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NOTES

Multiechinocandin- and Multiazole-Resistant *Candida parapsilosis* Isolates Serially Obtained during Therapy for Prosthetic Valve Endocarditis

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Echinocandins are approved for the treatment of candidal infections. In vitro they have been shown to be less potent against strains of *Candida parapsilosis* than against other *Candida* spp. This is the first case report describing the development of a secondary multidrug (echinocandin-azole)-resistant *Candida* strain during therapy.

Echinocandins are a new class of synthetic antifungals that act by noncompetitive inhibition of the synthesis of 1,3-β-D-glucan synthase (16). They have demonstrated clinical efficacy in candidemia, in esophageal candidiasis, and as salvage therapy in invasive aspergillosis (2, 9, 12, 14). The concern with echinocandins, as with any new antimicrobial agent, is the potential for the emergence of drug-resistant organisms. In vitro echinocandin resistance has been described and partially characterized (10). In addition, it appears that resistance may also have some element of cross-resistance to other echinocandins (10). Among the *Candida* species, *Candida parapsilosis* has been commonly isolated and shown to be less susceptible in vitro to echinocandins than other *Candida* species are (5, 7, 11, 13, 17).

Case report. A 51-year-old male was admitted with fever 7 months after aortic valve replacement and aortic repair. His blood cultures were positive for *C. parapsilosis*. The patient was a poor candidate for surgery and was treated with amphotericin B at 0.7 mg/kg/day and flucytosine at 25 mg/kg every 6 h. After 7 days, he development acute renal insufficiency and his antifungal regimen was changed to simultaneous caspofungin acetate at 50 mg/day intravenously (i.v.) along with fluconazole at 400 mg/day i.v. He improved and cleared the *C. parapsilosis* from his bloodstream. After completing 6 weeks of this combination therapy, he was discharged on fluconazole at 400 mg/day as lifelong suppressive therapy.

Three months later, he was readmitted with fever and chills and his blood cultures again were found to contain *C. parapsilosis* (isolate 2, Table 1). Fluconazole was discontinued, and i.v. caspofungin was restarted at 50 mg/day. Despite 10 days of therapy, his blood cultures remained positive. At that time, MICs were determined (Table 1) and on the basis of these, his therapy was changed to amphotericin B lipid complex (Abelcet) at 5 mg/kg/day i.v. After 1 week, he improved and his blood cultures became negative.

Yeast strains. *C. parapsilosis* was recovered from six separate blood cultures obtained from the patient on separate occasions. The identity of the *C. parapsilosis* isolate was confirmed by means of germ tube and chlamydospore formation and the API 32C testing kit (bioMérieux). Quality control isolates were used in every testing batch and included ATCC 20019 (*C. parapsilosis*), ATCC 6258 (*C. krusei*), and ATCC 90028 (*C. albicans*).

In vitro antifungal susceptibility assays. All six *C. parapsilosis* bloodstream isolates and the three quality control isolates were evaluated by the broth microdilution method in accordance with the National Committee for Clinical Laboratory Standards M27-A2 standard (15). The antifungal agents tested included fluconazole, voriconazole, amphotericin B, micafungin, anidulafungin, and caspofungin.

Electrophoretic karyotyping. Strain delineation (genotyping) of *C. parapsilosis* was established by electrophoretic karyotyping by contour-clamped homogeneous electric field electrophoresis, a form of pulsed-field gel electrophoresis (19). All gels were analyzed in duplicate to ensure reproducibility, as demonstrated elsewhere (19).

The MICs are shown in Table 1. The initial isolate was susceptible to all of the antifungals tested except micafungin, whose MIC was 8 μg/ml. The remaining isolates were obtained during the patient's second hospitalization. The serial *C. parapsilosis* isolates all demonstrated cross-resistance to fluconazole (MIC, >64 μg/ml) and voriconazole (MIC, >16 μg/ml). In addition, the isolates also demonstrated an increase in the MICs of micafungin and caspofungin (MIC, >16 μg/ml). Interestingly, the MIC of anidulafungin was essentially unchanged, with an increase from 1.0 to 2.0 μg/ml. The MICs of amphotericin B remained the same throughout the course of the infection at 0.25 to 0.5 μg/ml. The MICs for all sequential isolates were identical.

Genotyping of the *C. parapsilosis* isolates by the contour-clamped homogeneous electric field method revealed that all

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TABLE 1. In vitro susceptibilities of serial *C. parapsilosis* isolates[a]

Isolate	MIC (μg/ml)					
	Fluconazole	Voriconazole	Caspofungin	Micafungin	Anidulafungin	Amphotericin B
1 (1st admission)	1	0.03	2	8	1	0.25
2 (2nd admission)	>64	>16	>16	>16	2	0.5
ATCC 22019	2	0.03	1	4	0.5	0.5
ATCC 6258	32	0.25	1	1	0.25	0.5
ATCC 90028	0.25	0.03	0.5	0.03	0.03	0.125

[a] Interpretive criteria for susceptibility (breakpoints) to fluconazole <8 μg/ml, susceptible; 16 to 32 μg/ml, dose-dependent susceptible; >64 μg/ml, resistant. Interpretive criteria for voriconazole, anidulafungin, caspofungin, anidulafungin, and amphotericin B have not been developed. However, the susceptibility breakpoints for voriconazole and amphotericin B are considered to be <1 μg/ml. For the echinocandins, the MICs for 90% of the *Candida* strains tested are between 0.01 and 2 μg/ml (6a, 15).

six isolates (data not shown) had the same pattern and were thus identical strain types.

The echinocandins are a recently developed class of antifungals with a novel mechanism of action (2, 4). They have potent fungicidal activity against *Candida* species, including the fluconazole-resistant *Candida* strains (1, 3, 6, 8, 17, 18). There is minimal data available regarding the development of echinocandin resistance in fungi in the clinical setting (10). The initial investigators evaluated independent spontaneous *C. albicans* mutants resistant to L-733,560, a synthetic pneumocandin. They found that these mutants exhibited cross-resistance to other 1,3-β-D-glucan synthase inhibitors. In addition, the daughter strains of the resistant *C. albicans* mutants had glucan synthase activity that was more resistant to pneumocandins than that of the parent strains. These data seem to suggest that the resistant mutants may have an alteration in glucan synthase leading to cross-resistance among the echinocandin class of antifungals, but not to the azole or polyene class of antifungals.

An additional consideration when using echinocandins should be the fact that the entire class has been found to have less intrinsic in vitro activity against *C. parapsilosis* and *C. guilliermondii* than against other pathogenic *Candida* species (11, 13). Whether this decrease in susceptibility correlates with clinical failure remains to be established.

Our patient presented from the community with *C. parapsilosis* prosthetic valve endocarditis and is unique for a variety of reasons. First, community-acquired *C. parapsilosis* fungemia in a non-intravenous drug user is extremely uncommon. Second, the initial *C. parapsilosis* isolate that was obtained from the patient prior to the initiation of any antifungal therapy already revealed a high MIC of micafungin (8 μg/ml) while remaining susceptible to caspofungin and anidulafungin. This is also the first case describing the development of a secondary multidrug (fluconazole, voriconazole, caspofungin, and micafungin)-resistant isolate of *C. parapsilosis* during continuous antifungal therapy. Furthermore, despite the high-level resistance to caspofungin and micafungin, the isolates did not demonstrate the same increase in the MIC of anidulafungin (2 μg/ml). Furthermore, the isolates developed resistance to fluconazole, but also to voriconazole, to which the patient was not exposed. This again raises concerns regarding the potential for cross-resistance among azoles such as fluconazole and itraconazole and the newer generation of azoles, such as voriconazole and posaconazole.

Finally, the most significant factor that should have been considered in this case is the fact that the optimal management of this patient should have been surgical removal of the infected prosthetic valve, followed by prolonged antifungal therapy. Prosthetic valve endocarditis due to *Candida* has been associated with frequent medical treatment failures and high mortality rates.

The observations described in this report raise questions regarding the development, incidence, acquisition, and mechanisms of resistance in *Candida*. Although the expansion of our antifungal armamentarium is truly exciting, a great many issues remain unclear and the exploration of further avenues of research is necessary to address these concerns while improving our understanding of the various antifungals.

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