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Effects of Serum on In Vitro Susceptibility Testing of Echinocandins▽

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The effects of protein binding on the activities of caspofungin, anidulafungin, and micafungin were evaluated against *Candida* and *Aspergillus* species. Adding human serum sharply increased the MICs of micafungin and anidulafungin and modestly affected the MIC of caspofungin. The increase in MICs does not appear consistent with the rate of protein binding for the three compounds.

The echinocandins are a new class of lipopeptide antifungal agents that act by inhibiting the synthesis of (1,3)-β-D-glucan. Compounds of this class are relatively highly protein bound, with rates of 96% reported for caspofungin (5), 99.8% for micafungin (15), and ~99% for anidulafungin (Eraxis package insert; Pfizer). Protein binding may change the in vitro and in vivo activities of antimicrobial agents (17). The available data on the effects of protein binding on the antifungal activities of echinocandins are limited to reports of increased MICs for anidulafungin when tested against *Candida albicans* (Eraxis package insert; Pfizer), of increased MICs for micafungin when tested against *Candida* spp. and *Aspergillus fumigatus* (4, 16), of no effect on caspofungin MICs for one isolate of *C. albicans* (2), and of a potentiation of the effect of caspofungin versus *A. fumigatus* (3). As none of these reports have provided comparative data and the total number of isolates evaluated has been small, we now report the effect of 50% human serum on the activities of the echinocandins against a collection of *Candida* and *Aspergillus* isolates.

A total of 16 *Candida* isolates and 8 *Aspergillus* isolates were tested. The isolates included were *C. albicans* (two isolates), *C. parapsilosis* (five isolates), *C. krusei* (three isolates), *C. glabrata* (two isolates), *C. tropicalis* (two isolates), *C. lusitaniae* (two isolates), *A. fumigatus* (four isolates), *A. flavus* (two isolates), *A. terreus* (one isolate), and *A. niger* (one isolate). The two quality control isolates specified in the Clinical and Laboratory Standards Institute (CLSI) M27-A2 procedure (10), ATCC 6258 (*C. krusei*) and ATCC 22019 (*C. parapsilosis*), were included in each set of the test and the results compared with published control limits (1).

Caspofungin, micafungin, and anidulafungin were supplied by their respective manufacturers. Stock solutions were prepared by dissolving the compounds in dimethyl sulfoxide (anidulafungin) or water (caspofungin and micafungin). Following the principles of CLSI M27-A2, serial dilutions at twice the desired final concentration were prepared in double-strength test medium (RPMI 1640 medium buffered with 0.165 M morpholinepropanesulfonic acid [MOPS] to pH 7.0). Test trays were prepared in advance by dispensing 100 μl of serially diluted drug into 96-well microdilution plates and freezing the plates at −70°C. All three compounds were tested over a 20-fold dilution range from 64 to 0.00012 μg/ml.

The MICs of test drugs with *Candida* species and *Aspergillus* species were determined using the broth microdilution variants of CLSI M27-A2 and CLSI M38-A (9), respectively. Testing was performed both in standard medium (RPMI 1640, 0.165 M MOPS, pH 7.0) and in standard medium containing 50% pooled human serum (Sigma, St. Louis, MO). To achieve this, inocula were standardized spectrophotometrically and diluted either with sterile water or with 100% pooled human serum to final concentrations of 1×10^3 to 5×10^3 yeast or 0.4×10^4 to 5×10^4 conidia. Then, 100-μl volumes of these double-strength inocula were added to the 100-μl volumes of serially diluted drug in the microdilution trays. MICs of *Candida* and *Aspergillus* species were read after 24 and 48 h at 35°C as MIC-0, i.e., complete growth inhibition, and MIC-2, i.e., at least 50% growth inhibition. For *Aspergillus*, a minimum effective concentration was also determined microscopically (7) and found to be equivalent to the macroscopically determined MIC-2 reading. The MIC-2 readings are thus used for both fungal genera.

A line listing of the results seen after 24 h is shown in Table 1. The qualitative effects of serum on echinocandin susceptibility were similar for *Candida* species at 24 and 48 h and using endpoints of MIC-0 and MIC-2 (not shown); thus, the 24-h MIC-2 results are shown as being characteristic of the entire data set. For *Aspergillus* species, all MIC-0 readings were off-scale, and thus only the MIC-2 readings provide useful information; qualitative effects of serum on echinocandin susceptibility were similar at 24 and 48 h, as seen for *Candida* spp. A summary of the ratios of the MIC-2 values by time, genus, and drug is shown in Table 2. The addition of 50% serum consistently elevated the observed MICs for anidulafungin and micafungin. For caspofungin, on the other hand, an increased MIC was observed for some (but not all) isolates, and this effect was never as frequent as or of the magnitude of that seen for the other two agents. An analysis of the effect of serum for *Candida* and *Aspergillus* species (Table 3) showed that the effect of serum was similar for each drug across the range of tested species.

In this study, we have extended prior work by testing a broad range of *Candida* and *Aspergillus* spp. and by directly comparing the effects of serum on all three echinocandins (2, 3, 4, 16;

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TABLE 1. Comparison of MIC-2 values observed for RPMI and 50% serum

Species	Isolate code	Anidulafungin			Caspofungin			Micafungin		
		RPMI MIC-2 (μg/ml)	50% serum MIC-2 (μg/ml)	Ratio[a]	RPMI MIC-2 (μg/ml)	50% serum MIC-2 (μg/ml)	Ratio	RPMI MIC-2 (μg/ml)	50% serum MIC-2 (μg/ml)	Ratio
C. albicans	0012-025	0.0156	0.25	16	0.25	0.25	1	0.0156	1	64
	0021-076	0.5	128	256	0.5	4	8	0.5	64	128
C. glabrata	0022-012	0.0313	1	32	0.5	0.5	1	0.0156	1	64
	0049-038	0.0625	2	32	0.5	0.5	1	0.004	1	256
C. krusei	0001-058	0.0313	1	32	0.5	0.5	1	0.0625	2	32
	0013-054	0.125	4	32	0.5	2	4	0.125	8	64
	ATCC 6258	0.125	2	16	0.5	4	8	0.0625	8	128
C. lusitaniae	0013-002	0.002	1	512	0.25	0.25	1	0.008	1	128
	0015-049	0.125	1	8	0.5	1	2	0.0156	4	256
C. parapsilosis	0014-074	0.004	0.25	64	0.25	0.5	2	0.0156	0.5	32
	0011-013	0.0313	2	64	0.25	1	4	0.0156	4	256
	0026-003	2	128	64	1	8	8	0.5	64	128
	0036-030	1	128	128	1	8	8	0.5	128	256
	ATCC 22019	0.5	64	128	0.5	8	16	0.5	32	64
C. tropicalis	0022-021	0.0156	2	128	0.5	0.5	1	0.0156	2	128
	0050-028	1	128	128	0.5	2	4	0.25	32	128
A. terreus[b]	0031-087	0.0156	0.25	16	0.5	0.5	1	0.002	0.25	128
A. niger	0001-073	0.001	0.5	512	0.0004	0.25	512	0.002	0.25	128
A. fumigatus	0002-0016	2	128	64	8	128	16	0.0313	2	64
	0020-018	0.5	128	256	16	128	8	0.0156	0.5	32
	0002-020	0.008	0.5	64	0.5	128	256	0.004	0.5	128
	0002-021	0.002	128	64,000	1	128	128	0.001	1	1,024
A. flavus	0001-033	4	128	32	32	128	4	0.125	128	1,024
	0001-061	4	128	32	128	128	1	0.125	128	1,024

[a] Shown is the ratio of the MIC-2 values with/without serum.
[b] Growth rate was insufficient for *A. terreus* at 24 h; MIC-2 results were detected at 48 h.

Eraxis package insert [Pfizer]). The effect of serum was greatest for anidulafungin and micafungin, and this is entirely consistent with the results of prior studies on these two drugs (4, 16; Eraxis package insert [Pfizer]). Prior work with caspofungin against single isolates of *C. albicans* (2) and *A. fumigatus* (3) found that serum had no effect against the *Candida* isolate and enhanced activity against the *Aspergillus* isolate. These results are consistent with the absence of effect of serum seen for some isolates in our survey, and we did not see any enhancing activity with the addition of serum.

In summary, we have observed that serum strongly affects the observed MICs of anidulafungin and micafungin and modestly affects the MIC of caspofungin. This rank order of effects does not appear consistent with the rank order of protein binding for the three compounds and is thus a further demonstration of the relatively unpredictable nature of the effect of serum on antifungal effect. As related examples, terbinafine (>99% protein bound [13]) has a reduced in vitro potency in the presence of serum that has been correlated with reduced in

TABLE 2. Ratios of MIC-2 values with and without serum

Genus	Time (h)	Drug	Ratio of MIC-2 in standard medium with/without 50% serum[a]										
			0.5	1	2	4	8	16	32	64	128	256	512+
Candida	24	Anidulafungin					1	2	4	3	4	1	1
		Caspofungin		6	2	3	4	1					
		Micafungin							2	4	6	4	
	48	Anidulafungin		1				5	4	4	1	1	
		Caspofungin	3	2	3	6	2						
		Micafungin						2	1	9	2	2	
Aspergillus	24	Anidulafungin						1	2	2		1	2
		Caspofungin		2		1	1	1			1	1	1
		Micafungin							1	1	3		3
	48	Anidulafungin						2	4				2
		Caspofungin	NA										
		Micafungin	NA										

[a] NA, not applicable; for those drugs, most of the MIC-2 results with and without serum were out of range, and so ratio determinations were not meaningful.

TABLE 3. Relationship of MIC-2 values with/without serum to *Candida* and *Aspergillus* species

Drug	Species	Ratio of MIC-2 in standard medium with/without 50% serum										
		0.5	1	2	4	8	16	32	64	128	256	512
Anidulafungin	*C. albicans*						1				1	
	C. glabrata							2				
	C. krusei						1	2				
	C. lusitaniae					1						1
	C. parapsilosis								3	2		
	C. tropicalis									2		
	A. fumigatus								2		1	1
	A. flavus, others						1	2				1
Caspofungin	*C. albicans*		1			1						
	C. glabrata		2									
	C. krusei		1		1	1						
	C. lusitaniae		1	1								
	C. parapsilosis			1	1	2	1					
	C. tropicalis		1		1							
	A. fumigatus					1	1			1	1	
	A. flavus, others		2		1							1
Micafungin	*C. albicans*								1	1		
	C. glabrata								1		1	
	C. krusei							1	1	1		
	C. lusitaniae									1	1	
	C. parapsilosis							1	1	1	2	
	C. tropicalis									2		
	A. fumigatus							1	1	1		1
	A. flavus, others									2		2

vivo potency (8, 11, 14). On the other hand, although the potency of itraconazole (99.8% protein bound [6]) does appear affected in vitro or in vivo by the presence of serum, protein binding does not impede the drug's activity (12, 13, 18). For the echinocandin antifungal agents, the impact of these in vitro effects on in vivo activity is not yet understood and is likely not of clinical significance. However, we think that the different serum effects with these three echinocandins may explain the different dosage regimens of these drugs in clinical practice.

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