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Echinocandin Failure Case Due to a Previously Unreported *FKS1* Mutation in *Candida krusei*

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Echinocandins are the preferred therapy for invasive infections due to *Candida krusei*. We present here a case of clinical failure involving *C. krusei* with a characteristic *FKS1* hot spot mutation not previously reported in *C. krusei* that was isolated after 14 days of treatment. Anidulafungin MICs were elevated by ≥5 dilution steps above the clinical breakpoint but by only 1 step for a *Candida albicans* isolate harboring the corresponding mutation, suggesting a notable species-specific difference in the MIC increase conferred by this mutation.

Echinocandins are generally advised as first-line antifungal agents for the management of invasive candidiasis, especially where the local epidemiology reveals a significant proportion of species that are less susceptible or resistant to fluconazole (1–4). Not surprisingly, the shift in treatment recommendations from fluconazole to echinocandins has resulted in breakthrough infections. In recent years, the emergence of echinocandin resistance has been observed, as demonstrated by the increasing number of publications on treatment failure and breakthrough infections (5–10). Nonsynonymous mutations in hot spot regions of *FKS1* and/or *FKS2* are linked to decreased echinocandin susceptibility due to the altered amino acid composition of the target protein 1,3-β-D-glucan synthase (11). This correlation is well established and has been demonstrated in the most clinically relevant *Candida* species (7, 8, 11–20). Regarding *Candida krusei*, echinocandin resistance has significant therapeutic implications, as this species is inherently less susceptible or resistant to azoles, limiting the already narrow spectrum of antifungal treatment options (21). So far, alterations at amino acids F655 and L658 in hot spot 1 and R1368 in hot spot 2 of Fks1p have been reported in *C. krusei* isolates with elevated echinocandin MICs (12, 22, 23). Here, we present a clinical failure case of breakthrough infection caused by *C. krusei* strongly attributable to the acquisition of a resistant *FKS1* genotype that has not been previously described in *C. krusei*.

A 62-year-old woman with diffuse large B-cell lymphoma was admitted for chemotherapy in 2013. During prior admissions and as an outpatient, she had received fluconazole orally (200 mg daily) for a 2-month period until 1 week before this admission. Due to her febrile neutropenia, rising C-reactive protein (CRP) levels, and lack of response to broad-spectrum antibiotics, empirical fluconazole was resumed on day 2 (200 mg/day), followed by caspofungin starting on day 8 (50 mg/day after a loading dose of 70 mg). Blood cultures remained negative until a peripheral blood culture obtained at day 22 yielded a *C. krusei* isolate after 14 days of caspofungin therapy. The patient died from cerebral infarction and fungal infection on day 25 while still on caspofungin therapy and before identification or susceptibility patterns were available.

Susceptibility testing was done according to EUCAST definitive document EDef 7.2 (24) (anidulafungin, micafungin, and azoles) and by the Etest (caspofungin and amphotericin B [AMB]). *FKS1* gene sequencing was performed for the resistant *C. krusei* isolate (CPH-T5842), including reference strain ATCC 6258, and aligned to the *FKS1* wild-type sequence of *C. krusei* (NCBI accession no. EF426563) using CLC Main Workbench (CLC Bio, Denmark) as previously described (12). Susceptibility data for a *Candida albicans* isolate with the corresponding mutant *fks1* genotype (DPL-1012) and a clinical unrelated *FKS1* wild-type isolate (CPH-T53911) were included for comparison. Echinocandin susceptibility was evaluated using the EUCAST species-specific MIC breakpoints for anidulafungin (≤0.06 mg/liter, susceptible [S], and >0.06 mg/liter, resistant [R], for *C. krusei* and ≤0.03 mg/liter, S, and >0.03 mg/liter, R, for *C. albicans*) and micafungin (≤0.016 mg/liter, S, and >0.016 mg/liter, R, for *C. albicans*) and the revised CLSI breakpoints for the interpretation of caspofungin Etest results (≤0.25 mg/liter, S, and >0.5 mg/liter, R, for both species) (25). The EUCAST micafungin breakpoints have not yet been established for *C. krusei*; therefore, the common epidemiological cutoff (ECOFF) value (0.25 mg/liter for *C. krusei*) was used to discriminate between wild-type (WT) and non-WT susceptibility (26).

The *C. krusei* isolate displayed wild-type susceptibility to AMB (MIC, 0.5 mg/liter) and azoles (fluconazole MIC, >16 mg/liter; voriconazole MIC, 0.25 mg/liter) but was classified as resistant to all three echinocandins, with ≥5, ≥3, and 6 MIC dilution steps above the defined clinical breakpoints for anidulafungin, micafungin, and caspofungin, respectively (Table 1). *FKS1* sequencing revealed a D662Y alteration in Fks1p of the resistant *C. krusei* isolate. In comparison, a corresponding D648Y alteration in *C. albicans* Fks1p was associated with minor decreases in susceptibility, with elevations of 1, 2, and 2 MIC dilution steps above the clinical breakpoints for anidulafungin, micafungin, and caspofungin, respectively (Table 1).

Wild-type *C. krusei* displays higher echinocandin MICs (most pronounced for micafungin) than does *C. albicans*, potentially related to the naturally occurring amino acid difference I660 in *C.*

Received 22 January 2014 **Returned for modification** 17 March 2014
Accepted 27 March 2014
Published ahead of print 31 March 2014
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doi:10.1128/AAC.02367-14

TABLE 1 Species, hot spot sequences, and relevant susceptibility data for the echinocandin-resistant *Candida krusei* isolate and relevant reference isolates

Isolate no.	Species	MICs (mg/liter) and susceptibility data according to[a]: EUCAST Anidulafungin	EUCAST Micafungin	Etest: caspofungin	Fks1p hot spot 1[b] (aa no.-sequence)
ATCC 6258	*C. krusei*	0.03, S	0.125, WT	0.5, I	655-FLILSIRDP
CPH-T5842	*C. krusei*	>1, R (≥5)	>1, non-WT (≥3)	16, R (6)	655-FLILSI**R**Y**P**
CPH-T53911	*C. albicans*	0.008, S	0.008, S	0.125, S	641-FLTLSLRDP
DPL-1012	*C. albicans*	0.06, R (1)	0.06, R (2)	1, R (2)	641-FLTLSLR**Y**P

[a] Dilution steps above clinical breakpoints (anidulafungin and caspofungin) or ECOFF (micafungin) are shown in parentheses. S, susceptible; R, resistant; I, intermediate.
[b] The intrinsic amino acid (aa) isoleucine (I660) uniquely found in the *C. krusei* Fks1p hot spot region 1 is underlined, and the *FKS*-encoded substitution is in bold type.

krusei corresponding to L646 in *C. albicans* (Table 1) in the hot spot region of Fks1p not found in other *Candida* species. Hence, hot spot mutations in *C. krusei* may be of particular clinical importance. To our knowledge, the D662Y alteration has not been described in *C. krusei* (2, 12, 13, 22, 27, 28). The equivalent amino acid substitution has been described in a clinical isolate of *C. albicans* (D648Y) to be associated with intermediate MICs, as also demonstrated in this study (29). Similar discrete MIC elevations were found in *Candida glabrata* with the equivalent substitution D632Y (9, 30), while the combination with a nonsense mutation (D632Y plus R1377STOP) was associated with high echinocandin resistance (31). It is evident that the phenotypic resistance is dependent both on species and on *FKS* genotype, signifying the difficulties in therapeutic management when encountering acquired resistance. Nonetheless, the significantly elevated MICs for anidulafungin and caspofungin above the clinical breakpoints and the micafungin MICs above the ECOFF indicate that echinocandins are very unlikely to be effective against this *C. krusei* mutant strain (30). The MICs for the resistant isolate may even be underestimated due to the truncated upper end of the concentration range for anidulafungin and micafungin (2, 16, 30).

In conclusion, this case represents another breakthrough infection where the previous use of fluconazole prophylaxis probably selected for *C. krusei*, which in turn developed resistance to echinocandins while the patient was on therapy with caspofungin. This emphasizes the crucial importance of susceptibility testing for appropriate patient management, particularly for echinocandin-exposed patients. Future dissection of the hot spots of *FKS1* and *FKS2* may provide further knowledge on the interplay between *Candida* species and individual *FKS* mutations and their associated susceptibilities to echinocandin *in vitro* and *in vivo*. Due to the low rates of positive blood cultures, particularly during treatment, it may also highlight the potential value of molecular methods in detecting resistance genotypes directly from primary patient samples. This is specifically important in patients not responding to treatment, but it requires a species-specific algorithm developed for the translation of each unique alteration into a clinically meaningful susceptibility profile (32, 33).

ACKNOWLEDGMENTS

We thank Birgit Brandt for her invaluable technical assistance in the laboratory.

This study is supported by Gilead and the 2013 ESCMID research grant. D.S.P. is supported by the NIH and receives grant funding from Merck, Pfizer, and Astellas.

R.H.J has received travel grants from Pfizer, Gilead, Astellas, and MSD. M.C.A. has received research grants from Astellas, Gilead, MSD, and Pfizer, has been an advisor or consultant for Gilead, MSD, and Pfizer, and has received speakers' honoraria from Astellas, Gilead, MSD, and Pfizer for talks. U.S.J. and A.R. have received travel grants from Pfizer.

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