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NOTES

Emergence of a *Candida krusei* Isolate with Reduced Susceptibility to Caspofungin during Therapy

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Clinical failure associated with reduced susceptibility to caspofungin has been described in *Candida albicans* and *C. parapsilosis*. We report a case of *Candida krusei* infection that progressed despite caspofungin therapy. Reduced microbial susceptibility to all three echinocandins (caspofungin, anidulafungin, and micafungin) was noted but was not associated with mutations in *FKS1*.

The echinocandin family of antifungals, which include caspofungin (Merck & Co, Inc., West Point, PA), anidulafungin (Pfizer, New York, NY), and micafungin (Astellas Pharmaceuticals, Osaka, Japan), inhibit (1,3)-β-D-glucan synthase, thereby decreasing synthesis of glucan, an essential component of the fungal cell wall. Caspofungin was the first echinocandin to become available in the United States (reviewed in reference 4) and is approved for the treatment of oropharyngeal, esophageal, and invasive candidiasis and invasive aspergillosis refractory to other therapies and for empirical therapy of febrile neutropenia (1).

We treated a 35-year-old woman with relapsed acute myelogenous leukemia who developed candidemia with *Candida krusei* during neutropenia secondary to mitoxantrone and VP-16. Blood cultures from two consecutive days grew *C. krusei*. Caspofungin therapy (70 mg on the first day followed by 50 mg daily) was initiated. An ophthalmic exam did not reveal any abnormalities. Blood cultures obtained during the following week were all without growth. Seventeen days into caspofungin therapy, she developed pain, blurred vision, and floaters in her right eye accompanied by a sore throat and odynophagia. A repeat ophthalmic examination revealed findings consistent with fungal endophthalmitis in the right eye only (Fig. 1A). Her visual acuity in the right eye was preserved compared to that of the unaffected left eye. A vitreal tap was performed, and she received a single intravitreal injection of amphotericin B (0.5 μg). Her examination was otherwise notable for the presence of severe oropharyngeal thrush. Caspofungin was discontinued, and liposomal amphotericin B (AmBisome) therapy was begun at a dose of 5 mg/kg of body weight/day. Blood cultures and cultures from the vitreal fluid were without growth; throat swab cultures grew *C. krusei* and *Candida fermentati*. She received a total of 14 days of liposomal amphotericin B, at which time treatment was switched to oral voriconazole. Nearly complete resolution of her retinal lesion was apparent after 4 weeks of voriconazole (Fig. 1B).

Susceptibility testing of the *C. krusei* blood and throat isolates was performed using methods recommended by the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS; standard M27-A2) (8). Caspofungin, anidulafungin, and micafungin were obtained from the manufacturers. MICs for the blood and throat *C. krusei* isolates are listed in Table 1; a fourfold increase in MICs to caspofungin was noted for the throat isolate when measured in both RPMI 1640 at 48 h and AM3 medium at 24 h (9). MICs for the isolates of two other echinocandins, micafungin and anidulafungin, were also increased, but to lower absolute MIC. The *C. krusei* isolates had predictably high MICs to fluconazole and low MICs to voriconazole and amphotericin B. The *C. fermentati* isolate demonstrated a high MIC to caspofungin (>16 μg/ml) and low MICs to fluconazole (4 μg/ml), voriconazole (0.06 μg/ml), and amphotericin (0.125 μg/ml).

To assess the genetic relatedness of the *C. krusei* isolates, purified genomic DNA (MasterPure yeast DNA purification kit; Epicenter, Madison, WI) samples (0.5 μg) were digested with 1 U HinfI (Invitrogen, Carlsbad, CA) for 3 h at 37°C. Separation of DNA fragments was performed by electrophoresis in a 1% agarose gel for 10 h at 40 V, followed by visualization under UV illumination (11). The patient's blood and throat isolates had similar patterns (Fig. 2, lanes 2 and 3, respectively), while both differed from an unrelated *C. krusei* isolate (lane 1), suggesting that the patient's isolates were the same, or closely related, strains.

Amino acid mutations in a region of Fks1p, a component of the catalytic subunit of (1,3)-β-D-glucan synthase and the target of echinocandins (5), have been associated with caspofungin resistance in *C. albicans* and *C. krusei* clinical isolates (5, 10). To determine whether similar mutations were present in the *C. krusei* isolates, 2.4-kb amplicons of the *FKS1* open reading frame from the two *C. krusei* isolates were generated by PCR (high-fidelity *Taq*; Invitrogen, Carlsbad, CA) using primers previously described (10), followed by cloning (pCR-Blunt; Invitrogen, Carlsbad, CA) and sequencing. The deduced

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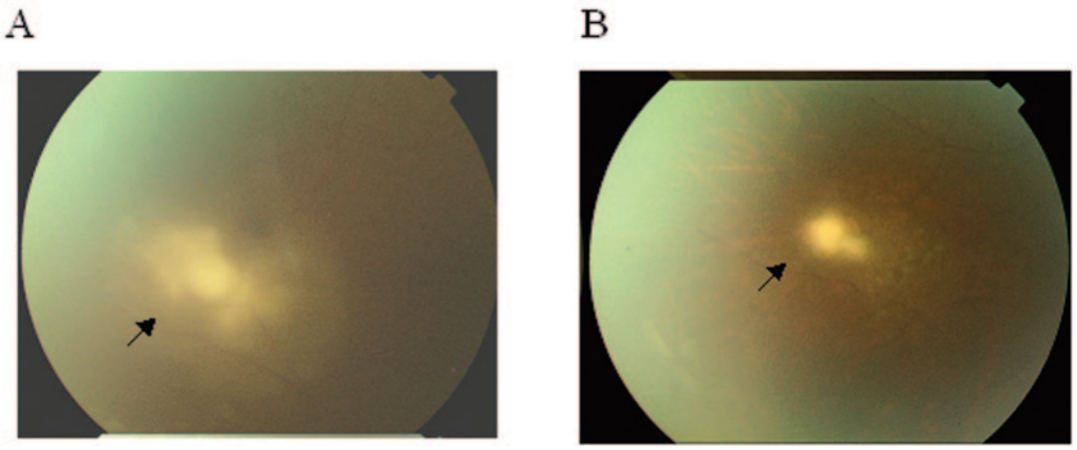

FIG. 1. Fundoscopic appearance of right eye lesion (indicated by the arrows) at presentation (A) and after 2 weeks of liposomal amphotericin B followed by 4 weeks of voriconazole (B).

amino acid sequences did not reveal significant amino acid differences between these isolates or between these isolates and an unrelated *C. krusei* Fks1p sequence (GenBank accession no. DQ017894; data not shown). These results suggest that the decreased caspofungin susceptibility of the throat isolate is not associated with a mutation in this region of Fks1p. We cannot rule out the possibility of contributing amino acid differences outside of the sequenced region.

This is the first report, to our knowledge, of the development of decreased susceptibility to caspofungin in a *C. krusei* clinical isolate over time in a patient receiving caspofungin therapy. The MICs obtained were considerably higher than those reported using similar methods (2). Moreover, the development of reduced susceptibility to caspofungin was associated with the onset of clinically apparent fungal endophthalmitis and oropharyngeal candidiasis. We cannot definitively prove that the endophthalmitis was due to progressive *C. krusei* since this patient's vitreal culture was negative; however, the development of clinically symptomatic oropharyngeal candidiasis during caspofungin therapy likely represents true antimicrobial "resistance" given the MICs of the *C. krusei* isolate recovered from the oropharynx. The finding that this patient's two *C. krusei* isolates displayed identical restriction endonuclease digestion patterns, yet differed from an unrelated *C. krusei* strain, argues against reinfection with a second, distinct caspofungin-resistant strain of *C. krusei*.

There exists little data correlating the development of reduced susceptibility to echinocandins in vitro and poor clinical outcomes in vivo. Refractory oropharyngeal candidiasis with *C. albicans* demonstrating increased MICs to caspofungin over time has been described in a patient with AIDS (6). Another report described persistent fungemia associated with the development of reduced susceptibility of *C. parapsilosis* to caspofungin in a patient treated for prosthetic valve endocarditis (7). Interestingly, while this *C. parapsilosis* isolate demonstrated increased MICs to micafungin and caspofungin, MICs to anidulafungin remained low. The *C. krusei* isolate described in this report demonstrated increased MICs to both micafungin and anidulafungin in addition to caspofungin, indicating the possible development of echinocandin "cross-resistance". As MIC interpretive breakpoints defining resistance to echinocandins are not yet defined, we cannot comment on whether these drugs would have been effective therapies.

Recently, five *Candida* isolates (four *C. albicans* isolates and one *C. krusei* isolate) with reduced susceptibility to caspofungin (CLSI MICs $>$ 4 μg/ml) collected from clinical studies were found to have point mutations in a specific region of their respective *FKS1* genes when compared to the known sequence for *C. albicans* (10). In that study, there were no matched, genetically similar isolates of *C. krusei* available for comparison. Although the isolates described in this report exhibited reduced susceptibility to three echinocandins, no causative mutations implicated in echinocandin resistance were found in the same region of *FKS1* of the isolates. Determining whether mutations exist in another region of *FKS1* or in other genes associated with antifungal resistance (3) will require further study.

In this report, we describe the emergence of *C. krusei* with reduced susceptibility to caspofungin as a potential cause of fungal endophthalmitis and oropharyngeal candidiasis. As

TABLE 1. Antifungal susceptibility testing of *Candida* isolates

Source of *C. krusei* isolate	MIC[a] (μg/ml) of:					
	Fluconazole	Voriconazole	Amphotericin B	Caspofungin	Micafungin	Anidulafungin
Blood	32	0.5	0.5	0.25/**2**	0.03/**0.5**	0.03/**0.25**
Throat	64	0.5	0.5	1/**8**	0.06/**4**	0.06/**4**

[a] MICs are shown as follows: fluconazole and voriconazole, MICs at which 80% of growth was inhibited (MIC_{80}); amphotericin B, MIC_{100}, interpreted after 48 h; caspofungin, micafungin, and anidulafungin susceptibilities reported as partial end points (lightface; MIC_{50}), measured in AM3 medium and interpreted after 24 h, and clear end points (boldface; MIC_{100}), measured in RPMI 1640 and interpreted after 48 h (8).

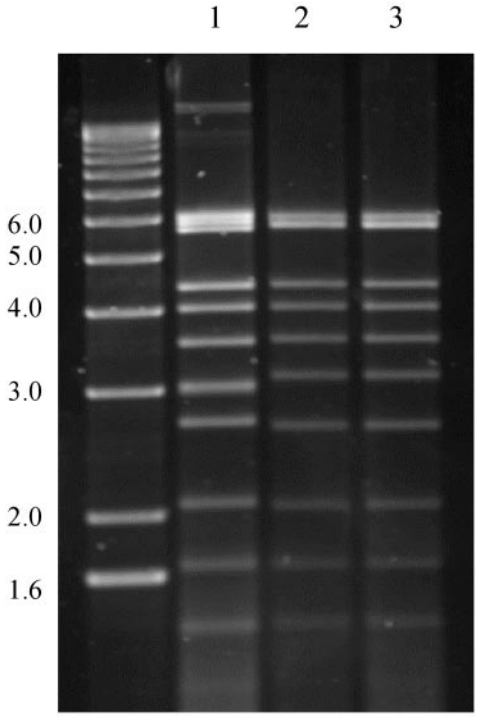

FIG. 2. HinfI restriction endonuclease digestion patterns of *C. krusei* isolates. Lane 1, unrelated *C. krusei* isolate; lane 2, patient's blood isolate; lane 3, patient's throat isolate. A DNA molecular size ladder (kb) is shown to the left of the gel.

echinocandin antifungals are being used more frequently for the treatment of invasive fungal infections, resistance to these medications is expected to be an increasingly prevalent problem. Understanding the mechanisms of, risk factors for, and clinical impact of echinocandin resistance will be an essential component in improving outcomes of therapy.

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