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Activities of Micafungin against 315 Invasive Clinical Isolates of Fluconazole-Resistant *Candida* spp.

S. A. Messer,[1] D. J. Diekema,[1,3] L. Boyken,[1] S. Tendolkar,[1] R. J. Hollis,[1] and M. A. Pfaller[1,2]*

Departments of Pathology,[1] Epidemiology,[2] and Medicine,[3] Roy J. and Lucille A. Carver College of Medicine, and College of Public Health, University of Iowa, Iowa City, Iowa 52242

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Micafungin is a new echinocandin exhibiting broad-spectrum activity against *Candida* spp. The activity of the echinocandins against *Candida* species known to express intrinsic or acquired resistance to fluconazole is of interest. We determined the MICs of micafungin and caspofungin against 315 invasive clinical (bloodstream and other sterile-site) isolates of fluconazole-resistant *Candida* species obtained from geographically diverse medical centers between 2001 and 2004. MICs were determined using broth microdilution according to the CLSI reference method M27-A2. RPMI 1640 was used as the test medium, and we used the MIC endpoint of prominent growth reduction at 24 h. Among the 315 fluconazole-resistant *Candida* isolates, 146 (46%) were *C. krusei*, 110 (35%) were *C. glabrata*, 41 (13%) were *C. albicans*, and 18 (6%) were less frequently isolated species. Micafungin had good in vitro activity against all fluconazole-resistant *Candida* spp. tested; the MICs at which 50% (MIC_{50}) and 90% (MIC_{90}) of isolates were inhibited were 0.03 μg/ml and 0.06 μg/ml, respectively. All the fluconazole-resistant *Candida* spp. were inhibited at a micafungin MIC that was ≤1 μg/ml. Among the most common fluconazole-resistant *Candida* spp. tested in the collection, *C. glabrata* exhibited the lowest micafungin MICs (MIC_{90}, ≤0.015 μg/ml), followed by *C. albicans* (MIC_{90}, 0.03 μg/ml) and *C. krusei* (MIC_{90}, 0.06 μg/ml). The new echinocandin micafungin has excellent in vitro activity against 315 invasive clinical isolates of fluconazole-resistant *Candida*, which represents the largest collection to date of fluconazole-resistant *Candida* isolates tested against micafungin. Micafungin may prove useful in the treatment of infections due to azole-resistant *Candida*.

In the United States, *Candida* spp. are the fourth most common cause of nosocomial bloodstream infection (13, 14). These infections result in increased mortality (5) and longer hospital stays (3, 18) with associated higher health care costs. Of special concern has been the emergence of non-*Candida albicans* species exhibiting resistance to the current spectrum of azoles (6, 8, 12–14). Treatment of azole-resistant infections has remained a challenge for clinicians and has driven the need for alternative agents having novel mechanisms of action.

The development of the echinocandin class of systemic antifungal agents has added to the limited number of drugs used against *Candida* spp. In contrast to the azoles, which inhibit ergosterol synthesis, echinocandins interrupt the synthesis of 1,3-β-D glucan, an important component of the fungal cell wall (2). The echinocandins have been shown to exhibit in vitro activity against both *Candida* spp. and *Aspergillus* spp. (4, 10, 11, 15, 17, 19).

Micafungin is the most recently available echinocandin, currently FDA approved for use in esophageal candidiasis and for the prophylaxis of invasive fungal infections in hematopoietic stem cell transplant patients. The activity of micafungin against clinical isolates of azole-resistant *Candida* spp. is of interest. We examined the in vitro activities of micafungin against 315 invasive clinical isolates of fluconazole-resistant *Candida* spp. from medical centers worldwide, using Clinical and Laboratory Standards Institute (CLSI, formerly the NCCLS) broth microdilution methods with RPMI 1640 broth, incubation for 24 h, and an MIC endpoint defined as a prominent reduction in growth (≥50% inhibition relative to control growth) (9). Caspofungin was also tested as a comparator that has FDA approval for the treatment of invasive candidiasis.

MATERIALS AND METHODS

Organisms. A total of 315 isolates of *Candida* spp. from blood or normally sterile body fluid were collected from diverse medical centers worldwide and sent to the University of Iowa for testing. The collection included 146 *C. krusei*, 110 *C. glabrata*, 41 *C. albicans*, 6 *C. parapsilosis*, 3 *C. tropicalis*, 3 *C. guillermondii*, and 2 yeast species isolates and 1 isolate each of *C. inconspicua*, *C. kefyr*, *C. lipolytica*, and *C. norvegensis*. Isolates were identified by standard methods (20) and stored in water vials at ambient room temperature until used in the study. At the time of testing, the isolates were subcultured twice on potato dextrose agar (Remel, Inc., Lenexa, KS) to achieve exponential growth and to ensure purity.

Antifungal agents. Standard antifungal powders of micafungin (Astellas Pharma, Osaka, Japan), caspofungin (Merck and Co., Whitehouse Station, PA), and fluconazole (Pfizer, Inc., New York, NY) were received from the respective manufacturers and stored according to instructions until stock solutions were prepared. Stock solutions were prepared in sterile water (micafungin, caspofungin) or dimethyl sulfoxide (fluconazole) and diluted in twofold increments as described in the CLSI document M27-A2 (7). Final dilutions were prepared in RPMI 1640 medium (Sigma, St. Louis, MO) buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid (MOPS; Sigma). Each antifungal agent was dispensed (0.1-ml aliquots, 2× final concentration) into 96-well round-bottomed microdilution panels (Sartedt, Inc., Newton, NC) by using a QuickSpense II system (Dynatech Laboratories, Chantilly, VA). The panels were sealed and stored at −70°C until thawed for use at the time of testing.

Inoculum preparation. Inocula were prepared by a spectrophotometric method as described in the CLSI M27-A2 guidelines. At least five isolated yeast colonies from a potato dextrose agar plate were sampled using a sterile applicator stick and diluted to a concentration of 1.0×10^3 to 5.0×10^3 cells/ml in RPMI 1640 medium. A 0.1-ml yeast inoculum was added to each well of the

* Corresponding author. Mailing address: Medical Microbiology Division, C606 GH, Department of Pathology, University of Iowa College of Medicine, Iowa City, IA 52242. Phone: (319) 384-9566. Fax: (319) 356-4916. E-mail: michael-pfaller@uiowa.edu.

TABLE 1. Activities of micafungin and caspofungin against 315 invasive clinical isolates of *Candida* spp. that are resistant to fluconazole[a]

Organism (no. of isolates)	Antifungal	MIC (μg/ml)			Cumulative % of isolates susceptible at an MIC of (μg/ml):						
		Range	50%	90%	0.03	0.06	0.12	0.25	0.5	1.0	2.0
C. albicans (41)	Micafungin	0.007–0.25	0.015	0.03	95	97	97	100			
	Caspofungin	0.007–2.0	0.03	0.06	61	95	95	97	97	97	100
C. glabrata (110)	Micafungin	0.007–0.06	0.015	0.015	97	100					
	Caspofungin	0.015–0.5	0.03	0.06	59	90	96	100			
C. krusei (146)	Micafungin	0.007–.25	0.06	0.06	26	93	99	100			
	Caspofungin	0.015–0.5	0.12	0.25	1	41	81	97	100		
Candida spp. (18)	Micafungin	0.007–1.0	0.25	1.0	33	38	44	61	77	100	
	Caspofungin	0.015–0.5	0.06	0.5	22	50	50	77	100		
All *Candida* spp. (315)	Micafungin	0.007–1.0	0.03	0.06	60	93	96	97	98	100	
	Caspofungin	0.007–2.0	0.06	0.25	30	66	86	97	99	99	100

[a] Resistance to fluconazole was indicated by a *C. krusei* or fluconazole MIC of >32 μg/ml.

thawed panel volumes of a 2×-concentration antifungal agent to bring the final inoculum to 0.5×10^3 to 2.5×10^3 cells/ml and the final drug concentrations to 1×. Final concentrations were 0.007 to 8.0 μg/ml for micafungin and caspofungin and 0.12 to 128.0 μg/ml for fluconazole.

Reading endpoints. The panels were incubated at 35°C, and MICs were determined for both 24 h and 48 h. MICs were determined by the visual method with the aid of a reading mirror, and the growth in each well was compared to that in drug-free growth control wells. The MIC was defined as the lowest concentration at which a prominent decrease in growth (approximately 50%) relative to the growth of the control occurred after 24 h of incubation (micafungin and caspofungin) or after 48 h of incubation (fluconazole). Interpretive criteria for fluconazole were those published by Rex et al. (16) and the CLSI (7): isolates for which fluconazole MICs are ≤8.0 μg/ml are susceptible, isolates for which MICs are 16 to 32 μg/ml are susceptible dose dependent, and isolates for which MICs are ≥64 μg/ml are resistant. The fluconazole breakpoints apply to all *Candida* species except *C. krusei*, which is considered intrinsically resistant regardless of the MIC recorded (7). Interpretive criteria for micafungin and caspofungin have not yet been established.

Quality control. Quality control was performed using *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019 for each batch of isolates tested, as recommended in CLSI document M27-A2 (1, 7).

RESULTS AND DISCUSSION

All 146 isolates of *C. krusei* were considered resistant to fluconazole, including 3 isolates for which fluconazole MICs were ≤8.0 μg/ml, 35 isolates for which the MICs were 16 to 32 μg/ml, and 108 isolates for which the MICs were ≥64 μg/ml. Table 1 summarizes the in vitro susceptibilities of 315 fluconazole-resistant *Candida* spp. Micafungin exhibited good in vitro activity against all fluconazole-resistant *Candida* isolates tested; the MICs at which 50% (MIC_{50}) and 90% (MIC_{90}) of the isolates were inhibited were 0.03 μg/ml and 0.06 μg/ml, respectively. All isolates were inhibited at ≤1.0 μg/ml. Among the most common fluconazole-resistant *Candida* encountered in the collection, *C. glabrata* was most susceptible (MIC_{90} ≤ 0.015 μg/ml), followed by *C. albicans* (MIC_{90}, 0.03 μg/ml) and *C. krusei* (MIC_{90}, 0.06 μg/ml).

Caspofungin was included in this study as a comparator that has already been FDA approved for the treatment of invasive candidiasis. The excellent in vitro activity of caspofungin against these clinically significant azole-resistant species confirms that reported previously (10, 15). Although micafungin appears to be somewhat more active than caspofungin against *C. krusei* (MIC_{90}, 0.06 μg/ml versus 0.25 μg/ml, respectively), all isolates of this species were inhibited by 0.25 to 0.5 μg/ml of both agents.

These findings support those of Ostrosky-Zeichner et al. (10), who reported similar activity of micafungin against *Candida* spp. despite using a more prolonged incubation time of 48 h. Micafungin exhibited potent activity against a large and geographically diverse collection of azole-resistant *Candida* species. The spectrum and potency of micafungin against these clinically important isolates compare favorably with those of caspofungin, an echinocandin that is licensed for the treatment of invasive candidiasis. The fungicidal nature of micafungin coupled with the maximum concentrations of the drug in serum (8 μg/ml after a 75-mg dose [19]), which exceed the MIC_{90}s for all of the azole-resistant isolates, makes it a promising systemic antifungal agent.

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