							
	PSC					2	3	3	1							
	VRC		1	2	3	1	1	1								
Trichosporon spp. (5)	ANF												5			
	CSF											2	3			
	MCF												5			
	FLC								2	2	1					
	ITR					1	1	2	1							
	PSC				2	1	1	1								
	VRC				3	2										
Aspergillus species																
A. flavus SC (19)	ANF	16	1	2												
	CSF	4	9	6												
	MCF	15	2	2												
	ITR							12	7							
	PSC						3	15	1							
	VRC							11	7	1						
A. niger SC (15)	ANF	10	3	2												
	CSF		5	8	1	1										
	MCF	12	1	2												
	ITR								8	6						
	PSC							9	5							
	VRC							8	4	1						
A. terreus SC (7)	ANF	3	2	1		1										
	CSF		3	3			1									
	MCF	5	1			1										
	ITR						1	3	3							
	PSC						4	3								
	VRC						1	6								
Non-*Aspergillus* molds																
Gibberella fujikuroi SC (6)	ANF										1		5			
	CSF												6			
	MCF												6			
	ITR							1				5				
	PSC						1	2	1		1	1				
	VRC		1						1	2	1	1				

[a] ANF, anidulafungin; MCF, micafungin; CSF, caspofungin; ITR, itraconazole; PSC, posaconazole; VRC, voriconazole.

combined with similarly derived data to form a more robust understanding of the MIC profiles of these unusual species.

Among the 169 isolates of the less-common species of *Candida* encountered during the 2010 to 2011 time period, we identified 15 different species (Table 6; see also the supplemental material), including 5 species (*C. dubliniensis*, *C. guilliermondii*, *C. kefyr*, *C. lusitaniae*, and *C. pelliculosa*) for which ECVs have been derived for the echinocandins and triazoles (11). In general, the MIC values obtained for both classes and these species conform to the WT MIC distributions described previously. Notable observations include decreased echinocandin susceptibility (MIC > ECV) in *C. guilliermondii* (1 isolate) to anidulafungin and micafungin (MIC >4 μg/ml), in *C. kefyr* (1 isolate) to caspofungin (MIC, >0.03 μg/ml) and micafungin (MIC, >0.12 μg/ml), and in *C. lusitaniae* (6 isolates) to micafungin (MIC, >0.5 μg/ml). The MIC values for the echinocandins against the very rare species of *Candida* were generally low (<0.5 μg/ml), with the exceptions of *C. fermentati* (Table 6), *C. haemulonii*, *C. lipolytica*, and *C. nivariensis* (see the supplemental material).

Cross-resistance (MIC > ECV) among the triazoles was detected in 4 isolates of *C. guilliermondii* (fluconazole MIC, >8 μg/ml; itraconazole MIC, >1 μg/ml; posaconazole MIC, >0.5 μg/ml; voriconazole MIC, >0.25 μg/ml), 1 isolate of *C. dubliniensis* (fluconazole MIC, >0.5 μg/ml; itraconazole MIC, >0.25 μg/ml; posaconazole MIC, >0.12 μg/ml; voriconazole MIC, >0.06 μg/ml), and 3 isolates of *C. lusitaniae* (fluconazole MIC, >2 μg/ml; itraconazole MIC, >0.5 μg/ml; posaconazole MIC, >0.12 μg/ml; voriconazole MIC, >0.03 μg/ml). Additional species in which fluconazole MICs appeared to be elevated (MIC, ≥8 μg/ml) included *C. catenulata* (MIC, 8 μg/ml), *C. fermentati* (MIC, 128 μg/ml), *C. inconspicua* (MIC, 32 μg/ml), and *C. lipolytica* (MIC, 16 μg/ml).

As expected, the echinocandins were inactive against many of the non-*Candida* yeasts (Table 6; see also the supplemental material). Echinocandin MIC results of ≤0.5 μg/ml were seen with *A. pullulans*, *D. fabryi*, *L. elongisporus*, and *S. cerevisiae*. In contrast, the triazoles, especially voriconazole, showed good activity against most of the non-*Candida* yeasts, with the exception of *R. mucilaginosa*. Among the 84 isolates of *C. neoformans* (Table 6), all (100.0%) were WT to fluconazole (ECV, 16 μg/ml) and voriconazole (ECV, 0.25 μg/ml); 97.6% were WT to posaconazole (ECV, 0.5 μg/ml), which is similar to our previous experience (12). The echinocandins were inactive against *C. neoformans* (Table 6).

The echinocandins were highly active against the non-*fumigatus* species of *Aspergillus* (Table 6; see also the supplemental material): only 1 isolate of *A. niger* SC and 1 of *A. terreus* SC exhibited an MEC of >0.06 μg/ml. Among the 4 species of *Aspergillus* for which triazole ECVs have been derived (*A. flavus*, *A. terreus*, *A. niger*, and *A. nidulans*), the MIC values obtained for both itraconazole and voriconazole conformed to the WT MIC distribution described by Espinel-Ingroff and colleagues (37). As seen with *A. fumigatus* (Table 5), the MIC distributions for posaconazole against *A. flavus* SC and *A. niger* SC were shifted 1 dilution higher than those described by Espinel-Ingroff et al. (37). Among the 4 remaining species of *Aspergillus*, only *A. sydowii* exhibited triazole MICs of >1 μg/ml (MIC, 2 μg/ml for both itraconazole and voriconazole).

Among the non-*Aspergillus* molds, only *P. variotii*, *Penicillium* spp., *Sarocladium* (*Acremonium*) *kiliense*, and *Trichoderma* spp. consistently exhibited echinocandin MECs of ≤0.06 μg/ml (Table 6; see also the supplemental material). Notably, MEC values of ≥8 μg/ml were observed for the vast majority of the *Fusarium* and *Mucorales* species in this molecularly characterized collection. Similarly, the triazoles showed poor activities against many of these rare molds.

Among the extended-spectrum triazoles, posaconazole stands apart from voriconazole in that it appears to be active against some clinical isolates of the mucoraceous molds both *in vitro* and *in vivo* (52, 53). Among the 4 molecularly identified isolates from the *Mucormycetes* genus in the present study, the voriconazole MICs were all 8 μg/ml, whereas the MICs for posaconazole ranged from 0.25 μg/ml (*R. oryzae*) to 1 μg/ml (*R. pusillus* and *R. microsporus* group) (see the supplemental material).

The typical antifungal susceptibility profiles of *Fusarium* spp. are of relative resistance to most antifungal agents (54). Among the small number of isolates in the present study, MICs were generally ≤4 μg/ml for both posaconazole and voriconazole against the 10 *Fusarium* isolates tested: *G. fujikuroi* (80% ≤4 μg/ml) (Table 6), *F. solani* (67%) (see the supplemental material), and *Fusarium* spp. (100%) (see the supplemental material). Although voriconazole and posaconazole exhibit only modest activity *in vitro* against isolates of *Fusarium*, both of these triazoles have been used successfully in some patients with amphotericin B-refractory fusariosis (55, 56).

S. apiospermum is generally considered to be resistant to amphotericin B, to which its clinical response is very poor (3), whereas both posaconazole and voriconazole have successfully been used for the treatment of central nervous system abscesses (57, 58). MICs for posaconazole ranged from 0.5 to 2 μg/ml and for voriconazole from 0.12 to 1 μg/ml against the 4 *S. apiospermum* isolates in the present study (see the supplemental material).

There are several important observations that can be made from this global survey. First, we have used molecular methods to document an amazing diversity of opportunistic fungal pathogens in four broad geographic regions of the world. Although the majority of IFIs were due to the five major species of *Candida* (*C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei*), *C. neoformans*, and *A. fumigatus*, IFIs were associated with the isolation of an additional 15 species of *Candida*, 9 species of non-*Candida* yeasts, 8 species of *Aspergillus*, and 23 species of non-*Aspergillus* molds.

As reported previously (14) for *Candida*, the species distributions and antifungal susceptibility profiles varied across geographic regions. Whereas the common species of *Candida* remained largely susceptible to both the triazoles and echinocandins, resistance to both classes of agents continued to be detected among isolates of *C. glabrata*. In contrast to the results from the 2009 SENTRY Program, the highest frequency of resistance to both echinocandins and azoles was observed among isolates from the Asia-Pacific region. Conversely, we have yet to detect echinocandin resistance in *C. glabrata* isolates from Latin America. By comparison with earlier susceptibility data from 2003 to 2007, isolates of *C. glabrata* in the present collection showed an approximately 3-fold increase in resistance to the echinocandins, and the resistant phenotypes were associated with mutations in *fks* ($P < 0.001$).

We have included the antifungal susceptibility profiles of those species from the SENTRY Program that have undergone sequence-based ID in an effort to provide MIC data not only for the relatively common species of *Candida* and *A. fumigatus* but also for those that may be less frequently encountered but still pose problems for selecting optimal antifungal therapies. In doing so, we have identified decreased susceptibilities to both echinocandin and triazoles in several additional species of *Candida*, non-*Candida* yeasts, and non-*Aspergillus* molds. Whereas the emergence of azole resistance among *A. fumigatus* has been reported by others (43), we identified only 2% of *A. fumigatus* isolates (all from North America) that warrant further investigation regarding acquired resistance mechanisms (MIC > ECV).

In summary, we provide additional information demonstrating the excellent activity of the echinocandins (anidulafungin, caspofungin, and micafungin) and newer triazoles (posaconazole and voriconazole) against *Candida* and *Aspergillus* spp., and of the triazoles against *C. neoformans* and other non-*Candida* yeasts. We have applied the new (lower) species-specific CLSI CBPs and ECVs for the echinocandins and triazoles to this contemporary (2010 to 2011) collection of *Candida* spp. and documented measurable but low levels of resistance or decreased susceptibility (non-WT; MIC > ECV) for most species. We provide additional validation of the echinocandin CBPs by demonstrating that the vast majority of *C. albicans* and *C. glabrata* isolates that were phenotypically resistant to one or more echinocandin possessed an acquired resistance mutation in *fks1* and/or *fks2*. Continued application of antifungal susceptibility testing combined with molecular characterization of species ID and resistance mechanisms for the available antifungal agents is critical in detecting the emergence of resistance among the ever-evolving spectrum of opportunistic fungal pathogens. Given the now-documented ability of *C. glabrata* to express resistance to both azoles and echinocandins (13, 59), this species must remain a focus of antifungal resistance surveillance in the coming years.

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REFERENCES

1. **Azie N, Neofytos D, Pfaller M, Meier-Kriesche HU, Quan SP, Horn D.** 2012. The PATH (Prospective Antifungal Therapy) Alliance registry and invasive fungal infections: update 2012. Diagn. Microbiol. Infect. Dis. **73:**293–300.
2. **Cleveland AA, Farley MM, Harrison LH, Stein B, Hollick R, Lockhart SR, Magill SS, Derado G, Park BJ, Chiller TM.** 2012. Changes in incidence and antifungal drug resistance in candidemia: results from population-based laboratory surveillance in Atlanta and Baltimore, 2008-2011. Clin. Infect. Dis. **55:**1352–1361.
3. **Cortez KJ, Roilides E, Quiroz-Telles F, Meletiadis J, Antachopoulos C, Knudsen T, Buchanan W, Milanovich J, Sutton DA, Fothergill A, Rinaldi MG, Shea YR, Zaoutis T, Kottilil S, Walsh TJ.** 2008. Infections caused by *Scedosporium* spp. Clin. Microbiol. Rev. **21:**157–197.
4. **Alexander BD, Pfaller MA.** 2006. Contemporary tools for the diagnosis and management of invasive mycoses. Clin. Infect. Dis. **43:**S15–S27. doi: 10.1086/504491.
5. **Balajee SA, Borman AM, Brandt ME, Cano J, Cuenca-Estrella M, Dannaoui E, Guarro J, Haase G, Kibbler CC, Meyer W, O'Donnell K, Petti CA, Rodriguez-Tudela JL, Sutton D, Velegraki A, Wickes BL.** 2009. Sequence-based identification of *Aspergillus*, *Fusarium*, and *Mucorales* species in the clinical mycology laboratory: where are we and where should we go from here? J. Clin. Microbiol. **47:**877–884.
6. **Castanheira M, Woosley LN, Diekema DJ, Jones RN, Pfaller MA.** 2013. *Candida guilliermondii* and other species of *Candida* misidentified as *Candida famata*: assessment by Vitek2, DNA sequencing analysis and matrix-assisted laser desorption ionization-time of flight mass spectrometry in two global surveillance programs. J. Clin. Microbiol. **51:**117–124.
7. **Pemán J, Cantón E, Quindós G, Eraso E, Alcoba J, Guinea J, Merino P, Ruiz-Pérez-de-Pipaon MT, Pérez-del-Molino L, Linares-Sicilia MJ, Marco F, García J, Rosell ó EM, Gómez G-de-la-Pedrosa E, Borrell N, Porras A, Yagüe G, FUNGEMYCA Study Group.** 2012. Epidemiology, species distribution and *in vitro* antifungal susceptibility of fungaemia in a Spanish multicentre prospective survey. J. Antimicrob. Chemother. **67:**1181–1187.
8. **Pfaller MA, Woosley LN, Messer SA, Jones RN, Castanheira M.** 2012. Significance of molecular identification and antifungal susceptibility of clinically significant yeasts and moulds in a global antifungal surveillance programme. Mycopathologia **174:**259–271.
9. **Pfaller MA, Neofytos D, Diekema D, Azie N, Meier-Kriesche HU, Quan SP, Horn D.** 2012. Epidemiology and outcomes of candidemia in 3648 patients: data from the Prospective Antifungal Therapy (PATH Alliance) registry, 2004–2008. Diagn. Microbiol. Infect. Dis. **74:**323–331.
10. **Linton CJ, Borman AM, Cheung G, Holmes AD, Szekely A, Palmer MD, Bridge PD, Campbell CK, Johnson EM.** 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.
11. **Pfaller MA, Diekema DJ.** 2012. Progress in antifungal susceptibility testing of *Candida* spp. by use of Clinical and Laboratory Standards Institute broth microdilution methods, 2010 to 2012. J. Clin. Microbiol. **50:**2846–2856.
12. **Pfaller MA, Castanheira M, Messer SA, Moet GJ, Jones RN.** 2011. Echinocandin and triazole antifungal susceptibility profiles for *Candida* spp., *Cryptococcus neoformans*, and *Aspergillus fumigatus*: application of new CLSI clinical breakpoints and epidemiologic cutoff values to characterize resistance in the SENTRY Antimicrobial Surveillance Program (2009). Diagn. Microbiol. Infect. Dis. **69:**45–50.
13. **Pfaller MA, Castanheira M, Lockhart SR, Ahlquist AM, Messer SA, Jones RN.** 2012. Frequency of decreased susceptibility and resistance to echinocandins among fluconazole-resistant bloodstream isolates of *Candida glabrata*. J. Clin. Microbiol. **50:**1199–1203.
14. **Pfaller MA, Moet GJ, Messer SA, Jones RN, Castanheira M.** 2011. Geographic variations in species distribution and echinocandin and azole antifungal resistance rates among *Candida* bloodstream infection isolates: report from the SENTRY Antimicrobial Surveillance Program (2008 to 2009). J. Clin. Microbiol. **49:**396–399.
15. **Rex JH, Pfaller MA, Galgiani JN, Bartlett MS, Espinel-Ingroff A, Ghannoum MA, Lancaster M, Odds FC, Rinaldi MG, Walsh TJ, Barry AL.** 1997. Development of interpretive breakpoints for antifungal susceptibility testing: conceptual framework and analysis of *in vitro-in vivo* correlation data for fluconazole, itraconazole, and *Candida* infections. Subcommittee on Antifungal Susceptibility Testing of the National Committee for Clinical Laboratory Standards. Clin. Infect. Dis. **24:**235–247.
16. **Rex JH, Pfaller MA, Walsh TJ, Chaturvedi V, Espinel-Ingroff A, Ghannoum MA, Gosey LL, Odds FC, Rinaldi MG, Sheehan DJ, Warnock DW.** 2001. Antifungal susceptibility testing: practical aspects and current challenges. Clin. Microbiol. Rev. **14:**643–658.
17. **Clinical and Laboratory Standards Institute.** 2008. Reference method for broth dilution antifungal susceptibility testing of yeasts—third edition. CLSI document M27-A3. Clinical and Laboratory Standards Institute, Wayne, PA.
18. **Clinical and Laboratory Standards Institute.** 2008. Reference method for broth dilution antifungal susceptibility testing of yeasts; 3rd informational supplement. CLSI M27-S3. Clinical and Laboratory Standards Institute, Wayne, PA.
19. **Clinical and Laboratory Standards Institute.** 2008. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi—second eddition. CLSI document M38-A2. Clinical and Laboratory Standards Institute, Wayne, PA.
20. **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.
21. **Baddley JW, Patel M, Jones M, Cloud G, Smith AC, Moser SA.** 2004. Utility of real-time antifungal susceptibility testing for fluconazole in the treatment of candidemia. Diagn. Microbiol. Infect. Dis. **50:**119–124.
22. **Grim SA, Berger K, Teng C, Gupta S, Layden JE, Janda WM, Clark NM.** 2012. Timing of susceptibility-based antifungal drug administration in patients with *Candida* bloodstream infection: correlation with outcomes. J. Antimicrob. Chemother. **67:**707–714.
23. **Hadley S, Martinez JA, McDermott L, Rapino B, Snydman DR.** 2002. Real-time antifungal susceptibility screening aids management of invasive yeast infections in immunocompromised patients. J. Antimicrob. Chemother. **49:**415–419.
24. **Karthaus M, Rüping MJ, Cornely OA, Steinbach A, Groll AH, Lass-Flörl C, Ostermann H, Ruhnke M, Vehreschild JJ.** 2011. Current issues in the clinical management of invasive *Candida* infections–the AGIHO, DMykG, ÖGMM and PEG web-based survey and expert consensus conference 2009. Mycoses **54:**e546–e556.
25. **Magill SS, Shields C, Sears CL, Choti M, Merz WG.** 2006. Triazole cross-resistance among *Candida* spp.: case report, occurrence among bloodstream isolates, and implications for antifungal therapy. J. Clin. Microbiol. **44:**529–535.
26. **Pappas PG, Kauffman CA, Andes D, Benjamin DK, Jr, Calandra TF, Edwards JE, Jr, Filler SG, Fisher JF, Kullberg BJ, Ostrosky-Zeichner L, Reboli AC, Rex JH, Walsh TJ, Sobel JD, Infectious Diseases Society of America.** 2009. Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. Clin. Infect. Dis. **48:**503–535.
27. **Parkins MD, Sabuda DM, Elsayed S, Laupland KB.** 2007. Adequacy of empirical antifungal therapy and effect on outcome among patients with

invasive *Candida* species infections. J. Antimicrob. Chemother. **60:**613–618.
28. **Perkhofer S, Mrazek C, Hartl L, Lass-Flörl C.** 2010. In *vitro* susceptibility testing in fungi: what is its role in clinical practice? Curr. Infect. Dis. Rep. **12:**401–408.
29. **Pfaller MA, Andes D, Diekema DJ, Espinel-Ingroff A, Sheehan D, CLSI Subcommittee for Antifungal Susceptibility Testing.** 2010. Wild-type MIC distributions, epidemiological cutoff values and species-specific clinical breakpoints for fluconazole and *Candida*: time for harmonization of CLSI and EUCAST broth microdilution methods. Drug Resist. Updat. **13:**180–195.
30. **Pfaller MA, Andes D, Arendrup MC, Diekema DJ, Espinel-Ingroff A, Alexander BD, Brown SD, Chaturvedi V, Fowler CL, Ghannoum MA, Johnson EM, Knapp CC, Motyl MR, Ostrosky-Zeichner L, Walsh TJ.** 2011. Clinical breakpoints for voriconazole and *Candida* spp. revisited: review of microbiologic, molecular, pharmacodynamic, and clinical data as they pertain to the development of species-specific interpretive criteria. Diagn. Microbiol. Infect. Dis. **70:**330–343.
31. **Pfaller MA, Diekema DJ, Andes D, Arendrup MC, Brown SD, Lockhart SR, Motyl M, Perlin DS, CLSI Subcommittee for Antifungal Testing.** 2011. Clinical breakpoints for the echinocandins and *Candida* revisited: integration of molecular, clinical, and microbiological data to arrive at species-specific interpretive criteria. Drug Resist. Updat. **14:**164–176.
32. **Arendrup MC.** 2010. Epidemiology of invasive candidiasis. Curr. Opin. Crit. Care. **16:**445–452.
33. **Castanheira M, Woosley LN, Diekema DJ, Messer SA, Jones RN, Pfaller MA.** 2010. Low prevalence of *fks1* hotspot 1 mutations in a worldwide collection of *Candida* strains. Antimicrob. Agents Chemother. **54:**2655–2659.
34. **Arendrup MC, Denning DW, Pfaller MA, Diekema DJ, Rex JH.** 2007. Does one voriconazole breakpoint suit all *Candida* species? J. Clin. Microbiol. **45:**2093–2094.
35. **Arendrup MC, Kahlmeter G, Rodriguez-Tudela JL, Donnelly JP.** 2009. Breakpoints for susceptibility testing should not divide wild-type distributions of important target species. Antimicrob. Agents Chemother. **53:**1628–1629.
36. **Pfaller MA, Boyken L, Hollis RJ, Kroeger J, Messer SA, Tendolkar S, Jones RN, Turnidge J, Diekema DJ.** 2010. Wild-type MIC distributions and epidemiological cutoff values for the echinocandins and *Candida* spp. J. Clin. Microbiol. **48:**52–56.
37. **Espinel-Ingroff A, Diekema DJ, Fothergill A, Johnson E, Pelaez T, Pfaller MA, Rinaldi MG, Canton E, Turnidge JD.** 2010. Wild-type MIC distributions and epidemiological cutoff values for the triazoles and six *Aspergillus* spp. for the CLSI broth microdilution method (M38-A2 document). J. Clin. Microbiol. **48:**3251–3257.
38. **Messer SA, Kirby JT, Sader HS, Fritsche TR, Jones RN.** 2004. Initial results from a longitudinal international surveillance programme for anidulafungin (2003). J. Antimicrob. Chemother. **54:**1051–1056.
39. **Espinel-Ingroff A, Chowdhary A, Cuenca-Estrella M, Fothergill A, Fuller J, Hagen F, Govender N, Guarro J, Johnson E, Lass-Flörl C, Lockhart SR, Martins MA, Meis JF, Melhem MS, Ostrosky-Zeichner L, Pelaez T, Pfaller MA, Schell WA, Trilles L, Kidd S, Turnidge J.** 2012. *Cryptococcus neoformans-Cryptococcus gattii* species complex: an international study of wild-type susceptibility endpoint distributions and epidemiological cutoff values for amphotericin B and flucytosine. Antimicrob. Agents Chemother. **56:**3107–3113.
40. **Hazen KC, Howell SA.** 2007. *Candida*, *Cryptococcus*, and other yeasts of medical importance, p 1762–1788. *In* Murray PR, Baron EJ, Jorgensen JH, Landry ML, Pfaller MA (ed), Manual of clinical microbiology, 9th ed. ASM Press, Washington, DC.
41. **Larone DH.** 2002. Medically important fungi: a guide to identification, 4th ed. ASM Press, Washington, DC.
42. **Leaw SN, Chang HC, Sun HF, Barton R, Bouchara JP, Chang TC.** 2006. Identification of medically important yeast species by sequence analysis of the internal transcribed spacer regions. J. Clin. Microbiol. **44:**693–699.
43. **Howard SJ, Cerar D, Anderson MJ, Albarrag A, Fisher MC, Pasqualotto AC, Laverdiere M, Arendrup MC, Perlin DS, Denning DW.** 2009. Frequency and evolution of azole resistance in *Aspergillus fumigatus* associated with treatment failure. Emerg. Infect. Dis. **15:**1068–1076.
44. **Rodriguez-Tudela JL, Alcazar-Fuoli L, Mellado E, Alastruey-Izquierdo A, Monzon A, Cuenca-Estrella M.** 2008. Epidemiological cutoffs and cross-resistance to azole drugs in *Aspergillus fumigatus*. Antimicrob. Agents Chemother. **52:**2468–2472.
45. **Pfaller MA, Messer SA, Boyken L, Tendolkar S, Hollis RJ, Diekema DJ.** 2008. Selection of a surrogate agent (fluconazole or voriconazole) for initial susceptibility testing of posaconazole against *Candida* spp.: results from a global antifungal surveillance program. J. Clin. Microbiol. **46:**551–559.
46. **Arendrup MC, Perlin DS, Jensen RH, Howard SJ, Goodwin J, Hope W.** 2012. Differential *in vivo* activity of anidulafungin, caspofungin, and micafungin against *Candida glabrata* with and without *fks* resistance mutations. Antimicrob. Agents Chemother. **56:**2435–2442.
47. **Howard SJ, Arendrup MC.** 2011. Acquired antifungal drug resistance in *Aspergillus fumigatus*: epidemiology and detection. Med. Mycol. **49**(Suppl 1):S90–S95.
48. **van der Linden JW, Snelders E, Kampinga GA, Rijnders BJ, Mattsson E, Debets-Ossenkopp YJ, Kuijper EJ, Van Tiel FH, Melchers WJ, Verweij PE.** 2011. Clinical implications of azole resistance in *Aspergillus fumigatus*, The Netherlands, 2007–2009. Emerg. Infect. Dis. **17:**1846–1854.
49. **Bueid A, Howard SJ, Moore CB, Richardson MD, Harrison E, Bowyer P, Denning DW.** 2010. Azole antifungal resistance in *Aspergillus fumigatus*: 2008 and 2009. J. Antimicrob. Chemother. **65:**2116–2118.
50. **Alanio A, Sitterl é E, Liance M, Farrugia C, Foulet F, Botterel F, Hicheri Y, Cordonnier C, Costa JM, Bretagne S.** 2011. Low prevalence of resistance to azoles in *Aspergillus fumigatus* in a French cohort of patients treated for haematological malignancies. J. Antimicrob. Chemother. **66:**371–374.
51. **Baddley JW, Marr KA, Andes DR, Walsh TJ, Kauffman CA, Kontoyiannis DP, Ito JI, Balajee SA, Pappas PG, Moser SA.** 2009. Patterns of susceptibility of *Aspergillus* isolates recovered from patients enrolled in the Transplant-Associated Infection Surveillance Network. J. Clin. Microbiol. **47:**3271–3275.
52. **Diekema DJ, Messer SA, Hollis RJ, Jones RN, Pfaller MA.** 2003. Activities of caspofungin, itraconazole, posaconazole, ravuconazole, voriconazole, and amphotericin B against 448 recent clinical isolates of filamentous fungi. J. Clin. Microbiol. **41:**3623–3626.
53. **van Burik JA, Hare RS, Solomon HF, Corrado ML, Kontoyiannis DP.** 2006. Posaconazole is effective as salvage therapy in zygomycosis: a retrospective summary of 91 cases. Clin. Infect. Dis. **42:**e61–e65.
54. **Alastruey-Izquierdo A, Cuenca-Estrella M, Monzón A, Mellado E, Rodríguez-Tudela JL.** 2008. Antifungal susceptibility profile of clinical *Fusarium* spp. isolates identified by molecular methods. J. Antimicrob. Chemother. **61:**805–809.
55. **Perfect JR, Marr KA, Walsh TJ, Greenberg RN, DuPont B, de la Torre-Cisneros J, Just-Nübling G, Schlamm HT, Lutsar I, Espinel-Ingroff A, Johnson E.** 2003. Voriconazole treatment for less-common, emerging, or refractory fungal infections. Clin. Infect. Dis. **36:**1122–1131.
56. **Raad II, Hachem RY, Herbrecht R, Graybill JR, Hare R, Corcoran G, Kontoyiannis DP.** 2006. Posaconazole as salvage treatment for invasive fusariosis in patients with underlying hematologic malignancy and other conditions. Clin. Infect. Dis. **42:**1398–1403.
57. **Mellinghoff IK, Winston DJ, Mukwaya G, Schiller GJ.** 2002. Treatment of *Scedosporium apiospermum* brain abscesses with posaconazole. Clin. Infect. Dis. **34:**1648–1650.
58. **Nesky MA, McDougal EC, Peacock JE, Jr.** 2000. *Pseudallescheria boydii* brain abscess successfully treated with voriconazole and surgical drainage: case report and literature review of central nervous system pseudallescheriasis. Clin. Infect. Dis. **31:**673–677.
59. **Pfeiffer CD, Garcia-Effron G, Zaas AK, Perfect JR, Perlin DS, Alexander BD.** 2010. Breakthrough invasive candidiasis in patients on micafungin. J. Clin. Microbiol. **48:**2373–2380.