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Clinical perspectives on echinocandin resistance among Candida species

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Abstract

Purpose of the review—We review and offer our clinical perspectives on the emergence of echinocandin-resistant Candida.

Recent findings—Candida *FKS* gene mutations attenuate echinocandin activity, but overall mutation rates among clinical isolates remain low (*C. glabrata*, ∼4%; other species, <1%). Rates are higher with prior echinocandin exposure, exceeding 50% among *C. glabrata* or *C. albicans* isolates causing breakthrough invasive candidiasis. The median duration of prior echinocandin exposure among *FKS* mutant isolates is ∼100 days. The clinical usefulness of echinocandin susceptibility testing is limited by the low overall prevalence of resistance, and uncertainties surrounding testing methods and interpretation of minimum inhibitory concentrations (MICs). In single-center studies, caspofungin resistance (defined using institution-specific MIC breakpoints) was 32-53% sensitive and 75-95% specific for predicting treatment outcomes of *C. glabrata* invasive candidiasis; corresponding values for the presence of an *FKS* mutation were 35-41% and 90-98%. Results were similar using anidulafungin and micafungin MICs. Clinical data are scarce for non-*C. glabrata* species.

Summary—Echinocandins remain preferred agents against invasive Candida infections. Susceptibility testing and *FKS* genotypic testing do not have roles in routine clinical practice, but may be useful in newly-diagnosed patients who are echinocandin-experienced or those who have not responded to echinocandin treatment.

Keywords

Echinocandin; Candida; *FKS* mutation; resistance; susceptibility testing

Introduction

Echinocandin antifungals (anidulafungin, caspofungin and micafungin) are agents of choice for the treatment of invasive candidiasis [1-3]. The drugs exert fungicidal activity against Candida species *in vitro* by inhibiting the synthesis of β-1,3-Dglucan, a major cell wall

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constituent [4]. Although there are some pharmacokinetic differences among the agents, practice guidelines consider the echinocandins to be interchangeable [1,2].

Widespread echinocandin usage has been accompanied by reports of emerging drug resistance among clinical Candida isolates, particularly among the haploid species *C. glabrata* [5,6]. While these reports are worrisome, uncertainty surrounds the extent and clinical significance of echinocandin-resistant Candida. Moreover, methods for detecting resistance, the roles of resistance testing in clinical practice, and the impact of emerging resistance on the management of invasive candidiasis are unclear. In this article, we review echinocandin resistance among Candida species and offer our clinical perspectives on the phenomenon.

FKS mutations among Candida species

The catalytic subunit of the echinocandin target enzyme, β-1,3-D-glucan synthase, is encoded by *FKS1*, *FKS2* and *FKS3* genes. Mutations in hot spot regions of *FKS1* (all Candida species) or *FKS2* (*C. glabrata*) result in amino acid substitutions that attenuate echinocandin activity [7]. Echinocandin inhibition of β-1,3-D-glucan synthase is reduced by 30- to several thousand-fold in isolates harboring *FKS* mutations [8-10]. Readers interested in the impact of specific *FKS* mutations on echinocandin susceptibility are referred to a recent review [7].

Five Candida species account for >95% of invasive candidiasis. Among these species, *C. albicans*, *C. glabrata*, *C. tropicalis* and *C. krusei* can acquire *FKS* mutations under selection pressure. Certain acquired *FKS* mutations result in highly-attenuated susceptibility *in vitro*, which correlates with poor treatment responses in mouse models of disseminated candidiasis [7-9,11-14]. *C. parapsilosis*, in contrast, harbors a naturally-occurring *FKS1* polymorphism that confers diminished echinocandin susceptibility [10], the clinical significance of which is not established [15-17].

The prevalence of *FKS* mutant Candida is not precisely defined (Table 1) [5,18-28]. Reported rates of 8–18% among *C. glabrata* isolates at certain high-risk centers may overstate prevalence since studies have been limited by incomplete access to medical records and/or a lack of systematic testing across consecutive isolates [5,19,20]. In a more recent study of 453 consecutive Candida bloodstream isolates from a single center, *FKS* mutations were detected in only 4% and <1% of *C. glabrata* and *C. albicans*, respectively [29]. These low rates are in general agreement with data from international repositories [18,30,31]. *FKS* mutant *C. tropicalis* and *C. krusei* been described in case reports [22,24-28], but rates among repository strains are low [18].

It is important to understand that *FKS* mutations arise in specific clinical settings, within which echinocandin resistance rates are significantly higher than in the broader population with invasive candidiasis. Most notably, *FKS* mutant *C. glabrata* and *C. albicans* are recovered almost exclusively from patients with prior echinocandin exposure [5,19-21]. The greatest risk is among patients who develop breakthrough infections during treatment, in whom ≥50% of *C. glabrata* or *C. albicans* isolates harbor mutations (Table 1) [22,32]. In contrast, *FKS* mutant isolates account for <10% of *C. glabrata* or *C. albicans* infections

among patients with remote echinocandin exposure [29]; risk is greatest for exposure within the preceding month [19].

In both breakthrough and remote settings, prolonged echinocandin exposure is typically required for emergence of mutations (median: ∼100 days; range: 7–450 days) [20,21,29,32]. By the same token, infections by wild-type *C. glabrata* have been reported after as many as 84 treatment days [21,22,29,32]. Other risk factors for *FKS* mutant *C. glabrata* include underlying gastrointestinal disorders, solid organ transplantation and recurrent invasive candidiasis [5,19,20,32]. Of note, ≥25% of *FKS* mutant *C. glabrata* may also exhibit fluconazole and/or amphotericin B resistance [5,23,29,33,34], in keeping with past exposure to multiple antifungal drug classes. Less is known about the emergence of *FKS* mutations among non-*C. glabrata* species, but it is likely that risk factors are similar. Breakthrough infections with *C. parapsilosis* are well-recognized [22,29], but acquired *FKS* hot spot mutations are not described.

Some *FKS* mutations may confer differential resistance to individual echinocandins [7,12], but agent-specific mutants are extremely rare [29]. The extent to which echinocandin resistance in Candida is mediated by *FKS* mutations versus other, less well-characterized mechanisms is not established. In a recent study, 25% of *C. glabrata* isolates that were resistant to multiple echinocandins did not harbor an *FKS* mutation [29].

Echinocandin susceptibility testing

At present, most hospital microbiology laboratories do not have the capacity to detect *FKS* mutations [35-37]. Echinocandin susceptibility is typically assessed by measuring minimum inhibitory concentrations (MICs), and comparing results to interpretive clinical breakpoints. Reference broth microdilution methods for testing echinocandins against Candida species have been developed by the Clinical Laboratory Standards Institute (CLSI) [38] and European Committee on Antimicrobial Susceptibility Testing (EUCAST) [39]. Drug- and species-specific breakpoints have been derived from data on MIC distributions, pharmacokinetic-pharmacodynamic (PK-PD) parameters, epidemiological cut-off values (ECVs), and, to a lesser extent, patient responses to echinocandin treatment (Table 2) [40,41]. MICs and clinical breakpoints are higher for *C. parapsilosis* than other species, in keeping with intrinsic *FKS* polymorphisms. A prominent limitation of echinocandin susceptibility testing is that CLSI and EUCAST clinical breakpoints often differ. Discrepancies in clinical breakpoints are one of several major issues that make the interpretation of echinocandin MICs difficult in the clinic.

Shortly after CLSI proposed interpretive criteria, it became apparent that caspofungin MICs varied significantly among laboratories using broth microdilution methods. Results of a large international study showed that modal caspofungin MICs generated in individual laboratories by either of the reference methods differed by ≥4 two-fold dilutions against *C. albicans*, *C. glabrata*, *C. tropicalis* and *C. krusei* [42]. In contrast, modal anidulafungin and micafungin MICs were distributed within narrow ranges. Given these data, EUCAST has not proposed caspofungin breakpoints, and recommends against testing this agent [41,43].

Variability in caspofungin MICs is most relevant for *C. glabrata*, as rates of non-susceptibility approach 100% at some centers applying CLSI breakpoints [20,44].

The overall clinical impact of caspofungin MIC variability is somewhat mitigated by the fact that an overwhelming majority of hospitals do not use reference broth microdilution methods, but rather commercialized assays such as Sensititre YeastOne (SYO, Trek Diagnostics, Cleveland, United States) and Etest (Biomerieux, Marcy-I'Etoile, France), or automated systems like Vitek 2 (Biomerieux) [45,46]. Echinocandin MICs generated by these methods and reference methods demonstrate good essential agreement (MICs within a two-fold dilution), but categorical agreement (defining isolates as susceptible, intermediate or resistant) using reference breakpoints may be lower [47,48]. In a recent study of hospitals that routinely perform susceptibility testing, inter-laboratory modal echinocandin MICs obtained by SYO (including those of caspofungin) were within a single two-fold dilution against each Candida species [45]. However, the use of CLSI breakpoints resulted in disproportionately high rates of caspofungin nonsusceptibility among *C. glabrata* and *C. krusei*, compared to other agents. In a follow-up study at one of the hospitals, *FKS* mutations were not detected among isolates that were non-susceptible to caspofungin but susceptible to other agents [29]. Therefore, commercialized assays like SYO may reduce the inter-laboratory variation in caspofungin MICs seen with reference methods; at the same time, CLSI and EUCAST interpretive criteria may not be valid in classifying MICs generated by these assays, particularly for caspofungin.

The distinction between clinical breakpoints and ECVs is another issue of confusion for many clinicians. Clinical breakpoints assign MICs at which the likelihood of treatment failure is increased, and thus they are designed to predict patient outcomes. An ECV, on the other hand, distinguishes between a population of wild-type, drug-susceptible isolates and a population that includes non-wild-type isolates with acquired resistance mechanisms. To this end, the ECV is designed to be a sensitive surveillance tool for detecting emergence of resistance. As in the case of the echinocandins, clinical breakpoint MICs and ECVs may not agree (Table 2) [49,50]. In prioritizing sensitivity to detect resistant mutants, ECVs may sacrifice the specificity needed to be useful clinically; at the same time, some mutations confer low-level resistance that does not impact treatment responses. Echinocandin ECVs are sensitive at detecting *FKS* mutant Candida [50,51], but it does not necessarily follow that they are useful in guiding treatment.

In fact, correlations between echinocandin MICs and treatment responses remain uncertain. In a comprehensive investigation of 746 Candida isolates from 6 caspofungin treatment studies for esophageal (4 trials; 515 isolates) or invasive candidiasis (2 trials; 231 isolates), there was no correlation between caspofungin MICs (as determined by broth microdilution) and patient outcomes [44]. Upon closer consideration, these results are not necessarily surprising since studies did not include patients with prior echinocandin exposure. Indeed, the absence of associations between MICs and outcomes in patients at low-risk for resistance highlights that microbiologic resistance is not the sole determinant of treatment response. For invasive candidiasis, factors like underlying disease, host immune function, severity of illness, source control, time to initiation of treatment, PK-PD parameters and isolate fitness may play more significant roles in a given patient than antifungal

susceptibility [52]. Studies seeking to demonstrate correlations between echinocandin MICs and outcomes should include patients from high-risk populations in which resistance is most likely to emerge.

Echinocandin MICs, *FKS* mutations and treatment outcomes

Several single-center studies have investigated MICs and *FKS* mutations as potential risk factors for echinocandin treatment failures among patients with invasive candidiasis. At three centers using various testing methods and either CLSI or institution-specific, receiver operator characteristic (ROC)-derived breakpoints, treatment failure rates among patients with invasive infections due to caspofungin-resistant or *FKS* mutant *C. glabrata* were 47%-79% and 60-90%, respectively (Table 3) [5,19-21,53]. Caspofungin resistance was 32-53% sensitive (percentage of patients in whom treatment failed who were infected with resistant isolates) and 75-95% specific (percentage of patients in whom treatment was successful who were infected with non-resistant isolates). The corresponding sensitivity and specificity for the presence of an *FKS* mutation were 35–41% and 90–98%, respectively [5,19-21]. In a study of cancer patients with *C. glabrata* candidemia that did not assess *FKS* mutations, caspofungin MICs determined by CLSI broth microdilution were inversely related to 28-day all-cause mortality (P=0.001); mortality rates among patients infected with resistant and non-resistant *C. glabrata* isolates by CLSI breakpoints were 57% (8/14) and 28% (22/79), respectively [34]. Caspofungin resistance or the presence of an *FKS* mutation was an independent risk factor for treatment failure in some, but not all of these studies [5,19-21,34,54].

Prior echinocandin exposure likewise was associated with unsuccessful treatment of invasive *C. glabrata* infections in several studies [5,20], and implicated as an independent risk factor in one study [19]. Echinocandin exposure, *FKS* mutations and phenotypic resistance are inter-related factors, which complicates assessment of their relative contributions to outcomes. An elevated MIC in the setting of prior echinocandin exposure was a useful surrogate for the detection of *FKS* mutations among *C. glabrata* isolates at one center, and an algorithm that considered these factors accurately predicted treatment responses (Figure) [21,53].

Data are limited for infections due to species other than *C. glabrata*, and for associations between anidulafungin and micafungin MICs and treatment responses. Elevated echinocandin MICs and *FKS* mutations among *C. albicans*, *C. krusei*, and *C. tropicalis* isolates have been linked to unsuccessful therapy in individual cases [22,24,55-58]. In clinical trials, outcomes among patients treated with echinocandins for *C. parapsilosis* candidemia have not been impaired, despite higher MICs and intrinsic *FKS* polymorphisms [15-17]; conclusive data on deep-seated *C. parapsilosis* infections such as endocarditis are lacking. An association was demonstrated between caspofungin resistance, as determined by broth microdilution and CLSI breakpoints, and excess mortality among cancer patients with candidemia due to non-*C. albicans* species [54]; however, these findings were based largely on resistant *C. glabrata*. In another study that used CLSI broth microdilution methods and clinical breakpoints, anidulafungin and micafungin MICs were superior to caspofungin MICs in predicting caspofungin treatment outcomes among patients with invasive *C.*

glabrata infections [53]. Of note, ROC-derived breakpoints for anidulafungin and micafungin performed better than CLSI breakpoints. Moreover, ROC-derived caspofungin breakpoints performed comparably to ROC-derived anidulafungin and micafungin breakpoints. For each of the agents, SYO or Etest MICs and ROC-derived breakpoints correlated with caspofungin treatment responses at least as well as broth microdilution MICs [21,53].

Perspectives on echinocandin resistance in clinical practice

Several conclusions from the published data on echinocandin resistance serve as a foundation for rational management strategies against invasive candidiasis: 1) *FKS* mutations remain rare among clinical Candida isolates; 2) Mutations are encountered almost exclusively among *C. glabrata* or *C. albicans* recovered from echinocandin treatment-experienced patients, most commonly during breakthrough infections; 3) Mutations are generally difficult to induce in clinical settings and typically require weeks to months of echinocandin exposure; 4) Most echinocandin treatment failures are not due to microbiologic resistance. Given these facts, we propose that echinocandins remain the preferred agents against the vast majority of invasive Candida infections, and susceptibility testing and/or screening for *FKS* mutations does not have a role in routine clinical practice.

For the subset of patients with echinocandin-breakthrough invasive candidiasis or infections following prolonged, remote drug exposure, a practical approach is to treat with an agent from an antifungal class for which the patient is treatment-naïve. This strategy is infeasible in a growing minority of echinocandin-experienced patients who have prior exposure to multiple antifungals. In such patients or in those with invasive candidiasis who do not respond to echinocandin treatment, susceptibility testing or *FKS* genotyping may be useful. At present, it is unclear if echinocandin MICs or *FKS* genotypes correlate more closely with treatment responses. Given uncertainties about susceptibility testing methods and precise clinical breakpoints, *FKS* mutations are easier to interpret; however, most hospital labs are not equipped to perform genotypic testing. An elevated echinocandin MIC in the context of prior drug exposure may be a useful proxy for the presence of an *FKS* mutation. Although MICs or *FKS* genotypes are not needed to manage most cases of invasive candidiasis, centers should test isolates for epidemiologic purposes and as surveillance for resistance.

Clinicians need to be aware of the susceptibility testing method employed in their hospital, and understand MIC distributions of individual echinocandins against common Candida species. Caspofungin MICs determined by broth microdilution and corresponding CLSI clinical breakpoints, in particular, may not be reliable. To place MIC results in some context, institutional MIC and resistance patterns for all agents should be compared to those published in the literature. Caspofungin MICs may be reliable at centers using SYO or other commercial assays, but local MIC distributions must be validated. If caspofungin MICs are skewed, anidulafungin or micafungin MICs may be used as surrogates for the class. This strategy is not satisfactory for the field in the long-term, however, as accurate caspofungin MICs are essential for determining differences in efficacy between agents, and for meaningful epidemiologic and PK-PD studies.

Conclusions

Echinocandin resistance among Candida species has emerged, and the challenges it poses to clinicians are likely to become more pronounced. As clinicians grapple with the issue, several questions merit immediate research investigation (Table 4). The emergence of echinocandin resistance has stemmed directly from the extensive use of these agents. Therefore, strict antifungal stewardship is the most powerful weapon for preserving echinocandin susceptibility.

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Key Points

- Mutations in hot spot regions of *FKS* genes lead to decreased echinocandin susceptibility among Candida species, but they remain uncommon outside of *C. glabrata* or *C. albicans* isolates recovered from patients with breakthrough infections or extensive drug exposure in the recent past.
- The clinical utility of echinocandin minimum inhibitory concentration (MIC) measurements is limited by the low overall prevalence of resistant Candida isolates, and uncertainties about optimal testing methods, inter-laboratory reproducibility (particularly for caspofungin), resistance breakpoints, and correlations with responses to echinocandin treatment among patients with invasive candidiasis.
- Most echinocandin treatment failures for invasive candidiasis are not due to drug resistance, but rather a combination of factors such as underlying disease, host immune function, severity of illness, source control, time to initiation of treatment, pharmacokinetic-pharmacodynamic parameters, and isolate fitness.
- Either an elevated echinocandin MIC or the detection of an *FKS* mutation may predict treatment failures among patients with invasive candidiasis due to *C. glabrata*, but the roles of genotypic or phenotypic testing in clinical practice are undefined.
- Echinocandins remain preferred agents against the vast majority of invasive Candida infections, and susceptibility testing and/or screening for *FKS* mutations is best reserved for echinocandin-experienced patients with newly diagnosed infections or those who have not responded to echinocandin treatment.

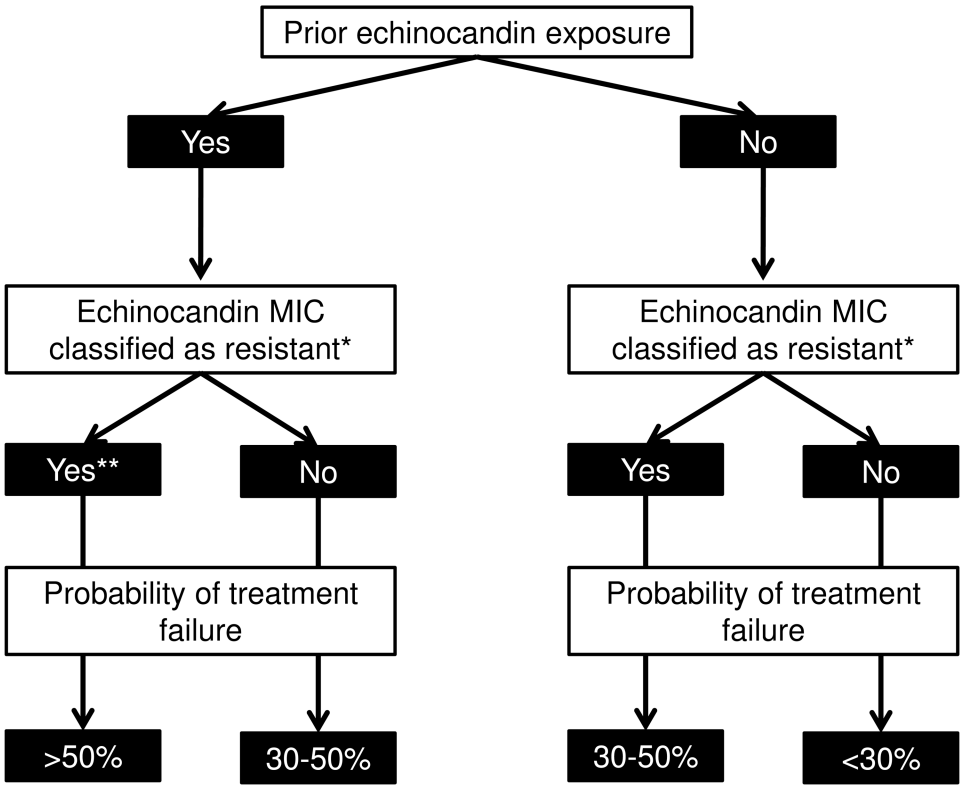

Figure. An algorithm for predicting echinocandin treatment responses among patients with *C. glabrata* invasive candidiasis based on prior drug exposure and MICs

The algorithm is based upon single-center data published for each of the echinocandins [21, 53]. Note that failure rates include unsuccessful treatment of newly-diagnosed invasive candidiasis and breakthrough infections. Rates are lower if only treatment of newly-diagnosed infections is considered.

* Resistance is classified according to institution-specific clinical breakpoints.

** Over 80% of *FKS* mutations are encountered in this arm.

Table 1

Rates of acquired *FKS* mutations among Candida isolates causing invasive infections

Species	Overall	Any prior echinocandin exposure[1]	Echinocandin breakthrough candidiasis	Echinocandin resistance among *FKS* mutants[2]	Reference(s)
C. albicans	1 – 3%	5%	50%	Approaching 100%	[18]
C. glabrata	4 – 18%	15 - 32%	67 - 75%	46 - 81%	[5, 18-23]
C. krusei	Case reports*	NA	NA	NA	[22, 24, 25]
C. tropicalis	Case reports*	NA	NA	NA	[22, 26-28]

*Insufficient data to determine rates; NA = Not available

[1]Includes remote exposure and ongoing exposure at time of breakthrough infections

[2]As defined by echinocandin-specific CLSI breakpoints

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Table 2

Clinical breakpoints and epidemiological cut-off values for echinocandins against Candida[1]

Agent	Species	MIC breakpoint (μg/mL)					
		CLSI			EUCAST[2]		ECV[3]
		S	I	R	S	R	
Anidulafungin	*C. albicans*	≤ 0.25	0.5	≥ 1	≤ 0.03	≥ 0.06	0.12
	C. glabrata	≤ 0.12	0.25	≥ 0.5	≤ 0.06	≥ 0.12	0.25
	C. krusei	≤ 0.25	0.5	≥ 1	≤ 0.06	≥ 0.12	0.12
	C. parapsilosis	≤ 2	4	≥ 8	≤ 0.002	> 4	4
	C. tropicalis	≤ 0.25	0.5	≥ 1	≤ 0.06	≥ 0.12	0.12
Caspofungin	*C. albicans*	≤ 0.25	0.5	≥ 1	Breakpoints have not been established due to significant inter-laboratory variation in MIC ranges for caspofungin		0.12
	C. glabrata	≤ 0.12	0.25	≥ 0.5			0.12
	C. krusei	≤ 0.25	0.5	≥ 1			0.25
	C. parapsilosis	≤ 2	4	≥ 8			1
	C. tropicalis	≤ 0.25	0.5	≥ 1			0.12
Micafungin	*C. albicans*	≤ 0.25	0.5	≥ 1	≤ 0.016	≥ 0.03	0.03
	C. glabrata	≤ 0.06	0.12	≥ 0.25	≤ 0.03	≥ 0.06	0.03
	C. krusei	≤ 0.25	0.5	≥ 1	IE	IE	0.12
	C. parapsilosis	≤ 2	4	≥ 8	≤ 0.002	> 2	4
	C. tropicalis	≤ 0.25	0.5	≥ 1	IE	IE	0.12

S = Susceptible, I = Intermediate, R = Resistant, IE = Insufficient evidence

[1] Data are presented for five Candida species that account for >95% of invasive candidiasis.

[2] An intermediate classification is not provided, but it is designated for values between the susceptible and resistant breakpoints.

[3] Epidemiological cut-off value (ECV), as defined using CLSI reference broth microdilution methods. ECV is the highest endpoint of the MIC distribution of the wild type population, as established using MIC distributions from multiple laboratories. Epidemiological cutoff values (ECOFFs) also have been proposed for anidulafungin by EUCAST [41], but they are not presented here to maintain clarity.

Table 3

Factors associated with echinocandin treatment failure for *C. glabrata* invasive candidiasis

Study (reference)	Variable associated with echinocandin treatment failure	Rate of failure	*P*-value	Multivariate *P*-value
Shields et al. 2012 [20]	Caspofungin MIC > 0.5 μg/mL (BMD)	60% (6/10)	0.009	NS
	Presence of *FKS* mutation	86% (6/7)	0.0004	0.002
	Prior echinocandin exposure	54% (7/13)	0.008	NS
	GI surgery within 30 days	42% (8/19)	0.031	NS
Shields et al. 2013 [21]	Caspofungin MIC > 0.25 μg/mL (Etest)	79% (11/14)	0.0001	--
	Presence of *FKS* mutation	90% (9/10)	0.0001	--
	Prior echinocandin exposure	62% (13/21)	0.002	--
Shields et al. 2013 [53]	Anidulafungin MIC > 0.12 μg/mL (BMD)	83% (5/6)	0.014	--
	Micafungin MIC > 0.06 μg/mL (BMD)	55% (6/11)	NS	--
Alexander et al. 2013 [5]	Caspofungin MIC ≥ 0.5 μg/mL (BMD)	73% (8/11)	NS	NS
	Micafungin MIC ≥ 0.25 μg/mL (BMD)	75% (6/8)	NS	NS
	Presence of *FKS* mutation	62% (8/13)	0.039	NS
Beyda et al. 2014 [19]	Caspofungin MIC ≥ 0.25 μg/mL (SYO)	47% (9/19)	0.04	NS
	Presence of *FKS* mutation	60% (6/10)	0.02	NS
	Echinocandin exposure within 60d	73% (8/11)	<0.01	<0.01
	Underlying GI disorder	45% (14/31)	<0.01	0.04

BMD = Broth microdilution; GI = Gastrointestinal; NS = Not significant; SYO = Sensititre YeastOne

Table 4

Pressing questions for research on echinocandin resistance among Candida species

Questions
• What are the optimal echinocandin susceptibility testing methods for hospital microbiology laboratories, and what are the most accurate clinical breakpoint MICs using these methods? • Do Sensititre YeastOne assays or other testing methods eliminate inter-laboratory variations in caspofungin MICs that are seen with reference broth microdilution methods? • What are the roles of echinocandin susceptibility testing and screening for *FKS* gene mutations in clinical practice? • What mechanisms other than *FKS* mutations mediate echinocandin resistance among Candida species? • Are there clinically meaningful differences between echinocandins, such as agent-specific resistance mechanisms or site-specific pharmacokinetics? • Can antifungal stewardship programs limit the emergence of echinocandin-resistant Candida? • Can rapid diagnostics and molecular resistance markers be incorporated into rational patient management strategies?