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Activity of Echinocandins and Triazoles Against a Contemporary (2012) Worldwide Collection of Yeast and Moulds Collected from Invasive Infections

M CASTANHEIRA, SA MESSER, PR RHOMBERG, RR DIETRICH, RN JONES, MA PFALLER
JMI Laboratories, North Liberty, IA, USA

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JMI Laboratories
North Liberty, IA, USA
www.jmilabs.com
ph. 319.665.3370, fax 319.665.3371
mariana-castanheira@jmilabs.com

AMENDED ABSTRACT

Background: Invasive fungal infections (IFIs) have emerged as major causes of morbidity and mortality, in particular among patients who are immunocompromised or hospitalized with serious underlying diseases. We evaluated 1,714 fungal clinical isolates causing IFIs against seven antifungal agents tested using CLSI reference broth microdilution methods (BMD).

Methods: 1,486 *Candida* spp., 107 *Aspergillus* spp., 34 non-*Candida* yeasts, 52 *Cryptococcus neoformans* (CN), and 35 rare moulds (rMO) isolates causing IFI were consecutively collected and susceptibility (S) tested by CLSI BMD in a central laboratory. Yeasts were identified (ID) using CHROMagar, biochemical methods and sequencing of ITS and/or 28S regions (IGS was used for a small subset). Moulds were ID by sequencing of 1 or 2 of the following genes: ITS, 28S, beta-tubulin, TEF.

Results: Echinocandin (EC) resistance (R) among *Candida* was low and R rates to anidulafungin, caspofungin, and micafungin varied from 0.0 to 2.6% among different species (**Table**). EC-R *C. glabrata* (CG) strains were shown to have *fks* mutations (*fks1* HS1 S663F or *fks2* HS1 F659 deletion) and fluconazole (FLC)-R was also observed in those strains. One *C. krusei* and one *C. dubliniensis* had L701M or S645P *fks1* HS1 mutations, respectively. R to FLC among isolates of CA (0.4%) was low and stable compared to previous years. *C. tropicalis* (CT) and CG had higher FLC-R rates, 8.4 and 6.9%, respectively. FLC-R CT were collected in five countries (USA, China, Germany, Belgium and Thailand). Voriconazole was active against all *Candida* spp. inhibiting 93.1 to 99.7% of isolates using species-specific breakpoints. All agents except for the ECs and posaconazole were active vs. CN and triazoles were active vs. other yeasts (MIC_{90}, 2 µg/ml). The ECs and triazoles were active vs. *Aspergillus* (MIC/MEC_{90} range, 0.015-2 µg/ml), but were not active vs. rMO (MIC/MEC_{90} range, 4->16 µg/ml).

Conclusions: IFIs are increasing worldwide due to the increase in populations at risk. Appropriate antifungal prophylaxis and therapeutic treatment play an important role in decreasing morbidity and mortality; and although the prevalence of R is low among fungal isolates, extended monitoring of R seems prudent.

	MIC/MEC_{50} / % S					
Species	Anidulafungin	Caspofungin	Micafungin	Fluconazole	Voriconazole	Posaconazole
C. albicans (711)	0.015/100.0	0.03/100.0	0.015/100.0	0.25/99.6	≤0.008/99.7	0.03/-[a]
C. glabrata (274)	0.06/97.4	0.06/97.4	0.015/98.9	8/-	0.25/-	1/-
C. parapsilosis (245)	2/88.6	0.5/100.0	1/98.8	1/91.8	0.015/97.1	0.06/-
C. tropicalis (131)	0.015/100.0	0.03/100.0	0.03/100.0	0.5/91.6	0.03/93.1	0.06/-
C. krusei (36)	0.06/97.2	0.12/97.2	0.12/97.2	32/-	0.25/97.2	0.25/-
C. neoformans (52)	>16/-	16/-	>16/-	4/-	0.06/-	0.12/-
A. fumigatus (68)	0.015/-	0.015/-	0.015/-	-/-	0.25/-	0.25/-
A. flavus SC (13)	≤0.008/-	0.015/-	≤0.008/-	-/-	0.5/-	0.5/-
A. niger SC (10)	≤0.008/-	0.015/-	≤0.008/-	-/-	0.5/-	0.5/-

a. "-"= not available.

INTRODUCTION

Invasive fungal infections (IFI) constitute an ever-increasing cause of morbidity and mortality among immunocompromised individuals, those who have undergone intra-abdominal surgery, neonates, and the elderly. In a retrospective case-control study, data from the 2004 Healthcare Cost and Utilization Project Nationwide Inpatient Sample was used to estimate the number, frequency and excess costs and mortality of IFIs in the United States (USA). The frequency of IFIs was estimated at 22 infections per 100,000 persons annually, representing 64,480 IFI cases per year and an excess mortality and length of stay (LOS) in the hospital of 10 and 11 days, respectively. These IFIs accrued $29,281 more in medical costs per infection, contributing an estimated 735,000 additional hospital days and $1.89 billion in additional hospital costs. The most frequent infections were invasive candidiasis (IC), other mycoses (e.g. pseudallescheriosis, fusariosis and other unspecified mycoses), and invasive aspergillosis (IA). Notably IC was associated with the highest attributable mortality (14%), excess LOS (17.2 days), and excess costs ($45,616) among nine different types of IFI.

Concurrent with increasing numbers of IFI, surveillance programs have become important in defining the species distribution and antifungal resistance profiles of the responsible pathogens and thus are providing information necessary for appropriate empiric antifungal therapy. Data from several sources show that mortality rates and resource utilization significantly increases when therapy is delayed or inadequate (suboptimal dose, resistant isolate), further underscoring the importance of detailed epidemiological data.

In the present study, we used the Clinical and Laboratory Standards Institute (CLSI) broth microdilution (BMD) methods and newly developed clinical breakpoints (CBPs) and epidemiological cutoff values (ECVs) to examine the rates of resistance and non-susceptibility (NS) among anidulafungin, fluconazole, voriconazole and comparator agents tested against 1,714 clinical isolates of yeasts and moulds collected from IFIs. Additionally, isolates of *Candida* spp. showing resistance to one or more echinocandin were examined for mutations in hot spot (HS) regions of *fks1* and *fks2*.

METHODS

Organisms. A total of 1,714 clinical isolates from patients with IFI were collected during 2012 from 72 laboratories located in North America (442 isolates, 12 sites), Europe (706 isolates, 30 sites), Latin America (217 isolates, 10 sites), and the Asia-Pacific Region (349 isolates, 20 sites) as part of a global surveillance program. In each case, collection was approved by the appropriate institutional review board. Each participating center recovered consecutive non-duplicated isolates from patients with bloodstream infections (BSI; 1,065 isolates) from normally sterile body fluids, tissues and abscesses (95 isolates), respiratory tract specimens (226 isolates) and other/unknown sites (327 isolates). Isolates were submitted to JMI Laboratories (North Liberty, Iowa, USA), where the ID was confirmed by morphological, biochemical, and molecular methods as described elsewhere.

Antifungal susceptibility testing. All yeast isolates were tested for in vitro susceptibility to the echinocandins (anidulafungin, caspofungin, micafungin) and the triazoles (fluconazole, itraconazole, posaconazole, voriconazole) using CLSI BMD according to the M27-A3 document. MIC results for all agents were read following 24-h of incubation when tested against *Candida* spp., whereas MIC endpoints for the triazoles were read after 48-h when tested against non-*Candida* yeasts. In vitro susceptibility testing of *Aspergillus* spp. and other moulds against the echinocandins and triazoles (itraconazole, posaconazole, voriconazole) was performed by BMD as described in CLSI document M38-A2. The triazole MICs and echinocandin minimum effective concentrations (MEC) were determined as described in the CLSI reference method. Recently revised CLSI clinical breakpoint (CBP) values (CLSI M27-S4 guideline) were used for the most common species and ECVs were applied for less common species of *Candida*, non-*Candida* yeasts, *Aspergillus* spp., or the non-*Aspergillus* moulds. Quality control was performed as recommended in CLSI documents M27-A3 and M38-A2 using *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019.

Sequencing of the *fks* HS regions. *Candida* spp. isolates with MIC values higher than the ECV had the hotspots of the genes encoding the 1,3 β-D-glucan synthase subunits sequenced. DNA extraction was performed using the Qiagen QIAamp DNA mini kit (Qiagen, Hilden, Germany) in the QiaCube automated platform (Qiagen). PCR amplification was carried on using previously described oligunucleotides for the *fks1* and *fks2* HSs 1 and 2. Amplicons were sequenced on both strands and the nucleotide sequences and deduced amino acid sequences were analyzed using the Lasergene software package (DNASTAR, Madison, WI).

RESULTS

- *C. albicans* (711) were very susceptible against the echinocandin compounds (**Table 1**) and all these isolates were considered susceptible against the current CLSI CBP criteria. Azole resistance was observed in 0.0 to 5.2% of the isolates (voriconazole and posaconazole, respectively) and only 0.3% of the isolates were considered fluconazole-resistant (**Table 1**).
- Echinocandin-resistance among *C. glabrata* was noted in all regions, but Latin America. The overall resistance rates to anidulafungin, caspofungin and micafungin were 1.5, 1.5 and 1.1% of the 274 isolates tested, respectively. Five *C. glabrata* strains displaying echinocandin MIC values greater than the ECV were sequenced for the *fks* HSs and four strains displayed mutations on *fks2* HS1 positions F659 and S663 (**Table 2**). Fluconazole resistance was noted among 6.9% of the *C. glabrata* strains (**Table 1**), and 2 of the 19 strains were also non-susceptible to echinocandins (data not shown).
- Applying current clinical breakpoints, all 245 *C. parapsilosis* isolates tested were categorized as echinocandin-susceptible (**Table 1**). Fluconazole, posaconazole and voriconazole inhibited 95.9, 99.6 and 100.0% of these isolates, respectively, at species-specific CBP recently published (**Table 1**).
- Azole-resistance was observed among *C. tropicalis* (131 isolates) in all regions (**Table 1**). Overall, fluconazole was active against 93.9% of the isolates. The echinocandins were very active against *C. tropicalis* strains (100.0% susceptible).
- Anidulafungin, caspofungin and micafungin inhibited 97.2, 97.2 and 100.0% of the *C. krusei* isolates at CPB criteria (**Table 1**). One isolate resistant to anidulafungin and caspofungin carried a L701M *fks1* HS1 substitution (**Table 2**). Posaconazole and voriconazole were active against 94.4 and 97.2% of the isolates using current CBPs.
- Among other Candida species, one *C. dubliniensis* and one *C. kefyr* displayed echinocandin MIC>ECV and both isolates carried at least one *fks* HS mutation (**Table 2**).
- Fluconazole and other triazoles displayed good activity against *C. neoformans* (52 isolates including *C. neoformans variant grubii* [47 strains] and *C. neoformans variant neoformans* [5 strains]; data not shown) and $MIC_{50/90}$ values were 4/8, 0.06/0.12 and 0.12/0.25 µg/ml for fluconazole, voriconazole and posaconazole, respectively. All strains had MIC values below ECV for fluconazole and 96.2 and 94.2% for voriconazole and posaconazole, respectively.
- The vast majority (98.5%) of *A. fumigatus* (68 isolates) had MIC values below the ECV established for voriconazole and itraconazole were considered wildtype (**Table 3**). Against posaconazole, 95.6% of the isolates were categorized as wildtype.

Table 1. Frequency of antifungal resistance among clinical isolates of *Candida* spp. by geographic region in the 2012 SENTRY Surveillance Program.

		No. of isolates (% resistant) to each antifungal agent by region				
Candida species	Antifungal agent	North America	Europe	Latin America	Asia-Pacific	Total
C. albicans	Anidulafungin	162 (0.0)	345 (0.0)	84 (0.0)	120 (0.0)	711 (0.0)
	Caspofungin	162 (0.0)	345 (0.0)	84 (0.0)	120 (0.0)	711 (0.0)
	Micafungin	162 (0.0)	345 (0.0)	84 (0.0)	120 (0.0)	711 (0.0)
	Fluconazole	162 (0.0)	345 (0.3)	84 (1.2)	120 (0.0)	711 (0.3)
	Posaconazole	162 (7.4)	345 (4.3)	84 (4.8)	120 (5.0)	711 (5.2)
	Voriconazole	162 (0.0)	345 (0.0)	84 (0.0)	120 (0.0)	711 (0.0)
C. glabrata	Anidulafungin	96 (2.1)	97 (1.0)	13 (0.0)	68 (1.5)	274 (1.5)
	Caspofungin	96 (3.1)	97 (1.0)	13 (0.0)	68 (0.0)	274 (1.5)
	Micafungin	96 (2.1)	97 (1.0)	13 (0.0)	68 (0.0)	274 (1.1)
	Fluconazole	96 (13.5)	97 (4.1)	13 (0.0)	68 (2.9)	274 (6.9)
	Posaconazole	96 (6.2)	97 (1.0)	13 (0.0)	68 (1.5)	274 (2.9)
	Voriconazole	96 (15.6)	97 (5.1)	13 (0.0)	68 (5.9)	274 (8.8)
C. parapsilosis	Anidulafungin	55 (0.0)	94 (0.0)	41 (0.0)	55 (0.0)	245 (0.0)
	Caspofungin	55 (0.0)	94 (0.0)	41 (0.0)	55 (0.0)	245 (0.0)
	Micafungin	55 (0.0)	94 (0.0)	41 (0.0)	55 (0.0)	245 (0.0)
	Fluconazole	55 (7.3)	94 (2.1)	41 (7.3)	55 (1.8)	245 (4.1)
	Posaconazole	55 (0.0)	94 (0.0)	41 (0.0)	55 (1.8)	245 (0.4)
	Voriconazole	55 (0.0)	94 (0.0)	41 (0.0)	55 (0.0)	245 (0.0)
C. tropicalis	Anidulafungin	25 (0.0)	42 (0.0)	20 (0.0)	44 (0.0)	131 (0.0)
	Caspofungin	25 (0.0)	42 (0.0)	20 (0.0)	44 (0.0)	131 (0.0)
	Micafungin	25 (0.0)	42 (0.0)	20 (0.0)	44 (0.0)	131 (0.0)
	Fluconazole	25 (8.0)	42 (4.8)	20 (0.0)	44 (9.1)	131 (6.1)
	Posaconazole	25 (8.0)	42 (7.1)	20 (5.0)	44 (13.6)	131 (9.2)
	Voriconazole	25 (4.0)	42 (4.8)	20 (0.0)	44 (9.1)	131 (5.3)
C. krusei	Anidulafungin	10 (0.0)	20 (5.0)	3 (0.0)	3 (0.0)	36 (2.8)
	Caspofungin	10 (0.0)	20 (5.0)	3 (0.0)	3 (0.0)	36 (2.8)
	Micafungin	10 (0.0)	20 (0.0)	3 (0.0)	3 (0.0)	36 (0.0)
	Posaconazole	10 (0.0)	20 (10.0)	3 (0.0)	3 (0.0)	36 (5.6)
	Voriconazole	10 (0.0)	20 (5.0)	3 (0.0)	3 (0.0)	36 (2.8)

Table 2. Summary of *fks* alterations detected in echinocandin-resistant strains of *Candida* spp., 2012.

					1,3-β-D-Glucan synthase alterations in:			
		MIC (µg/ml)			*fks1*		*fks2*	
Organism	Region	Anidulafungin	Caspofungin	Micafungin	HS1	HS2	HS1	HS2
C. glabrata	North America	0.25	0.5	0.06	WT	WT	F659Y	WT
C. glabrata	North America	2	1	1	WT	WT	F659del	WT
C. glabrata	North America	2	2	1	WT	WT	S663F	WT
C. glabrata	Europe	2	0.5	1	WT	WT	S663P	WT
C. glabrata	APAC	1	0.25	0.03	A/T1929	WT	WT	WT
C. dubliniensis	Europe	2	2	1	S645P	WT	ND	ND
C. kefyr	North America	2	0.5	1	WT	WT	S663P	WT
C. krusei	Europe	1	1	0.06	L701M	ND	ND	ND

Table 3. Frequency of decreased susceptibility of *Aspergillus fumigatus* to azole antifungal agents by geographic region using CLSI epidemiological cutoff values (ECV) tested using CLSI broth microdilution methods (CLSI, M38-A2).

		No. of isolates (% non-wildtype) to each antifungal agent by region				
Antifungal agent	ECV[a] (µg/ml)	North America	Europe	Latin America	Asia-Pacific	Total
Itraconazole	1	19 (0.0)	31 (0.0)	9 (0.0)	9 (11.1)	68 (1.5)
Posaconazole	0.5	19 (10.5)	31 (0.0)	9 (0.0)	9 (11.1)	68 (4.4)
Voriconazole	1	19 (0.0)	31 (0.0)	9 (0.0)	9 (11.1)	68 (1.5)

a. ECVs as published by Espinel-Ingroff et al (2010).

CONCLUSIONS

- The echinocandins displayed excellent potency against contemporary isolates of *Candida* species. Resistance among *C. glabrata* was greater than other species, but resistance rates are still very low (1.0 to 2.0%) and most resistant strains carried *fks* mutations.
- Fluconazole and other azoles were very active against *C. neoformans* strains. Additionally, mould-active azoles and echinocandins had good activity when tested against *A. fumigatus*.
- Although resistance to antifungal agents among clinical isolates of opportunistic fungi is generally considered uncommon, both increased resistance and breakthrough infections have been reported among patients with long-term exposure to even the newest of antifungal agents. These facts highlight the importance of continued monitoring of antifungal resistance.

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