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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)[▽]

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Antifungal testing results from the SENTRY Antimicrobial Surveillance Program (2008 to 2009) were analyzed for regional variations of invasive *Candida* species infections. Among 2,085 cases from the Asian-Pacific (APAC) (51 cases), Latin American (LAM) (348 cases), European (EU) (750 cases), and North American (NAM) (936 cases) regions, *Candida albicans* predominated (48.4%), followed by *C. glabrata* (18.0%), *C. parapsilosis* (17.2%), *C. tropicalis* (10.5%), and *C. krusei* (1.9%). Resistance to echinocandins (anidulafungin [2.4%] and micafungin [1.9%]) and azoles (3.5 to 5.6%) was most prevalent among *C. glabrata* isolates, as determined using recently established CLSI breakpoint criteria. *C. glabrata* isolates were more common in NAM (23.5%), and *C. albicans* isolates were more common in APAC (56.9%), with *C. parapsilosis* (25.6%) and *C. tropicalis* (17.0%) being more prominent in LAM. Emerging resistance patterns among *C. glabrata* cases in NAM require focused surveillance.

Among the systemically active antifungal agents with potencies against *Candida* spp., the echinocandins micafungin and anidulafungin were approved by the U.S. Food and Drug Administration (FDA) for the treatment of candidemia and other forms of invasive candidal infections in 2005 and 2006, respectively, and posaconazole was approved for the prevention of invasive fungal infections in 2006. Although the variation in *Candida* species causing bloodstream infection (BSI) and the frequency of resistance to fluconazole and voriconazole by geographic region have been described earlier (6, 15, 17), similar data are lacking for anidulafungin, micafungin, and posaconazole. Given the widespread use of both the echinocandins and azoles, coupled with reports of emerging resistance to both of these classes of antifungal agents (10, 17, 19, 21), there is a need for ongoing surveillance to monitor for evolving anidulafungin, micafungin, and posaconazole resistance among *Candida* isolates.

We have performed global antifungal surveillance to monitor trends in antifungal susceptibility of clinical isolates of *Candida* spp. since 1997 (18). We now report recent (2008-2009) data from the SENTRY Antimicrobial Surveillance Program (Fungal Objective) describing the *in vitro* activities of anidulafungin, micafungin, posaconazole, fluconazole, and voriconazole tested against contemporary clinical isolates of *Candida* spp. from BSI worldwide. In addition, we compared these data for micafungin to the MIC distribution from North American 2004-2005 surveillance (in the years before the widespread availability of micafungin) (13). In this analysis, SENTRY Program investigators have employed the recently revised species-specific Clinical and Laboratory Standards Institute (CLSI) breakpoints for anidulafungin and micafungin (16) and for fluconazole (12).

A total of 2,085 clinical *Candida* isolates obtained from 79 medical centers in the Asian-Pacific (16 centers; 51 isolates), European (25 centers; 750 isolates), Latin American (10 centers; 348 isolates), and North American (28 centers; 936 isolates) regions between January 2008 and December 2009 were tested as part of the SENTRY Program (9, 18). The collection included 1,010 strains of *C. albicans*, 376 strains of *C. glabrata*, 359 strains of *C. parapsilosis*, 218 strains of *C. tropicalis*, 40 strains of *C. krusei*, 33 strains of *C. lusitaniae*, 16 strains of *C. dubliniensis*, 8 strains of *C. guilliermondii*, 6 strains of *C. kefyr*, 3 strains each of *C. famata* and *C. lipolytica*, 2 strains each of *C. rugosa*, *C. sake*, and *C. pelliculosa*, and 1 strain each of *C. lambica*, *C. utilis*, *C. haemulonii*, *C. norvegensis*, and *C. inconspicua* (Table 1). All isolates were obtained from blood or other normally sterile body sites and represented individual infectious episodes. The prior (comparator) yeast collection of 718 invasive isolates was sampled between 2004 and 2005 from 60 North American medical centers as part of the ARTEMIS surveillance program (13). The isolates were collected at individual study sites and were sent to JMI Laboratories (North Liberty, IA) for central reference laboratory identification and susceptibility testing as described previously (9). The isolates were identified by standard methods and stored as water suspensions until used in the study. Before testing, each isolate was passaged at least twice on Sabouraud dextrose agar (Remel, Lenexa, KS) and CHROMagar *Candida* medium (Becton Dickinson, Sparks, MD).

Broth microdilution (BMD) testing was performed in accordance with the guidelines in CLSI document M27-A3 (4). MICs were determined visually after 24 h of incubation for anidulafungin, micafungin, and fluconazole and after 48 h for posaconazole and voriconazole as the lowest concentration of

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TABLE 1. Species distribution of *Candida* bloodstream infection isolates across geographic regions: SENTRY Surveillance Program, 2008 to 2009

Species	% of isolates by species and geographic region (n^b)				
	Asia-Pacific (51)	Latin America (348)	Europe (750)	North America (936)	Total (2,085)
C. albicans	56.9	43.6	55.2	43.4	48.41
C. glabrata	13.7	5.2	15.7	23.5	18.0
C. parapsilosis	13.7	25.6	13.7	17.1	17.2
C. tropicalis	11.7	17.0	7.3	10.5	10.5
C. krusei	2.0	1.4	2.5	1.6	1.9
C. lusitaniae	0.0	0.9	1.2	2.2	1.6
C. dubliniensis	0.0	0.3	0.8	1.0	0.8
C. guilliermondii	0.0	1.7	0.1	0.1	0.4
Misc.[a]	2.0	1.6	1.7	0.6	1.2

[a] Miscellaneous species including 6 isolates of *C. kefyr*, 2 each of *C. rugosa*, *C. sake*, and *C. pelliculosa*, 3 each of *C. famata* and *C. lipolytica*, and 1 each of *C. lambica*, *C. utilis*, *C. haemulonii*, *C. norvegensis*, and *C. inconspicua.*
[b] *n*, no. tested.

each drug that caused a significant diminution (≥50%) of growth below control levels. We used the recently revised CLSI breakpoints to identify strains resistant to anidulafungin, micafungin, and fluconazole (12, 16): anidulafungin and micafungin MIC values at >0.5 μg/ml were defined as resistant for *C. albicans*, *C. tropicalis*, and *C. krusei*, and MIC values at >4 μg/ml were considered resistant for *C. parapsilosis*; anidulafungin MICs at >0.5 μg/ml and micafungin MIC values at >0.12 μg/ml were defined as resistant for *C. glabrata*; fluconazole MIC results of >4 μg/ml were declared resistant for *C. albicans*, *C. tropicalis*, and *C. parapsilosis*, and MIC values at >32 μg/ml were considered resistant for *C. glabrata*. The CLSI resistance breakpoint for voriconazole (>2 μg/ml) was also applied to posaconazole for all species (20). Quality control was performed by testing the CLSI-recommended strains *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019 (5).

Table 1 displays the species distribution of invasive *Candida* sp. isolates from 2008 to 2009. *C. albicans* was most common in the Asian-Pacific region (56.9%) and least common in North America (43.4%), whereas *C. glabrata* was most common in North America (23.5%) and least encountered in Latin America (5.2%). *C. parapsilosis* and *C. tropicalis* were most common in Latin America (25.6 and 17.0%, respectively), and *C. krusei* was more common in Europe (2.5%). No anidulafungin or micafungin resistance was detected in any species from the Asian-Pacific and Latin American regions (Table 2). Similarly, no resistance to posaconazole or voriconazole was observed among isolates of *C. albicans* and *C. parapsilosis* from any region. Resistance to anidulafungin (3.2%), micafungin (2.7%), and the azoles (5.5 to 8.2%) was most prominent among iso-

TABLE 2. Frequency of antifungal resistance among *Candida* bloodstream infection isolates by geographic region: SENTRY Surveillance Program, 2008 to 2009

Species	Antifungal agent	No. (%) of isolates resistant to each antifungal agent by region[a]				
		Asia-Pacific	L. America	Europe	N. America	Total
C. albicans	Anidulafungin	29 (0.0)	161 (0.0)	414 (0.2)	406 (0.0)	1,010 (0.1)
	Micafungin	29 (0.0)	161 (0.0)	414 (0.2)	406 (0.0)	1,010 (0.1)
	Fluconazole	29 (3.4)	161 (0.0)	414 (0.0)	406 (0.0)	1,010 (0.1)
	Posaconazole	29 (0.0)	161 (0.0)	414 (0.0)	406 (0.0)	1,010 (0.0)
	Voriconazole	29 (0.0)	161 (0.0)	414 (0.0)	406 (0.0)	1,010 (0.0)
C. glabrata	Anidulafungin	7 (0.0)	18 (0.0)	131 (1.5)	220 (3.2)	376 (2.4)
	Micafungin	7 (0.0)	18 (0.0)	131 (0.8)	220 (2.7)	376 (1.9)
	Fluconazole	7 (0.0)	18 (0.0)	131 (2.3)	220 (8.2)	376 (5.6)
	Posaconazole	7 (0.0)	18 (0.0)	131 (1.5)	220 (5.5)	376 (3.7)
	Voriconazole	7 (0.0)	18 (0.0)	131 (0.0)	220 (5.9)	376 (3.5)
C. parapsilosis	Anidulafungin	7 (0.0)	89 (0.0)	103 (0.0)	160 (0.0)	359 (0.0)
	Micafungin	7 (0.0)	89 (0.0)	103 (0.0)	160 (0.0)	359 (0.0)
	Fluconazole	7 (0.0)	89 (6.7)	103 (3.9)	160 (5.0)	359 (5.0)
	Posaconazole	7 (0.0)	89 (0.0)	103 (0.0)	160 (0.0)	359 (0.0)
	Voriconazole	7 (0.0)	89 (0.0)	103 (0.0)	160 (0.0)	359 (0.0)
C. tropicalis	Anidulafungin	6 (0.0)	59 (0.0)	55 (0.0)	98 (1.0)	218 (0.5)
	Micafungin	6 (0.0)	59 (0.0)	55 (0.0)	98 (0.0)	218 (0.0)
	Fluconazole	6 (0.0)	59 (1.7)	55 (3.6)	98 (4.1)	218 (3.2)
	Posaconazole	6 (0.0)	59 (0.0)	55 (0.0)	98 (2.0)	218 (0.9)
	Voriconazole	6 (0.0)	59 (1.7)	55 (3.6)	98 (2.0)	218 (2.9)
C. krusei[b]	Anidulafungin	1 (0.0)	5 (0.0)	19 (0.0)	15 (0.0)	40 (0.0)
	Micafungin	1 (0.0)	5 (0.0)	19 (0.0)	15 (0.0)	40 (0.0)
	Posaconazole	1 (0.0)	5 (0.0)	19 (0.0)	15 (0.0)	40 (0.0)
	Voriconazole	1 (100.0)[c]	5 (0.0)	19 (0.0)	15 (0.0)	40 (2.5)

[a] Resistance is defined as an MIC > 0.5 μg/ml for anidulafungin and micafungin versus *C. albicans*, *C. tropicalis* and *C. krusei*, an MIC > 4 μg/ml versus *C. parapsilosis*, an MIC > 0.5 μg/ml for anidulafungin and an MIC > 0.12 μg/ml for micafungin and *C. glabrata*, an MIC > 4 μg/ml for fluconazole versus *C. albicans*, *C. tropicalis*, and *C. parapsilosis* an MIC > 32 μg/ml versus *C. glabrata*, and an MIC > 2 μg/ml for posaconaole and voriconazole for all species. L., Latin; N., North.
[b] All isolates of *C. krusei* were defined as resistant to fluconazole as per CLSI criteria.
[c] Represents one isolate only.

TABLE 3. Comparison of *in vitro* susceptibilities of BSI isolates of *Candida* collected before (2004 to 2005) and after (2008 to 2009) the clinical introduction of micafungin in North America[a]

Species	Yr	No. of isolates tested	No. of isolates by MIC (μg/ml)										
			0.007	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8
C. albicans	2004-2005[b]	358	36	232	77	13							
	2008-2009	406	8	111	211	76							
C. glabrata	2004-2005[b]	195	7	167	12	5	1	2		1			
	2008-2009	220	2	26	110	72	4	1	2		2		1
C. parapsilosis	2004-2005[b]	101						5	14	44	38		
	2008-2009	160			1	1	1	1	12	97	47		
C. tropicalis	2004-2005[b]	50	2	13	14	17	2		1	1			
	2008-2009	98		6	37	45	7	2	1				
C. krusei	2004-2005[b]	14			1	10	2	1					
	2008-2009	15					9	6					

[a] All isolates tested using CLSI broth microdilution methods.
[b] Data compiled from the work of Pfaller et al. (13).

lates of *C. glabrata* from North America (Table 2). In addition to *C. glabrata*, resistance to fluconazole was observed in *C. parapsilosis* and *C. tropicalis*, respectively, from the Latin American (6.7 and 1.7%), European (3.9 and 3.6%), and North American (5.0 and 4.1%) regions. Voriconazole-resistant isolates were found for *C. tropicalis* from Latin America (1.7%), Europe (3.6%), and North America (2.0%). Cross-resistance between fluconazole and voriconazole and between all three triazoles was seen among *C. tropicalis* isolates from Latin American, Europe, and North America, respectively.

Table 3 provides a comparison of the MIC distribution for micafungin according to species of *Candida* collected before and after the clinical introduction of micafungin in North America. Results from the two time periods were obtained with CLSI reference methods in different laboratories; however, quality control procedures were rigorously performed during both studies, suggesting that testing conditions did not influence the differences noted.

Although no resistant strains of *C. albicans* or *C. krusei* were observed in either time period, a one doubling dilution shift toward a higher modal MIC was seen in 2008 and 2009 for both of these species. A similar shift was detected for *C. glabrata* with the emergence of six (2.7%) resistant strains. Even though the number of resistant *C. glabrata* strains was small, the recent report of five episodes of breakthrough fungemia (*C. glabrata*) on micafungin treatment with elevated MIC values (4 to 8 μg/ml) and *fks* mutations suggests that this species requires monitoring with respect to both azole and echinocandin resistance (21). The micafungin MIC distributions for *C. parapsilosis* were comparable in the two time periods, and although a shift was noted for C. *tropicalis*, no resistant strains of either species were detected in the most recent sample. It is noteworthy that in addition to cases of breakthrough fungemia due to micafungin-resistant *C. glabrata*, Pfeiffer et al. (21) also reported similar cases due to *C. parapsilosis* in patients receiving at least three doses of this echinocandin. None of the isolates of *C. parapsilosis* were found to harbor *fks* hot-spot mutations; however, MIC values of >2 μg/ml were documented for five of the six isolates tested (21). These investigators were unable to identify the echinocandin resistance mechanism in these *C. parapsilosis* isolates.

The BSI data for these contemporary *Candida* species confirm previous findings that both species distribution and antifungal resistance patterns vary across geographic regions. It is notable that although fluconazole resistance was detected in only a small proportion of *C. albicans* (0.1%), *C. tropicalis* (3.2%), and *C. parapsilosis* (5.0%) isolates, these species accounted for 34% of the 93 fluconazole-resistant isolates. This finding is similar to that of Oxman et al. (10), who cautioned that simple species identification may not be sufficient to predict the fluconazole susceptibility patterns.

Although rates of resistance to anidulafungin, micafungin, and the azoles were quite low for all of the identified *Candida* species in the Asian-Pacific, Latin American, and European regions, the presence of resistance to both antifungal classes among North American BSI isolates of *C. glabrata* is a growing concern. Whereas azole resistance in North American *C. glabrata* isolates is well known (1, 11, 14), the recent reports of isolates with echinocandin resistance due to *fks* mutations are most troubling (2, 3, 7, 8, 21, 22). The emergence of breakthrough infections due to either *C. glabrata* with *fks* mutations or wild-type *C. parapsilosis* with a naturally occurring polymorphism in the *fks* gene has generally been related to prolonged echinocandin exposure (21). Such infections appear to be sporadic and generally uncommon, but the modest shifts in the MIC distribution of echinocandins (micafungin) for *C. glabrata* demonstrated in the present study should be monitored more closely.

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