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Wild-Type MIC Distributions and Epidemiological Cutoff Values for Selected Triazole and Echinocandin Antifungal Agents for Six Uncommon Species of *Candida* as Determined by CLSI Broth Microdilution Methods

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ABSTRACT

Background: Epidemiological cutoff values (ECVs) provide a sensitive means for detecting emerging resistance and they have not been established for less common, clinically important, species such as *C. dubliniensis* (CD), *C. guilliermondii* (CGU), *C. kefyr* (CKF), *C. lusitaniae* (CL), *C. orthopsilosis* (COR), and *C. pelliculosa* (CPL). We determined species-specific ECVs for anidulafungin (ANF), caspofungin (CSF), fluconazole (FLC), posaconazole (PSC), and voriconazole (VRC) versus six species.

Methods: 819 invasive clinical isolates of CD (70 isolates), CGU (198), CKF (112), CL (280), COR (102), and CPL (57) were collected from 2001-2010 worldwide (653 isolates from ARTEMIS and 166 from SENTRY). Susceptibility testing against six compounds using CLSI broth microdilution (BMD; 24-h incubation) was performed. Isolates were identified by standard biochemical methods and/or molecular sequencing.

Results: The modal MICs (in µg/ml) for ANF,CSF, FLC, PSC, and VRC, respectively, were as follows: for CD (0.03, 0.06, 0.25, 0.03, 0.008), CGU (2, 0.5, 2, 0.12, 0.06), CKF (0.06, 0.015, 0.25, 0.06, 0.008), CL (0.5, 0.25, 0.5, 0.06, 0.008), COR (1, 0.12, 0.5, 0.03, 0.015), and CPL (0.015, 0.015, 2, 1, 0.12). The ECVs are summarized in the Table but could not be determined for CPL versus ANF.

	ECV (µg/ml: % MIC ≤ ECV)				
Species	ANF	CSF	FLC	PSC	VOR
C. dubliniensis	0.12 (95.2)	0.12 (97.8)	0.5 (95.7)	0.12 (98.6)	0.06 (100.0)
C. guilliermondii	4 (100.0)	2 (96.0)	8 (95.0)	0.5 (97.5)	0.25 (98.0)
C. kefyr	0.25 (98.9)	0.03 (98.0)	1 (99.1)	0.25 (99.1)	0.015 (100.0)
C. lusitaneae	2 (100.0)	1 (99.6)	2 (96.1)	0.25 (98.6)	0.03 (96.6)
C. orthopsilosis	2 (100.0)	0.5 (100.0)	2 (98.0)	0.25 (97.1)	0.06 (98.0)
C. pelliculosa	ND[a]	0.12 (94.4)	4 (98.2)	2 (98.2)	0.25 (98.2)

Conclusions: In the absence of species-specific clinical breakpoints these wild-type (WT) MIC distributions and ECVs will be useful for monitoring the emergence of reduced susceptibility to antifungals among these less common *Candida* spp. Overall, the activity profiles of both triazoles and two echinocandins were quite favorable against these species. However, activity did vary by drug-species combination.

INTRODUCTION

Presently there are more than 200 species of *Candida*, of which 30 to 40 are known to cause human infections. The Clinical and Laboratory Standards Institute (CLSI) has recently established species-specific clinical breakpoints (CBPs) for triazole and echinocandin antifungal agents tested by broth microdilution (BMD) and these values may be used to identify those isolates from the five most common Candidal species (*Candida albicans, C. glabrata, C. tropicalis, C. parapsilosis,* and *C. krusei*) that are likely to respond to treatment with a given antifungal agent administered at approved dosing schedule.

The low frequency of occurrence and the lack of clinical data preclude the establishment of a CBP for the less commonly cultured Candidal species. However, several of these rarer species have been observed to occur in nosocomial clusters and/or to exhibit innate or acquired resistance to one or more established antifungal agents. Thus, it is prudent to develop criteria, such as epidemiological cutoff values (ECVs) to differentiate wild-type (WT) strains (those without mutational or acquired resistance mechanisms) from non-WT strains (those having mutational or acquired resistance mechanisms) as a means of tracking the emergence of reduced susceptibility.

In the present study, we analyzed the extensive global databases from two independent antifungal surveys, the ARTEMIS Program and SENTRY Antimicrobial Surveillance Program, to establish ECVs for each less common but yet prevalent Candidal species and antifungal agent.

MATERIALS AND METHODS

Organisms: A total of 819 clinical isolates obtained from more than 60 medical centers worldwide from 2001 through 2010 were tested (653 isolates, from ARTEMIS and 166 from SENTRY Program). The collection included 70 isolates of *C. dubliniensis*, 198 isolates of *C. guilliermondii,* 112 isolates of *C. kefyr,* 280 isolates of *C. lusitaniae*, 102 isolates of *C. orthopsilosis*, and 57 isolates of *C. pelliculosa* (Table 1). All isolates were obtained from blood or other normally sterile sites and represented the incident isolates from individual infectious episodes. The isolates were collected at individual study sites and were sent to the University of Iowa (ARTEMIS isolates, Iowa City, Iowa, USA) and JMI Laboratories (SENTRY Program isolates, North Liberty, Iowa, USA) for identification and reference susceptibility testing as described previously. The isolates were identified by standard methods supplemented by molecular identification as needed and stored as water suspensions until used in the study. Prior to being tested, each isolate was passaged at least twice onto potato dextrose agar (Remel, Lenexa, Kansas, USA) and CHROMagar *Candida* medium (Becton Dickinson and company, Sparks, Maryland, USA) to ensure purity and viability.

Antifungal susceptibility testing: BMD testing was performed in accordance with the guidelines in CLSI document M27-A3 (2008), using RPMI 1640 medium, inoculum of 0.5 X 10^3 to 2.5 X 10^3 cells/ml, and incubation at 35ºC. MIC values were determined visually, after 24-h of incubation, as the lowest concentration of drug that caused a significant diminution (≥50% inhibition) of growth relative to that of the growth control. Quality control was performed by testing CLSI-recommended strains of *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019.

Definitions: The definitions of WT organisms and ECVs were those outlined previously. A WT organism is defined as a strain which does not harbor any acquired resistance to the particular antimicrobial agent being examined. The typical MIC distribution for WT organisms covers three to five doubling dilutions surrounding the modal MIC. Inclusion of WT strains in the present study was ensured by testing only the incident isolate for each infection episode.

The ECVs for fluconazole, posaconazole, voriconazole, anidulafungin and caspofungin and the six species of *Candida* were obtained as described previously, by considering the WT MIC distribution, the modal MIC for each distribution, and the inherent variability of the test (usually within one doubling dilution). In general, the ECV should encompass at least 95% of isolates in the WT distribution. Organisms with acquired or mutational resistance mechanisms may be included among those for which the MIC results are higher than the ECV.

RESULTS

- The WT MIC distributions for anidulafungin, caspofungin, fluconazole, posaconazole and voriconazole and each of the six rarer species of *Candida* are shown in Table 1.
- Among the echinocandins, the modal MIC values at 24-h of incubation ranged from 0.015 to 2 µg/ml for anidulafungin and 0.06 to 0.25 µg/ml for caspofungin, with higher values for *C. guilliermondii* (Table 2).
- For the azoles, the modal MIC at 24-h incubation ranged from 0.25 to 2 µg/ml for fluconazole, 0.03 to 1 µg/ml for posaconazole and 0.008 to 0.12 µg/ml for voriconazole (Table 2). The highest values were observed from *C. pelliculosa* and *C. guilliermondii* (fluconazole).
- Overall, *C. dubliniensis* and *C. kefyr* were the species displaying the lowest 24-h endpoint ECVs for echinocandins and azoles and the majority of strains having MIC values below the determined ECV (Table 1 and 2; 95.2-100.0% below ECV for *C. dubliniensis* and 97.5-100.0% for *C. kefyr*).
- Echinocandins and fluconazole ECVs for *C. guilliermondii* were elevated (4, 2 and 8 µg/ml for anidulafungin, caspofungin, fluconazole, respectively; Table 1), but ≥95.0% of the strains had MIC results below these values.
- *C. lusitaniae and C. orthopsilosis* had similar ECV values for all antifungal agents tested, with modestly elevated values for the echinocandins (0.5-2 µg/ml) when compared to other more susceptible species, such as *C. dubliniensis*. Fluconazole, posaconazole and voriconazole ECVs were 2, 0.25 and 0.03-0.06 µg/ml, respectively (Table 2).
- The ECVs proposed demonstrated that ≥94.4% of strains were within the susceptible WT population of MIC results (Table 1, lowest for caspofungin and *C. pelliculosa*).

Table 1. WT MIC distributions of azole and echinocandin antifungal agents for six uncommon species of *Candida* obtained using CLSI BMD methods.[a]

		No. of isolates with MIC (µg/ml)													
Species	Antifungal agent (no. tested)	≤0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	ECV (%)[c]
C. dubliniensis	Anidulafungin (63)		5	26	24	5[b]	3									0.12 (95.2)
	Caspofungin (45)		4	16	22	2[b]	1									0.12 (97.8)
	Fluconazole (70)					20	24	23[b]		1	1			1		0.5 (95.7)
	Posaconazole (70)	1	7	28	25	8[b]		1								0.12 (98.6)
	Voriconazole (46)	43	2	1[b]												0.03 (100.0)
C. guilliermondii	Anidulafungin (126)				1	5	7	5	41	53	14[b]					4 (100.0)
	Caspofungin (176)			1	13	21	34	66	28	6[b]		3	4			2 (96.0)
	Fluconazole (196)							8	32	88	52	6[b]	5	3	2	8 (95.0)
	Posaconazole (197)			3	16	24	78	53	18[b]	3	2					0.5 (97.5)
	Voriconazole (198)	2	23	71	74	15	9[b]	3			1					0.25 (98.0)
C. kefyr	Anidulafungin (89)		2	20	39	26	1[b]	1								0.25 (98.9)
	Caspofungin (101)	18	72	9[b]	1	1										0.03 (98.0)
	Fluconazole (113)					16	61	27	8[b]	1						1 (99.1)
	Posaconazole (112)	1	5	26	40	30	9[b]	1								0.25 (99.1)
	Voriconazole (101)	84	17[b]													0.015 (100.0)
C. lusitaniae	Anidulafungin (206)		1			7	21	74	86	17	–[b]					2 (100.0)
	Caspofungin (276)		1	5	9	81	98	67	14[b]		1					1 (99.6)
	Fluconazole (272)					47	81	99	34	9[b]				1	1	2 (99.3)
	Posaconazole (279)	3	39	76	112	29	16[b]	1	3							0.25 (98.6)
	Voriconazole (233)	185	33	7[b]	1		5	2								0.03 (96.6)
C. orthopsilosis	Anidulafungin (52)					3	12	28	9[b]							2 (100.0)
	Caspofungin (91)	1		3	17	37	25	8[b]								0.5 (100.0)
	Fluconazole (102)					3	30	46	9	12[b]		1		1		2 (98.0)
	Posaconazole (102)		14	28	26	20	11[b]	3								0.25 (97.1)
	Voriconazole (102)	30	43	16	11[b]	1	1									0.06 (98.0)
C. pelliculosa	Anidulafungin (31)	5	18	5	1	2										–
	Caspofungin (54)	1	23	19	5	3[b]	2	1								0.12 (94.4)
	Fluconazole (57)							3	4	31	18[b]	1				4 (98.2)
	Posaconazole (57)				2	7	9	13	20	5[b]	1					2 (98.2)
	Voriconazole (57)	1	3	2	18	27	5[b]	1								0.25 (98.2)

a. All MICs determined after 24-h incubation (CLSI, 2008a and b).
b. Proposed ECV.
c. Percentage of isolates at ≤ ECV (µg/ml).

Table 2. Current breakpoints and proposed epidemiological cut-off values for 11 *Candida* spp. (reportable reading conditions, 50% diminution at 24-h).[a]

	Breakpoints (mcg/ml)				ECV (mcg/ml)	
Organism	Susceptible	S-DD	Intermediate	Resistant	WT	Non-WT
C. albicans						
Caspofungin	≤0.25		0.5	≥1	≤0.12	>0.12
Anidulafungin	≤0.25		0.5	≥1	≤0.12	>0.12
Fluconazole	≤2.0	4	-	≥8	≤0.5	>0.5
Voriconazole	≤0.12	-	0.25-0.5	≥1	≤0.03	>0.03
Posaconazole	-	-	-	-	≤0.06	>0.06
C. parapsilosis						
Caspofungin	≤2		4	≥8	≤1	>1
Anidulafungin	≤2		4	≥8	≤4	>4
Fluconazole	≤2	4	-	≥8	≤2	>2
Voriconazole	≤0.12	-	0.25-0.5	≥1	≤0.12	>0.12
Posaconazole	-	-	-	-	≤0.25	>0.25
C. tropicalis						
Caspofungin	≤0.25		0.5	≥1	≤0.12	>0.12
Anidulafungin	≤0.25		0.5	≥1	≤0.12	>0.12
Fluconazole	≤2.0	4	-	≥8	≤2	>2
Voriconazole	≤0.12	-	0.25-0.5	≥1	≤0.06	>0.06
Posaconazole	-	-	-	-	≤0.12	>0.12
C. glabrata						
Caspofungin	≤0.12		0.25	≥0.5	≤0.12	>0.12
Anidulafungin	≤0.12		0.25	≥0.5	≤0.25	>0.25
Fluconazole	-	≤32	-	≥64	≤32	>32
Voriconazole	-	-	-	-	≤0.5	>0.5
Posaconazole				-	≤2	>2
C. krusei						
Caspofungin	≤0.25		0.5	≥1	≤0.25	>0.25
Anidulafungin	≤0.25		0.5	≥1	≤0.12	>0.12
Fluconazole	-	-	-	-	≤64	>64
Voriconazole	≤0.5	-	1	≥2	≤0.5	>0.5
Posaconazole	-	-	-	-	≤0.5	>0.5

a. Abbreviations: S-DD, susceptible-dose dependent; WT, wild-type; non-WT, non- wild-type.

	Breakpoints (mcg/ml)				ECV (mcg/ml)	
Organism	Susceptible	S-DD	Intermediate	Resistant	WT	Non-WT
C. dubliniensis						
Caspofungin	-	-	-	-	≤0.12	>0.12
Anidulafungin	-	-	-	-	≤0.12	>0.12
Fluconazole	-	-	-	-	≤0.5	>0.5
Voriconazole	-	-	-	-	≤0.03	>0.03
Posaconazole	-	-	-	-	≤0.12	>0.12
C. guilliermondii						
Caspofungin	≤2		4	≥8	≤2	>2
Anidulafungin	≤2		4	≥8	≤4	>4
Fluconazole	-	-	-	-	≤8	>8
Voriconazole	-	-	-	-	≤0.25	>0.25
Posaconazole	-	-	-	-	≤0.5	>0.5
C. kefyr						
Caspofungin	-	-	-	-	≤0.03	>0.03
Anidulafungin	-	-	-	-	≤0.25	>0.25
Fluconazole	-	-	-	-	≤1	>1
Voriconazole	-	-	-	-	≤0.015	>0.015
Posaconazole	-	-	-	-	≤0.25	>0.25
C. lusitaniae						
Caspofungin	-	-	-	-	≤1	>1
Anidulafungin	-	-	-	-	≤2	>2
Fluconazole	-	-	-	-	≤2	>2
Voriconazole	-	-	-	-	≤0.03	>0.03
Posaconazole	-	-	-	-	≤0.25	>0.25
C. orthopsilosis						
Caspofungin	-	-	-	-	≤0.05	>0.5
Anidulafungin	-	-	-	-	≤2	>2
Fluconazole	-	-	-	-	≤2	>2
Voriconazole	-	-	-	-	≤0.06	>0.06
Posaconazole	-	-	-	-	≤0.25	>0.25
C. pelliculosa						
Caspofungin	-	-	-	-	≤0.12	>0.12
Fluconazole	-	-	-	-	≤4	>4
Voriconazole	-	-	-	-	≤0.25	>0.25
Posaconazole	-	-	-	-	≤2	>2

CONCLUSIONS

- These distributions show the overall favorable susceptibility of these less common species to both triazole and echinocandin classes of antifungal agents. As noted previously, *C. guilliermondii, C. lusitaniae,* and *C. orthopsilosis* were less susceptible to fluconazole and the echinocandins when compared to the other three species.
- Given the similar ECV results for each antifungal agent calculated for both common and uncommon species of *Candida,* it is tempting to assign the same CBPs to the rarer species (see *C. guilliermondii* in Table 2). This possibility must be considered by international standards organizations (CLSI, EUCAST), but will require considerably more clinical outcomes data.

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