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Development of Caspofungin Resistance following Prolonged Therapy for Invasive Candidiasis Secondary to *Candida glabrata* Infection▽

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We report a case of *Candida glabrata* invasive candidiasis that developed reduced susceptibility to caspofungin during prolonged therapy. Pre- and posttreatment isolates were confirmed to be isogenic, and sequencing of hot spots known to confer echinocandin resistance revealed an F659V substitution within the *FKS2* region of the glucan synthase complex.

The echinocandins have become first-line therapy in many centers for the treatment of invasive candidiasis due to their proven efficacy, the infrequency of side effects, and the favorable drug interaction profile (12, 16, 20, 23). However, reduced susceptibility to these agents has been reported in patients receiving therapy for invasive candidiasis and is primarily due to mutations within highly conserved regions of *FKS1* and *FKS2*, genes encoding subunits of the glucan synthase enzyme complex (8, 9, 21). We report a case of invasive candidiasis caused by *Candida glabrata* that developed reduced susceptibility to caspofungin during a prolonged course of therapy with this agent.

A 41-year-old previous orthotopic liver recipient, who had no previous antifungal exposure, developed *C. glabrata* candidemia 8 months after transplantation. Intravenous caspofungin (70-mg load, followed by 50 mg daily) was initiated, and the fungemia cleared within 24 h. Yet cultures of multiple sites remained positive: bronchoalveolar lavage cultures, thought to represent colonization, were positive on days 23 and 52 of therapy; peritoneal fluid and an abdominal wall abscess were positive on day 40; and blood cultures returned positive on day 53. Dialysis dependence, hepatic dysfunction, and drug interaction concerns precluded alternative antifungal agents. The patient died on day 61 of caspofungin therapy after the development of multiorgan failure. Broth microdilution testing performed according to CLSI (formerly NCCLS) standard M27-A2 methodology (17) demonstrated reduced caspofungin susceptibility (MICs of 2 and 8 μg/ml at 24 and 48 h, respectively) for *C. glabrata* isolate 7755 recovered from the peritoneal fluid on day 40 compared to isolate 7754 (MIC of 0.25 μg/ml) recovered from the blood prior to antifungal therapy.

Random amplification of polymorphic DNA using previously described methods and primers (AP50-1, OPA-18, and OPE-18) (1, 2) strongly suggested strain isogenicity for isolates 7754 and 7755 recovered from this patient. Band patterns were identical for these two isolates with each of the three primers used, while differences in band intensity and location were observed compared to the unrelated isolate 0562 with primers OPA-18 and AP50-1 (Fig. 1).

Conserved regions of the glucan synthase enzyme complex hot spot regions were identified within the *C. glabrata* genome sequence (http://cbi.labri.fr/Genolevures/index.php) for *C. glabrata FKS1* (Cg*FKS1*) (CAGL0G01034g) and Cg*FKS2* (CAGL0K04037g). Genomic DNA was exracted using a commercially available kit (MasterPure yeast DNA purification kit; Epicentre Biotechnologies, Madison, WI), and regions of interest were sequenced with primers prepared at the UTHSCSA Advanced Nucleic Acid Core facility (Table 1). Sequence analysis of susceptible isolate 7754 revealed wild-type sequences in hot spots 1 and 2 of Cg*FKS1* and Cg*FKS2*. However, a mutation within hot spot 1 of Cg*FKS2* that conferred an F659V amino acid substitution in CgFks2p was found in isolate 7755 with reduced caspofungin susceptibility.

Although rare, recent reports have illustrated the potential for echinocandin resistance to emerge during therapy (7, 10, 13, 14, 22). Many of these reports have identified mutations within genes encoding subunits of the glucan synthase complex, and all mutations described to date reside within highly conserved regions of *FKS1* or its homolog, *FKS2* (5, 6, 11, 21). *Candida albicans* isolates comprise the majority of these cases, with mutations leading to codon changes F641S, S645F, S645Y, S645P, and R1361H (13, 14, 21). Additionally, a mutation resulting in amino acid change R1361G within the *FKS1* homolog in *Candida krusei* has been described (8).

Reduced echinocandin susceptibility and clinical failure have also been reported with *C. glabrata*. One case report detailed the emergence of caspofungin resistance and clinical failure after prolonged therapy, a finding supported by both in vitro and in vivo studies (10). However, no sequence analysis of either Cg*FKS1* or Cg*FKS2* was reported. Conversely, another study described a mutation within Cg*FKS2* resulting in an F659V codon change. Although no clinical information was

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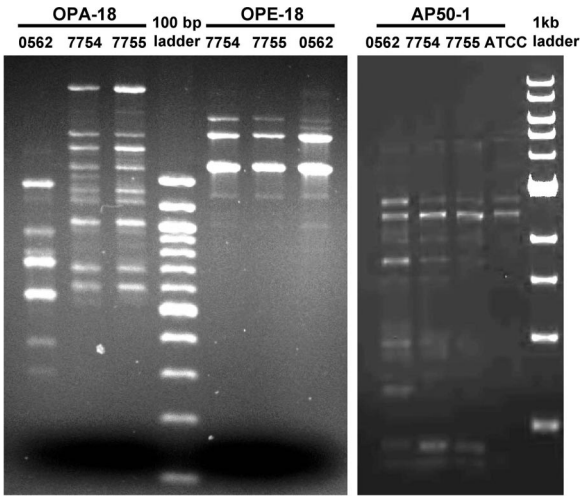

FIG. 1. Random amplification of polymorphic DNA gel patterns for *C. glabrata* isolates 7754, 7755, and 0562 obtained with primers OPA-18, OPE-18, and AP50-1.

provided, this mutation was proven to confer caspofungin resistance (9). The same mutation within C*gFKS2* was also found in isolate 7755 in our patient and was associated with a marked increase in caspofungin MICs. Similarly, another recent case report also demonstrated a mutation within hot spot 1 of Fks1p in a *C. glabrata* isolate during caspofungin therapy leading to reduced susceptibility and clinical failure (3).

Despite the 8- to 32-fold increases in the caspofungin 24- and 48-h MICs for isolate 7755, anidulafungin and micafungin maintained potency against both 7754 and 7755 (Table 2). However, this difference in potencies between caspofungin and the other echinocandins was no longer present when susceptibility testing was repeated in the presence of 50% human serum. In this setting, the 24- and 48-h MICs for anidulafungin and micafungin increased 8- to 32-fold against isolate 7754 and 4- to 16-fold for isolate 7755. The prospect of using a different echinocandin when caspofungin resistance is encountered has been proposed (7, 10, 22) and is based on enhanced potency of anidulafungin and micafungin against *Candida* isolates observed in vitro (4, 18). Unfortunately, these observations have not translated into improved efficacy in murine models of invasive fungal infections. In these studies, in vivo efficacy correlated better with in vitro potency when tested in the presence of human serum (19, 25). The effect of serum on the activity of echinocandins is not fully understood. One potential explanation proposes the observed reduction in susceptibility is due to significant protein binding associated with these agents. Although this reduction in potency may be secondary to protein binding, significantly higher drug activity has been measured for micafungin than that predicted by the free drug concentration using protein binding data (15). The clinical relevance of reduced in vitro potency for the echinocandins in the presence of serum is unknown.

TABLE 1. Primer sequences used for amplification and sequencing of the hot spot regions within C*gFKS1* and C*gFKS2*

Primer	Sequence
FKS1	
HS1	
Forward	5′-CCATTGGGTGGTCTGTTCACG
Reverse	5′-GATTGGGCAAAGAAAGAAATACGAC
Sequencing	5′-CTCAAACCTTCACTGCCTC
HS2	
Forward	5′-GGTATTTCAAAGGCTCAAAAGGG
Reverse	5′-ATGGAGAGAACAGCAGGGCG
Sequencing	5′-CGGTATGAATGCCCTATTACG
FKS2	
HS1	
Forward	5′-GTGCTCAACATTTATCTCGTAGG
Reverse	5′-CAGAATAGTGTGGAGTCAAGACG
Sequencing	5′-GCTTCTCAGACTTTCACCG
HS2	
Forward	5′-CGTAGACCGTTTCTTGACTTC
Reverse	5′-CTTGCCAATGTGCCACTG
Sequencing	5′-TCTTGACTTTCTACTATGCG

TABLE 2. Caspofungin, anidulafungin, and micafungin MICs in the presence and absence of 50% human serum

C. glabrata isolate	MIC (μg/ml) with no human serum/50% human serum[a]					
	Caspofungin		Anidulafungin		Micafungin	
	24 h	48 h	24 h	48 h	24 h	48 h
7754	0.25/0.5	0.25/0.5	0.125/1	0.125/2	0.125/1	0.125/4
7755	2/2	8/8	0.5/4	1/4	0.25/4	0.5/4

[a] MICs were read at 24 and 48 h as the lowest concentration of drug resulting in a significant (≥50%) decrease in turbidity compared to the growth control.

Continued exposure to antimicrobials is often associated with the development of resistance, and as our case and previous reports illustrate, this also may lead to the development of echinocandin resistance in *Candida* species, including non-*C. albicans* isolates, during continued drug pressure with members of this antifungal class. A heightened suspicion for reduced echinocandin susceptibility as a possible cause of patient failure is needed as the use of these agents continues to increase.

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