Caspofungin-Resistant *Candida tropicalis* Strains Causing Breakthrough Fungemia in Patients at High Risk for Hematologic Malignancies[▽]

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We identified three cases of *C. tropicalis* strains causing breakthrough fungemia in allogeneic stem cell recipients receiving caspofungin prophylaxis and treatment. Three genetically unrelated isolates with high echinocandin MICs were identified. Each strain carried a characteristic mutation conferring an amino acid substitution within Fks1p hot spot 1.

Echinocandins inhibit fungal cell wall biosynthesis through noncompetitive inhibition of 1,3-D-glucan synthase (GS) (4). These agents are widely used for treatment and prophylaxis of candidiasis in selected high-risk patients (2, 15). Although resistance in *Candida* species is uncommon (13, 14), clinical isolates of *Candida* spp. having increased echinocandin MICs and amino acid substitutions in Fksp have been described to occur in candidemic patients for whom caspofungin-based therapy has failed (1, 3, 6, 8, 11, 13). *Candida tropicalis* is an important pathogen in patients with hematologic malignancies (9). Recently, it was reported that a patient with acute myelogenous leukemia developed caspofungin-resistant *C. tropicalis* fungemia (12). Herein, we describe three additional cases of caspofungin-resistant *C. tropicalis* candidemia in patients at

high risk for hematologic malignancies and characterize the resistance mechanism.

From March 2001, when caspofungin was introduced, until February 2007, we identified 40 *C. tropicalis* bloodstream isolates in 37 patients at the M. D. Anderson Cancer Center (isolates T1 to T40). The isolates were characterized as *C. tropicalis* by conventional and molecular methods (16). Three patients at high risk for hematologic malignancies who developed *C. tropicalis* fungemia breakthrough during caspofungin treatment were identified during this period, representing 8% (3/37) of the cases. Breakthrough *C. tropicalis* fungemia was defined as *C. tropicalis* septicemia during caspofungin administration (empirical or prophylactic).

Patient 1 (isolate T19). An 84-year-old male with acute

TABLE 1. In vitro susceptibility testing and GS inhibition profiles for echinocandin drugs^a

Strain	Organism	Fks1p hot spot 1	MIC (μg/ml)			IC ₅₀ (ng/ml) for indicated drug and kinetic phase					
			ANF	CSF	MCF	ANF		CSF		MCF	
						I	II	I	II	I	II
ATCC 750	<i>C. tropicalis</i>	FLTLSLRDP	0.06	0.25	0.06	4.661		2.331		7.545	
T7 ^b	<i>C. tropicalis</i>	FLTLSLRDP	0.12	0.50	0.06	2.689		7.652		6.168	
T3 ^c	<i>C. tropicalis</i>	FLTLS/PLRDP	2.00	4.00	2.00	0.320	233.2	0.306	369.6	0.149	289.8
T19 ^d	<i>C. tropicalis</i>	FLTLS/PLRDP	1.00	4.00	2.00	0.574	407.5	0.296	263.6	0.217	283.8
T26 ^e	<i>C. tropicalis</i>	LLTLSLRDP	0.50	1.00	0.50	31.62		76.88		75.66	
ATCC 90028	<i>C. albicans</i>	FLTLSLRDP	0.03	0.12	0.03	1.830		0.500		18.80	
205	<i>C. albicans</i>	FLTLPLRDP	2.00	8.00	4.00	989.0		245.4		1085.0	

^a ANF, anidulafungin; CSF, caspofungin; MCF, micafungin. Bold letters represent amino acid changes.

^b This was a clinical echinocandin-susceptible isolate from another patient in this study. Azole MICs: fluconazole, 0.25 μg/ml; itraconazole, 0.25 μg/ml; voriconazole, 0.06 μg/ml; and posaconazole 0.25 μg/ml.

^c Azole MICs: fluconazole, 8 μg/ml; itraconazole, 0.5 μg/ml; voriconazole, 1 μg/ml; and posaconazole, 0.25 μg/ml.

^d Azole MICs: fluconazole, 32 μg/ml; itraconazole, 1 μg/ml; voriconazole, 2 μg/ml; and posaconazole 1 μg/ml.

^e Azole MICs: fluconazole, 0.5 μg/ml; itraconazole, 0.125 μg/ml; voriconazole, 0.03 μg/ml; and posaconazole, 0.06 μg/ml.

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FIG. 1. Echinocandin inhibition profiles for product-entrapped GCs. Enzyme complexes were obtained from reference *C. tropicalis* ATCC 750 (gray diamonds), *C. tropicalis* T19 (black squares), *C. tropicalis* T26 (triangles), and echinocandin-resistant *C. albicans* 205 (gray circles) strains. Inhibition curves and IC_{50} s for anidulafungin (ANF) (A), caspofungin (CSF) (B), and micafungin (MCF) (C) (in nanograms per milliliter) were obtained using a sigmoidal response (variable-slope) curve (for strains ATCC 750, T26, and 205) and a two-site competition-fitting algorithm (for strain T19).

lymphocytic leukemia started on intravenous caspofungin (50 mg/day) for persistent (despite broad-spectrum antibacterials) febrile neutropenia. Three sets of blood cultures obtained on days 15 and 16 of caspofungin treatment revealed low-grade (6 CFU) fungemia caused by *C. tropicalis*. During the course of

fungemia, the patient remained clinically stable, afebrile, and normotensive. The patient cleared his cultures 1 day after removal of his central venous catheter and initiation of intravenous voriconazole treatment (caspofungin was discontinued). He did well clinically after completing 2 weeks of voriconazole treatment and showed no evidence of recurrence until his death 8 months later from leukemia.

Patient 2 (isolate T3). A 59-year-old male with large-cell lymphoma who received a matched allogeneic hematopoietic stem cell transplant had a complicated course with renal failure and persistent *Acinetobacter* bacteremia. The patient was on intravenous caspofungin at 50 mg/day as a posttransplant prophylaxis. Because of a persistent fever, all catheters were removed. However, on day 44 of caspofungin treatment, a peripheral blood culture was positive for both *Pseudomonas aeruginosa* and *C. tropicalis*. Antifungal treatment was changed to liposomal amphotericin B treatment (5 mg/kg of body weight/day). He cleared his blood cultures and showed no evidence of recurrent candidemia until his death 3 months later from lymphoma.

Patient 3 (isolate T26). A 45-year-old nonneutropenic male with a prior history of Hodgkin's lymphoma, renal cell carcinoma, and esophageal cancer had multiple courses of bacterial pneumonia. In addition, the patient developed catheter-related *Candida parapsilosis* fungemia and was treated with caspofungin at 50 mg/day, with clinical and mycologic responses. However, the patient developed recurrent fevers on day 21 of caspofungin. The catheter tip cultures grew 50 CFU/ml of *C. tropicalis* and 30 CFU/ml of *Staphylococcus epidermidis*. His antibiotics were adjusted, and caspofungin was switched to fluconazole (6 mg/kg/day). Although follow-up

FIG. 2. DNA sequencing chromatograms and amino acid sequence alignment for *C. tropicalis* strains. (A) *C. tropicalis* Fks1p hot spot 1 strain ATCC 750. (B) Strain T3. (C) Strain T26. Arrows show mutations. (D) Sequence alignments of the Fks1p hot spot 1 region (boxed) in different *Candida* species. Line 1, *C. tropicalis* wild-type allele (GenBank accession no. pending); line 2, *C. tropicalis* T26 strain (F-to-L change); line 3, *C. tropicalis* T3 strain mutant allele (S-to-P change); line 4, *C. albicans* (GenBank accession no. XP_721429); line 5, *C. parapsilosis* (ABX80511); line 6, *C. metapsilosis* (ABY67254); line 7, *C. orthopsilosis* (ABY67253); line 8, *C. glabrata* (XP_446406); line 9, *C. krusei* (AAY40291).

blood cultures became negative, he eventually succumbed to respiratory failure secondary to ventilator-associated pneumonia.

Echinocandin susceptibility testing of the 40 *C. tropicalis* strains was performed following CLSI (formerly NCCLS) document M27-A2 (10), with modifications (14). Echinocandin drugs exhibited potent in vitro activities against 92% (37/40) of the *C. tropicalis* strains tested, with MIC geometric means of 0.47 $\mu\text{g/ml}$ (MIC₉₀, 0.5; range, 0.02 to 4.0), 0.13 $\mu\text{g/ml}$ (MIC₉₀, 0.12 $\mu\text{g/ml}$; range, 0.06 to 2.0), and 0.23 $\mu\text{g/ml}$ (MIC₉₀, 0.12; range, 0.06 to 2.0) for caspofungin, anidulafungin, and micafungin, respectively. However, the strains isolated from the patients described above showed 4- to 33-fold-higher MICs than a reference strain and exceeded or were at the maximum range reported for large collections of clinical isolates (14) (Table 1). The reduced in vitro susceptibilities to echinocandin drugs were confirmed in GS enzyme assays. Following GS isolation, echinocandin inhibition kinetics yielding 50% inhibitory concentrations (IC₅₀s) were obtained (6, 11). The product of the reaction mixture was characterized as 1,3- β -glucan (5). A standard inhibition curve (monophasic, with one IC₅₀) was obtained for the reference strain (ATCC 750), three clinical susceptible strains, and the T26 strain. However, T26 GS yielded higher IC₅₀s for all echinocandin drugs (Fig. 1 and Table 1). In contrast, biphasic inhibition kinetics (with two IC₅₀s) was obtained for T3 and T19 GS enzymes (Fig. 1 and Table 1) (T3 data not shown). The highest IC₅₀ was at least 50-fold higher than the IC₅₀s obtained from susceptible GS enzymes. The presence of two inflection points in kinetic curves suggests mixed populations of wild-type and mutant GS enzymes (6, 11). Consistent with this notion, DNA sequence analysis of *C. tropicalis FKS1* (GenBank accession no. EU676168) revealed a homozygous T-to-C mutation (in strain T26) and heterozygous T-to-C mutations (in strains T3 and T19) (equivalent to nucleotides T1923 and T1935 in *Candida albicans FKS1* [GenBank accession no. XM 716336], respectively) (Fig. 2). These nucleotide changes resulted in deduced Phe-to-Leu (in the T26 strain) and Ser-to-Pro (in the T3 and T19 strains) amino acid changes. These amino acid substitutions are located in the highly conserved hot spot 1 region of Fks1p (Fig. 2), associated with echinocandin resistance in *Candida* spp. (3, 6, 11). These results provide additional support for Fks1p modification as a common mechanism for echinocandin resistance in *Candida* spp. (1, 3, 5, 6, 11, 13).

Echinocandin resistance is uncommon (13), creating skepticism regarding the value of routine MIC determination for the management of candidemic patients with these agents. Moreover, there is no compelling evidence that the rates of response to echinocandins for *Candida* isolates having elevated echinocandin MICs are inferior to the rates for isolates having low echinocandin MICs (7). Our report contributes to such knowledge, as it links *FKS1* mutation, high echinocandin MICs, and clinical failures of echinocandin-based therapy. Moreover, in our cases of caspofungin-resistant *C. tropicalis*, the clinical courses were rather benign, in contrast to what has been pub-

lished before with respect to *C. tropicalis* fungemia in patients at high risk for hematologic malignancies (9).

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