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Caspofungin Activity against Clinical Isolates of Fluconazole-Resistant *Candida*

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A total of 7,837 clinical isolates of *Candida* were tested against fluconazole, and 351 resistant (fluconazole MIC ≥ 64 μg/ml) isolates were identified (4% of the total tested). All fluconazole-resistant isolates were inhibited by caspofungin at concentrations that can be exceeded by standard doses (MIC at which 90% of the isolates were inhibited, 1 μg/ml; 99% of the MICs were ≤2 μg/ml).

Invasive candidiasis is a common and devastating infection. The annual incidence of bloodstream infections due to *Candida* spp. in the United States ranges from 6 to 10 infections per 100,000 persons, and the mortality attributable to such infections is estimated to be 30 to 50% (3, 4, 9, 10, 23, 29, 33; R. A. Hajjeh, 6th ASM Conf. *Candida* Candidasis, abstr. S-6, p. 15, 2002). The optimal treatment of candidemia requires a high index of suspicion and the early use of systemically active antifungal agents (25, 26). Although antifungal resistance among invasive isolates of *Candida* is not common (16–19, 25, 26), it remains a concern, especially for *Candida glabrata* and *C. krusei* (20-22, 25). Both of these species are known to express intrinsic (*C. krusei*) or acquired (*C. glabrata*) resistance to fluconazole and may also demonstrate decreased susceptibility to amphotericin B (16, 18, 20, 22, 25, 27). Thus, there is a great need for systemically active agents with fungicidal activity against species expressing resistance to fluconazole.

Caspofungin is an echinocandin with potent fungicidal activity against many species of *Candida* (2, 5, 6, 21). Recently, caspofungin was shown to be equivalent to (and less toxic than) amphotericin B in the treatment of patients with invasive candidiasis (13). Caspofungin is now approved for the treatment of candidemia and esophageal candidiasis as well as for salvage therapy for invasive aspergillosis (13, 31).

Caspofungin and other echinocandins have been shown to exhibit potent activity against fluconazole-resistant *Candida* spp. (5, 7, 12, 15). In the interest of augmenting the data on this phenomenon, we have determined the in vitro activity of caspofungin against 351 strains of fluconazole-resistant *Candida* spp. This set of strains constitutes the largest collection of fluconazole-resistant *Candida* isolates from blood or sterile sites yet tested against caspofungin.

We tested 7,837 clinical isolates of *Candida* obtained between 1992 and 2002 from over 160 medical centers worldwide (see Table 1). More than 85% of the isolates were from blood or a normally sterile body fluid (cerebrospinal fluid, pleural fluid, or peritoneal fluid), and each isolate represented an individual infectious episode. The isolates were identified by standard methods (32) and stored as water suspensions until they were used. Prior to testing, each isolate was passaged at least twice on potato dextrose agar (Remel, Lenexa, Kans.).

Standard antifungal powders of caspofungin (Merck & Co., Whitehouse Station, Pa.) and fluconazole (Pfizer, Inc., New York, N.Y.) were obtained from their respective manufacturers. Stock solutions of caspofungin and of fluconazole were prepared in sterile water. Serial twofold dilutions were prepared exactly as outlined in NCCLS document M27-A2 (14). Final dilutions in RPMI 1640 medium (Sigma, St. Louis, Mo.) buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid (MOPS) buffer (Sigma) were prepared. Aliquots (0.1 ml) of each antifungal agent at two times the final dilution were dispensed into wells of plastic microdilution trays with a Quick Spense II system (Dynatech Laboratories, Chantilly, Va.). The trays were sealed and frozen at −70°C until they were used.

Broth microdilution testing was performed as described previously (21) and in accordance with the guidelines in NCCLS document M27-A2 (14) with an inoculum concentration of $(1.5 \pm 1.0) \times 10^3$ cells/ml and RPMI 1640 medium buffered to pH 7.0 with MOPS. A 0.1-ml yeast inoculum was added to each

TABLE 1. Frequency of fluconazole resistance[a] among *Candida* species

Species	No. of isolates tested	No. (%) of fluconazole-resistant isolates[b]
C. albicans	5,345	47 (1)
C. glabrata	1,432	121 (8)
C. parapsilosis	434	4 (1)
C. tropicalis	319	3 (1)
C. krusei	171	171 (100)
C. guilliermondii	56	3 (5)
C. lusitaniae	42	1 (2)
C. pelliculosa	14	0 (0)
Candida spp.[c]	24	1 (4)
All species	7,837	351 (4)

[a] Determined by NCCLS M27-A2 broth microdilution.
[b] MIC, ≥64 μg/ml.
[c] *C. dubliniensis* (two isolates), *C. famata* (three isolates), *C. kefyr* (seven isolates), *C. lipolytica* (four isolates), *C. rugosa* (five isolates), and *C. zeylanoides* (three isolates).

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TABLE 2. In vitro activity of caspofungin against 351 clinical isolates of *Candida* spp. resistant to fluconazole[a]

Species	No. of isolates tested	Cumulative % of isolates susceptible at MIC (μg/ml)[b] of:							
		0.03	0.06	0.12	0.25	0.5	1	2	4
C. albicans	47	0	0	30	66	74	96	98	100
C. glabrata	121	1	45	68	82	95	100		
C. krusei	171	38	38	38	39	47	88	99	
Candida spp.[c]	12	25	25	42	58	67	67	92	100
All species	351	20	35	47	58	68	92	99	100

[a] Fluconazole resistance was determined by NCCLS M27-A2 broth microdilution and fluconazole interpretive criteria (resistance MIC, ≥64 μg/ml).
[b] Caspofungin MICs were determined by the NCCLS M27-A2 broth microdilution method.
[c] *C. tropicalis* (three isolates), *C. guilliermondii* (three isolates), *C. parapsilosis* (four isolates), *C. lusitaniae* (one isolate), and *C. lipolytica* (one isolate).

well of the microdilution trays. The final concentrations of the antifungal agents were 0.007 to 8 μg/ml for caspofungin and 0.12 to 128 μg/ml for fluconazole. The trays were incubated at 35°C, and MIC end points were read after 48 h. Drug-free and yeast-free controls were included.

Following incubation, the wells were examined with a reading mirror, and the growth in each well was compared with the growth in the control well. The MIC of caspofungin was defined as the concentration resulting in complete inhibition of growth (21), and the MIC of fluconazole was defined as the lowest concentration that produced a prominent decrease in turbidity (approximately 50%) relative to that of the drug-free control well. Importantly, susceptibility testing methods for caspofungin, unlike those for fluconazole, have not been standardized yet. Our choice of complete growth inhibition as the MIC end point reflects both the fungicidal activity of caspofungin against *Candida* (2, 5, 6), compared to the fungistatic activity of fluconazole, and our experience with testing large numbers of organisms, including selected glucan synthesis mutants. However, the methods we used in this study may differ from future standardized methods.

The interpretive criterion for fluconazole resistance was that published by Rex et al. (24) and the NCCLS (14): an MIC of ≥64 μg/ml. Isolates of *C. krusei* were considered resistant to fluconazole irrespective of the MIC (14). For quality control, the NCCLS-recommended strains *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019 were tested (1, 14).

Table 1 summarizes the frequency of fluconazole resistance among 7,837 clinical isolates of *Candida* spp. All 171 isolates of *C. krusei* were considered resistant to fluconazole, including 6 isolates for which the fluconazole MICs were ≤8 μg/ml, 105 for which the MICs were 16 to 32 μg/ml, and 60 for which the MICs were ≥64 μg/ml.

Among the 351 fluconazole-resistant isolates, 99% were inhibited by caspofungin at an MIC of ≤2 μg/ml, and no caspofungin MICs greater than 4 μg/ml were observed (Table 2). Although in vitro susceptibility testing of caspofungin is not yet standardized, it is notable that testing by the method employed in the present study revealed that caspofungin MICs for all laboratory-derived glucan synthesis mutants of *C albicans* and *C. krusei* with in vivo resistance to caspofungin (11) were ≥8 μg/ml (8 μg/ml for one mutant and ≥32 μg/ml for seven mutants) (data not shown). Given that peak plasma drug concentrations in excess of 16 μg of caspofungin per ml may be achieved with a dosage of 1 mg/kg daily (8, 28), it is evident that the concentrations of the drug in plasma may exceed by fourfold or more the caspofungin MICs for fluconazole-resistant isolates of *Candida* (5, 6).

Our data also demonstrate the low frequency of fluconazole resistance among bloodstream isolates of *Candida* spp. (Table 1). Fluconazole resistance was extremely rare among isolates of *C. albicans* (1%), *C. tropicalis* (1%), *C. parapsilosis* (1%), *C. lusitaniae* (2%), and miscellaneous *Candida* spp. (0 to 4%). Furthermore, only 8% of 1,432 isolates of *C. glabrata* exhibited resistance to fluconazole.

In summary, we have used a large international collection of clinical *Candida* isolates to demonstrate both a low incidence of fluconazole resistance and the excellent potency of caspofungin against those *Candida* strains that are fluconazole resistant.

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