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 DDef 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.
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 (D632Y plus R1377STOP) was associated with high echinocandin resistance (31).

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).

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REFERENCES


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

<table>
<thead>
<tr>
<th>Isolate no.</th>
<th>Species</th>
<th>MICs (mg/liter) and susceptibility data according toa:</th>
<th>Fks1p hot spot b (aa no.-sequence)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATCC-6258</td>
<td>C. krusei</td>
<td>0.03, S 0.125, WT 0.5, I 655-FLTLSLRDP</td>
<td>E343–E353. <a href="http://dx.doi.org/10.1086/596757">http://dx.doi.org/10.1086/596757</a>.</td>
</tr>
<tr>
<td>CPH-T5842</td>
<td>C. krusei</td>
<td>&gt;1, R (≥5) &gt;1, non-WT (≥3) 16, R (6) 655-FLTLSLRYP</td>
<td>D648Y substitutión is in bold type.</td>
</tr>
<tr>
<td>CPH-T53911</td>
<td>C. albicans</td>
<td>0.008, S 0.008, S 0.125, S 641-FLTLSLRDP</td>
<td>E343–E353. <a href="http://dx.doi.org/10.1086/596757">http://dx.doi.org/10.1086/596757</a>.</td>
</tr>
<tr>
<td>DPL-1012</td>
<td>C. albicans</td>
<td>0.06, R (1) 0.06, R (2) 1, R (2) 641-FLTLSLRYP</td>
<td>D648Y substitutión is in bold type.</td>
</tr>
</tbody>
</table>

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 Fks1p hot spot region 1 is underlined, and the FKS-encoded substitution is in bold type.

Echinocandin-Resistant C. krusei Related to FKS1


