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Impact of New Antifungal Breakpoints on Antifungal Resistance in *Candida* Species

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We reviewed our antifungal susceptibility data for micafungin, anidulafungin, fluconazole, and voriconazole against *Candida* species and compared resistance rates determined by the previous and recently revised CLSI antifungal breakpoints. With the new breakpoints, resistance was significantly increased for micafungin (from 0.8% to 7.6%), anidulafungin (from 0.9% to 7.3%), and voriconazole (from 6.1% to 18.4%) against *Candida glabrata*. Resistance was also increased for fluconazole against *Candida albicans* (from 2.1% to 5.7%).

The Clinical and Laboratory Standards Institute (CLSI) recently revised the azole and echinocandin clinical breakpoints against *Candida* species (1). These new breakpoints are now both drug and species specific, whereas the previous breakpoints were not. For most species, with the exception of *Candida parapsilosis* and *Candida guilliermondii* against the echinocandins, the breakpoints have been lowered, such that previously susceptible MICs are now classified as resistant. In addition, *Candida glabrata* isolates are no longer considered susceptible to fluconazole but are rather classified only as either dose-dependent susceptible or resistant. The breakpoint revisions were made based on information from clinical studies, case reports describing clinical failure at MIC values below the previous breakpoints, results from pharmacokinetic/pharmacodynamic studies, and epidemiologic cutoff values (2, 3). Additionally, a goal of harmonizing the antifungal breakpoints with those set by EUCAST was sought. Epidemiologic cutoff values for individual species are used to optimize the detection of non-wild-type strains and, thus, the acquisition of resistance mechanisms and to prevent the breakpoints from dividing wild-type populations (2–4). The impact of the revised clinical breakpoints regarding categorical placement of *Candida* strains as resistant is unknown. Our objective was to evaluate what effect the new antifungal clinical breakpoints may have on azole and echinocandin resistance patterns in *Candida* species.

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The antifungal susceptibility database in the Fungus Testing Laboratory at the University of Texas Health Science Center at San Antonio was reviewed. Susceptibility data for fluconazole, voriconazole, anidulafungin, and micafungin against *Candida albicans*, *C. glabrata*, *Candida tropicalis*, *Candida krusei*, and *C. parapsilosis* isolates sent to our laboratory for testing between 1 January 2008 and 31 December 2012 were reviewed. During this period, antifungal powders were obtained from the appropriate manufacturers (Pfizer and Astellas) and stock solutions were prepared in water for agents with aqueous solubility or in dimethyl sulfoxide (DMSO) as recommended (1, 5). Susceptibility testing was performed by broth microdilution methodology in RPMI according to the CLSI M27-A3 guidelines (5), and a 50% inhibition of growth compared to the growth control well was used as the endpoint for all agents. *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019 served as the quality control isolates for each testing run, and the results were consistently within the range specified by the CLSI. Isolates were classified as resistant based on both the previous and the recently revised CLSI clinical breakpoints (Table 1) (1). For voriconazole against *C. glabrata*, the epidemiologic cutoff value of 0.5 μg/ml was used for the new threshold for resistance, as the CLSI has not set clinical breakpoints for this triazole against this species (1, 6, 7). Thus, *C. glabrata* isolates with a voriconazole MIC above this value (i.e., ≥1 μg/ml) were classified as resistant. Differences in resistance rates between the previous and revised breakpoints were assessed for significance by Fisher's exact test, and a P value of ≤0.05 was considered significant.

The number of each *Candida* species tested with each drug, the MIC range, the MIC_{50} and MIC_{90} values, and the percentage of isolates classified as resistant per the previous and revised breakpoints are shown in Table 2, and the MIC distributions are shown in Table 3. As shown in these tables and in Fig. 1, resistance did increase with the new breakpoints. For the echinocandins anidulafungin and micafungin, the most marked changes occurred against *C. glabrata*. For this species, the number of isolates classified as resistant increased from 1 (0.9%) to 8 (7.3%) of 110 isolates ($P = 0.0353$) for anidulafungin and from 3 (0.8%) to 27 (7.6%) of 354 isolates ($P < 0.0001$) for micafungin when the new CLSI clinical breakpoints were applied. Our rates of micafungin and anidulafungin resistance with the new CLSI clinical breakpoints are higher than those recently reported in the SENTRY study (1.3% and 1.6%, respectively) (6). However, others have found higher rates of echinocandin resistance for this species, with rates of micafungin and anidulafungin resistance reported to range from 9% to 12% between 2007 and 2010 at a large medical center in the United States (8). Against the other *Candida* species, the number of isolates considered to be resistant remained relatively low, and for anidulafungin against *C. parapsilosis*, the number actually decreased slightly from 2 to 0 isolates (2.4% to 0%), although this

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TABLE 1 Previous and recently revised CLSI antifungal clinical breakpoints for resistance for fluconazole, voriconazole, anidulafungin, and micafungin against *Candida* species[a]

Antifungal and CBP	Breakpoint (μg/ml) against:				
	C. albicans	*C. glabrata*	*C. tropicalis*	*C. krusei*	*C. parapsilosis*
Anidulafungin					
Previous	≥4	≥4	≥4	≥4	≥4
Revised	≥1	≥0.5	≥1	≥1	≥8
Micafungin					
Previous	≥4	≥4	≥4	≥4	≥4
Revised	≥1	≥0.25	≥1	≥1	≥8
Fluconazole					
Previous	≥64	≥64	≥64	≥64	≥64
Revised	≥8	≥64	≥8	≥8	≥8
Voriconazole					
Previous	≥4	≥4	≥4	≥4	≥4
Revised	≥1	≥1	≥1	≥2	≥1

[a] The epidemiologic cutoff value was used for voriconazole against *C. glabrata*. CBP, clinical breakpoints.

reduction was not statistically significant. This was not unexpected against this species, as the CLSI echinocandin breakpoints against *C. parapsilosis* were raised from ≥4 μg/ml as nonsusceptible to ≥8 μg/ml as resistant.

The azoles fluconazole and voriconazole were also affected by the revised breakpoints. Against *C. albicans*, the number of isolates classified as resistant to fluconazole by the new breakpoints significantly increased from 25 (2.1%) to 68 (5.7%) of a total of 1,196 strains tested ($P < 0.0001$) (Table 2 and Fig. 1) compared to the previous threshold for resistance. This rate of fluconazole resistance in *C. albicans* is higher than what was recently reported in isolates from North American institutions in the SENTRY study (0.6%) (6). There were also trends for increased resistance to fluconazole with the new breakpoints against *C. tropicalis* (19 to 32 of 327 isolates [6% to 9.9%]; $P = 0.0557$) and *C. parapsilosis* (3 to 11 of 497 isolates [0.6% to 2.2%]; $P = 0.0793$). A similar observation was found for voriconazole against *C. albicans* (17 to 27 of 593 isolates [2.9% to 4.6%]; $P = 0.17$), although this difference was not significant. In contrast, only minor increases in voriconazole resistance against *C. tropicalis* (30 to 36 of 205 isolates [14.6% to 17.6%]) and *C. krusei* (4 to 7 of 98 isolates [4.1% to 12.2%]) were observed when the new breakpoints were applied. A significant increase in the number of *C. glabrata* isolates that were classified as resistant to voriconazole (32 to 96 of 522 isolates [6.1% to 18.4%]; $P < 0.0001$) was observed when the epidemiologic cutoff value was used. The revised CLSI breakpoints for the azoles are supported by epidemiologic cutoff values and the results of various pharmacodynamic analyses (9–12), as well as by the cross-resistance observed among different members of this class in *Candida* species (13–16). For most species, our rates of fluconazole and voriconazole resistance were higher than those reported in the SENTRY study (6). This may be a reflection of the nature of our reference laboratory in that we may often be sent isolates from patients exposed to antifungals who are failing therapy. Thus, some of the isolates we receive for susceptibility testing may be more likely to be non-wild-type strains. However, the overall MIC distributions from our study shown in Table 3 are similar to those reported by others, including those from large surveillance studies (6, 7, 17).

TABLE 2 MIC ranges, MIC_{50} and MIC_{90} values, and percentages of isolates classified as resistant based on the previous and recently revised CLSI clinical breakpoints for antifungals[a]

Value for each species and drug	*Candida albicans*			*Candida glabrata*			*Candida tropicalis*			*Candida krusei*			*Candida parapsilosis*		
Antifungal	AFG	MFG	FLU	AFG	MFG	FLU	AFG	MFG	FLU	AFG	MFG	FLU	AFG	MFG	FLU
No. of strains	118	433	1,196	110	354	882	49	170	327	28	65	98	83	269	496
MIC range	≤0.015–0.25	≤0.015–8	≤0.125–>64	≤0.015–4	≤0.015–4	≤0.125–>64	≤0.015–8	≤0.015–8	≤0.125–>64	0.03–0.25	≤0.015–0.25	0.03–4	≤0.015–4	≤0.015–2	≤0.125–>64
MIC_{50}	0.015	0.015	0.125	0.06	0.03	4	0.03	0.03	0.5	0.125	0.125	0.25	1	0.5	0.25
MIC_{90}	0.06	0.03	0.5	0.25	0.06	32	0.125	0.06	8	0.25	0.25	1	2	1	0.5
% resistant to previous CBP	0	0.2	2.1	0.9	0.8	7.9	2.0	1.8	5.8	0	0	4.1	2.4	0	0.6
% resistant to new CBP	0	0.5	5.7	7.3	7.6	7.9	18.4	2.0	1.8	9.8	17.6	0	0	7.1	0	0	2.2	2.0
				0.9	0.8	6.1	2.0	1.8	5.8	14.6	0	0	0	4.1	2.4	0	0.6	0.7
				2.9	2.9	4.6	0.03–>16	0.03–>16	0.06	16	0.125	0.25	0.5	1	2	0.5	0.06	0.03–>16
				0.03–>16	0.015–4	0.03–>16	0.03	0.03	0.125–>64	0.03–0.25	0.125	0.25	1	0.5	1	0.25	0.03	0.03
				0.03	0.03	0.125	0.125	0.06	0.06	0.125	0.25	0.5	2	1	2	0.5	0.06	0.06
				0.125	0.06	2	0.125	0.06	16	0.25	0.25	1	4	2	4	1	0.25	0.25
				0.25	0.06	32	0.25	0.06	16	0.25	0.25	1	4	2	4	1	0.25	0.25
				0.9	0.8	7.9	2.0	1.8	14.6	0	0	4.1	2.4	0	0.6	0.7	2.2	2.0

[a] CBP, clinical breakpoints; AFG, anidulafungin; MFG, micafungin; FLU, fluconazole; VOR, voriconazole.

TABLE 3 Comparison of *in vitro* susceptibilities of anidulafungin, micafungin, fluconazole, and voriconazole against *Candida* isolates

Species and agent[a]	No. of isolates tested	% of isolates at an MIC (μg/ml) of:												
		≤0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	≥64
C. albicans														
AFG	118	60.2	29.7	4.2	3.4	2.5								
MFG	433	75.5	21.0	1.4	0.5	0.5	0.7		0.2		0.2			
FLU	1,196				69.6	17.4	3.2	1.3	1.0	1.8	1.5	1.3	0.8	2.1
VOR	593		84	4.9	3.2	2.4	1.0	0.8	0.8	0.5	0.5	1.9		
C. glabrata														
AFG	110	2.7	26.4	29.1	27.3	7.3	2.7	1.8	1.8	0.9				
MFG	354	48.0	39.0	3.4	2.0	2.3	2.0	1.1	1.4	0.8				
FLU	882				0.2	1.8	7.0	12.6	27.2	27.3	6.7	4.1	5.1	7.9
VOR	522		14.6	24.5	22.2	11.9	8.4	7.5	4.8	4.8	1.1	0.2		
C. tropicalis														
AFG	49	26.5	30.6	24.5	12.2	4.1					2.0			
MFG	170	21.2	64.1	8.8	2.9		1.2				1.8			
FLU	327				10.1	37.3	26.6	8.5	5.5	2.4	1.2	2.1	0.6	5.8
VOR	205		39.0	21.0	12.7	6.3	3.4	1.5	1.5	1.5	0.5	12.6		
C. krusei														
AFG	28		21.4	14.3	57.1	7.1								
MFG	65	3.1	6.1	13.8	58.5	18.5								
VOR	98		4.1	3.1	25.5	41.8	11.2	7.1	3.1	4.1				
C. parapsilosis														
AFG	83	1.2			2.4	10.8	28.9	38.6	15.7	2.4				
MFG	269	0.7	1.5	0.7	3.3	21.6	43.1	27.9	1.1					
FLU	496				8.9	56.1	25.8	2.8	3.0	1.2	1.2		0.4	0.6
VOR	298		88.9	4.0	3.4	1.0	0.7	0.7	0.7	0.3		0.3		

[a] AFG, anidulafungin; MFG, micafungin; FLU, fluconazole; VOR, voriconazole.

The results from our laboratory demonstrate that the rates of resistance for the echinocandins and the azoles may be increased with the use of the new CLSI antifungal breakpoints. These changes were especially marked for micafungin, anidulafungin, and voriconazole against *C. glabrata* and for fluconazole against *C. albicans*. One limitation of this study is that we did not determine if the isolates harbored mechanisms of acquired antifungal resistance. Although this information is important, it would not change how an isolate is classified as susceptible or resistant by the CLSI breakpoints, something which is determined by the pheno-

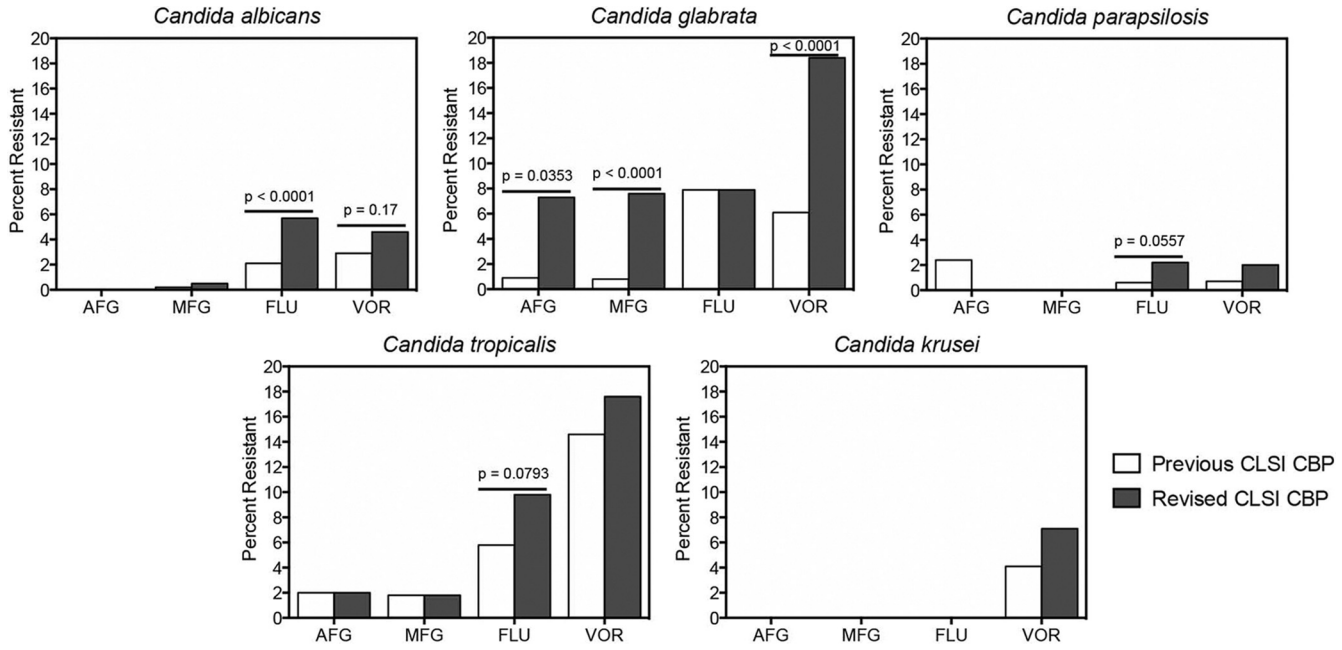

FIG 1 Percentage of *Candida* isolates per species classified as resistant to anidulafungin (AFG), micafungin (MFG), fluconazole (FLU), and voriconazole (VOR) per the previous and recently revised CLSI antifungal clinical breakpoints (CBP).

typic MIC. The clinical relevance of our findings is unknown. Studies have indicated that *in vitro* resistance may be indicative of clinical failure (8, 18, 19). The results from other laboratories and institutions are needed to confirm our observations.

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