Novel FKS Mutations Associated with Echinocandin Resistance in Candida Species

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We studied three clinical isolates of Candida spp. (one C. tropicalis isolate and two C. glabrata isolates) from patients with invasive candidiasis. The first isolate emerged during echinocandin treatment, while the others emerged after the same treatment. These strains harbored an amino acid substitution in Fksp never linked before with reduced echinocandin susceptibility in C. tropicalis or in C. glabrata. The molecular mechanism of reduced susceptibility was confirmed using a 1,3-β-d-glucan synthase inhibition assay.

Since the introduction of the echinocandin drugs in 2001, these antifungals are becoming the preferred choice for invasive candidiasis treatment (13, 14, 16, 24). Candida tropicalis and Candida glabrata are important pathogens causing invasive disease, especially in immunocompromised patients (12, 22). Echinocandin drugs target the Fksp subunits of the 1,3-β-d-glucan synthase complex and inhibit fungal cell wall biosynthesis (4). Echinocandin clinical failures are rare events. Yet, as the number of patients exposed to echinocandin therapy increases, the probability for resistance increases. In all the published cases of C. tropicalis and C. glabrata with a reduced echinocandin susceptibility (RES) phenotype, amino acid substitutions in two conserved regions of the Fksp (hot spots) were reported (2, 6, 11, 21, 25).

From March 2004 to March 2009, 285 cases of candidemia were identified at Summa Health System’s hospitals. Eighty-nine cases were identified as C. glabrata and 17 as C. tropicalis. During this period, two C. glabrata isolates and one C. tropicalis isolate were considered nonsusceptible to caspofungin by the CLSI susceptibility breakpoint (MIC ≥ 2 μg/ml) (3, 23), representing an RES incidence of 2.2% (2/89) and 5.8% (1/17) for C. glabrata and C. tropicalis, respectively. In this work, we describe the molecular mechanism responsible for the RES phenotype observed in these three clinical cases.

Case 1 (C. tropicalis strain CT-C1). A 28-year-old female diabetic and renal transplant recipient showed erosive esophagitis. She developed respiratory failure (3rd hospital day) and was treated empirically with broad-spectrum antibacterials. Her tracheal aspirate culture was positive for C. tropicalis. She developed pancytopenia (9th hospital day) and was treated empirically with broad-spectrum antibacterials for 2 weeks. His pulmonary infiltrates progressed. His respiratory cultures showed C. glabrata and C. albicans. Anidulafungin (100 mg/daily) was started (21st hospital day) and continued for 3 weeks, with no clinical improvement. After a respiratory failure, his blood and urine cultures were positive for Klebsiella pneumoniae and C. glabrata (>100,000 CFU/ml), respectively. Echinocandin MICs were determined (Table 1), and the treatment was switched to liposomal amphotericin B, with significant clinical improvement.

Characterisation of clinical isolates. Susceptibility testing was performed according to the recommendations of CLSI document M27-A3 (3). C. glabrata ATCC 90030, C. tropicalis ATCC 750, C. albicans ATCC 90028, C. albicans 205, and C. albicans 177 (3, 7) were used to compare the MICs for our clinical isolates. The strains CT-C1, CG-C2, and CG-C3 showed 16- to >500-fold-higher MICs than the reference strains (Table 1). 1,3-β-d-Glucan synthase complexes were isolated, and inhibition kinetics values (50% inhibitory concentrations [IC50]) were obtained to confirm the RES phenotype.
Clinical C. albicans region of the Fksp associated with RES in (11, 18) and are located in the highly conserved hot spot 1. However, equivalent substitutions were described for C. albicans (strains 177 and 205) (Table 1). Also, the 1,3-D-glucan synthase complexes isolated from our strains showed decreased susceptibility testing and 1,3-D-glucan synthase complex inhibition profiles for anidulafungin, caspofungin, and micafungin drugs, elevated MIC, and echinocandin clinical failure.

In summary, we report three patients who developed invasive Candida species infection despite echinocandin treatment, showing that RES is an emerging problem as echinocandin use continues to increase (2, 6, 8–11, 15, 18–20, 25). Furthermore, we demonstrate the clear linkage between Fksp substitutions, 1,3-D-glucan synthase complex resistance to echinocandin drugs, elevated MIC, and echinocandin clinical failure.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Organism</th>
<th>Wild-type FKS or FKS with nucleotide substitution</th>
<th>Fksp hot spot 1a</th>
<th>MIC (µg/ml)b</th>
<th>IC50 (µg/ml)c</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ANF</td>
<td>CSF</td>
</tr>
<tr>
<td>ATCC 750</td>
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<td>Wild type</td>
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<td>CG-C2</td>
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<td>FLIIIPLDRDP</td>
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<td>CG-C3</td>
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<td>205</td>
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<td>1.33</td>
<td>8.00</td>
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</tbody>
</table>

a Bold letters represent amino acid changes. Shown are the sequences of Fks1p hot spot 1 for all strains except 03-Cg, in which the mutation was present in Fks2p.
b MIC values represent the geometric means of results from three repetitions.
c IC50 values represent the arithmetical means of results from three repetitions.
d ANF, anidulafungin; CSF, caspofungin; MCF, micafungin.

The C. glabrata FKS1 and FKS2 genes (GenBank accession no. XM_446406 and XM_448401, respectively) from strains CG-C2 and CG-C3 were sequenced. These isolates showed S629P and S663P amino acid substitutions in Fks1p and Fks2p, respectively (Table 1). These amino acid substitutions have not been previously reported for C. tropicalis or C. glabrata. However, equivalent substitutions were described for C. albicans (7, 11, 18) and are located in the highly conserved hot spot 1 region of the Fksp associated with RES in Candida spp. (21).

Our isolates showed MIC values comparable with those of clinical C. albicans strains harboring equivalent FKS mutations (strains 177 and 205) (Table 1). Also, the 1,3-D-glucan synthase complexes isolated from our strains showed decreased echinocandin sensitivity (a higher IC50), demonstrating that FKS hot spot mutations are sufficient and necessary to produce an RES phenotype. However, the IC50 shifts did not show a strict correlation with MIC values, especially for the 01-Ct strain. This phenomenon was observed before for C. albicans and C. tropicalis, suggesting that factors other than 1,3-D-glucan synthase complex inhibition may influence echinocandin MIC values (1, 6, 7, 17).

FIG. 1. Echinocandin inhibition profiles for product-entrapped 1,3-D-glucan synthase complexes obtained using a sigmoidal-response (variable-slope) curve. Echinocandin inhibition kinetics yielding 50% inhibitory concentrations (IC50) were obtained and are expressed in nanograms per milliliter. (A) Inhibition curves and 50% inhibitory concentrations (IC50) for micafungin (MCF) and for 1,3-D-glucan synthase complexes obtained from reference C. tropicalis ATCC 750 (750) and C. tropicalis CT-C1 strains. (B) Anidulafungin (ANF) titration curves for 1,3-D-glucan synthase complexes isolated from reference C. glabrata ATCC 90030, C. glabrata CG-C2, and C. glabrata CG-C3 strains.
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REFERENCES


