Variation in Species Distribution and Antifungal Resistance Among *Candida* Bloodstream Infection Isolates In Four Geographic Regions

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ABSTRACT

Background: Geographic variations in species and resistance (R) rates to fluconazole (FLC) have been described previously in Candida bloodstream infection (CBI) isolates. Similar information is not known for echinocandins, such as anidulafungin (ANF), caspofungin (CSF) and micafungin (MCF), nor newer triazoles (posaconazole [PSC] and voriconazole [VRC]).

Methods: Species distribution and antifungal-R profiles of CBI isolates from the SENTRY Program (2008-2009) were analyzed by geographic region. CLSI MICs were obtained and CLSI breakpoints were applied for: ANF, CSF, and MCF MICs >0.5 mg/L were R for *C. albicans* (Ca), *C. glabrata* (Cg), *C. tropicalis* (Ct) and *C. krusei* (Ck); MICs >4 mg/L were R for *C. parapsilosis* (Cp); FLC MICs >4 mg/L were R for Ca, Cp and Ct; MICs >32 mg/L were R for Cg; and PSC and VRC MICs >2 mg/L were R for all species.

Results: 2,085 CBI isolates were from Asia-Pacific (APAC; 51 isolates), Latin America (LAM; 348), European (EU; 750) and North American (NAM; 936). 48.4% were Ca, 18.0% Cg, 17.2% Cp, 10.5% Ct, and 1.9% Ck. Ca was more common in APAC (56.9%) and least found in NAM (43.4%). Cg was more common in NAM (23.5%) and least common in LAM (5.2%). Cp and Ct were more common in LAM (25.6 and 17.0%, respectively). No R to ANF, CSF, or MCF was detected in APAC and LAM. Likewise, no R to PSC or VRC was observed among Ca, Cg, Cp and Ck in APAC, LAM, and EU. R to echinocandins and azoles was most prominent among Cg isolates with highest R rates to ANF (3.2%), CSF (4.5%), MCF (1.4%), FLC (8.2%) PSC (0.5%) and VRC (1.4%) found in NAM. In addition to Cg, FLC-R was observed among Cp and Ct isolates, respectively, from LAM (6.7 and 1.7%), EU (3.9 and 3.6%), and NAM (5.0 and 4.1%).

Conclusions: Species distribution and R patterns significantly varied by geographic region. Cg CBI isolates can exhibit R to newer azoles and echinocandins, with highest R rates observed in NAM. R rates to all antifungals were generally lower in APAC and LAM.

INTRODUCTION

Among the systemically active antifungal agents with potencies against *Candida* spp., the echinocandins micafungin and anidulafungin were approved by the United States-Food and Drug Administration (US-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 has been described earlier, similar data is 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, there is a need for ongoing surveillance to monitor for evolving anidulafungin, micafungin and posaconazole resistance among *Candida.*

In this study, we report recent (2008-2009) data from the SENTRY Antimicrobial Surveillance Program (Fungal Objective) describing the in vitro activity of anidulafungin, micafungin, posaconazole, fluconazole and voriconazole tested against contemporary clinical isolates of *Candida* spp. from BSI worldwide. In addition, we compare these data for micafungin to the MIC distribution from North American 2004-2005 surveillance (in the years before the widespread availability of micafungin). In this analysis, SENTRY Program investigators have employed the recently revised species-specific Clinical and Laboratory Standards Institute (CLSI) breakpoints for micafungin and fluconazole.

MATERIALS AND METHODS

Organisms and study sites: A total of 2,085 clinical *Candida* isolates obtained from 79 medical centers in the Asia-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. The collection included 1,010 strains of *C. albicans,* 376 of *C. glabrata*, 359 of *C. parapsilosis*, 218 of *C. tropicalis*, 40 of *C. krusei*, 33 of *C. lusitaniae*, 16 of *C. dubliniensis*, eight of *C. guilliermondii*, six of *C. kefyr*, three each of *C. famata* and *C. lipolytica*, two each of *C. rugosa, C. sake,* and *C. pelliculosa* and one 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. The isolates were collected at individual study sites and were sent to JMI Laboratories (North Liberty, Iowa, USA) for central reference laboratory identification and susceptibility testing as described previously. 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 onto Sabouraud dextrose agar (Remel, Lenexa, Kansas, USA) and CHROMagar™ *Candida* medium (Becton Dickinson, Sparks, Maryland, USA).

Susceptibility test methods: Broth microdilution (BMD) testing was performed in accordance with the guidelines in CLSI document M27-A3. 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 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: anidulafungin and micafungin MIC values at >0.5 mg/L were defined as resistant for *C. albicans, C. tropicalis* and *C. krusei* and MIC values at >4 mg/L were considered resistant for *C. parapsilosis;* anidulafungin MICs at >0.5 mg/L and micafungin MIC values at >0.12 mg/L were defined as resistant for *C. glabrata;* fluconazole MIC results of >4 mg/L were declared resistant for *C. albicans, C. tropicalis,* and *C. parapsilosis* and MIC values at >32 mg/L were considered resistant for *C. glabrata*. The CLSI resistance breakpoint for voriconazole (>2 mg/L) was also applied to posaconazole for all species. Quality control was performed by testing CLSI-recommended strains *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019.

RESULTS

- Table 1 displays the species distribution of invasive *Candida* spp. isolates from 2008 to 2009. *C. albicans* was most common in the Asia-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 Asia-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 (5.5-8.2%) was most prominent among isolates of *C. glabrata* from North America (Table 2).
- 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 among *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.
- Micafungin MIC distribution for *C. albicans* or *C. krusei* (Table 3) demonstrated a one doubling dilution shift toward a higher modal MIC in 2008-2009 when compared to the 2004-2005 period (prior to micafungin release). A similar shift was detected for *C. glabrata* with the emergence of six (2.7%) resistant strains.
- The micafungin MIC distributions for *C. parapsilosis* and *C. tropicalis* were comparable in both time periods and no resistant strains of either species were detected in the most recent sample.

Table 1. Species distribution of *Candida* bloodstream infection isolates across geographic regions: SENTRY Surveillance Program 2008-2009.

Species	% of isolates (no. tested) by species and geographic region				
	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.*

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

		% of isolates resistant (R) to each antifungal by region[a]									
		APAC		L. America		Europe		N. America		Total	
Species	Agent	N	%R	N	%R	N	%R	N	%R	N	%R
C. albicans	Anidulafungin	29	0.0	161	0.0	414	0.2	406	0.0	1010	0.1
	Micafungin	29	0.0	161	0.0	414	0.2	406	0.0	1010	0.1
	Fluconazole	29	3.4	161	0.0	414	0.0	406	0.0	1010	0.1
	Posaconazole	29	0.0	161	0.0	414	0.0	406	0.0	1010	0.0
	Voriconazole	29	0.0	161	0.0	414	0.0	406	0.0	1010	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	5	0.0	19	0.0	15	0.0	40	2.5

a. Resistance (R) defined as an MIC >0.5 mg/L for anidulafungin and micafungin versus *C. albicans, C. tropicalis* and *C. krusei* and as an MIC >4 mg/L versus *C. parapsilosis;* R defined as an MIC >0.5 mg/L for anidulafungin and as an MIC >0.12 mg/L for micafungin and *C. glabrata*; R defined as an MIC >4 mg/L for fluconazole versus *C. albicans, C. tropicalis,* and *C. parapsilosis* and as an MIC >32 mg/L versus *C. glabrata*; R defined as an MIC >2 mg/L for posaconaole and voriconaole for all species.
b. All isolates of *C. krusei* were defined as resistant to fluconazole as per CLSI criteria.

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

Species	Year	No. tested	No. of isolates by MIC (mg/L):									
			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 Pfaller et al. (2006).

CONCLUSIONS

- These contemporary *Candida* species BSI data confirm previous findings that 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.
- Although rates of resistance to anidulafungin, micafungin and the azoles were quite low for all of the identified *Candida* species in the Asia-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.

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