Rapid Emergence of Echinocandin Resistance in *Candida glabrata* Resulting in Clinical and Microbiologic Failure.

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Running Title: Rapid Echinocandin Resistance in *Candida glabrata*

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ABSTRACT: We report a case of *Candida glabrata* candidemia that developed resistance to micafungin within 8 days of starting therapy in a patient without previous echinocandin exposure or other known risk factors for clinical or microbiological failure. Pre- and post-resistant isolates were confirmed to be isogenic, and sequencing of hot spots known to confer echinocandin resistance revealed a phenylalanine deletion at codon 659 within *FKS2*. 
Echinocandins are now first line agents for the treatment of invasive candidiasis (1-4). Their fungicidal activity, tolerability, perceived lack of resistance issues, and clinical superiority over fluconazole for candidemia have made them a cornerstone of anti-Candida therapy (5). However, there are increasing reports of Candida spp. becoming resistant to the echinocandins, usually in immunocompromised patients receiving prolonged therapy (6, 7). In addition, there is debate over the recent changes to the CLSI echinocandin breakpoints for Candida spp., which significantly lowered the threshold for resistance for many species including C. glabrata (8). We describe a case of rapid emergence of echinocandin resistance and clinical failure in a patient with C. glabrata fungemia who lacked the usual risk factors for echinocandin resistance (6, 9, 10).

Case. A female patient in her mid 50s presented to the emergency department with acute pyelonephritis and persistent vomiting for the past 6 days. Her medical history was significant for a cerebrovascular accident 5 years ago, poorly controlled type 2 diabetes, and hypertension. Blood and urine cultures obtained in the emergency department grew E. coli that was resistant to ciprofloxacin and trimethoprim-sulfamethoxazole. In the emergency department her blood glucose was >500mg/dL, she was treated for an anion gap metabolic acidosis, and she received vancomycin and piperacillin-tazobactam. She improved initially but was transferred to the medical intensive care unit (MICU) due to hypotension and respiratory failure requiring intubation. On hospital day 3, antibiotics were deescalated to ceftriaxone when culture and susceptibility results returned. The patient developed acute kidney injury that required dialysis through a left femoral vein catheter but otherwise experienced a relatively uncomplicated ICU stay. On HD 11 she was transferred to the medical service, but on HD 13 she developed fever to 102.9F and was restarted on vancomycin and piperacillin-tazobactam. No bacterial pathogens were isolated for the remainder of her hospital stay. The next day fluconazole 200mg daily was initiated. On HD 15 blood cultures from HD 13 were positive for Candida spp., which was later identified as C. glabrata using the YST card with Vitek 2 and was susceptible dose-dependent.
(SDD) to fluconazole with an MIC of 16mcg/mL and susceptible to micafungin, anidulafungin and caspofungin with MICs of 0.015mcg/mL, 0.06, and 0.06 respectively (Trek Sensititre YeastOne broth microdilution panel). On HD 15, fluconazole was discontinued and the patient was started on micafungin 100mg daily. The day the blood culture turned positive for yeast all vascular catheters were removed and bilateral thrombi in her internal jugular veins were identified. Blood cultures from HD14 and HD15 were also positive for *C. glabrata*. On HD 16 the patient acutely decompensated and was transferred back to the MICU. No blood cultures were drawn on HD 16 but cultures from HD 17 and 18 were negative. On HD 18 the patient was had a fever of 102.9° F and vascular catheters were again exchanged. A transesophageal echocardiogram, ophthalmologic exam, as well as ultrasound studies to evaluate for DVT were all negative. On HD23 blood cultures were performed due to a low grade fever of 99.4° F in dialysis, and subsequently became positive for *C. glabrata*. Susceptibility testing of the *C. glabrata* isolate from HD 23 revealed that the micafungin MIC had increased to 0.5mcg/mL. Anidulafungin and caspofungin MICs had also increased to 0.5 and 1.0 respectively while fluconazole remained SDD with an MIC of 8mcg/mL. Blood cultures from HD 26,27,30, and 33 were all eventually positive with *C. glabrata*. The blood cultures drawn on hospital day 34 remained negative. Fluconazole 400mg daily was added to the micafungin therapy on HD28 when the patient again became febrile and tachycardic. On HD 34 and 35 she developed a fever >102° F with severe rigors and chills and a WBC of 20,000/μl. An indium scan performed on HD 34 failed to identify any foci of infection and on HD 35 the micafungin and fluconazole were discontinued in favor of liposomal amphotericin B (L-AmB) at a dose of 5mg/kg daily. She then uneventfully completed 2 weeks of L-AmB therapy and was discharged home to complete 3 months of fluconazole therapy. All blood cultures drawn after hospital day 33 remained negative.

The *C. glabrata* isolates from HD13 prior to micafungin therapy (isolate CG1) and HD23 after 8 days of therapy (CG2) were obtained from the clinical microbiology laboratory. Genomic DNA was extracted,
and random amplification of polymorphic DNA (RAPD) analysis was performed using previously described primers (ERIC2, M13, OPE18, and OPA18) (11-14). RAPD profiles were compared with each other and against genomic DNA extracted from a control isolate (C. glabrata ATCC 2001) on 1.2% w/v agarose gels visualized with UV light following ethidium bromide staining. As shown in Figure 1, RAPD analysis strongly suggested strain isogenicity for isolates CG1 and CG2.

To identify mutations within conserved regions of genes encoding subunits of the glucan synthase enzyme complex known to confer echinocandin resistance, hot spot regions in C. glabrata FKS1 and FKS2 (GenBank accession no. XM-446406 and XM-448401) were amplified by PCR and purified (10, 15). The purified PCR product was then sequenced at the UTHCSA Advanced Nucleic Acids Core facility using Big Dye Terminator Cycle sequencing (Applied Biosystems, Inc., Foster City, CA) and analyzed with an ABI Prism 3100 genetic analyzer. Sequences were aligned using the MacVector software package (MacVector, Inc., Cary, NC) and compared to the control sequence (C. glabrata CBS138). The FKS1 hot spot 1 showed a single silent point mutation from the control strain. In CG2, the hot spot 1 and hot spot 2 regions of FKS2 both had base pair differences that also resulted in silent mutations. However, a three base pair deletion was also detected in hot spot 2 of FKS2 resulting in a single amino acid deletion (F659del) that did not further change the reading frame (Figure 2). Point mutations in the hot spot regions of FKS1 and FKS2 that result in amino acid changes have been shown to reduced the catalytic capacity of the (1,3)-β-d-glucan synthase enzyme complex and reduce echinocandin potency in C. glabrata (15). Although the deletion of phenylalanine at codon 659 has been associated with a weak phenotype in vitro, our case and those reported by others suggest that this mutation can be associated with microbiological resistance and clinical failure (6, 9). The linkage of the F659del mutation with resistance in other studies, and the isogenic nature of a sensitive isolate from the same patient that did not contain the deletion in our study, led us to conclude that the resistant strain was selected from the same population as the sensitive strain after echinocandin exposure.
Our case demonstrates that echinocandin resistance in *C. glabrata* can emerge rapidly, occurring in this patient within 8 days, and this type of resistance can occur in strains without previous echinocandin exposure that are not azole resistant. Other reports of clinical failure associated with *FKS* mutations in *C. glabrata* have noted previous or prolonged echinocandin exposure and severe immunosuppression as potential risk factors for microbiological failure (7, 9, 10). Our patient had none of these risk factors, though she did have uncontrolled diabetes, and rapidly developed clinical failure secondary to microbiological resistance. The rapid development of resistance in a patient without previous echinocandin exposure is surprising. In a recent report by Shields et al., patients with invasive candidiasis due to *C. glabrata* who failed therapy were significantly more likely to have prior echinocandin exposure or be infected with an isolate harboring an *FKS* mutation (9). In contrast, treatment failure was rare in patients without previous echinocandin exposure, and *FKS* mutations were only found in isolates collected from patients with previous exposure to this class of antifungals. Also of note, the new CLSI clinical breakpoint for micafungin against *C. glabrata* accurately reflected the clinical course of the patient, as the emergence of CG2 with a micafungin MIC of 0.5 mcg/mL resulted in both clinical and microbiologic failure and correctly predicted the presence of a mutation in the target *FKS* gene. This report highlights the need for clinicians and microbiologists to remain vigilant for the emergence of resistance in *C. glabrata* infections being managed with echinocandin therapy.
REFERENCES


Figure 1. RAPD gel patterns for C. glabrata isolates CG1, CG2, and control (C) obtained using primers ERIC2, M13, OPE-18, and OPA-18. DNA ladder (L) is shown in the left lane of each gel.
Figure 2: Timeline of days of positive blood cultures relative to antifungal therapy administered

Day 1  - Admission

Day 13  - Initial C. glabrata + blood cultures obtained

Day 14  - Fluconazole 200 mg daily initiated. Blood cultures + for C. glabrata

Day 15  - Fluconazole D/Cd – Micafungin 100 mg daily initiated. Blood cultures + for C. glabrata

Days 17 & 18 - Blood cultures negative. Patient remains on Micafungin 100 mg daily

Day 23  - Patient remains on Micafungin. Blood culture + for C. glabrata (Micafungin resistant)

Day 26  -
- All performed blood cultures + for C. glabrata
- Patient remains on Micafungin
- Fluconazole 400 mg daily started (day 28)

Day 35  - Blood cultures negative
- Micafungin and fluconazole discontinued
- Liposomal amphotericin B 5 mg/kg daily started