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Rate of *FKS* Mutations among Consecutive *Candida* Isolates Causing Bloodstream Infection

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Precise *FKS* mutation rates among *Candida* species are undefined because studies have not systematically screened consecutive, disease-causing isolates. The Sensititre YeastOne (SYO) assay measures echinocandin MICs against *Candida* with less variability than reference broth microdilution methods. However, clinical breakpoint MICs may overstate caspofungin nonsusceptibility compared to other agents. Our objectives were to determine *Candida FKS* mutation rates by studying consecutive bloodstream isolates and to determine if discrepant susceptibility results were associated with *FKS* mutations. *FKS* hot spots were sequenced in echinocandin-intermediate and -resistant isolates and those from patients with breakthrough candidemia or ≥3 days of prior echinocandin exposure. Overall, 453 isolates from 384 patients underwent susceptibility testing; 16% were echinocandin intermediate or resistant. Intermediate susceptibility rates were higher for *Candida glabrata* than for other species ($P < 0.0001$) and higher for caspofungin than for other agents ($P < 0.0001$). Resistance rates were similar between agents. *FKS* mutations were detected in 5% of sequenced isolates and 2% of isolates overall. Corresponding rates among *C. glabrata* isolates were 8% and 4%, respectively. Among *Candida albicans* isolates, rates were 5% and <1%, respectively. Mutations occurred exclusively with prior echinocandin exposure and were not detected in other species. Isolates with discrepant susceptibility results did not harbor *FKS* mutations. Mutation rates among isolates resistant to ≥2, 1, and 0 agents were 75%, 13%, and 0%, respectively. In conclusion, *FKS* mutations were uncommon among non-*C. glabrata* species, even with prior echinocandin exposure. Discrepancies in echinocandin susceptibility by SYO testing were not driven by mutations and likely reflect imprecise caspofungin clinical breakpoints.

Echinocandin resistance is emerging among clinical *Candida* isolates, particularly those of the haploid species *Candida glabrata* (1–3). Resistance is mediated through point mutations in hot spot regions of the *FKS1* and *FKS2* genes, which encode the echinocandin target enzyme β-1,3-D-glucan synthase. *FKS* mutations are associated with echinocandin treatment failures and high mortality rates among patients with invasive candidiasis (1–5). Exposure to the echinocandins almost always precedes the emergence of *FKS* mutations and development of resistance (3, 6, 7). Up to 32% of *C. glabrata* isolates from patients with prior echinocandin exposure harbor *FKS* mutations; risk is greatest with breakthrough infections during echinocandin prophylaxis or treatment (6). Overall rates of *FKS* mutant *Candida* are imprecisely defined. Rates of 8 to 18% have been reported among *C. glabrata* isolates from patients at high-risk centers (1, 2); however, these data may overstate mutation rates, since the studies were limited by incomplete access to medical records and a lack of systematic testing of consecutive isolates (1). The other major *Candida* species (*Candida albicans*, *C. parapsilosis*, *C. tropicalis*, *C. krusei*, and *C. guilliermondii*) account for 60 to 80% of invasive candidiasis (8), but *FKS* mutations have been described only in case series and reports (9). To date, no study has systematically screened sequential *Candida* isolates for the presence of *FKS* mutations.

In the clinical microbiology laboratory, resistance is typically assessed by measuring drug MICs and comparing results to reference breakpoints. Reference broth microdilution testing methods and clinical breakpoint MICs for echinocandins against *Candida* species have been developed by the Clinical and Laboratory Standards Institute (CLSI) (10) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) (11). Unfortunately, several features of the broth microdilution reference methods have limited their utility in clinical practice. First, there is significant interlaboratory variability in caspofungin MICs (12), which has prevented EUCAST but not CLSI from proposing interpretive criteria for caspofungin. Second, echinocandin MICs have not been shown to correlate consistently with outcomes among patients with invasive candidiasis who are treated with these agents (13). Third, the reference methods are not used in most clinical microbiology laboratories (14), which instead employ commercial assays such as Sensititre YeastOne (SYO; Trek Diagnostics) and Etest (bioMérieux) or automated systems like the Vitek 2 (bioMérieux) antifungal testing instrument.

We recently showed that the SYO assay, as employed by clinical labs in routine practice, may reduce interlaboratory variability in caspofungin MICs (14). However, echinocandin MIC clinical breakpoints are not validated for commercial methods, and results may overstate nonsusceptibility. We demonstrated that application of CLSI breakpoints results in disproportionately high rates of caspofungin nonsusceptibility among *C. glabrata* and *C.*

Received 14 August 2015 **Returned for modification** 31 August 2015
Accepted 12 September 2015

Accepted manuscript posted online 21 September 2015

Citation Shields RK, Nguyen MH, Press EG, Cumbie R, Driscoll E, Pasculle AW, Clancy CJ. 2015. Rate of *FKS* mutations among consecutive *Candida* isolates causing bloodstream infection. Antimicrob Agents Chemother 59:7465–7470. doi:10.1128/AAC.01973-15.

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TABLE 1 Susceptibility profile of three echinocandins based on MICs determined by SYO

Species	*n*	No. (%) of isolates[a]									
		Intermediate[b]			Resistant[c]			Intermediate or resistant to any EC	MIC > SYO-specific ECV[d]		
		ANF	CSP	MCF	ANF	CSP	MCF		ANF	CSP	MCF
C. albicans	169	2 (1)	6 (4)	0 (0)	0 (0)	1 (0.6)	1 (0.6)	8 (5)	4 (2)	7 (4)	9 (5)
C. glabrata	167	1 (0.6)	44 (26)	2 (1)	7 (4)	13 (8)	5 (3)	58 (35)	9 (5)	13 (8)	13 (8)
C. parapsilosis	71	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)	0 (0)
C. tropicalis	38	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (5)
C. krusei	6	0 (0)	3 (50)	0 (0)	0 (0)	1 (17)	0 (0)	4 (67)	0 (0)	0 (0)	0 (0)
C. guilliermondii	2	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total	453	3 (1)	53 (12)	3 (1)	7 (2)	15 (3)	6 (1)	71 (16)	13 (3)	20 (4)	24 (5)

[a] ANF, anidulafungin; CSP, caspofungin; EC, echinocandin; ECV, epidemiological cutoff value; MCF, micafungin.
[b] Intermediate susceptibility was adapted from CLSI criteria. For *C. albicans*, *C. tropicalis*, and *C. krusei*, MICs were 0.5 μg/ml; for *C. parapsilosisi* and *C. guilliermondii*, MICs were 4 μg/ml; and for *C. glabrata*, MICs were 0.25 μg/ml for anidulafungin and caspofungin and 0.12 μg/ml for micafungin.
[c] Resistance was adapted from CLSI criteria. For *C. albicans*, *C. tropicalis*, and *C. krusei*, MICs were ≥1 μg/ml; for *C. parapsilosisi* and *C. guilliermondii*, MICs were ≥8 μg/ml; and for *C. glabrata*, MICs were ≥0.5 μg/ml for anidulafungin and caspofungin and ≥0.25 μg/ml for micafungin.
[d] Epidemiologic cutoff values were obtained from reference 16. The ECVs for anidulafungin MICs against *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. krusei*, and *C. guillermondii* were 0.12, 0.12, 4, 0.5, 0.25, and 4 μg/ml, respectively. The corresponding values for caspofungin were 0.25, 0.25, 2, 0.25, 1, and 2 μg/ml, respectively. The corresponding values for micafungin were 0.06, 0.03, 4, 0.06, 0.25, and 2 μg/ml, respectively.

krusei compared to other agents (14). For example, 18% and 19% of *C. glabrata* isolates in our study were identified as intermediate or resistant to caspofungin but susceptible to anidulafungin and micafungin, respectively (14). Indeed, categorical discrepancies occurred most frequently among *C. glabrata* and *C. krusei* isolates classified as caspofungin intermediate, anidulafungin susceptible, and micafungin susceptible (14). The significance of discrepant susceptibility results is unknown, and it is unclear if categorical discrepancies are driven biologically by agent-specific *FKS* mutations (15) or if they are an artifact of imprecise clinical breakpoints.

The objectives of this study were to determine *FKS* mutation rates across *Candida* species by systematic sequencing of at-risk isolates and to determine if discrepant echinocandin susceptibility results were associated with agent-specific *FKS* mutations.

MATERIALS AND METHODS

Consecutive cases of candidemia at the University of Pittsburgh Medical Center Presbyterian Hospital from October 2009 to December 2014 were evaluated. A unique case of candidemia was defined as a blood culture yielding *Candida* that was more than 30 days after any prior positive blood culture with *Candida*. For candidemia caused by more than one *Candida* species, each species was considered a separate case for analysis. Antifungal susceptibility testing was performed with SYO panels according to the manufacturer's recommendations (TREK Diagnostic Systems, Cleveland, OH, USA). *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019 were used as quality controls. Results were included only when both control isolates were within acceptable CLSI MIC ranges for all agents (10). MICs were interpreted in accordance with recently published CLSI M27-S4 clinical breakpoints (10). Ten cases of candidemia due to uncommon species (4 *Candida lusitaniae*, 3 *C. dubliniensis*, 2 *C. kefyr*, and 1 *C. famata* isolate) were excluded from the study because CLSI breakpoints have not been established.

We employed a targeted, systematic screening approach to identify *FKS* mutations, which were detected using previously described methods (3, 6, 7). In short, DNA was extracted, hot spots of *FKS1* (all species) and *FKS2* (*C. glabrata* only) were amplified by PCR, and purified DNA was sequenced for any isolate meeting any of the following criteria: (i) isolation from a patient with ≥3 days of prior echinocandin exposure, (ii) isolation from a patient receiving ≥3 days of echinocandin therapy at the time of positive blood culture (i.e., breakthrough candidemia), or (iii) an echinocandin MIC classified as intermediate or resistant by CLSI breakpoints (10). Rates of *FKS* mutations and resistance were compared by chi-squared or Fisher's exact tests, as appropriate. Significance was defined as a two-tailed *P* value of <0.05.

RESULTS

Characteristics of *Candida* isolates and antifungal susceptibilities. A total of 453 *Candida* isolates from 384 unique patients with candidemia were included in the analysis. More than 1 isolate was included from patients with candidemia due to multiple species ($n = 11$), relapsing candidemia occurring >30 days after a previous episode ($n = 14$), or both ($n = 19$). *C. albicans* and *C. glabrata* (37% each) were the most common species encountered, followed by *C. parapsilosis* (16%), *C. tropicalis* (8%), *C. krusei* (1%), and *C. guilliermondii* (<1%).

Sixteen percent (71/453) of isolates were classified as intermediate or resistant to an echinocandin by CLSI breakpoints (Table 1). Rates of intermediate susceptibility were higher for caspofungin (12%, 53/453) than anidulafungin (1%, 3/453; $P < 0.0001$) or micafungin (1%, 3/453; $P < 0.0001$). Intermediate susceptibility was more common among isolates of *C. glabrata* (26%, 44/167) than other species (3%, 10/286; $P < 0.0001$). Ninety-eight percent (43/44) and 95% (42/44) of caspofungin-intermediate *C. glabrata* isolates were susceptible to anidulafungin and micafungin, respectively.

Rates of resistance did not differ significantly for caspofungin (3%, 15/453), anidulafungin (2%, 7/453; $P = 0.12$), and micafungin (1%, 6/453; $P = 0.07$) (Table 1). Caspofungin resistance was identified among 17% (1/6), 8% (13/167), and 0.6% (1/169) of *C. krusei*, *C. glabrata*, and *C. albicans* isolates, respectively. Resistance to anidulafungin or micafungin was not detected among *C. kruseii* isolates. Anidulafungin and micafungin resistance was identified among 4% (7/167) and 3% (5/167) of *C. glabrata* isolates, respectively, and 0% (0/169) and 0.6% (1/169) of *C. albicans* isolates, respectively.

Three percent (13/453), 4% (20/453), and 5% (24/453) of isolates demonstrated MICs above recently proposed SYO-specific

TABLE 2 *Candida* isolates at risk for *FKS* gene mutations

Species	No. (%) of isolates			Median (range) duration of exposure in days	No. (%) of isolates		
	Total	Intermediate or resistant to any EC[a]	With prior EC exposure		Breakthrough	At risk[b]	Harboring *FKS* mutations
C. albicans	169	8 (5)	20 (12)	30 (3–190)	2 (1)	27 (16)	1 (0.6)
C. glabrata	167	58 (35)	41 (25)	18 (4–450)	6 (4)	77 (46)	6 (4)
C. parapsilosis	71	1 (1)	28 (39)	67 (8–211)	4 (6)	29 (41)	0 (0)
C. tropicalis	38	0 (0)	7 (18)	22 (4–400)	2 (5)	7 (18)	0 (0)
C. krusei	6	4 (67)	0 (0)		0 (0)	4 (67)	0 (0)
C. guilliermondii	2	0 (0)	0 (0)		0 (0)	0 (0)	0 (0)
Total	453	71 (16)	96 (21)	27 (3–450)	15 (3)	144 (32)	7 (2)

[a] As defined in Table 1. EC, echinocandin.
[b] At-risk isolates include those from patients with prior or ongoing (i.e., breakthrough) echinocandin exposure or echinocandin-intermediate or -resistant isolates.

epidemiologic cutoff values (ECVs) for anidulafungin, caspofungin, and micafungin, respectively (16) (Table 1). Rates of MICs above the ECV were comparable between echinocandin agents and ranged from 5 to 8% and 2 to 5% among *C. glabrata* and *C. albicans* isolates, respectively. Micafungin MICs were above the ECV for 2 *C. tropicalis* isolates; otherwise, none of the isolates from other species exhibited an echinocandin MIC above the ECV.

The overall rate of fluconazole resistance (excluding *C. krusei*) was 10% (46/447). Rates of fluconazole resistance were 19% (31/167), 13% (5/38), 6% (4/71), 4% (6/169), and 0% (0/2) among *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, *C. albicans*, and *C. guilliermondii* isolates, respectively. Twenty-six percent (15/58) and 25% (2/8) of echinocandin-intermediate or -resistant *C. glabrata* and *C. albicans* isolates were resistant to fluconazole, respectively.

Twenty-one percent (96/453) of *Candida* isolates were recovered from patients with prior echinocandin exposure; 3% (15/453) of isolates were classified as breakthrough (Table 2). Thirty-nine percent (28/71) of *C. parapsilosis* isolates were associated with prior echinocandin exposure, compared to 25% of *C. glabrata* isolates (41/166; $P = 0.03$) and 12% of *C. albicans* isolates (20/169; $P < 0.0001$). Prior exposure was more common among *C. glabrata* isolates than *C. albicans* isolates ($P = 0.003$). Twenty-four percent (23/96) and 13% (48/357) of isolates collected from patients with and without prior echinocandin exposure were classified as intermediate or resistant to an echinocandin, respectively ($P = 0.02$).

FKS mutations. *FKS* hot spots were sequenced in 144 "at-risk" isolates: 71 isolates were either intermediate or resistant to an echinocandin by CLSI criteria (23 were also recovered from patients with prior echinocandin exposure), and 73 isolates were recovered from patients with prior echinocandin exposure but were susceptible to all echinocandins (Table 2). Five percent (7/144), 7% (7/96), and 10% (7/71) of at-risk isolates, isolates from patients with prior echinocandin exposure, and isolates that were intermediate or resistant to ≥1 echinocandin harbored *FKS* hot spot mutations, respectively (Table 2). Thirty percent (7/23) of isolates intermediate or resistant to an echinocandin from patients with prior exposure were *FKS* mutants. Mutations were not identified in isolates associated with prior echinocandin exposure but susceptible to all three echinocandins. Mutations were identified exclusively among *C. albicans* and *C. glabrata* isolates from patients with prior echinocandin exposure (Table 3). Among such isolates, the *C. albicans* and *C. glabrata* species-specific rates of *FKS* mutations were 5% (1/20) and 15% (6/41), respectively. Fifty percent (1/2) of breakthrough *C. albicans* and 67% (4/6) of breakthrough *C. glabrata* isolates harbored mutations.

Based on caspofungin MICs, 100% (1/1) and 46% (6/13) of resistant *C. albicans* and *C. glabrata* isolates, respectively, harbored mutations. No caspofungin-intermediate *C. albicans* (0/6; $P = 0.14$) or *C. glabrata* (0/44; $P < 0.0001$) isolates harbored mutations. All *FKS* mutants were caspofungin resistant. Rates of anidulafungin and micafungin resistance among *FKS* mutant *Candida* isolates were 71% (5/7) and 57% (4/7), respectively. Median caspofungin (3 versus 0.12 μg/ml), anidulafungin (0.5 versus 0.03 μg/ml), and micafungin (0.19 versus 0.015 μg/ml) MICs against *FKS* mutant *C. glabrata* isolates were higher than those

TABLE 3 Characteristics of *FKS* mutant *Candida* isolates

Species	*FKS* mutation	MIC (μg/ml)[a]				No. of days of:		
		ANF	CSP	MCF	FLUC	Prior echinocandin exposure	Prior azole exposure	Echinocandin breakthrough
C. albicans	S645P	0.5 (I)	>8 (R)	2 (R)	>256 (R)	68	103	Yes
C. glabrata	D632Y	0.5 (R)	2 (R)	0.25 (R)	2 (S-DD)	239	None	Yes
	F659del	2 (R)	>8 (R)	4 (R)	128 (R)	46	139	No
	S663P	2 (R)	>8 (R)	8 (R)	256 (R)	155	94	Yes
	F659S	0.5 (R)	1 (R)	0.06 (S)	>128 (R)	7	22	Yes
	F659L	0.06 (S)	1 (R)	0.06 (S)	8 (S-DD)	117	108	No
	R636S	0.5 (R)	4 (R)	0.12 (I)	1 (S-DD)	450	227	Yes

[a] ANF, anidulafungin; CSP, caspofungin; FLUC, fluconazole; MCF, micafungin. The CLSI interpretation of the MIC is in parentheses. I, intermediate; R, resistant; S, susceptible; S-DD, susceptible, dose dependent.

against wild-type isolates ($P < 0.0001$ for each). Across species, *FKS* mutation rates were 75% (6/8), 13% (1/8), and 0% (0/437) among isolates resistant to ≥2, 1, and 0 echinocandins, respectively. Among isolates classified as intermediate or resistant to ≥2, 1, and 0 echinocandins, mutation rates were 69% (6/9), 2% (1/62), and 0% (0/382), respectively.

FKS mutations were present in 56% (5/9), 46% (6/13) and 46% (6/13) of *C. glabrata* isolates for which anidulafungin, caspofungin, and micafungin MICs were above the ECV, respectively. The corresponding rates for *C. albicans* isolates were 25% (1/4), 14% (1/7) and 11% (1/9). Eighty-seven percent (6/7), 100% (7/7), and 100% (7/7) of *FKS* mutant *Candida* isolates exhibited anidulafungin, caspofungin, and micafungin MICs above the ECV, respectively.

No *FKS* mutations were identified among an additional 40 isolates (27 *C. glabrata*, 8 *C. albicans*, 3 *C. tropicalis*, and 2 *C. parapsilosis* isolates) that did not meet sequencing criteria (negative controls). Assuming that none of the remaining isolates were *FKS* mutants, the overall mutation rate across all species was 2% (7/453). Overall *FKS* mutation rates were 4% (6/167) and 0.6% (1/169) among *C. glabrata* and *C. albicans*, respectively.

DISCUSSION

This is the first study to report the rates of *FKS* hot spot mutations across the major *Candida* species recovered sequentially from patients at a single center. Several findings are particularly noteworthy. First, echinocandin resistance and *FKS* gene mutations were uncommon during consecutive cases of candidemia and were encountered exclusively among *C. glabrata* and *C. albicans* isolates recovered from patients with prior echinocandin exposure. Second, we found no evidence to support agent-specific *FKS* mutations among isolates with discrepant echinocandin susceptibility results. Most notably, none of the isolates that tested as intermediate to caspofungin but susceptible to other agents by CLSI breakpoints harbored an *FKS* mutation. Finally, 75% of isolates that were classified as resistant to two or more echinocandins were *FKS* mutants, implicating hot spot mutations as a predominant, but not exclusive, mechanism of resistance. Taken together, our data provide new insights into echinocandin resistance and carry important implications for the use of these agents in clinical practice.

It is striking that only 2% (7/453) of all *Candida* isolates, and 5% (7/144) of isolates considered to be at risk for resistance, harbored *FKS* mutations. The corresponding rates for *C. glabrata* and *C. albicans* were 4 and 8% and <1 and 5%, respectively. The low frequency of gene mutations identified here is consistent with data from previous studies of isolates in an international repository (17, 18). Our *C. glabrata* rates are significantly lower than the recently reported rates of 8 to 18% at two major U.S. centers (1, 2), which may reflect institutional differences or the lack of systematic screening strategies in the earlier studies. On balance, the cumulative data indicate that *FKS* mutations and echinocandin resistance are important clinical problems, but the phenomena need to be placed in context. Indeed, selection for *FKS* gene mutations generally occurs in highly specific, echinocandin treatment-experienced patients (1–3, 6, 7). Isolates recovered from patients with breakthrough infections are at significantly greater risk than isolates from patients with more distant echinocandin exposure. In fact, only 4% of nonbreakthrough *C. glabrata* and *C. albicans* isolates that were associated with past echinocandin exposure were *FKS* mutants (2/35 and 0/18, respectively). Moreover, durations of prior exposure preceding resistance are typically quite extensive. It is likely that both the duration and timing of echinocandin exposure facilitate the emergence of echinocandin-resistant mutants (19).

Almost 26% of *C. glabrata* isolates were classified as caspofungin intermediate but susceptible to anidulafungin or micafungin using CLSI breakpoints (43/167 and 42/167, respectively). These discrepancy rates were slightly higher than the corresponding rates of 16% and 17%, respectively, that were reported in our earlier multicenter study (14). The number of *C. krusei* isolates was small, but 50% (3/6) were caspofungin intermediate and susceptible to the other agents. There is some evidence that certain *FKS* mutations may confer differential relative resistance to individual echinocandins (15, 20). However, the fact that none of our caspofungin-intermediate *C. glabrata* or *C. krusei* isolates had an *FKS* mutation indicates that categorical discrepancies in echinocandin susceptibility, in general, are not driven by such mutations but are more likely artifacts of imprecise caspofungin breakpoints.

Due to the interlaboratory variability in caspofungin MICs obtained with reference broth microdilution methods, recommendations have been made to use anidulafungin or micafungin MICs as a surrogate for the echinocandin class (21, 22). We found that anidulafungin or micafungin resistance was slightly more sensitive than caspofungin resistance for detecting *FKS* mutations (71% [5/7] and 67% [4/6], respectively, versus 47% [7/15]). However, 100% (7/7) of *FKS* mutants were caspofungin resistant, whereas only 71% (5/7) and 57% (4/7) were anidulafungin and micafungin resistant, respectively. Of note, the sensitivity and specificity of resistance to ≥2 agents for identifying *FKS* mutant *Candida* were 75% (6/8) and >99% (444/445), respectively, compared to 13% (1/8) and 99% (439/445) for resistance to one agent. Therefore, the best approach to identifying *FKS* mutations may be to consider MICs of all three echinocandins, rather than any single agent. In this regard, SYO panels and other commercial assays that provide results for each of the echinocandins may offer advantages for clinical microbiology laboratories.

Each of the mutations we identified has been linked with echinocandin resistance (20). At the same time, *FKS* mutations were not the sole determinants of diminished echinocandin susceptibility, as 25% of isolates resistant to ≥2 agents were not mutants. The mechanisms of resistance in these isolates are unclear, but they may involve modulation of membrane sphingolipids (23) and upregulation of cell wall chitin and/or other cell wall compensatory mechanisms (24). Furthermore, chromosomal instability during stress leads to increased genetic diversity, which enables isolates to develop rapid resistance to multiple antifungal drug classes (19). Along these lines, it is noteworthy that 57% of *FKS* mutant isolates reported here were also resistant to fluconazole; all four fluconazole-resistant isolates were recovered from patients with prior azole exposure (Table 3). Multidrug-resistant *C. glabrata* isolates, in particular, are a serious threat to the antifungal armamentarium, as at least 10% of fluconazole-resistant isolates are reported to harbor *FKS* mutations (25). Our data add to accumulating evidence that resistance to echinocandins is associated with an increased likelihood of azole resistance, and vice versa (2, 25, 26).

ECVs are designed to distinguish between a population of wild-type, drug-susceptible isolates and a population that includes non-wild-type isolates with acquired resistance mecha-

nisms. A recent multicenter study assigned echinocandin ECVs against *Candida* species by using the SYO assay (16). In keeping with data from the multicenter study, we found that the species-specific ECVs correctly classified almost all of our *FKS* mutant *C. glabrata* and *C. albicans* isolates. Follow-up studies are needed to determine the value of MICs, clinical breakpoints, ECVs, and the presence of *FKS* mutations in predicting outcomes of echinocandin treatment among patients with invasive candidiasis.

In conclusion, this study provides important perspectives on echinocandin resistance among *Candida* species. These drugs are now the first choice for treatment of most cases of candidemia (27–30). Reports of the emergence of echinocandin-resistant and multidrug-resistant *Candida* isolates (in particular, *C. glabrata*) are concerning, but our data suggest that *FKS* mutations remain rare and are fairly difficult to induce in the clinic. Clinicians should maintain suspicion for resistance among patients with extensive prior echinocandin exposure, especially those with breakthrough infections or more recent treatment courses. In these settings, echinocandin MICs and screening for *FKS* mutations may help guide treatment decisions (3, 6, 21). Outside of these settings, however, resistance is extremely uncommon, and it is reasonable for clinicians to assume that each of the agents retains activity.

ACKNOWLEDGMENTS

This work was supported by investigator-initiated grants from Astellas and Merck and by the National Institutes of Health through grant numbers K08AI114883 and KL2TR000146 awarded to R.K.S.

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