MAJOR ARTICLE

# Increasing Echinocandin Resistance in *Candida glabrata*: Clinical Failure Correlates With Presence of *FKS* Mutations and Elevated Minimum Inhibitory Concentrations

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**(See the Editorial Commentary by Ostrosky-Zeichne on pages 1733–4.)**

***Background.*** Fluconazole (FLC) resistance is common in *C. glabrata* and echinocandins are often used as first-line therapy. Resistance to echinocandin therapy has been associated with *FKS1* and *FKS2* gene alterations.

***Methods.*** We reviewed records of all patients with *C. glabrata* bloodstream infection at Duke Hospital over the past decade (2001–2010) and correlated treatment outcome with minimum inhibitory concentration (MIC) results and the presence of *FKS* gene mutations. For each isolate, MICs to FLC and echinocandins (anidulafungin, caspofungin, and micafungin) and *FKS1* and *FKS2* gene sequences were determined.

***Results.*** Two hundred ninety-three episodes (313 isolates) of *C. glabrata* bloodstream infection were analyzed. Resistance to echinocandins increased from 4.9% to 12.3% and to FLC from 18% to 30% between 2001 and 2010, respectively. Among the 78 FLC resistant isolates, 14.1% were resistant to 1 or more echinocandin. Twenty-five (7.9%) isolates harbored a *FKS* mutation. The predictor of a *FKS* mutant strain was prior echinocandin therapy (stepwise multivariable analysis, odds ratio, 19.647 [95% confidence interval, 7.19–58.1]). Eighty percent (8/10) of patients infected with *FKS* mutants demonstrating intermediate or resistant MICs to an echinocandin and treated with an echinocandin failed to respond or responded initially but experienced a recurrence.

***Conclusions.*** Echinocandin resistance is increasing, including among FLC-resistant isolates. The new Clinical and Laboratory Standards Institute clinical breakpoints differentiate wild-type from *C. glabrata* strains bearing clinically significant *FKS1*/*FKS2* mutations. These observations underscore the importance of knowing the local epidemiology and resistance patterns for *Candida* within institutions and susceptibility testing of echinocandins for *C. glabrata* to guide therapeutic decision making.

***Keywords.*** *Candida glabrata*; echinocandin; susceptibility; resistance; micafungin.

Echinocandins are frequently used as first-line therapy for invasive candidiasis because of their favorable safety profile and broad-spectrum anti-*Candida* activity [1]. In 2007, the Clinical and Laboratory Standards Institute (CLSI) proposed clinical interpretive breakpoints (CBPs) for minimum inhibitory concentration (MIC) testing of the echinocandins (anidulafungin [ANF], caspofungin [CSF], and micafungin [MCF]) against *Candida* species [2]. The CBP for susceptibility was an MIC $\leq 2$ μg/mL for all 3 echinocandins and all species of *Candida* [3]. A resistant breakpoint was not definable owing to the lack of echinocandin resistance in the population of *Candida* isolates at that time. Over time it became apparent that a CBP of $\leq 2$ μg/mL might not

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reliably identify isolates with resistance mechanisms associated with treatment failure [4–6].

Echinocandins inhibit β-(1,3)-D-glucan synthase (GS) by targeting GS *FKS* subunits, which are encoded by 3 genes, *FKS1*, *FKS2*, and *FKS3* [7–9]. Resistance to echinocandin therapy has been associated with amino acid substitutions in *FKS1* (*Candida albicans*, *Candida tropicalis*, *Candida krusei*, *Candida glabrata*) and *FKS2* (*C. glabrata*) [5, 10–14]. Notably, *Candida* isolates with mutations in *FKS1* and/or *FKS2* genes recovered from patients with clinically resistant infections have not necessarily had echinocandin MICs >2 μg/mL [4–6, 11, 14–24]; kinetic studies of the GS enzyme complex suggested that the pattern of decreased enzyme sensitivity to inhibition in FKS mutants extended across all 3 echinocandins and that a lower MIC cutoff (0.25–0.5 μg/mL) would be more sensitive in detecting strains with *FKS1/FKS2* mutations [5, 6, 25]. Although treatment outcome information was available for only 48 (ANF), 49 (CSF), and 33 (MCF) *C. glabrata*–invasive infection episodes at the time, the CLSI lowered echinocandin CBPs for *Candida* species to susceptibility at ≤0.12 μg/mL for ANF and CSF and ≤0.06 μg/mL for MCF to ensure maximal detection of *FKS* mutant strains among clinical isolates [23].

To further examine echinocandin resistance in *C. glabrata*, we reviewed clinical data on all patients who developed *C. glabrata* bloodstream infection (BSI) at Duke Hospital over a 10-year period (2001–2010) and correlated treatment outcome with MICs and the presence of *FKS* gene alterations.

## MATERIALS AND METHODS

### Patients and Microbiologic Methods

The Duke Hospital institutional review board approved this retrospective cohort study. Subjects were identified via review of microbiology laboratory culture logs. Subject information was obtained by medical records review. MICs to fluconazole (FLC) and all echinocandins were determined by the CLSI broth microdilution method [2]. If >1 phenotype was noted on the culture, colonies representing each phenotype were tested. *Candida glabrata* genomic DNA was extracted from cells grown in YPD (2% yeast extract, 4% Bacto peptone, 4% dextrose) broth medium with a Q-Biogene (Irvine, California) FastDNA kit. Hot spots 1 and 2 of *FKS1* and *FKS2* were amplified using polymerase chain reaction (PCR), as previously described [5]. Standard Sanger DNA sequencing of purified PCR amplicons was performed with a CEQ Dye Terminator Cycle Sequencing Quick Start Kit (Beckman Coulter, Fullerton, California) according to the manufacturer's recommendations. Sequence analysis was performed with CEQ 8000 Genetic Analysis System Software (Beckman Coulter) and BioEdit Sequence Alignment Editor (Ibis Therapeutics, Carlsbad, California). The following definitions were applied:

- Directed antifungal therapy: antifungal administered after the yeast recovered was identified as *C. glabrata*.
- Episode of infection: >14 days since last positive blood culture with *C. glabrata*. The first and last (if recovered >7 days after the first) blood isolates from each patient episode were tested when available.
- Breakthrough candidemia: blood culture collected after ≥3 days of antifungal therapy positive for *C. glabrata*.
- CLSI CBPs, resistant: ANF and CSF ≥0.5 μg/mL, MCF ≥0.25 μg/mL, FLC ≥64 μg/mL; susceptible: ANF and CSF ≤0.12 μg/mL, MCF ≤0.06 μg/mL.
- Initial therapy: first antifungal administered on or after collection date of first positive blood culture.
- Mortality outcome: all-cause mortality within 10 and 30 days of directed antifungal therapy.
- Overall clinical outcome: documented negative blood culture within 10 and 30 days of directed antifungal therapy and no relapse or recurrence of *C. glabrata* BSI.
- Persistence: failure to clear blood after ≥5 days of directed antifungal therapy.
- Recurrence: blood culture positive for *C. glabrata* after initially clearing blood but while still on antifungal therapy.
- Relapse: blood culture positive for *C. glabrata* after completing the planned directed course of therapy for an initial *C. glabrata* BSI.
- Successful treatment outcome: negative blood culture within 10 and 30 days of directed antifungal therapy.

### Statistical Methods

Descriptive statistics were used to characterize the study population. Univariable analysis was performed for the association of clinical/microbiologic variables and the outcomes of 10- and 30-day treatment success, 10- and 30-day mortality, and presence of an *FKS* mutant strain, an FLC-resistant strain, and an echinocandin (CSF or MCF assessed independently)–resistant strain. Chi-square tests of association were performed for categorical variables. Candidate variables included age, 18–64 vs ≥65 years; pediatric (age <18 years) vs adult; race; underlying disease (hematopoietic stem cell transplant [HSCT], solid organ transplant [SOT], acute leukemia/lymphoma, solid organ malignancy, other immunosuppression, major surgery within 30 days); renal dysfunction (acute renal failure requiring hemodialysis or end-stage renal disease); end-stage liver disease, birth weight <1 kg; neutropenia; intensive care unit (ICU) status and presence of central venous catheter at the time of initial positive blood culture; prior antifungal therapy (azole, echinocandin, polyene, azole) within 30 days of initial positive blood culture; episode type (breakthrough on antifungal therapy vs primary infection); breakthrough drug class (azole, echinocandin, polyene); disseminated disease status; drug class

used as directed therapy (echinocandin vs azole, polyene vs other, polyene vs azole, polyene vs echinocandin); and presence of an *FKS* mutation, FLC-resistant MIC, and an echinocandin-resistant MIC. Variables with a *P* value ≤.2 were considered for inclusion in the multivariable logistic regression models; variables with a *P* value ≤.05 were included in the final logistic regression models. Modeling techniques included forward and backward selection and consideration of 2-way interaction terms. Ten- and 30-day treatment outcomes were restricted to patients who were evaluable at 10 and 30 days; 10- and 30-day mortality was determined in all treated patients. All analyses were performed with SAS version 9.2.

## RESULTS

### Cohort and Demographics

Over the 10-year study period (2001–2010), 274 patients had 293 unique episodes of *C. glabrata* BSI. Demographics are shown in Table 1. The most common underlying diseases predisposing subjects to candidemia were major surgery within the preceding 30 days (38.7%) and immunosuppression (51.1%), including malignancy; 14 patients had multiple underlying immunosuppressive conditions, most commonly malignancy or transplant and diabetes mellitus.

Thirty percent (88/293) of episodes broke through antifungal therapy; among 79 episodes for which the breakthrough agent was known, azoles were the most common (69.6%; 36 FLC, 10 VRC, 5 posaconazole, 4 itraconazole) followed by echinocandins (24.1%; 16 MCF, 3 CSF) and polyenes (3.8%; 1 amphotericin B deoxycholate, 2 amphotericin B lipid complex) ($P \leq .0001$). Two (2.5%) episodes broke through on combination therapy (an azole plus an echinocandin).

A total of 313 *C. glabrata* isolates were analyzed. From the 273 "first" *C. glabrata* BSI episodes, 291 isolates were analyzed including 277 initial and 14 subsequent/serial isolates. Among the 19 "second" episodes, 21 isolates were analyzed including 18 initial and 3 subsequent isolates. One patient had a third episode from which a single isolate was evaluated.

### MIC/Susceptibility Results

As shown in Figure 1, resistance to 1 or more echinocandins increased from 4.9% (3/61) to 12.3% (9/73) between 2001 and 2010, respectively, and was 6.7% [21/313] overall. FLC resistance increased from 18% to 30.1% between 2001 and 2010, respectively, and was 24.9% (78/313) overall. Among the 78 FLC-resistant isolates, 11 (14.1%) were resistant to 1 or more echinocandins and 8 (10.3%) were resistant to all echinocandins.

### *FKS* Mutations and MIC Correlation

Twenty-five (7.9%) *C. glabrata* isolates harbored an *FKS* mutation and 64%, 68%, and 68% of the *FKS1/FKS2* mutants identified were classified as nonsusceptible (intermediate or resistant) to ANF, CSF, and MCF, respectively. From 2001 to 2010, *FKS* mutants were increasingly associated with higher MICs (Figure 1). Twenty-five percent (2/8) of isolates with *FKS* mutations recovered between 2001 and 2006 were at or above the CBP for resistance, compared with 88.2% (15/17) of *FKS* mutants recovered between 2007 and 2010 ($P = .003$, Fisher exact test).

**Table 1. Patient Demographics**

| Variable | All Patients (N = 274) |
|---|---|
| Age, y, median (range) | 59 (15 d–95 y) |
| Race | |
| Caucasian | 183 (66.7) |
| African American | 82 (30) |
| Asian | 4 (1.5) |
| Indian | 4 (1.5) |
| Other | 1 (0.3) |
| Male sex | 134 (48.9) |
| Underlying disease[a] | |
| Hematopoietic stem cell transplant | 23 (8.4) |
| Solid organ transplant | 26 (9.5) |
| Acute leukemia/lymphoma | 28 (10.2) |
| Solid organ malignancy[b] | 38 (13.8) |
| Other immunosuppression[c] | 25 (9.1) |
| Major surgery within 30 d | 106 (38.7) |
| Renal dysfunction | |
| End-stage renal disease | 31 (11.3) |
| Acute renal failure | 48 (17.5) |
| Liver disease | 21 (7.7) |
| Extremely low birth weight infant (<1 kg) | 2 (0.7) |
| Neutropenia (total white blood cell count <500 cells/μL)[d] | 22 (8.0) |
| Days of hospitalization at time of candidemia, mean (range)[d] | 18.2 (–4 to 165) |

Data are presented as No. (%) unless otherwise specified.

[a] Subjects may have had >1 underlying disease.

[b] Received chemotherapy within the prior 3 months.

[c] Steroids (>15 mg prednisone or equivalent) daily for >30 days, diabetes, congenital immunosuppressive condition, human immunodeficiency virus/AIDS.

[d] Based on the initial (index) positive blood culture; neutropenic status is unknown for 4 patients.

### Treatment and Mortality Outcomes and Predictors

Among 257 of 293 episodes treated with antifungal therapy, echinocandins were the agents administered as directed therapy for the majority (175; 68.1%), followed by azoles (17.5%), polyenes (12.8%), or combination therapy (1.6%). One hundred fifty-three and 119 episodes were treated with echinocandin monotherapy and evaluable at 10 and 30 days, respectively; 96.3% (52/54) and 89.1% (33/37) of episodes with a

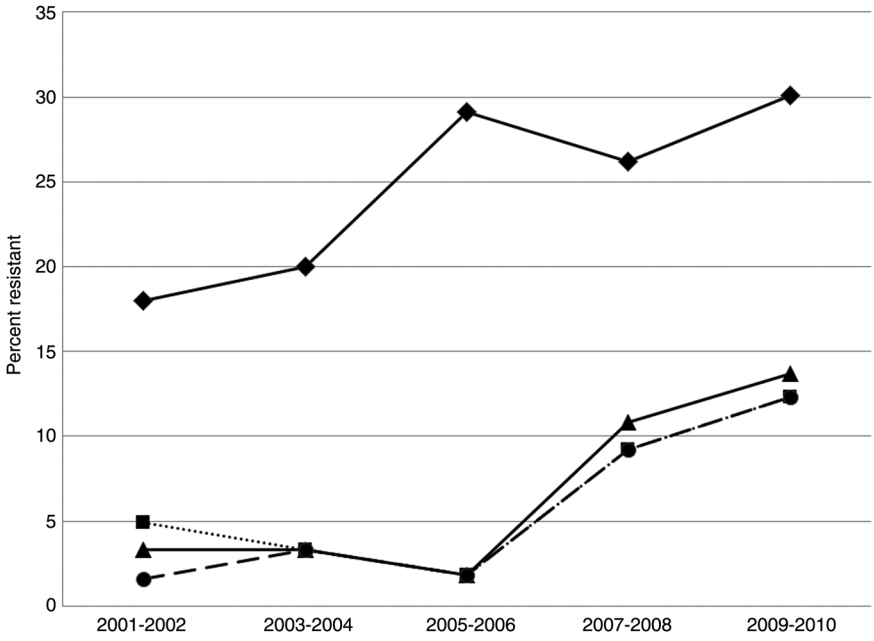


**Figure 1.** Temporal trends in antifungal resistance of *Candida glabrata* isolates to fluconazole (solid line with diamonds), anidulafungin (short dashed line with squares), caspofungin (solid line with triangles), and micafungin (long dashed line with circles).

susceptible CSF MIC were successfully treated (ie, candidemia was cleared) with CSF at 10 and 30 days, respectively, whereas 94.2% (81/86) and 97.3% (72/74) of episodes with a susceptible MCF MIC were successfully treated with MCF at 10 and 30 days, respectively. Among those treated with the echinocandin to which the isolate was subsequently found to be resistant, 0 of 1 and 8 of 9 cleared their blood at 10 days with CSF and MCF monotherapy, respectively.

Alternatively, Table 2 summarizes overall clinical outcome (ie, patients who cleared their candidemia and did not suffer relapse or recurrent infection) for episodes involving isolates resistant to 1 or more echinocandins and episodes involving *FKS* mutant strains. Thirteen of 23 episodes involving isolates with a resistant MIC were treated with echinocandin monotherapy; 5 of these 13 episodes responded to therapy and did not relapse or recur, whereas 8 (all with a *FKS* mutation) did not respond. Twelve of the 17 (70.6%) episodes involving *FKS1/FKS2* mutants demonstrating a resistant MIC to at least 1 of the echinocandins, including the 8 of 10 treated with echinocandin monotherapy, either failed to respond or responded initially but recurred.

Treatment and mortality outcomes for several key variables based on univariable analysis are presented in Table 3. Based on the full multivariable model (Table 4), independent predictors of 10-day mortality were malignancy (odds ratio [OR], 2.86; 95% confidence interval [CI], 1.23–6.25) and ICU status (OR, 3.50; 95% CI, 1.90–6.67). Independent predictors of 30-day mortality were prior azole therapy (OR, 2.58; 95% CI, 1.45–4.64), polyene treatment (OR, 2.52; 95% CI, 1.12–5.78), end-stage liver disease (OR, 4.1; 95% CI, 1.4–12.8), malignancy (OR, 2.62; 95% CI, 1.16–6.02), and ICU status (OR, 3.31; 95% CI, 1.87–5.92).

### Predictors of *FKS* Mutations and Resistant MICs

Based on a full multivariable model, predictors of a *FKS* mutant strain were SOT status, multiple episodes of *C. glabrata* BSI, and prior echinocandin therapy. In stepwise multivariable analysis, the sole predictor of a *FKS* mutant strain was prior echinocandin therapy (median, 19 days; OR, 19.647; 95% CI, 7.19–58.1). Predictors of CSF-resistant MICs were SOT (OR, 9.26; 95% CI, 1.55–64.08), breakthrough infection (OR, 7.85; 95% CI, 1.75–49.41), and receipt of prior echinocandin therapy (OR, 36.95; 95% CI, 9.66–198.65), and predictors of MCF-resistant MICs were SOT (OR, 15.59; 95% CI, 2.01–164.4) and prior receipt of an echinocandin (OR, 9.67; 95% CI, 2.11–53.26). Predictors of FLC resistance were history of HSCT (OR, 2.6; 95% CI, 1.02–6.84) and prior antifungal therapy (OR, 4.03; 95% CI, 2.01–8.36). In separate models controlling for HSCT and considering individual drug classes of prior exposure, prior azole (OR, 3.17; 95% CI, 1.23–8.38) and prior echinocandin (OR, 3.00; 95% CI, 1.31–6.76) therapy were significantly associated with presence of FLC-resistant MICs.

## DISCUSSION

In this large cohort of *C. glabrata* clinical isolates recovered over the past 10 years, we observed emerging and clinically

**Table 2. Treatment and Outcomes for Patients Infected With *Candida glabrata* Isolates Harboring *FKS* Mutations or Echinocandin Resistance**

| Patient (Episode No.) | *FKS* Mutation | MIC (μg/mL) | | | | Directed Therapy (Dose, Total mg/d) | Overall Clinical Outcome (Investigator Adjudicated) |
|---|---|---|---|---|---|---|---|
| | | ANF | CSF | MCF | FLC | | |
| 1 (1) | I1379V | 0.06 | 0.06 | 0.03 | 8 | MCF (100) | Responded |
| 2 (1) | I1379V | 0.06 | 0.06 | 0.03 | 8 | CSF (35), dose adjusted for liver failure | Responded |
| 3 (1) | R631G | 0.12 | 0.12 | 0.06 | 128 | MCF (100) | Responded |
| 4 (2) | R665S | 0.12 | 0.5 | 0.12 | 128 | MCF (150) | Nonresponse |
| 5 (2) | R665G | 0.25 | 0.5 | 0.12 | 128 | MCF (100) | Responded but recurred |
| 6 (2) | P667T | 1 | 1 | 0.12 | 8 | MCF (100) | Responded |
| 7 (1) | D632E | 1 | 0.5 | 0.25 | 128 | MCF (100) | Nonresponse |
| 7 (2) | D632E | 1 | 1 | 0.25 | 128 | MCF (100) | Responded |
| 8 (1) | F659V | 1 | 4 | 0.25 | 64 | LAMB (700) | Responded but recurred |
| 8 (2) | F659V | 1 | 2 | 0.25 | 32 | LAMB (700) + POS (800) | Responded |
| 9 (1) | F625S | 2 | 2 | 0.5 | 32 | MCF (100) | Nonresponse |
| 10 (1) | F625S | 2 | 2 | 0.5 | 128 | ABLC (440) | Nonresponse |
| 11 (1) | I634V | 0.06 | 0.06 | 0.03 | 8 | None | Nonevaluable |
| 12 (1) | I634V | 0.06 | 0.06 | 0.03 | 8 | AMB (3.2) | Responded |
| 13 (1) | I634V | 0.06 | 0.06 | 0.015 | 8 | VRC (150) | Responded |
| 14 (1) | I634V | 0.06 | 0.06 | 0.03 | 8 | CSF (50) | Responded |
| 15 (2) | S663P | 1 | 2 | 0.5 | 8 | MCF (100) | Responded but relapsed |
| 16 (2) | S663P | 4 | >8 | >8 | 128 | MCF (150) | Nonresponse |
| 17 (1) | S663P | 2 | 8 | 0.5 | 8 | MCF (100) | Nonresponse |
| 18 (1) | S663P | 0.25 | 0.5 | 0.12 | 16 | MCF (100) + FLC (400) then VRC (400) | Responded but relapsed |
| 18 (2) | S663P | 4 | >8 | 4 | 128 | MCF (150) + VRC (400) | Nonresponse |
| 19 (1) | S663P | 1 | 8 | 0.5 | 16 | LAMB (350) | Responded |
| | S663P | 1 | 1 | 0.5 | 128 | | |
| 20 (1) | S629P | 4 | >8 | 4 | 128 | VRC (400) | Responded |
| 21 (2) | delF658 | 1 | >8 | 2 | 4 | CSF (50) | Responded but relapsed |
| 22 (1) | None | 0.5 | 0.5 | 0.25 | 1 | ABLC (375) | Responded |
| 23 (1) | None | 0.5 | 0.5 | 0.06 | 4 | ABLC (300) + CSF (50) | Responded |
| | None | 0.5 | 0.25 | 0.06 | 128 | | |
| 24 (1) | None | 1 | 4 | 1 | 4 | ABLC (350) | Responded |
| 25 (1) | None | 1 | 0.5 | 1 | 4 | MCF (100 then 150) | Responded |
| 26 (1) | None | 0.12 | 0.12 | 0.25 | 16 | MCF (100) | Responded |
| 27 (1) | None | 0.12 | 0.12 | 0.5 | 8 | MCF (100) | Responded |

Abbreviations: ABLC, amphotericin B lipid complex; AMB, amphotericin B deoxycholate; ANF, anidulafungin; CSF, caspofungin; FLC, fluconazole; LAMB, liposomal amphotericin B; MCF, micafungin; MIC, minimum inhibitory concentration; POS, posaconazole; VRC, voriconazole.

significant resistance to echinocandins and FLC. Furthermore, and consistent with other reports, resistance to echinocandins and azoles appeared to coemerge in the same isolates. Of 747 *C. glabrata* isolates from 90 hospitals worldwide between 2001 and 2006, <1% demonstrated resistant MICs to any echinocandin [26], but by the second half of the decade, 9.3%, 9.3%, and 8.0% of *C. glabrata* isolates were resistant to ANF, CSF and MCF, respectively, including among FLC-resistant *C. glabrata* isolates [27].

Although there are no data to suggest that cross-resistance occurs between the azoles and echinocandins, extensive echinocandin use over the past decade, driven by broadening azole

**Table 3. Univariable Assessment of Several Key Factors on Treatment and Mortality Outcome for Treated *Candida glabrata* Episodes (n = 257)**

| | Treatment Success, Evaluable | | Mortality, Among All Treated | |
|---|---|---|---|---|
| Variable | 10 d (n = 218) | 30 d (n = 172) | 10 d (n = 257) | 30 d (n = 257) |
| All episodes | 203/218 (93.1) | 163/172 (94.8) | 59/257 (22.9) | 93/257 (36.2) |
| Episode type | | | | |
| Primary | 140/148 (94.6) | 117/123 (95.1) | 38/178 (21.3) | 54/178 (30.3)[a] |
| Breakthrough[b] | 63/70 (90.0) | 46/49 (93.9) | 21/79 (26.6) | 40/79 (50.6)[a] |
| Disseminated | | | | |
| Yes | 49/54 (90.7) | 41/46 (89.1) | 10/63 (15.9) | 19/63 (30.2) |
| No | 154/164 (93.9) | 122/126 (96.8) | 48/193 (24.9) | 79/193 (37.8) |
| Directed therapy drug class | | | | |
| Azole | 32/36 (88.9) | 29/31 (93.5) | 11/47 (23.4) | 15/47 (31.9)[c] |
| Echinocandin[d] | 144/155 (92.9) | 114/121 (94.2) | 38/177 (21.5) | 60/177 (33.9)[c] |
| Polyene | 27/27 (100) | 20/20 (100) | 10/33 (30.3) | 19/33 (57.6)[c] |
| *FKS* mutation | | | | |
| Yes | 16/20 (80)[e] | 10/12 (83.3) | 3/20 (15) | 3/12 (15) |
| No | 187/198 (94.4)[e] | 154/161 (95.7) | 25/198 (12.6) | 21/161 (13) |

Data are presented as No. (%) unless otherwise specified.

[a] Thirty-day mortality for breakthrough vs primary infection was statistically significant ($P = .0037$).

[b] Among 79 breakthrough infections for which the antifungal agent being administered at the time of breakthrough was known, azoles were the most common breakthrough agent, followed by echinocandins and polyenes (69.6%, 24.1%, and 3.8%, respectively; $P \leq .0001$). Two episodes (2.5%) broke through on combination therapy (an azole and an echinocandin).

[c] Thirty-day mortality for an azole vs echinocandin vs polyene as directed therapy was statistically significant (3-way $\chi^2$ $P = .025$)

[d] Includes 2 episodes for which an echinocandin was administered as part of combination therapy with amphotericin B lipid complex (1) and voriconazole (1).

[e] Significantly fewer episodes involving an isolate with an *FKS* mutation had treatment success at 10 days compared with those without a mutation ($P = .0391$).

resistance, has provided substantial selection pressure for the development of multidrug resistance (ie, resistance to ≥2 classes of antifungal agents) in *C. glabrata*. In support of this, our data indicate that prior azole therapy predicted FLC resistance, prior echinocandin therapy predicted FLC and echinocandin (CSF, MCF) resistance, and breakthrough infection status (most commonly FLC or an echinocandin) predicted echinocandin (CSF) resistance. Additionally, 6 patients infected with echinocandin-susceptible *C. glabrata* isolates during their initial infection episode and treated with an echinocandin developed a second episode of infection with a *C. glabrata FKS* mutant, and prior echinocandin use was the sole predictor of a *FKS* mutation.

The new (lower) CBPs for echinocandins were predictive of clinical response. Combining treatment outcomes from this study with that of prior studies, 93.7% (90/96) and 92.4% (109/118) of infectious episodes with susceptible CSF or MCF MICs, respectively, had successful treatment outcomes at 10 days [23]. Moreover, resistant echinocandin MICs delineated isolates less likely to respond. Among the 13 episodes in our cohort involving isolates with a resistant MIC and treated with echinocandin monotherapy, 5 (38.5%) responded, whereas 8 (61.5%) did not respond or responded initially but relapsed or recurred. The 8 episodes that failed all involved an *FKS* mutant. Thus, it appears that elevated MICs serve as a sensitive but nonspecific phenotypic screen for the presence of clinically significant *FKS* mutations and the presence of an elevated MIC, and a characteristic *FKS* mutation in an isolate correlates with reduced clinical outcome. Alterations in *FKS* genes reduce by different degrees the sensitivity of the GS enzyme to inhibition and manifest phenotypically with different magnitudes of change in MIC value [28, 29], and the new CBPs differentiate *FKS* mutations based on their clinical relevance. Overall, in vitro echinocandin susceptibility results were well within the "90/60" predictive utility rule for in vitro susceptibility testing: Infections due to susceptible isolates respond to therapy approximately 90% of the time, whereas infections due to resistant isolates respond up to 60% of the time. This rule takes into account that many factors, including host clinical variables, influence the likelihood of successful therapy [30].

The shift to non-*albicans Candida*, particularly *C. glabrata*, has long been a cause for alarm among clinicians [31–33]. While the annual percentage (median, 23.5%) of bloodstream infections caused by *C. glabrata* at our hospital remained stable over the study period, the findings of increasing azole and echinocandin resistance among *C. glabrata* indicates that multidrug resistance in *C. glabrata* is the next threat to implementing

**Table 4. Variables Significantly Impacting Treatment Outcome or Mortality**

| | Treatment Success | | Mortality | |
|---|---|---|---|---|
| | 10-Day | 30-Day | 10-Day | 30-Day |
| Variable | *P* Values (Univariable Analysis) | | | |
| Adult vs pediatric (age <18 y) | NS | NS | .02 | NS |
| Underlying disease | NS | NS | NS | NS |
| Malignancy | NS | NS | .023[a] | .033[a] |
| End-stage liver disease | NS | NS | .0392 | .0124[a] |
| Acute renal failure | NS | NS | NS | .0295 |
| Breakthrough infection | NS | NS | NS | .0018 |
| Directed therapy drug class | | | | |
| Polyene vs other | NS | NS | NS | .0073[a] |
| Polyene vs azole | NS | NS | NS | .0223 |
| Polyene vs echinocandin | NS | NS | NS | .0099 |
| *FKS* mutation | .0391 | NS | NS | .0374 |
| Echinocandin-resistant MIC | | | | |
| Caspofungin | NS | NS | NS | .0119 |
| Micafungin | NS | NS | NS | .025 |
| Prior antifungal Therapy | NS | NS | NS | .005 |
| Azole | NS | NS | NS | .0017[a] |
| Under intensive care[b] | NS | NS | <.0001[a] | <.001[a] |

Abbreviation: MIC, minimum inhibitory concentration.

[a] Variables that remained significant in multivariable analysis.

[b] Located in an intensive care unit at the time of initial positive blood culture collection.

effective empiric treatment of patients at risk for *Candida* BSI. This potential threat comes at a time when consensus guidelines encourage the use of echinocandins as first-line therapy for candidemia [1] and the prevailing attitude is that given the safety and potency of the echinocandins, any increase in non-*albicans Candida* species and associated resistance to FLC is largely irrelevant. Although the latter sentiment may be completely appropriate in an institution such as the University of Iowa Hospitals and Clinics where, despite a steady increase in the frequency of BSI due to *C. glabrata* over 3 decades, there were no instances of echinocandin resistance detected in the post-echinocandin era [34], it may be problematic at other institutions such as ours where resistance to FLC and the echinocandins now exceeds 30% and 12%, respectively. Such tremendous variation in resistance rates between centers emphasizes that it is essential to have knowledge of the local *Candida* species distribution and antifungal resistance rates to guide initial therapy for *Candida* BSI [29, 35–37]. Moreover, all *Candida* isolates from blood and normally sterile sites should be identified to the species level and tested for susceptibility to FLC and the echinocandins using standardized reference methods.

One argument offered to lessen concerns regarding the emergence of antifungal resistance in *Candida* species is that antimicrobial resistance often comes at a fitness cost to the organism [38–40]. Whereas resistance to echinocandins has been associated with decreased fitness and virulence in isolates of *C. glabrata*, gain of function mutations in the transcription factor CgPdr1p of *C. glabrata*, as well as loss of mitochondrial functions, not only mediate antifungal (azole) resistance but also enhance virulence in animal models [41–43]. Thus, *C. glabrata* is proving to be both hypermutable and possibly hypervirulent and certainly worthy of our continued respect as an invasive fungal pathogen.

There are obvious limitations to our data. Although this is the largest *C. glabrata*–infected cohort with MICs and clinical data reported to date, our ability to fully elucidate the clinical relevance of *FKS* resistant mutants was limited. We included many of the most critical clinical factors known to influence outcomes in our modeling, but determining *Candida*-attributable mortality is difficult in such complex and critically ill patients, and we cannot exclude the possibility that the presence of an *FKS* mutant or higher echinocandin MICs are surrogate markers of other clinical factors that contribute to poor clinical outcome.

In summary, these data document the broad emergence over time of resistance to both FLC and echinocandins in clinical isolates of *C. glabrata* from a single tertiary care center. Prior exposure to echinocandins was predictive of resistance to echinocandins as well as the presence of *FKS* mutations, both of which were associated with clinical failure. In vitro susceptibility testing of the echinocandins using CLSI methods and the new CBPs can be used to predict the presence of clinically significant *FKS* gene alterations among *C. glabrata* strains [23]. These findings emphasize the importance of both rapid identification to the species level as well as antifungal susceptibility testing of isolates from patients with candidemia. This is especially important in high-risk patients with recurrent *Candida* isolation and in those who are receiving or were previously exposed to antifungal treatment [1, 31, 32].

## Notes

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