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Use of Anidulafungin as a Surrogate Marker To Predict Susceptibility and Resistance to Caspofungin among 4,290 Clinical Isolates of *Candida* by Using CLSI Methods and Interpretive Criteria

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This study addressed the application of anidulafungin as a surrogate marker to predict the susceptibility of *Candida* to caspofungin due to unacceptably high interlaboratory variation of caspofungin MIC values. CLSI reference broth microdilution methods and species-specific interpretive criteria were used to test 4,290 strains of *Candida* (eight species), including 71 strains with documented *fks* mutations. Caspofungin MIC values were compared with those of anidulafungin to determine the percentage of categorical agreement (CA) and very major (VME), major (ME), and minor error rates, as well as the ability to detect *fks* mutants. For all 4,290 isolates the CA was 97.1% (0.2% VME and ME, 2.5% minor errors) using anidulafungin as the surrogate. Among the 62 isolates of *Candida albicans* (4 isolates), *C. tropicalis* (5 isolates), *C. krusei* (4 isolates), *C. kefyr* (2 isolates), and *C. glabrata* (47 isolates) that were nonsusceptible (NS; either intermediate [I] or resistant [R]) to both caspofungin and anidulafungin, 52 (83.8%) contained a mutation in *fks1* or *fks2*. Eight mutants of *C. glabrata*, two of *C. albicans*, and one each of *C. tropicalis* and *C. krusei* were classified as susceptible (S) to both antifungal agents. The remaining 7 mutants (2 *C. albicans* and 5 *C. glabrata*) were susceptible to one of the agents and either intermediate or resistant to the other. Using the epidemiological cutoff value (ECV) of 0.12 μg/ml for both caspofungin and anidulafungin to differentiate wild-type (WT) from non-WT strains of *C. glabrata*, 42 of the 55 (76.4%) *C. glabrata* mutants were non-WT and 8 of the 55 (14.5%) were WT for both agents (90.9% concordance). Anidulafungin can accurately serve as a surrogate marker to predict S and R of *Candida* to caspofungin.

The echinocandins (anidulafungin, caspofungin, and micafungin) are all considered first-line agents for the treatment of invasive candidiasis, including candidemia (1–3). Numerous *in vitro* studies document comparable activities of these agents against a broad range of *Candida* species when tested using the broth microdilution methods of the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (4–10). Both the CLSI and EUCAST have established clinical MIC breakpoints (CBPs) and epidemiological cutoff values (ECVs) for anidulafungin and micafungin tests against common species of *Candida* (10–12). These interpretive criteria have been shown to be predictive of *in vivo* outcome and also serve to differentiate wild-type (WT) strains (no intrinsic or acquired resistance mutations) from non-WT strains that harbor acquired resistance mutations in the *fks* genes encoding the glucan synthase target enzyme (4, 10, 13, 14). Whereas the CLSI has also developed CBPs for *Candida* and caspofungin (13), the EUCAST has not done so and presently does not recommend caspofungin MIC testing for clinical decision making due to unacceptably high variation among the caspofungin MIC values obtained from different centers (4, 5, 11, 15). Notably, a recent CLSI analysis of the caspofungin MIC distributions from 17 different laboratories documented variation in the WT modal MIC values of as much as five doubling dilutions (e.g., 0.015 to 0.5 μg/ml) (16). Similar variation was shown with the EUCAST method across four different species (and seven laboratories) (16). In contrast, the variation in both anidulafungin and micafungin WT modal values was within ±1 doubling dilution step for eight different species and 15 laboratories (10). The reasons for such variation in caspofungin MIC values from center to center remain unclear, but they may involve solubility issues, adherence of drug to the plastic microdilution wells, storage conditions, or MIC endpoint reading (5, 16).

The extreme intra- and interlaboratory variations in caspofungin MIC results are of great concern and suggest that the more reliable MIC testing of *Candida* species using either anidulafungin or micafungin as a surrogate for caspofungin may be preferred for clinical *in vitro* testing of echinocandins (4, 16, 17). Indeed, the EUCAST currently recommends that anidulafungin be used for determining the *in vitro* susceptibility of *Candida* to the echinocandin class (6). A recent analysis of cross-resistance between micafungin and caspofungin using the CLSI method has demonstrated the potential for micafungin results to predict the susceptibility and resistance of *Candida* spp. to caspofungin (14). Given these results and in the interest of harmonization between the CLSI and EUCAST methods for testing the echinocandins against *Candida*, we have explored the potential of anidulafungin to serve as a surrogate marker for evaluating the susceptibility of *Candida* to caspofungin.

In the present study, we utilized a large, multiyear database of susceptibility results, all determined by CLSI broth microdilution methods and including results for 71 *fks* mutant strains. This collection provides a robust analysis of cross-resistance between

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TABLE 1 MIC distributions of anidulafungin and caspofungin versus *Candida* spp., including strains with *fks* mutations, using reference CLSI methods

Species (no. tested)	Antifungal agent	No. of isolates (no. with *fks* mutation) at indicated MIC (μg/ml)										
		≤0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	≥8
C. albicans (2,307)	Anidulafungin	121	574	866	547 (1)	174 (2)	13 (1)	2 (1)	4 (3)	6		
	Caspofungin	38	625	1,054	538	23	12 (2)	11(2)	3 (2)	2 (2)	1	
C. glabrata (655)	Anidulafungin		1	72 (1)	282 (6)	228 (4)	30 (6)	4 (3)	21 (20)	9 (8)	8 (7)	
	Caspofungin		37	334 (1)	210 (6)	20 (3)	10 (6)	13 (10)	7 (6)	8 (8)	2 (2)	14 (13)
C. parapsilosis (539)	Anidulafungin		1	1			7	14	110	364	42	
	Caspofungin	1	1	1	17	35	208	219	49	7	1	
C. tropicalis (515)	Anidulafungin	9	110	270	92	21 (1)	6	2 (1)	2 (2)	3		
	Caspofungin	5	164	239	93	5 (1)	4		2 (1)	2 (3)		1
C. krusei (124)	Anidulafungin		4	55	52	9		3 (2)	1 (1)			
	Caspofungin		1	1	53	38	21	6	3 (2)	1 (1)		
C. guilliermondii (64)	Anidulafungin					2	2	2	30	23	5	
	Caspofungin			1	2	7	15	28	8			3
C. lusitaniae (57)	Anidulafungin					7	21	28	1			
	Caspofungin			1	1	24	28	2		1		
C. kefyr (29)	Anidulafungin			2	14	12				1 (1)		
	Caspofungin	4	23	1				1 (1)				

anidulafungin and caspofungin and additionally indicates the usefulness of anidulafungin as a surrogate marker for evaluating caspofungin susceptibility and resistance among WT and non-WT *Candida* spp.

MATERIALS AND METHODS

Organisms. We tested a total of 4,290 isolates of *Candida* spp. obtained from >100 medical centers worldwide (9, 13, 18). The collection included 2,307 isolates of *C. albicans*, 655 isolates of *C. glabrata*, 539 isolates of *C. parapsilosis*, 515 isolates of *C. tropicalis*, 124 isolates of *C. krusei*, 64 isolates of *C. guilliermondii*, 57 isolates of *C. lusitaniae*, and 29 isolates of *C. kefyr*. All were incident isolates from individual patients and were obtained from blood or other normally sterile body fluids. Among the included isolates of *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei*, and *C. kefyr* were 71 isolates (8 *C. albicans*, 55 *C. glabrata*, 4 *C. tropicalis*, 3 *C. krusei*, and 1 *C. kefyr*) with documented *fks* resistance mutations. The isolates were identified by the use of Vitek and API yeast identification systems (bioMérieux, Inc., Hazelwood, MO) supplemented with conventional methods as needed (19). The isolates were stored as water suspensions until use. Prior to testing, each isolate was passaged at least twice on potato dextrose agar (Remel, Lenexa, KS) and CHROMagar *Candida* (Becton, Dickinson, Sparks, MD) to ensure purity and viability. The presence or absence of a mutation in the hot-spot (HS) regions of *fks1* and *fks2* (*C. glabrata* only) were determined as described previously (20, 21).

Antifungal susceptibility testing. All isolates were tested for *in vitro* susceptibility to anidulafungin and caspofungin using CLSI broth microdilution methods (12, 22). The MIC results for each agent were read following 24 h of incubation. In all instances, the MIC values were determined visually as the lowest concentration of the drug that caused significant growth diminution (12, 22).

We used the recently revised CBPs to identify the strains of the six most common species of *Candida* (*C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. krusei*, and *C. guilliermondii*) that were susceptible (S), intermediate (I), or resistant (R) to anidulafungin and caspofungin (12, 23): anidulafungin and caspofungin MIC values of ≤0.25 μg/ml, 0.5 μg/ml, and ≥1 μg/ml were considered to indicate S, I, and R, respectively, for *C. albicans*, *C. tropicalis*, and *C. krusei*; MIC results of ≤0.12 μg/ml, 0.25 μg/ml, and ≥0.5 μg/ml were categorized as S, I, and R, respectively, for *C. glabrata*; and MIC results of ≤2 μg/ml, 4 μg/ml, and ≥8 μg/ml were considered to indicate S, I, and R, respectively, for *C. parapsilosis* and *C. guilliermondii*. In addition to the CBPs for these species, ECVs were established to provide a sensitive means of separating WT from non-WT strains (those that possess an intrinsic or acquired resistance mutation). The ECVs for anidulafungin and caspofungin for each species are 0.12 μg/ml for *C. albicans* and *C. tropicalis*, 0.25 μg/ml and 0.12 μg/ml for *C. glabrata*, 4 μg/ml and 1 μg/ml for *C. parapsilosis*, 0.12 μg/ml and 0.25 μg/ml for *C. krusei*, and 4 μg/ml and 2 μg/ml for *C. guilliermondii*, respectively (23). CBPs have yet to be established for anidulafungin and caspofungin and less common species such as *C. lusitaniae* and *C. kefyr*. The anidulafungin and caspofungin ECVs for these two species are 2 μg/ml and 1 μg/ml for *C. lusitaniae* and 0.25 μg/ml and 0.03 μg/ml for *C. kefyr*, respectively (23). *Candida* isolates for which anidulafungin or micafungin MIC results exceed the ECVs are considered to be non-WT and may harbor acquired mutations in the *fks* genes (24).

Quality control was performed as recommended in CLSI documents M27-A3 (22) and M27-S4 (12) using *C. krusei* strain ATCC 6258 and *C. parapsilosis* strain ATCC 22019.

Analysis of results. All MIC results for anidulafungin were directly compared with those for caspofungin by regression statistics and by scattergram (data not shown). The error rate bounding method to minimize intermethod interpretive error was also applied using the interpretive criteria described above. The acceptable error rate limits were those cited in CLSI document M23-A3 (25) and in other studies (26, 27).

The definitions of the errors used in this analysis are as follows: a very major error (VME), or a false-susceptible error, was a susceptible result for the surrogate marker (anidulafungin) and a resistant result for caspofungin; a major error (ME), or a false-resistant error, was a resistant result for anidulafungin and a susceptible result for caspofungin; and minor errors occurred when the result for one of the agents was susceptible or

TABLE 2 Use of anidulafungin to predict susceptibility patterns of caspofungin, using 4,290 clinical isolates of *Candida* spp. from a global surveillance program[a]

Species (no. tested)	Anidulafungin category	No. (%) in caspofungin category		
		S	I	R
C. albicans (2,307)	S	2,282 (98.9)	10 (0.4)	3 (0.1)
	I	9 (0.1)	1 (0.1)	
	R	7 (0.3)		3 (0.1)
C. glabrata (655)	S	576 (87.9)	4 (0.6)	3 (0.5)
	I	23 (3.5)	2 (0.3)	5 (0.8)
	R	2 (0.3)	4 (0.6)	36 (5.5)
C. parapsilosis (539)	S	496 (92.0)	1 (0.2)	
	I	42 (7.8)		
	R			
C. tropicalis (515)	S	508 (98.6)		
	I	1 (0.2)		1 (0.2)
	R	1(0.2)		4 (0.8)
C. krusei (124)	S	114 (91.9)	6 (4.8)	
	I			3 (2.5)
	R			1 (0.8)
C. guilliermondii (64)	S	58 (90.6)		1 (1.6)
	I	3 (4.7)		2 (3.1)
	R			
C. lusitaniae (57)	WT	56 (98.2)		1 (1.8)
	Non-WT			
C. kefyr (29)	WT	27 (93.1)		
	Non-WT			2 (6.9)

[a] MIC interpretive criteria for each species as shown in reference 23. Abbreviations: S, susceptible; I, intermediate; R, resistant; WT, wild type; non-WT, non-wild type.

TABLE 3 Absolute CA and error rate when the anidulafungin result was used to predict the caspofungin susceptibility of *Candida* spp.[a]

Species (no. tested)	CA (%)	VME (%)	% errors	
			Major	Minor
C. albicans (2,307)	99.1	0.1	0.3	0.5
C. glabrata (655)	93.7	0.5	0.3	5.5
C. parapsilosis (539)	92.0	0.0	0.0	8.0
C. tropicalis (515)	99.4	0.0	0.2	0.4
C. krusei (124)	92.7	0.0	0.0	7.3
C. guilliermondii (64)	90.6	1.6	0.0	7.8
C. lusitaniae (57)	98.2	1.8	0.0	0.0
C. kefyr (29)	100.0	0.0	0.0	0.0
All *Candida* (4,290)	97.1	0.2	0.2	2.5

[a] CA, categorical agreement; VME, very major error.

resistant and that for the other agent was intermediate. In general, for an agent to be considered a reliable surrogate marker, the VME rate should be ≤1.5% of all results, and the absolute categorical agreement (CA) between methods should be ≥90% (25, 28, 29). In addition to the above analysis, we also discuss *fks* mutant detection among *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei*, and *C. kefyr* using the CBPs and ECVs for each echinocandin.

RESULTS AND DISCUSSION

Table 1 shows the MIC distribution profiles for anidulafungin and caspofungin determined for *Candida* spp. (4,290 strains) using broth microdilution methods (22). Overall, 4,146 isolates (96.6%) were S, 84 isolates (2.0%) were I, and 60 isolates (1.4%) were categorized as R to anidulafungin. Similarly, 4,197 isolates (97.8%) were S, 28 isolates (0.7%) were I, and 65 isolates (1.5%) were R to caspofungin. The modal MIC values were 0.03 μg/ml for both anidulafungin (1,266 results [29.5%]) and caspofungin (1,632 results [38.0%]). There was a strong positive correlation ($r = 0.85$) between the anidulafungin and caspofungin MIC values (data not shown). Overall, the essential agreement (EA; MIC ± 2 $\log_2$ dilutions) was 92.7%. Decreased potencies for both anidulafungin and caspofungin were observed among *C. parapsilosis* (modal MIC values, 2 μg/ml and 0.5 μg/ml, respectively) and *C. guilliermondii* (modal MIC values, 1 μg/ml and 0.5 μg/ml, respectively). The highest rates of R to both agents were observed with *C. glabrata*: 6.4% were anidulafungin R and 6.8% were caspofungin R. Among the 42 isolates of *C. glabrata* that were R to anidulafungin, 38 isolates (90.5%) possessed a mutation in *fks1* or *fks2*, and among 44 isolates that were caspofungin R, 39 isolates (88.6%) possessed a mutation in the *fks* gene (Table 1).

The extent of cross-resistance between anidulafungin and caspofungin can be seen more clearly in Table 2. Of the 4,146 isolates that were S to anidulafungin, 4,117 (99.3%) were also caspofungin S. There were eight isolates that were S to anidulafungin and R to caspofungin; of those, three each were *C. albicans* and *C. glabrata*, one was *C. guilliermondii*, and one was *C. lusitaniae*; one each of the *C. albicans* and *C. glabrata* isolates contained an *fks* mutation. Among the 60 isolates that were anidulafungin R, 46 isolates (76.7%) were also R, four (6.7%) were I, and 10 (16.6%) were S to caspofungin. Among the 84 isolates categorized as I to anidulafungin, 14 (16.7%) were either I or R to caspofungin. There were 23 isolates of *C. glabrata* (27.4%) and 42 isolates of *C. parapsilosis* (50.0%) that were anidulafungin I but caspofungin S. Thus, 99.3% of the anidulafungin-S isolates and 44.4% of the anidulafungin-nonsusceptible (NS; I plus R) isolates were S and NS, respectively, to caspofungin.

When the anidulafungin test result category (S, I, or R) was used to predict the caspofungin category, the absolute CA between the test results was 97.1%, with only 0.2% VME (falsely susceptible) and ME (falsely resistant) and a 2.5% minor error rate, e.g., acceptable (Table 3). Among the eight species of *Candida* tested, the CA was ≥90% (range, 90.6 to 100.0%) for all species. Generally, the discords in the categorical results were minor errors. VMEs were seen more often with *C. glabrata*, *C. guilliermondii*, and *C. lusitaniae*.

Clearly, it is important to detect those isolates of *Candida* that harbor an acquired mutation in the *fks* gene (13, 24), and in that regard, anidulafungin performs very well as a surrogate marker. Among all 71 *fks* mutant strains, 9 (12.7%) were S to both anidulafungin and caspofungin and 53 (74.6%) were I or R to both, for an overall concordance of 87.3%. Furthermore, for the 8 isolates of *C. albicans* with a mutation in *fks1*, 4 (50.0%) were either intermediate or resistant to both agents, 2 isolates of *C. albicans* were susceptible to anidulafungin and either intermediate (1 isolate) or resistant to caspofungin (1 isolate), and 2 isolates were susceptible to both agents (Table 4). There were a total of 55 isolates of *C. glabrata* that contained a mutation in *fks1* or *fks2* (Tables 1 and 4). Of these, 8 isolates (14.5%) were S to both anidu-

TABLE 4 Isolates of *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei*, and *C. kefyr* harboring *fks* mutations

Species	Amino acid change(s) corresponding to *fks* mutation	MIC (μg/ml)	
		Anidulafungin	Caspofungin
C. albicans	S629P	1	2
	F641Y	0.25	1
	F641S	0.12	0.5
	S645P	1	2
	S645F	0.5	0.5
	S645Y	1	1
	D648Y	0.06	0.25
	P649H	0.12	0.25
C. glabrata	F625Y	0.25	0.12
	F625S	1	0.5
	F625S	2	2
	F625S	2	2
	S629P	2	16
	S629P	2	16
	S629P	2	16
	S629P, R631S	4	2
	L630I	0.03	0.03
	R631G	0.12	0.12
	D632Y	0.25	0.12
	D632E	1	0.5
	D632E	1	1
	I634V	0.06	0.06
	I634V	0.06	0.06
	I634V	0.06	0.06
	I634V	0.06	0.06
	F659S	0.5	0.5
	F659S	0.25	0.25
	F659S	1	0.5
	F659V	1	8
	F659V	1	2
	F659V	1	4
	F659V	1	2
	F659Y	1	1
	F659Y	1	2
	L662W	2	1
	L662W	1	0.5
	S663F	0.5	0.25
	S663F	0.5	0.25
	S663P	1	1
	S663P	4	16
	S663P	2	2
	S663P	4	4
	S663P	1	2
	S663P	4	16
	S663P	2	8
	S663P	0.25	0.5
	S663P	4	16
	S663P	1	8
	S663P	0.25	0.5
	S663P	4	16
	S663P	1	8
	S663P	1	1
	S663P	4	16
	S663Y	1	0.5
	L664R	1	0.5
	R665G	0.25	0.5
	R665S	0.12	0.5
	D666Y	0.12	0.25
	P667T	1	1
	ΔF658	1	16
	I1379V	0.06	0.06
	I1379V	0.06	0.06
	P1371S	0.12	0.25
C. tropicalis	F641S	1	1
	F641S	0.12	0.12
	S645P	1	2
	S645P	0.5	2
C. krusei	F655C	0.5	1
	R1361G	1	16
	L701M	0.03	0.25
C. kefyr	S663P	2	0.5

lafungin and caspofungin, 3 isolates (5.5%) were S to anidulafungin and either I or R to caspofungin, 2 isolates (3.6%) were I to anidulafungin and S to caspofungin, and 42 (76.4%) were I or R to both agents (Table 4). The overall concordance between the two methods (testing of anidulafungin versus caspofungin) using CLSI CBPs to classify *fks* mutant strains of *C. glabrata* as S or NS was 90.9%. Using the ECV of 0.12 μg/ml for both anidulafungin and caspofungin to classify these *fks* mutant strains of *C. glabrata* as WT or non-WT, 8 strains (14.5%) were WT and 42 strains (76.4%) were non-WT for both agents (overall concordance of 90.9%).

There were four strains of *C. tropicalis* and three of *C. krusei* that contained an *fks* mutation. Of these, three strains of *C. tropicalis* and two strains of *C. krusei* were R to both agents, whereas one strain of *C. tropicalis* was S to both agents and one strain of *C. krusei* was susceptible to both anidulafungin and caspofungin. Notably, the L701M mutation in the last strain is not localized to the HS region, and its relation to the resistance mechanism is uncertain. The single isolate of *C. kefyr* with an *fks* mutation was classified as non-WT to both agents using the ECVs of 0.25 μg/ml and 0.03 μg/ml for anidulafungin and caspofungin, respectively.

The most frequently encountered *fks* mutations in this collection corresponded to position S663 (19 isolates), followed by F659 (9 isolates), S645 (5 isolates), and S629 (5 isolates), and 4 isolates each contained mutations corresponding to positions F625, F641, and I634 (Table 4). Previous reports indicate that isolates of *C. glabrata* with the S663F mutation respond *in vivo* to high doses of either micafungin or caspofungin but not anidulafungin, whereas isolates with S629P mutation fail to respond to even the highest dose of any of the three echinocandins (30). These findings are supported by those of Spreghini et al. (31), who found in a comparison between anidulafungin, caspofungin, and micafungin that the *in vivo* response to both anidulafungin and caspofungin required much higher doses than that of micafungin against two R mutants of *C. glabrata* bearing specific mutations in the *fks2* HS region. Mutations at positions S663 and F659 in *C. glabrata* have been associated with breakthrough infections in patients receiving echinocandin therapy (32–34), whereas patients infected with *C. glabrata* strains containing the I1379V and I634V mutations (i.e., S to both anidulafungin and caspofungin) tended to respond to

echinocandin therapy (32). Regarding the *C. albicans* mutants in this study, three of the eight *fks* mutants contained the S645P mutation, which cannot be treated with conventional doses of any echinocandin (35). Taken together, these results suggest a linkage between the increased echinocandin MIC results, specific *fks* mutations, and the potential for a successful clinical outcome (30–32).

Previously, we conducted a similar analysis using micafungin to predict the susceptibilities of *Candida* spp. to caspofungin as a proof of concept regarding the use of surrogate markers or class representatives for antifungal susceptibility testing of the echinocandins (14). Micafungin functioned similarly to anidulafungin, with an overall CA of 98.8% (0.2% VME, 0.2% ME, and 0.8% minor errors). Thus, MIC testing with either anidulafungin or micafungin is highly predictive of caspofungin categorical results, and both reagents reliably detect clinically important *fks* mutations.

In addition to providing a strategy for predicting caspofungin S and R among *Candida* spp., these results provide further evidence for cross-resistance among the echinocandins (6, 7, 32, 36–38). By using a large global collection of clinically important *Candida* spp., including *fks* mutant strains, we validated concerns originating from single-center case series and provided further evidence for considering *C. glabrata* as the species most likely to demonstrate cross-resistance among the echinocandins. Furthermore, these results support the position of the EUCAST in designating anidulafungin as a reliable reagent for antifungal susceptibility testing of echinocandins against *Candida* spp.

Anidulafungin functioned well as a surrogate marker for caspofungin S and R when applied to this extensive collection of clinically significant isolates of *Candida* spp. The CA of 97.1%, with only 0.2% VME among 4,290 isolates tested, easily meets the recognized acceptable criteria for a reliable surrogate marker in antimicrobial susceptibility testing (27, 29). The excellent concordance between the anidulafungin and caspofungin results in categorizing *fks* mutants also provides further validation of this approach. As noted for the previous comparison of micafungin and caspofungin results (14), the major limitation of this study, given the interlaboratory variability of caspofungin MICs, is the fact that the data were obtained from only two laboratories. This raises concerns regarding the generalizability of the findings to other laboratories. These concerns may be lessened by the inclusion of a large number of *fks* mutants in the study. Furthermore, it should be noted that the MIC distributions for caspofungin and each species of *Candida* showed modal MICs that approximated the overall modes (not the lowest or the highest) reported by Espinel-Ingroff et al. (16).

In conclusion, we have demonstrated the existence of cross-R and -S between anidulafungin and caspofungin, with the greatest emphasis on *C. glabrata*. In the face of unreliable caspofungin MIC results as documented elsewhere (16), either anidulafungin or micafungin results may be used to predict the S of *Candida* spp. to caspofungin. Arguably, the most important role of *in vitro* susceptibility testing is to predict the resistance of the infecting organism to the agent under consideration for use in a patient (39). The occurrence of false-R and false-S errors with this application of the class representative concept to the echinocandin antifungal agents was very low and may be considered to be acceptable for use of anidulafungin as a surrogate class marker. The excellent CA documented in this study was further supported by the high level of concordance in identifying strains of *Candida* with clinically important *fks* resistance mutations. Further efforts to clarify and correct the issues of caspofungin testing using the CLSI and EUCAST broth microdilution methods remain a priority for future research.

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