M-1373

Use of Micafungin as a Surrogate Marker to Predict Susceptibility and Resistance to Caspofungin Among 3,749 Clinical Isolates of *Candida* using CLSI Methods and Interpretive Criteria

MA PFALLER, SA MESSER, DJ DIEKEMA, RN JONES, M CASTANHEIRA

JMI Laboratories, North Liberty, Iowa, USA; University of Iowa, Iowa City, Iowa, USA

ICAAC 2013

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

ABSTRACT

Background: Echinocandins are now well established as first-line agents for the treatment of candidemia. Whereas all three echinocandins have been shown to have comparable *in vitro* activity against *Candida* spp., concerns have been raised regarding the use of caspofungin (CSF) MIC testing for clinical decision making due to unacceptably high variation among MIC values from different centers.

Methods: We investigated the potential for use of micafungin (MCF) as a surrogate marker to predict the susceptibility of *Candida* to CSF using CLSI methods and species-specific interpretive criteria (IC). We analyzed reference broth microdilution MIC results for 3,749 strains of *Candida* (eight species), including 58 strains with *fks* mutations. CSF MICs and species-specific IC were compared with those of MCF to determine the % categorical agreement (CA) and very major (VME), major (ME) and minor (MI) error rates as well as the ability to detect *fks* mutant strains of *C. albicans* (3 mutants) and *C. glabrata* (55 mutants).

Results: For all 3,749 isolates, the % CA was 98.9% (0.2% VME and ME, 0.7% minor errors) using MCF as the surrogate marker. Among the 45 isolates of *C. albicans* (three isolates) and *C. glabrata* (42 isolates) that were non-susceptible (NS; either intermediate or resistant) to both CSF and MCF, 41 (91%) contained a mutation in *fks1*/*fks2*. An additional 10 mutants of *C. glabrata* were classified as S by both antifungal reagents (91% concordance overall). Using the epidemiological cutoff values (ECVs) of 0.12 µg/ml for CSF and 0.03 µg/ml for MCF to differentiate wild-type (WT) from non-WT strains of *C. glabrata*, 81% of the *C. glabrata* mutants were non-WT for both reagents (96% concordance).

Conclusion: MCF may serve as a valid surrogate marker to predict susceptibility and resistance of *Candida* to CSF, minimizing technical variations observed between laboratories using CSF-based reagents.

INTRODUCTION

The echinocandins, caspofungin and micafungin, are now well established as first-line agents for the treatment of candidemia and other forms of invasive candidiasis (IC). The *in vitro* activity of both caspofungin and micafungin against *Candida* spp. has been documented using reference methods and clinically relevant interpretive breakpoints for broth microdilution (BMD) MIC testing of *Candida* spp. have been established by the Clinical and Laboratory Standards Institute (CLSI). Whereas CLSI has developed clinical breakpoints (CBPs) for the six most common species of *Candida* tested against anidulafungin, caspofungin and micafungin, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) has elected to establish CBPs for anidulafungin and micafungin, but not for caspofungin. Furthermore, EUCAST does not currently recommend caspofungin MIC testing for clinical decision making due to unacceptably high variation among caspofungin MIC values produced in different centers. This high level of variation in caspofungin MIC results is evident not only with the EUCAST BMD method, but also with that of CLSI methods. The reasons for such center-to-center variation in caspofungin MIC values is unclear, but may involve the lot-to-lot variation in the potency of caspofungin powder, the use of DMSO versus water as a solvent, reagent storage conditions, or MIC endpoint determination.

In the present study, we utilized a large database of susceptibility test results, all determined by CLSI BMD methods and including results for 58 *fks* mutant strains, to provide a robust analysis of cross-resistance between two echinocandins agents and additionally to examine the usefulness of micafungin as a surrogate marker for evaluating caspofungin susceptibility and resistance among *Candida* spp.

MATERIALS AND METHODS

Organisms. A total of 3,749 clinical isolates of *Candida* spp. obtained from more than 100 medical centers worldwide were tested. The collection included the following species and number of isolates: 2,002 isolates of *C. albicans*, 566 isolates of *C. glabrata*, 539 isolates of *C. parapsilosis*, 422 isolates of *C. tropicalis*, 102 isolates of *C. krusei*, 52 isolates of *C. guilliermondii*, 42 isolates of *C. lusitaniae*, and 24 isolates of *C. kefyr*. All were incident isolates from individual patients and were obtained from blood or other normally sterile body fluids. Among the isolates of *C. albicans* and *C. glabrata* included in the study were a total of 58 isolates (3 *C. albicans* and 55 *C. glabrata*) with documented *fks* resistance mutations. 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.

Antifungal susceptibility testing. All isolates were tested for *in vitro* susceptibility to caspofungin and micafungin using CLSI BMD methods (M27-A3 guidelines). The MIC results for both agents were read following 24-h of incubation. In all instances, the MIC values were determined visually as the lowest concentration of drug that caused significant growth diminution levels. We used the recently revised CBPs to identify strains of the six most common species of *Candida* (*C. albicans, C. glabrata, C. parapsilosis, C. tropicalis, C. krusei*, and *C. guilliermondii*; CLSI M27-S4 [2012]) that were susceptible (S), intermediate (I) or resistant (R) to caspofungin and micafungin and epidemiological cutoff values (ECVs) that have been established in order to provide a sensitive means of separating wild-type (WT) strains from non-WT (possess an intrinsic or acquired resistance mutation) strains.

Quality control was performed as recommended in CLSI documents M27-A3 and M27-S4 using the strains *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019.

Analysis of results. All MIC results (in µg/ml) for micafungin 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. Acceptable error limits used in this comparison were those cited by CLSI (M23-A3 and M100-S23) and by other authors.

The definitions of errors used in this analysis were as follows: a very major error (VME), or a false-susceptible error, was a S result for the surrogate marker micafungin and a R result for caspofungin; a major error (ME), or a false-resistant error, was a R result for micafungin and a S result for caspofungin; and minor errors occurred when the result for one of the agents was S or R and that for the other agent was I. In general, for an agent to be considered a reliable surrogate, the VME rate should be ≤1.5% of all results, and the absolute categorical agreement (CA) between methods should be ≥90%. In addition to the above analysis, we will also examine the ability of the CBPs and ECVs for each echinocandin to detect *fks* mutants of *C. albicans* and *C. glabrata*.

RESULTS

- The modal MIC for micafungin was 0.015 µg/ml (1,323 results, 35.3% of total), compared to 0.03 µg/ml (1,362 results, 36.3% of total) for caspofungin (**Table 1**). Overall, the essential agreement (MIC value ±2 dilutions) was 96.8%.
- Decreased potencies of both micafungin and caspofungin were observed among *C. parapsilosis* (modal MICs of 1 µg/ml and 0.5 µg/ml, respectively) and *C. guilliermondii* (modal MICs of 0.25 µg/ml and 0.5 µg/ml, respectively; **Table 1**).
- Among the 3,690 isolates that were S to micafungin, 3,668 (99.4%) were also S to caspofungin. There were only six isolates that were S to micafungin and R to caspofungin (**Table 2**), four were *C. glabrata* and two were *C. guilliermondii*: two of the four isolates of *C. glabrata* contained an *fks* mutation.
- Among 43 isolates that were R to micafungin, 36 (83.7%) were R and only seven (16.3%) were S to caspofungin. Similarly, 13 of 16 (81.3%) isolates categorized as I to micafungin were either I or R to caspofungin. Thus 99.4% of the micafungin-susceptible and 83.1% of the micafungin-non-susceptible (NS; I plus R) isolates were S and NS, respectively, to caspofungin (**Table 2**).
- Absolute CA between the test results was 98.9% with a very acceptable 0.2% VME (false-susceptible error) and ME (false-resistant error), and a 0.7% minor error rate (**Table 3**).
- Among the eight species of *Candida* tested, the CA was 93% or better (range, 93.1 to 100.0%) for all species. VME were seen with *C. glabrata*, *C. krusei*, and *C. guilliermondii*; however, only the VME rate involving *C. guilliermondii* (3.8%) exceeded the allowable VME rate of ≤1.5% (**Table 3**).
- The highest rates of resistance to both agents was observed with *C. glabrata*: 5.8% R to micafungin and 7.9% R to caspofungin. Among 33 isolates of *C. glabrata* that were R to micafungin, 30 (90.9%) possessed a mutation in *fks1* or *fks2* and among 45 isolates that were R to caspofungin, 40 (88.9%) possessed a mutation in *fks* (**Tables 1** and **2**).
- There were a total of 55 isolates of *C. glabrata* that contained a mutation in *fks1* or *fks2* (**Tables 1** and **4**). Of these, 10 (18.2%) were S to both micafungin and caspofungin, five (9.1%) were S to micafungin and either I or R to caspofungin, and 40 (72.7%) were I or R to both agents.
- For the three isolates of *C. albicans* with a mutation in *fks1*, all were either I or R to micafungin and R to caspofungin. Using the micafungin ECV for *C. albicans* of 0.03 µg/ml, all three isolates would be classified as non-WT indicating that they were likely to contain an acquired resistance mutation.

Table 1. MIC distributions of caspofungin and micafungin versus *Candida* spp. including strains with *fks* mutations using CLSI methods.

Species (no. tested)	Antifungal agent	No. (no. with *fks* mutation) of isolates at MIC (µg/ml)										
		≤0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	≥8
C. albicans (2002)	Caspofungin	33	539	892	502	22	10	1	2 (2)	1 (1)		
	Micafungin	177	1357	375	79	4	1	1 (1)	8 (2)			
C. glabrata (566)	Caspofungin		34	273 (1)	189 (6)	16 (3)	9 (5)	14 (11)	7 (6)	8 (8)	2 (2)	14 (13)
	Micafungin	29	437 (3)	39 (7)	14 (5)	14 (10)	11 (9)	10 (10)	2 (1)	3 (3)	5 (5)	2 (2)
C. parapsilosis (539)	Caspofungin	1	1	1	17	35	208	219	49	7	1	
	Micafungin		1			2	25	93	285	133		
C. tropicalis (422)	Caspofungin	4	137	191	82	2	4		1			1
	Micafungin	7	126	169	101	14	2	1	2			
C. krusei (102)	Caspofungin		1		44	32	18	6	1			
	Micafungin		3	9	74	15	1					
C. guilliermondii (52)	Caspofungin			1	2	4	13	22	7		1	2
	Micafungin		1	2	3	4	23	18	1			
C. lusitaniae (42)	Caspofungin			1	1	20	18	2				
	Micafungin				2	25	13	2				
C. kefyr (24)	Caspofungin	3	20	1								
	Micafungin			9	15							

Table 2. Use of micafungin to predict susceptibility patterns of caspofungin, using 3,749 clinical isolates of *Candida* spp. from a global surveillance program[a,b].

Species (no. of isolates tested)	Micafungin susceptibility category	No. (%) in caspofungin category[a]		
		S	I	R
C. albicans (2002)	S	1992 (99.5)	1 (0.05)	
	I			1 (0.05)
	R	6 (0.3)		2 (0.1)
C. glabrata (566)	S	509 (90.0)	6 (1.1)	4 (0.7)
	I	3 (0.5)	3 (0.5)	8 (1.4)
	R			33 (5.8)
C. parapsilosis (539)	S	538 (99.8)	1 (0.2)	
	I			
	R			
C. tropicalis (422)	S	419 (99.4)		
	I			1 (0.2)
	R	1 (0.2)		1 (0.2)
C. krusei (102)	S	95 (93.1)	6 (5.9)	1 (1.0)
	I			
	R			
C. guilliermondii (52)	WT	49 (94.2)	1 (2.0)	2 (3.8)
	Non-WT			
C. lusitaniae (42)	WT	42 (100.0)		
	Non-WT			
C. kefyr (24)	WT	24 (100.0)		
	Non-WT			

a. S, susceptible; I, intermediate; R, resistant; WT, wild-type; non-WT, non-wild-type.
b. MIC interpretive criteria for each species as shown in Pfaller and Diekema (2012).

Table 3. Absolute categorical agreement (CA) and error rate when the micafungin result was used to predict the caspofungin susceptibility of *Candida* spp.

Species	No. of isolates tested	%[a]			
		CA	VME	ME	Minor
C. albicans	2,002	99.6	0.0	0.3	0.1
C. glabrata	566	96.3	0.7	0.0	3.0
C. parapsilosis	539	99.8	0.0	0.0	0.2
C. tropicalis	422	99.6	0.0	0.2	0.2
C. krusei	102	93.1	1.0	0.0	5.9
C. guilliermondii	52	94.2	3.8	0.0	2.0
C. lusitaniae	42	100.0	0.0	0.0	0.0
C. kefyr	24	100.0	0.0	0.0	0.0
All *Candida*	3,749	98.9	0.2	0.2	0.7

a. CA, categorical agreement; VME, very major error; ME, major error.

CONCLUSIONS

- The absolute categorical agreement between micafungin and caspofungin was 98.9%, with only 0.2% VME among 3,749 isolates tested. These results easily meet recognized criteria for a reliable surrogate marker as applied to antibacterial susceptibility testing. The excellent concordance between the micafungin and caspofungin results in categorizing the *fks* mutants provides further validation of this interpretive approach.
- In a clinical laboratory environment where unreliable caspofungin MIC results occur, a prediction of caspofungin susceptibility by a medical center currently performing antifungal susceptibility testing of micafungin can be accomplished by using the micafungin result as a surrogate marker.

ACKNOWLEDGEMENT

The antifungal global surveillance programs which served as the source of data were supported by Pfizer Inc and Astellas Pharma Inc.

REFERENCES

1. Arendrup MC, Garcia-Effron G, Lass-Florl C, Lopez AG, Rodriguez-Tudela JL, Cuenca-Estrella M, Perlin DS (2010). Echinocandin susceptibility testing of *Candida* species: comparison of EUCAST EDef 7.1, CLSI M27-A3, Etest, disk diffusion, and agar dilution methods with RPMI and isosensitest media. *Antimicrob Agents Chemother* 54: 426-439.
2. Arendrup MC, Rodriguez-Tudela JL, Park S, Garcia-Effron G, Delmas G, Cuenca-Estrella M, Gomez-Lopez A, Perlin DS (2011). Echinocandin susceptibility testing of *Candida* spp. Using EUCAST EDef 7.1 and CLSI M27-A3 standard procedures: analysis of the influence of bovine serum albumin supplementation, storage time, and drug lots. *Antimicrob Agents Chemother* 55: 1580-1587.
3. Castanheira M, Woosley LN, Pfaller MA, Diekema DJ, Messer SA, Jones RN (2010). Low prevalence of *fks1* hotspot 1 mutations in a worldwide collection of *Candida* spp. *Antimicrob Agents Chemother* 54: 2655-2659.
4. Clinical and Laboratory Standards Institute (2008). *M23-A3. Development of in vitro susceptibility testing criteria and quality control parameters: third edition*. Wayne, PA: CLSI.
5. Clinical and Laboratory Standards Institute (2008). *M27-A3. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts: third edition*. Wayne, PA: CLSI.
6. Clinical and Laboratory Standards Institute (2012). *M27-S4. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts: 4th Informational Supplement*. Wayne, PA: CLSI.
7. Ferraro MJ, Jorgensen JH (1995). *Instrument-based antibacterial susceptibility testing.* In *Manual of Clinical Microbiology, 6th ed.* Washington, DC: ASM Press.
8. Pfaller MA, Boyken L, Hollis RJ, Kroeger J, Messer SA, Tendolkar S, Jones RN, Turnidge J, Diekema DJ (2010). Wild-type MIC distributions and epidemiological cutoff values for the echinocandins and *Candida* spp. *J Clin Microbiol* 48: 52-56.
9. Pfaller MA, Diekema DJ (2012). Progress in antifungal susceptibility testing of *Candida* spp. by use of Clinical and Laboratory Standards Institute broth microdilution methods, 2010 to 2012. *J Clin Microbiol* 50: 2846-2856.