Amphotericin B Induction with Voriconazole Consolidation as Salvage Therapy for *FKS*-Associated Echinocandin Resistance in *Candida glabrata* Septic Arthritis and Osteomyelitis

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This Journal section presents a real, challenging case involving a multidrug-resistant organism. The case authors present the rationale for their therapeutic strategy and discuss the impact of mechanisms of resistance on clinical outcome. Two expert clinicians then provide a commentary on the case.

ABSTRACT We report the case of a 61-year-old female with Crohn's disease dependent on total parenteral nutrition who developed a central venous catheter bloodstream infection and septic arthritis, complicated further by osteomyelitis and persistent *Candida glabrata* fungemia. Fluconazole treatment led to persistent infection, and micafungin therapy failed with development of *FKS*-associated resistance. Infection responded after initiation of amphotericin B plus voriconazole. Echinocandin resistance is increasingly recognized, suggesting a role for alternative antifungal therapies.

KEYWORDS *Candida glabrata*, *FKS* mutation, echinocandin

CASE PRESENTATION

A 61-year-old Caucasian American female with short bowel syndrome (SBS) secondary to fistulizing Crohn's underwent arthroscopic incision and drainage for left knee septic arthritis (defined as day 1) (Fig. 1). She was dependent on total parenteral nutrition (TPN) provided through a peripherally inserted central venous catheter (PICC). On day 4, *Candida glabrata* was recovered from blood (Bactec 9240; Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and from bone and synovial fluid cultures (BBL; Becton, Dickinson and Company, Franklin Lakes, NJ). Her empirical regimen of vancomycin and piperacillin-tazobactam was changed to micafungin 100 mg administered intravenously (i.v.) every 24 h. All subsequent blood cultures remained negative, and routine susceptibility testing was not performed as she had no prior antifungal therapy exposure. On day 8, she received a fluconazole 800-mg loading dose administered i.v. followed by 400 mg i.v. every 24 h as maintenance therapy. Intravenous dosing was continued due to potentially reduced oral therapy absorption concerns with her underlying SBS. The central venous catheter was exchanged over a guidewire, and she was discharged on day 10 in stable clinical condition to complete a 6-month course of therapy.

The patient was readmitted 395 days later for an undifferentiated fever and left knee effusion. Arthroscopic needle aspirate revealed growth of *C. glabrata*, which prompted reinitiation of micafungin 100 mg administered i.v. every 24 h. She again underwent arthroscopic incision and drainage. Antifungal susceptibility testing (Sensititre Yeast

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Timeline of major clinical events

Day (s)	Event(s)
Day 1	Initial hospitalization and arthroscopic incision and drainage
Day 4	Isolation of <i>Candida glabrata</i> and initiation of micafungin
Day 8	Conversion to intravenous fluconazole
Day 10	Exchange of PICC line, hospital discharge, and continued fluconazole
Day 180	Completion of fluconazole therapy
Day 395	Hospital readmission, isolation of <i>Candida glabrata</i> , and initiation of micafungin
Day 400	Repeat arthroscopic incision and drainage
Day 401	Repeat arthroscopic incision and drainage
Day 404	Hospital discharge and continue micafungin
Day 490	Hospital readmission, isolation of <i>Candida glabrata</i> , and conversion to fluconazole
Day 520	Demonstration of FKS mutation and initiation of induction liposomal amphotericin B
Day 550	Conversion to maintenance intravenous voriconazole
Day 730	Completion of voriconazole

FIG 1 Timeline of major clinical events.

One; Trek Diagnostic Systems, United Kingdom) of the synovial fluid culture initially identified *C. glabrata* with amphotericin B, fluconazole, posaconazole, and voriconazole MICs of 0.50 $\mu\text{g/ml}$, 8.00 $\mu\text{g/ml}$ (dose-dependently susceptible), 1.00 $\mu\text{g/ml}$ (susceptible), and 0.25 $\mu\text{g/ml}$, respectively. Bone cultures again demonstrated growth of *C. glabrata*. On day 404, she was discharged with a planned duration of micafungin therapy of 6 to 12 months. After 86 days of micafungin, the patient was again readmitted with increased pain and effusion and subsequently underwent removal of aspirate from the left knee through the use of an arthroscopic needle, with synovial fluid cultures again demonstrating growth of *C. glabrata*. Antifungal susceptibility testing was repeated, and levels of sensitivity to anidulafungin and micafungin were found to be intermediate (MICs of 0.25 $\mu\text{g/ml}$ and 0.12 $\mu\text{g/ml}$, respectively), while the level of sensitivity to caspofungin showed resistance (MIC of 1.00 $\mu\text{g/ml}$) (Table 1). At that time, micafungin was discontinued, and fluconazole treatment was initiated (800 mg i.v. administered once, followed by 400 mg every 24 h).

CHALLENGE QUESTION

Which antimicrobial(s) would be best for the patient presented in the case?

- Amphotericin B
- Amphotericin B plus posaconazole
- Amphotericin B plus voriconazole
- Posaconazole
- Voriconazole

TABLE 1 Antifungal susceptibility profile^a

Agent	Antifungal class	MIC ($\mu\text{g/ml}$) for 1 st synovial fluid cultures	Interpretation ^b	MIC ($\mu\text{g/ml}$) for 2 nd synovial fluid cultures	Interpretation ^b
Amphotericin B	Polyene	0.50	NA	0.50	NA
Anidulafungin	Echinocandin	ND	ND	0.25	Intermediate
Caspofungin	Echinocandin	ND	ND	1.00	Resistant
Fluconazole	Azole	8.00	Dose-dependently sensitive	2.00	Dose-dependently sensitive
Itraconazole	Azole	ND	ND	0.25	Dose-dependently sensitive
Micafungin	Echinocandin	ND	ND	0.12	Intermediate
Posaconazole	Azole	1.00	Sensitive	0.25	Sensitive
Voriconazole	Azole	0.25	NA	0.12	NA

^aNA, no interpretive criteria; ND, no data.

^bReference: Clinical and Laboratory Standards Institute. M60: performance standards for antifungal susceptibility testing of yeasts; supplement—1st ed.; CLSI, Wayne, PA, USA, 2017.

TABLE 2 *C. glabrata* FKS mutation sequence results^a

Location	Nucleotide change	Amino acid change
FKS1 hot spot 1	Wild type	Wild type
FKS1 hot spot 2	T4314C	Y1438Y (wild type)
FKS2 hot spot 1	T1976A	F659Y
FKS2 hot spot 1	T2119C	L707L (wild type)
FKS2 hot spot 1	A2358G	L786L (wild type)
FKS2 hot spot 2	C4104T	N1368N (wild type)
FKS2 hot spot 2	G4320A	V1440V (wild type)

^aF, phenylalanine; Y, tyrosine.

TREATMENT AND OUTCOME

Molecular testing was performed for FKS mutations and demonstrated the presence of FKS2 hot spot 1 mutation F659Y associated with echinocandin resistance (Table 2). Amphotericin B lipid complex (LAMB) treatment (5 mg/kg of body weight/dose i.v. every 24 h) was initiated. These dynamics created a challenging clinical dilemma, in which a seemingly unresolvable conflict existed between antifungal MIC susceptibility data, FKS mutation testing results, persistent *C. glabrata* infection, and a failing antifungal regimen.

Candida glabrata has emerged as the second leading pathogen associated with candidemia, surpassing *C. parapsilosis* and *C. tropicalis* (1–5). Intrinsic low-level and acquired high-level azole (e.g., fluconazole) resistance has led to recommendations by the Infectious Diseases Society of America (IDSA) for preferential use of an echinocandin (e.g., anidulafungin, caspofungin, and micafungin) as primary therapy for treatment of *C. glabrata* infections (2, 6, 7). Echinocandins are fungicidal lipopeptides that inhibit synthesis of the major cell wall component β -(1,3)-D-glucan by noncompetitive binding to the multimeric catalytic functional subunit of 1,3- β -D-glucan synthase protein complex encoded by three putative related genes, *FKS1*, *FKS2*, and *FKS3* (named for FK506 hypersensitivity originally discovered in *C. albicans* and *Saccharomyces cerevisiae*) (1–8). The haploid nature of *C. glabrata* is associated with a greater propensity for acquiring resistant mutations, and *C. glabrata* is the first species for which resistance to echinocandins has been detected (1–10). Diploid species, such as *C. albicans* and *C. krusei*, can display silent (heterozygous) mutations or clinically significant (homozygous) mutations (4, 7, 9, 10).

Mutations in the *FKS1* and *FKS2* genes associated with reduced susceptibilities to the echinocandin class occur in two highly conserved regions, of eight and nine amino acids, designated hot spot 1 and hot spot 2 (1–10). In general, amino acid substitutions decrease enzyme velocity (V_{max}) and increase echinocandin MICs and 50% inhibitory concentrations (IC_{50}) but do not change the enzyme drug binding affinity (K_m) (1). Among the reported mutations, not all are of equal levels of clinical significance (e.g., correlation with MICs) or of fitness cost to the organism (1, 2, 4, 8–10). Echinocandin-resistant *C. glabrata* isolates can be detected by standard methodologies (e.g., broth microdilution or disk diffusion assay) with guidance from the Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) and by utilizing current epidemiologic MIC distribution, pharmacokinetic, and pharmacodynamic data and analysis of the presence or absence of FKS mutations (3–6, 8–10). Molecular methods (e.g., PCR, molecular beacon probe-based melting curve assays) are the only reliable means for mutation detection that also show improved laboratory turnaround time as well as reduced interlaboratory MIC data variability (1–5, 8).

Population-based estimates of the frequencies of *C. glabrata* isolates harboring FKS mutations are limited; however, rates of 3.3% to 31.1% have been reported (2–4, 8–10). Coresistance to fluconazole and echinocandin agents has been reported in as many as 10% to 11% of *C. glabrata* isolates (3, 6, 8). Among patients receiving an echinocandin (e.g., for treatment of breakthrough infections), rates have ranged between 8% and 32% (2, 3, 7, 8, 10). Patients were more likely to have breakthrough infections with an

FKS mutant species if they had a prior episode of *C. glabrata* candidemia, echinocandin exposure with initial candidemia, TPN within 30 days of infection, and an underlying gastrointestinal disorder or surgical procedure (3, 7, 8). The patient described in this case was intermittently subjected to 94 total days of micafungin treatment and also had episodes of recurrent septic arthritis infection before phenotypic and genotypic resistance was documented (Tables 1 and 2). It is likely that the patient's long-term need for TPN and indwelling central venous catheter may have created the initial infection; however, the underlying septic arthritis and osteomyelitis were most likely responsible for the persistent nidus of infection. Her underlying gastrointestinal disorder may also be a contributing factor in the emergence of *FKS* mutant *C. glabrata* subpopulations. Finally, fluconazole monotherapy could have also contributed to the development of resistance.

There are no published guidelines or controlled trial data to assist clinicians with optimal therapy for treatment of *FKS* mutant *C. glabrata* infections. Clinical data on the use of amphotericin B and voriconazole for these types of infections are limited. Alexander and colleagues evaluated the outcome of antifungal therapy in a 10-year retrospective study among 21 patient episodes of *FKS* mutant *C. glabrata* infections and reported that 66.7% of patients had responded to monotherapy with various agents (amphotericin B, caspofungin, micafungin, or voriconazole) (7). Those authors also reported that 35.7% of the patients who initially responded to monotherapy suffered relapses (7). One patient episode, involving the F659V mutation, was reported to have recurred after LAMB monotherapy but responded to the combination of LAMB and posaconazole (7). Our case patient's *FKS* mutant *C. glabrata* isolate was found to harbor a F659Y mutation, which has been previously described (1, 2, 4, 8–10). Therefore, on the basis of this information as well as concerns regarding oral absorption, LAMB treatment was initiated for 4 weeks followed by 6 months of voriconazole treatment. All subsequent cultures remained negative.

After prior therapies had failed due to resistance, LAMB treatment followed by voriconazole salvage therapy contributed to a positive clinical outcome and created a stable condition for our patient with persistent *C. glabrata* infection. Since a robust head-to-head comparison between antifungal agents is unlikely given the relative rarity of infections and host complexity, observational reports will need to be used to inform decisions regarding how and when to administer these agents most effectively. Although additional studies are needed to validate the optimal approach to treatment of *FKS* mutant *C. glabrata* infections, treatment using LAMB with or without voriconazole appears to be a reasonable option.

COMMENTARY

Antifungal resistance has become an emerging global phenomenon, most notably among *Candida auris* and other nonalbicans *Candida* species, causing concern not only among clinicians but also among hospital epidemiologists and infection prevention specialists. The absence of rapid testing for the diagnosis of invasive candidiasis and for routine susceptibility testing presents a significant impediment to the timely and appropriate management of this condition. Moreover, the judicious and timely use of antifungal therapy is critical to the effective management of IC, and the overuse of these antifungal compounds can lead to acquired resistance (7, 11).

Wright and colleagues report an interesting case of recurrent septic arthritis due to *C. glabrata* which developed progressive antifungal resistance while the patient was receiving prolonged therapy. The case underscores multiple challenges in approaching this emerging problem. Who is at risk? How does one limit the emergence of this phenomenon? What is the best management regimen for invasive candidiasis in the setting of echinocandin resistance?

C. glabrata has emerged as the second most common cause of candidemia and invasive candidiasis in much of the developed world (12). It is also the species most likely to demonstrate echinocandin resistance, thus strongly suggesting that the sce-

nario described in the case report will become more common. The patient described here demonstrated many of the features that one would expect in a clinical situation that results in breakthrough infection with echinocandin resistance, in particular, immunosuppression and long-term antifungal usage (13, 14). Prolonged and/or repeated exposure appears to have a significant influence on the development of both phenotypic and genotypic echinocandin resistance.

The clinician is faced with at least two dilemmas. What constitutes too much exposure? Is prophylactic use of echinocandins a risk factor for the development of resistance? The answer to the first question is unclear; the answer to the second is an unequivocal “yes.” The increased reliance on echinocandins for antifungal prophylaxis in high-risk patients raises substantial concern over the development of increasing rates of resistance.

The recent global emphasis on the emergence of antibacterial resistance has resulted in a concerted effort in many regions of the world to decrease the frequency and duration of antibacterial therapy under a variety of conditions, often in the form of adopting an approach that provides a minimum duration of therapy for common disorders (e.g., 5 days of therapy for community-acquired pneumonia) (15). These recommendations are driven by concerns over increasing rates of resistance for common bacterial pathogens and the specter of multidrug-resistant organisms. Perhaps the time has come for the mycology world to adopt a “less is more” approach to many invasive fungal infections. In an era of heightened focus on antimicrobial stewardship, one cannot deny that antifungal stewardship has lagged behind in our efforts to rein in the use of all antimicrobials.

Data pertaining to treatment duration for fungal infections are scarce. In the recent Infectious Diseases Society of America treatment guidelines for invasive candidiasis, most duration recommendations are based on low-quality evidence (11). With regard to the case reported here, there is a wide range of recommendations of duration of therapy for osteomyelitis (6 to 12 months), based almost entirely on anecdotal evidence, and thus a large potential window for improvement and limiting unnecessary exposure to antifungals and promotion of resistance.

In order to move forward, several issues must first be addressed. We must develop reliable rapid non-culture-based testing for invasive candidiasis. The recent development of the T2Candida assay is a step forward, but that assay would ideally be paired with rapid molecular-method-based susceptibility testing. Until such methods are available, other innovative methods must suffice. For instance, emerging methods of subculturing of positive blood culture broth directly onto Etest strips or anidulafungin-impregnated agar can more rapidly identify resistance (16). Surrogate markers to determine response to therapy in clinical trials, as opposed to more traditional clinical or culture based methods, are critically needed. Finally, new antifungal compounds with new mechanisms of action to combat the rising tide of antifungal resistance are desperately needed.

There is therapeutic help on the way. There are currently several new antifungals under development with activity versus antifungal-resistant *Candida* species. Two of these agents are now in clinical trials. Ibrexafungerp (SCY078) is an oral glucan synthase inhibitor with activity versus most *Candida* species, including many echinocandin-resistant isolates (17). APX001 (formerly E1210) is a novel agent that inhibits the Gwt1 enzyme responsible for cross-linking cell wall mannoproteins to β -1,6-glucan compromising the structural integrity. It also retains activity in the setting of echinocandin resistance (18). These are promising new agents, but the battle against the emergence of antifungal resistance will be won or lost by taking an integrated approach to invasive candidiasis: using thoughtful stewardship of new and existing agents guided by more meaningful clinical and diagnostic data.

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