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IDSA 2010
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Species Distribution and Frequency of Antifungal Resistance Variations Among *Candida* Bloodstream Infections Isolates by Patient Age: Report from the SENTRY Program

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SENTRY ANTIMICROBIAL SURVEILLANCE

Amended Abstract

Background: Variations in *Candida* species causing bloodstream infections (BSI) and frequency of resistance (R) to fluconazole (FLC) by patient age has been described before. Similar data however, is not known for echinocandins, such as anidulafungin (ANF), caspofungin (CSF), and micafungin (MCF) nor the newer azoles, (posaconazole [PSC] and voriconazole [VRC]).

Methods: We analyzed 24-h CLSI MICs from the SENTRY Program to compare antifungal profiles and species distribution of *Candida* BSI isolates by patient age. MIC results were obtained for 6 antifungal agents and revised CLSI breakpoints were used to identify R strains: ANF, CSF, and MCF MICs >0.5 µg/mL were R for *C. albicans* (Ca), *C. glabrata* (Cg), *C. tropicalis* (Ct) and *C. krusei* (Ck); MICs >4 µg/mL were R for *C. parapsilosis* (CP); FLC MICs >4 µg/mL were R for Ca, Cp, and Ct; MICs >32 µg/mL were R for Cg; and PSC and VRC MICs>2 µg/mL were R for all species.

Results: 1,239 Candidemia isolates were obtained from 79 hospitals (five countries) in 2008-2009: 50.0% were Ca, 17.4% were Cg, 17.4% were Cp, 9.8% were Ct and 1.8% were Ck. Ca was most common in 60-79 yr age group (52.3%) and least common in 80-99 yr age group (46.7%), whereas Cg was most common in 80-99 yr age group (28.6%). Cp and Ct occurred most often in 0-19 yr age group (28.5 and 12.9%, respectively), and Ck was most common in 20-39 yr age group (3.5%). No R to echinocandins was detected among Ca, Cp, and Ct in all age groups. Likewise, no R to PSC or VRC was observed among Ca, Cp or Ck isolates. R to azoles and echinocandins was most prominent in Cg with the highest R rates to ANF (16.7%), CSF (16.7), MCF (16.7), FLC (16.7), and PSC (5.6) found in isolates from 20-39 yr age group. No R (any drug) was detected in Cg isolates from the youngest (0-19 yr) and oldest (80-99 yr) age groups.

Conclusions: Both species distribution and antifungal R patterns varied widely with patient age. Cg BSI isolates may show R to both azoles and echinocandins, with the highest rates of refractory isolates observed in patients aged 20-39 yr followed by the 40-59 yr age group.

Introduction

Invasive candidiasis (IC; candidemia and other deep-seated infections including disseminated candidiasis, hepatic candidiasis, endocarditis and meningitis) is associated with considerable morbidity and mortality. Rapid initiation of appropriate antifungal therapy is essential for the management of IC and has been shown to reduce mortality. Due to the spectrum and fungicidal potency against *Candida* spp., an echinocandin antifungal agent (anidulafungin, caspofungin, micafungin) is recommended as first-line therapy for most patients with IC with de-escalation to fluconazole based on the susceptibility of the infecting organism to this agent.

Although much is known regarding the geographic variation in the species of *Candida* causing IC and the associated susceptibility of these isolates to fluconazole, considerably less is understood regarding the variation in the infecting species and their susceptibilities to the newer azoles (posaconazole, voriconazole) and echinocandins according to patient age. In the present study, we update this information using the fungal portion of the SENTRY Antimicrobial Surveillance Program database from 2008-2009, including species variation according to patient age and the associated resistance profiles for the contemporary echinocandin and azole antifungal agents.

Methods

Organisms and study sites: Between January 2008 and December 2009, a total of 2,085 BSI isolates of *Candida* spp. from 79 medical centers throughout the world were submitted to JMI Laboratories (North Liberty, Iowa, USA) for identification and antifungal susceptibility testing with fluconazole, posaconazole, voriconazole, anidulafungin, caspofungin, and micafungin. The isolates represented consecutive incident isolates from patients with candidemia treated at hospitals 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. Patient ages were provided for 1,239 (59%) isolates of *Candida*.

The isolates were identified by standard methods and stored as water suspensions until used in this study. Before testing, each isolate was passaged on Sabouraud dextrose agar (Remel, Lenexa, Kansas, USA) and CHROMagar (Beckton Dickinson, Sparks, Maryland, USA) to ensure purity and viability.

Susceptibility test methods: Broth microdilution (BMD) testing was performed in accordance with the guidelines in the Clinical and Laboratory Standards Institute (CLSI) document M27-A3 (CLSI, 2008). Minimal inhibitory concentrations (MIC) were determined visually after 24h incubation for the echinocandins and fluconazole and after 48h 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 the echinocandins and fluconazole: anidulafungin, caspofungin, and micafungin MICs at >0.5 µg/mL were considered resistant for *C. albicans*, *C. tropicalis* and *C. krusei* and MIC results at >4 µg/mL were defined as resistant for *C. parapsilosis*; anidulafungin and caspofungin MICs at >0.5 µg/mL and micafungin MICs at >0.12 µg/mL were considered resistant for *C. glabrata*; fluconazole MIC values >4 µg/mL were considered resistant for *C. albicans, C. parapsilosis* and *C. tropicalis* and MICs at >32 µg/mL were called resistant for *C. glabrata*. All isolates of *C. krusei* were declared as resistant to fluconazole. The CLSI resistant breakpoint for voriconazole (MIC, >2 µg/mL) was also defined as resistant for posaconazole for all species. Quality control was performed by testing CLSI-recommended strains *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019 (CLSI, 2008).

Results

- Among the 1,239 tested *Candida* BSI isolates, 620 (50%) were *C. albicans*, 215 (17.4%) were *C. glabrata*, 215 (17.4%) were *C. parapsilosis*, 122 (9.8%) were *C. tropicalis*, 22 (1.8%) were *C. krusei,* and only 45 (3.6%) were other species (Table 1).
- Comparing the frequency of isolation for different species by age group, we found identical rank orders for the groups 40 to 59, 60 to 79, and 80 to 99 years of age: *C. albicans* > *C. glabrata* > *C. parapsilosis* > *C. tropicalis* > *C. krusei* (Table 1).
- *C. albicans* was the most common species in all age groups. The proportions of BSIs due to this species was ≥50% in the groups 0 to 19 (50.0%), 20 to 39 (51.7%) and 60 to 79 (52.3%) years of age and <50% in the groups 40 to 59 (47.5%) and 80 to 99 (46.7%) years of age. The dominant causes of *Candida* BSI in the pediatric and adolescent age groups (0 to 19 years) were *C. albicans* and *C. parapsilosis* and very few infections were due to *C. glabrata* and *C. krusei* (Table 1).
- *C. parapsilosis* was detected more frequently than *C. glabrata* in the 20 to 39 year age group.
- *C. glabrata* increased with patient age: the lowest proportion (2.0%) was noted in the 0 to 19 year age group (0.5% in the <10 year old subgroup) and the highest proportion (28.6%) was seen in the 80 to 99 year age group (Table 1).
- *C. tropicalis* was most common in the 0 to 19 year age group (12.9%) and least common in the 80 to 99 year age group (3.8%), whereas *C. krusei* was most common in the 20 to 39 year age group (3.5%) and least common in the 0 to 19 year age group (0.8%; Table 1).
- Resistance to anidulafungin, caspofungin, or micafungin was not detected among isolates of *C. albicans, C. parapsilosis*, or *C. tropicalis* from all age groups (Table 2). Complete susceptibility to posaconazole and voriconazole was observed among isolates of *C. albicans, C. parapsilosis,* and *C. krusei* from all age groups.
- Resistance to both azoles and echinocandins was most prominent among isolates of *C. glabrata* with the highest resistance rates to anidulafungin (16.7%), caspofungin (16.7%), micafungin (16.7%), fluconazole (16.7%), posaconazole (5.6%) and voriconazole (11.1%) found in isolates from the 20 to 39 year age group (Table 2).
- Resistance to fluconazole was observed among isolates of *C. parapsilosis* from the 20 to 39 (15.8%), 40 to 59 (10.0%) and 60 to 79 (1.8%) year age groups and among *C. tropicalis* from the 20 to 39 (16.7%), 60 to 79 (2.4%) and 80 to 99 (25.0%) year age groups (Table 2).
- Cross-resistance between fluconazole and voriconazole was observed in isolates of *C. tropicalis* from the 20 to 39 (16.7%), 60 to 79 (2.4%) and 80 to 99 (25.0%) year age groups.
- No resistance to the echinocandins was found in isolates of *C. krusei* with the exception of a single caspofungin-resistant strain in the 40 to 59 year age group (Table 2).

Table 1. Species distributions of *Candida* bloodstream isolates stratified by patient age group, SENTRY Antimicrobial Surveillance Program (2008-2009).

	% by age (yr [no. isolates tested])					
Species	0-19 (256)	20-39 (116)	40-59 (326)	60-79 (436)	80-99 (105)	Total (1,239)
C. albicans	50.0	51.7	47.5	52.3	46.7	50.0
C. glabrata	2.0	15.5	21.8	20.9	28.6	17.4
C. parapsilosis	28.5	16.4	15.3	12.6	17.1	17.4
C. tropicalis	12.9	10.4	9.5	9.6	3.8	9.8
C. krusei	0.8	3.5	2.1	1.4	2.9	1.8
Misc.[a]	5.8	2.5	3.8	3.2	0.9	3.6

a. Miscellaneous species including *C. dubliniensis* (13 isolates), *C. guilliermondii* (eight isolates), *C. kefyr* (six isolates), *C. famata* (three isolates), *C. lipolytica* (three isolates), *C. rugosa* (two isolates), *C. sake* (two isolates), *C. pelliculosa* (two isolates), and one isolate each of *C. lambica, C. utilis, C. haemulonii, C. norvegensis,* and *C. inconspicua*.

Table 2. Frequency of antifungal resistance among *Candida* BSI isolates by patient age group: SENTRY Antimicrobial Surveillance Program (2008-2009).

		% of isolates resistant[b] to each antifungal by patient age group (yrs)											
		0-19		20-39		40-59		60-79		80-99		Total	
Species	Antifungal agent	No.[b]	%	No.[b]	%	No.[b]	%	No.[b]	%	No.[b]	%	No.[b]	%
C. albicans	Anidulafungin	128	0.0	60	0.0	155	0.0	228	0.0	49	0.0	620	0.0
	Caspofungin	128	0.0	60	0.0	155	0.0	228	0.0	49	0.0	620	0.0
	Micafungin	128	0.0	60	0.0	155	0.0	228	0.0	49	0.0	620	0.0
	Fluconazole	128	0.0	60	0.0	155	0.0	228	0.0	49	0.0	620	0.0
	Posaconazole	128	0.0	60	0.0	155	0.0	228	0.0	49	0.0	620	0.0
	Voriconazole	128	0.0	60	0.0	155	0.0	228	0.0	49	0.0	620	0.0
C. glabrata	Anidulafungin	5	0.0	18	16.7	71	7.0	91	0.0	30	0.0	215	3.7
	Caspofungin	5	0.0	18	16.7	71	7.0	91	2.2	30	0.0	215	4.7
	Micafungin	5	0.0	18	16.7	71	4.2	91	0.0	30	0.0	215	2.8
	Fluconazole	5	0.0	18	16.7	71	11.3	91	3.3	30	0.0	215	6.5
	Posaconazole	5	0.0	18	5.6	71	4.2	91	2.2	30	3.3	215	3.3
	Voriconazole	5	0.0	18	11.1	71	5.6	91	2.2	30	0.0	215	3.7
C. parapsilosis	Anidulafungin	73	0.0	19	0.0	50	0.0	55	0.0	18	0.0	215	0.0
	Caspofungin	73	0.0	19	0.0	50	0.0	55	0.0	18	0.0	215	0.0
	Micafungin	73	0.0	19	0.0	50	0.0	55	0.0	18	0.0	215	0.0
	Fluconazole	73	0.0	19	15.8	50	10.0	55	1.8	18	0.0	215	4.2
	Posaconazole	73	0.0	19	0.0	50	0.0	55	0.0	18	0.0	215	0.0
	Voriconazole	73	0.0	19	0.0	50	0.0	55	0.0	18	0.0	215	0.0
C. tropicalis	Anidulafungin	33	0.0	12	0.0	31	0.0	42	0.0	4	0.0	122	0.0
	Caspofungin	33	0.0	12	0.0	31	0.0	42	0.0	4	0.0	122	0.0
	Micafungin	33	0.0	12	0.0	31	0.0	42	0.0	4	0.0	122	0.0
	Fluconazole	33	0.0	12	16.7	31	0.0	42	2.4	4	25.0	122	3.3
	Posaconazole	33	0.0	12	0.0	31	0.0	42	2.4	4	0.0	122	0.8
	Voriconazole	33	0.0	12	16.7	31	0.0	42	2.4	4	25.0	122	3.3
C. krusei	Anidulafungin	2	0.0	4	0.0	7	0.0	6	0.0	3	0.0	22	0.0
	Caspofungin	2	0.0	4	0.0	7	14.3	6	0.0	3	0.0	22	0.5
	Micafungin	2	0.0	4	0.0	7	0.0	6	0.0	3	0.0	22	0.0
	Posaconazole	2	0.0	4	0.0	7	0.0	6	0.0	3	0.0	22	0.0
	Voriconazole	2	0.0	4	0.0	7	0.0	6	0.0	3	0.0	22	0.0

a. Resistance (R) defined as a MIC at >0.5 µg/mL for anidulafungin, caspofungin and micafungin versus *C. albicans, C. tropicalis,* and *C. krusei* and as a MIC of >4 µg/mL versus *C. parapsilosis*; R defined as a MIC at >0.5 µg/mL for anidulafungin and caspofungin and as a MIC at >0.12 µg/mL for micafungin versus *C. glabrata*; R defined as a MIC of >4 µg/mL for fluconazole versus *C. albicans, C. tropicalis,* and *C. parapsilosis* and as a MIC >32 µg/mL versus *C. glabrata;* and R defined as a MIC of >2 µg/mL for posaconaole and voriconazole for all species.
b. No. = number of organisms tested.

Conclusions

- Resistance to the systemically active echinocandin and azole antifungal agents was very uncommon among *C. albicans*, *C. parapsilosis, C. tropicalis,* and *C. krusei* isolates from all age groups.
- A trend towards an increased proportion of BSI isolates of *C. glabrata* with increasing patient age was noted; and resistance to azoles and echinocandins was evident in *C. glabrata* isolates from the 20 to 39 and 40 to 59 year age groups.
- Although prolonged exposure to antifungal agents may account for resistance in *C. glabrata* isolates, the haploid nature of *C. glabrata* makes it particularly adept at acquiring resistance mutations.
- These findings point to the importance of antifungal susceptibility testing in both surveillance programs and for local patient cases.

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