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Breakthrough Invasive Candidiasis in Patients on Micafungin▽†

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For *Candida* species, a bimodal wild-type MIC distribution for echinocandins exists, but resistance to echinocandins is rare. We characterized isolates from patients with invasive candidiasis (IC) breaking through ≥3 doses of micafungin therapy during the first 28 months of its use at our center: MICs were determined and hot-spot regions within *FKS* genes were sequenced. Eleven of 12 breakthrough IC cases identified were in transplant recipients. The median duration of micafungin exposure prior to breakthrough was 33 days (range, 5 to 165). Seventeen breakthrough isolates were recovered: *FKS* hot-spot mutations were found in 5 *C. glabrata* and 2 *C. tropicalis* isolates; of these, 5 (including all *C. glabrata* isolates) had micafungin MICs of >2 μg/ml, but all demonstrated caspofungin MICs of >2 μg/ml. Five *C. parapsilosis* isolates had wild-type *FKS* sequences and caspofungin MICs of 0.5 to 1 μg/ml, but 4/5 had micafungin MICs of >2 μg/ml. The remaining isolates retained echinocandin MICs of ≤2 μg/ml and wild-type *FKS* gene sequences. Breakthrough IC on micafungin treatment occurred predominantly in severely immunosuppressed patients with heavy prior micafungin exposure. The majority of cases were due to *C. glabrata* with an *FKS* mutation or wild-type *C. parapsilosis* with elevated micafungin MICs. MIC testing with caspofungin identified all mutant strains. Whether the naturally occurring polymorphism within the *C. parapsilosis FKS1* gene responsible for the bimodal wild-type MIC distribution is also responsible for micafungin MICs of >2 μg/ml and clinical breakthrough or an alternative mechanism contributes to the nonsusceptible echinocandin MICs in *C. parapsilosis* requires further study.

Invasive candidiasis (IC) is an important, life-threatening infection in hospitalized patients. The echinocandins (micafungin, caspofungin, and anidulafungin) are the newest class of medications approved for the prophylaxis and treatment of IC. They act via noncompetitive inhibition of β-1,3-glucan synthase, the enzyme responsible for producing β-1,3-D-glucan in the fungal cell wall (41). These drugs have low toxicity and few drug-drug interactions and possess a broad spectrum of antifungal activity against *Candida* species, including those resistant to fluconazole. In clinical trials, the echinocandins have demonstrated noninferiority for the treatment of IC versus amphotericin B deoxycholate, liposomal amphotericin B, and fluconazole (25, 32, 44). The echinocandins are considered interchangeable for clinical use, and a recent study comparing micafungin to caspofungin for IC supports this notion (38). Based on the accumulated experience, echinocandins are now considered a first-line therapeutic choice for IC (37).

The echinocandins exhibit a bimodal MIC distribution among *Candida* species. MICs of *C. parapsilosis*, *C. guilliermondii*, and *C. famata* MICs (MIC_{90}, 0.25 to 2 μg/ml) are up to 133 times higher than those of *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei*, *and C. kefyr* (MIC_{90}, 0.015 to 0.25 μg/ml) (42). However, this difference has not translated into consistent clinical failure (25, 38, 44), and the MIC breakpoint for echinocandin susceptibility was set at ≤2 μg/ml, which is inclusive of 99% of the wild-type distribution of all *Candida* species (9). Organisms with MICs of >2 μg/ml are considered "nonsusceptible," but the breakpoint for resistance has yet to be determined owing to the paucity of clinical isolates available from patients failing echinocandin therapy and with MICs of >2 μg/ml.

As echinocandin use has escalated, cases of echinocandin breakthrough IC have been described (6, 7, 13, 25, 39, 50), and nonsusceptible isolates (MIC > 2 μg/ml) have been recovered from patients who demonstrated treatment failure (9). Moreover, several of these nonsusceptible isolates possess nonsynonymous point mutations in genes encoding the β-1,3-glucan synthase enzyme complex (Fksp) (4, 13, 39, 47). These specific *FKS* "hot-spot" mutations reduce the susceptibility of the β-1,3-glucan synthase enzyme complex to echinocandin drugs, supporting a biological mechanism of resistance (14).

In February 2006, micafungin became the formulary echinocandin at our hospital, a tertiary care center with multiple intensive care units, two dedicated hematopoietic stem cell transplant (HSCT) units, and an active solid organ transplant (SOT) service. Multiple patients with breakthrough IC while receiving micafungin therapy were noted. These cases were reviewed, and the *Candida* isolates recovered from these patients were screened for *FKS* gene mutations; results were correlated with MIC values.

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MATERIALS AND METHODS

Definitions. Breakthrough IC was defined as a positive culture for *Candida* spp. collected from a normally sterile site in a patient receiving micafungin for 3

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days (minimum of 3 doses of drug). Episodes were further characterized as new disease versus recurrence of previously documented disease based on clinical and microbiologic characteristics. For patients with previously documented disease, IC was considered breakthrough only if the primary therapy was successful (i.e., negative culture from the original site of infection, if available, and clinical resolution of symptoms and signs of infection). Cases of primary treatment failure, defined by persistently positive culture, were excluded. The source of breakthrough infection was determined by investigator adjudication. Specifically, because catheter cultures are not performed at our institution, we defined catheter-related candidemia as a patient who had an indwelling central venous catheter (CVC) at the time of breakthrough candidemia and no other apparent source for bloodstream infection (with the exception of the catheter).

Chart review. This study was approved by the Duke University Medical Center (DUMC) Institutional Review Board. Pharmacy records were queried to determine the denominator which included all patients who received at least 3 doses of micafungin. The DUMC Clinical Microbiology Laboratory database was queried to identify patients with *Candida* spp. isolated from sterile body sites during the study period, and the lists were cross-referenced. Medical records were reviewed to confirm cases and to extract pertinent clinical information.

Susceptibility testing. Isolates were originally recovered by the BACTEC 9240 or BacT/Alert 3D blood culture system. *Candida* sp. isolates were retrieved from frozen storage (−80°C) and reidentified by classical methods, and susceptibility testing was performed in duplicate using the CLSI M27-A3 broth microdilution method (9). An echinocandin MIC of >2 μg/ml was considered nonsusceptible (9).

Molecular identification. Initial identification was confirmed by sequencing of the 5.8S RNA gene and adjacent internal transcribed spacer regions 1 and 2 (52). Molecular identification was performed in order to avoid misidentification with the novel anamorphic related species of *C. glabrata* [*C. bracarensis* (10) and *C. nivariensis* (1)] and *C. parapsilosis* [*C. orthopsilosis* and *C. metapsilosis* (45)].

Genotyping. The *Candida FKS1* and *FKS2* genes were sequenced in the "hot-spot" regions by the Sanger methodology using a CEQ 8000 Beckman Coulter genetic analysis system. GenBank accession numbers and *FKS* region sequences are displayed in the supplemental material.

MLST. Two pairs of isolates underwent multilocus sequence typing (MLST) as previously described (11, 46), using the *Candida tropicalis* Multi Locus Sequence Typing website developed by Keith Jolley, sited at the University of Oxford, Oxford, United Kingdom (19a), funded by the Wellcome Trust (accessed 11 April 2010).

Statistical analysis. Descriptive statistics were determined. The Wilcoxon rank sum test was employed for between-group comparisons. Statistical analyses were performed using the SAS 9.2 software program (SAS Institute, Cary, NC). Two-sided *P* values of 0.05 were used to determine statistical significance.

RESULTS

From February 2006 through May 2008, 649 patients received at least 3 doses of micafungin. Twelve patients (1.8%) with breakthrough infection met our predefined case definition and are summarized in Table 1. Case patients had a mean age of 43 years (range, 18 to 65) and included 7 males and 5 females. Underlying diseases included receipt of HSCT (n = 5), orthotopic liver transplant (OLT) (n = 3), bilateral orthotopic lung transplant (BOLT) (n = 3), and ventral hernia repair with chronic mesh infection (n = 1). Of the HSCT recipients, four had received allogeneic HSCT and had graft-versus-host disease (GVHD). Two patients were neutropenic, including the single autologous HSCT recipient.

Micafungin breakthrough occurred a median of 41 days following transplantation (range, 2 to 284 days). The median numbers of days to breakthrough IC following HSCT and SOT were 190 days and 30 days, respectively (difference between groups, P = 0.15). At the time of breakthrough infection, all case patients were receiving micafungin (100 mg) intravenously daily. The median total micafungin exposure in the 6 months preceding breakthrough IC was 33 days (range, 5 to 165), while the median contiguous micafungin exposure prior to breakthrough was 20 days (range, 5 to 165 days). Neither the contiguous nor the total micafungin exposure differed significantly between micafungin-nonsusceptible versus susceptible isolates (P = 0.51 and P = 0.53, respectively) or isolates with versus without *FKS* hot-spot mutations (P = 0.42 and P = 0.18, respectively).

Indications for micafungin administration included prophylaxis (4 cases), empirical therapy for sepsis (3 cases), treatment for diagnosed *Candida* infection (3 cases), and febrile neutropenia (2 cases). In all cases, empirical therapy was continued as prophylaxis after initial cultures for fungus were negative.

Because several patients had multiple pathogens recovered from various sites, 25 total *Candida* isolates were targeted for analysis, including 19 recovered at the time of breakthrough, of which 17 were available for testing (Table 2). Two breakthrough isolates were unavailable for analysis (*C. parapsilosis* from biliary fluid, patient 8; *C. albicans* from pleural fluid, patient 10), and two other isolates were likely the same strain captured from different sites. These two isolates, *C. tropicalis* from pleural fluid and from blood in patient 11, were recovered 1 day apart and had identical *FKS* gene sequences, MICs within one dilution for all susceptibility tests performed, including azoles, and an identical, unusual MLST (ST6) (*Candida tropicalis* Multi Locus Sequence Typing website developed by Keith Jolley, sited at the University of Oxford [19a], funded by the Wellcome Trust; accessed 11 April 2010). Patient 8 had a second episode of IC with *C. glabrata* 15 days after the initial breakthrough episode with *C. parapsilosis*. This occurred after micafungin was discontinued in favor of liposomal amphotericin B plus fluconazole combination therapy, but given the recent prior *C. parapsilosis* breakthrough with micafungin therapy, these *C. glabrata* isolates were also analyzed. MLST of both *C. glabrata* isolates revealed ST3, a common global *C. glabrata* strain type (29) (Multi Locus Sequence Typing website developed by David Aanensen, sited at Imperial College, London, funded by the Wellcome Trust; accessed 11 April 2010). The isolates also had identical *FKS* gene sequences and echinocandin MICs but different morphology types and disparate azole MICs (fluconazole and voriconazole MICs [μg/ml] for the 1st isolate were 16 and 0.75, while MICs for the 2nd isolate were >256 and >32, respectively). Thus, each isolate is presented individually. Details of the MLST analyses are presented in the supplemental material.

Breakthrough yeasts were most commonly recovered from blood (8/12 cases) or blood and another site (2/12 cases). In the other two cases, the breakthrough isolate was recovered from pleural fluid (1/12 cases) and ascites plus abdominal fascia (1/12 cases). Among all HSCT recipients, a central venous catheter (CVC) was implicated by the investigators as the likely source of breakthrough infection. The source was judged to be the abdomen for all liver transplant recipients and the thorax for 2 of the 3 lung transplant recipients.

The most common breakthrough isolates were *C. parapsilosis* (7 isolates) and *C. glabrata* (6 isolates), followed by *C. tropicalis* (3 isolates), *C. albicans* (1 isolate), *C. dubliniensis* (1 isolate), and *C. krusei* (1 isolate). Per Table 2, of the 17 breakthrough isolates available for testing, 10 (59%), 7 (41%), and 11 (65%) were nonsusceptible (MIC > 2 μg/ml) to micafungin, caspofungin, and anidulafungin, respectively. Five of 6 (83%) *C. parapsilosis* isolates and 5/6 (83%) *C. glabrata* isolates were micafungin nonsusceptible; all other *Candida* spp. re-

TABLE 1. Clinical characteristics of patients with *Candida* breakthrough infection receiving micafungin at DUMC, February 2006 to May 2008[a]

Pt	Age (yr)	Sex	Underlying disease in host	Indication for micafungin	Breakthrough *Candida* spp.	Time from transplant to breakthrough infection[b] (days)	Contiguous micafungin exposure (days)	Total micafungin exposure[c] (days)	Site(s) of culture	Likely source of infection	Breakthrough infection treatment/outcome	Patient outcome (days postbreakthrough)
1	47	M	MM s/p auto-HSCT	FN	*C. parapsilosis*; *C. krusei*	16	5	5	Blood	Abdomen or CVC	Failed ABLC (5 d) and CVC removal	Died (9): *Pseudomonas*/*Candida* sepsis
2	20	M	DBA s/p allo-HSCT w/GVHD	FN	*C. parapsilosis*	41	22	22	Blood	CVC	Failed various combination antifungals including LAMB, FLU, VORI, and MCF and CVC removal	Died (22): *C. parapsilosis*/VRE sepsis
3	21	M	NHL, CTCL s/p allo-HSCT w/GVHD	Prophylaxis	*C. glabrata*	246	165	165	Blood	CVC	Success w/VOR (14 d) and CVC removal	Died (19): VZV encephalitis, recurrent CTCL
4	18	F	ALL/MDS s/p 2nd allo-HSCT w/GVHD	Treatment for *C. parapsilosis*-infected lung hematoma	*C. parapsilosis*	284	14	104	Blood	CVC	Success w/LAMB (8 wks) and CVC removal (continued as prophylaxis)	Died (73): MOF; respiratory failure with parainfluenza virus
5	25	F	MLD s/p allo-HSCT w/GVHD	Treatment for *C. glabrata* vaginitis	*C. glabrata*	190	20	43	Blood	Vagina or CVC	Success w/LAMB (8 d) and CVC removal after failing combination FLU/VOR + MCF for 44 d	Died (62): MOF, progressive MLD
6	45	M	OLT w/primary graft failure	Prophylaxis	*C. glabrata*	73	21	43	Blood	Abdomen	Died prior to knowledge of breakthrough infection	Died (1): graft failure, *C. glabrata* sepsis
7	49	M	OLT #3 w/primary graft failure	Empirical for sepsis, continued as prophylaxis	*C. glabrata*; C. *parapsilosis*	2	8	22	Ascites; abdominal fascia	Abdomen	Success w/53 d of ABLC	Died (115): graft failure, VRE/*Pseudomonas* sepsis
8	60	M	OLT #2 w/primary graft failure	Empirical for sepsis, continued as prophylaxis	*C. parapsilosis*; *C. glabrata*[d]	7; 21	19; 0	19; 22	Blood/biliary fluid; blood	Abdomen	Failed combination LAMB + FLU	Died (12): graft failure, *C. glabrata* sepsis
9	54	F	BOLT	Prophylaxis	*C. parapsilosis*	25	20[e]	20	Blood	CVC or infected clot	Success w/21 d of LAMB after failing 15 d of VOR and CVC removal	Died (62): MOF, VRE sepsis
10	64	M	BOLT	Treatment for *C. albicans* and *C glabrata* fungemia	*C. albicans*; unspecified yeast	34	28	28	Pleural fluid; lung tissue[f]	Pleural space/ lung	Success w/MCF (150 mg/d) + inhaled ABLC followed by 150 d FLU	Survived; alive and well at day 480
11	65	F	BOLT	Empirical for sepsis after prior treatment for IC	*C. dubliniensis*; *C. tropicalis*	102	10	56	Pleural fluid; blood/ pleural fluid	Pleural space	Died after only 1 dose of ABLC	Died (2): disseminated candidiasis
12	45	F	Ventral hernia infection	Prophylaxis	*C. tropicalis*	NA	37	37	Blood	Abdomen or CVC	Success w/ABLC (14 d) and CVC removal	Died (24): polymicrobial (bacterial) sepsis

[a] ABLC, amphotericin B lipid complex; ALL, acute lymphoblastic leukemia; allo, allogeneic; AML, acute myelogenous leukemia; ANF, anidulafungin; auto, autologous; BOLT, bilateral orthotopic lung transplant; CAS, caspofungin; CTCL. cutaneous T-cell lymphoma; CVC, central venous catheter; d, day(s); DBA, diamond-blackfan anemia; F, female; FN, febrile neutropenia; GVHD, graft-versus-host disease; HSCT, hematopoietic stem cell transplant; IC, invasive candidiasis; LAMB, liposomal amphotericin B; M, male; MCF, micafungin; MDS, myelodysplastic syndrome; MLD, metachromatic leukodystrophy; MM, multiple myeloma; MOF, multiorgan failure; NA, not applicable; NHL, non-Hodgkin lymphoma; OLT, orthotopic liver transplant; Pt, patient number; s/p, status post; VRE, vancomycin-resistant enterococcus; VZV, varicella-zoster virus; w/, with.

[b] For patients that received >1 transplant, the date of the most recent transplant was used. In all cases involving multiple *Candida* species except case 8, isolates were recovered within 2 days of each other.

[c] Total micafungin exposure over the 6 months prior to breakthrough IC.

[d] For patient 8, *C. glabrata* isolates were recovered 14 days after the initial *C. parapsilosis* breakthrough infection; this occurred after MCF was discontinued in favor of LAMB plus FLU combination therapy. The *C. glabrata* isolates are not included in the analysis of days from transplant to breakthrough infection or duration of contiguous micafungin exposure. MLST of both *C. glabrata* isolates revealed ST3, a common global *C. glabrata* strain type. The isolates also had identical FKS gene sequences and echinocandin MICs but different morphology types and disparate azole MICs (fluconazole and voriconazole MICs [μg/ml] for the 1st isolate were 16 and 0.75, while MICs for the 2nd isolate were >256 and >32, respectively). Thus, each isolate is presented individually.

[e] For patient 9, the first 10 days of echinocandin therapy were with anidulafungin, followed by 10 days of micafungin.

[f] Patient 10 had *C. albicans* recovered from pleural fluid and intra-alveolar yeast visualized on histopathology of lung tissue.

TABLE 2. *In vitro* susceptibilities and genotypes of breakthrough isolates[a]

Patient no.	Breakthrough *Candida* species	Site of culture	MIC (μg/ml)[b]			Fksp amino acid substitution[c]
			ANF	CAS	MCF	
1	*C. parapsilosis*	Blood	4	1	4	None
	C. krusei	Blood	0.25	1	0.25	1: H675H/Q[d]
2	*C. parapsilosis*	Blood	8	1	8	None
3	*C. glabrata*	Blood	8	>16	8	1: S629P
4	*C. parapsilosis*	Blood	4	1	4	None
5	*C. glabrata*	Blood	4	>16	4	2: S663P
6	*C. glabrata*	Blood	0.25	0.25	0.25	None
7	*C. parapsilosis*	Abdominal fascia	2	0.5	1	None
	C. glabrata	Ascites	4	4	4	2: S663F
8	*C. parapsilosis*	Blood	4	0.5	4	None
	C. parapsilosis[h]	Biliary fluid				
	C. glabrata[e]	Blood	8	>16	8	2: S663P
	C. glabrata[e]	Blood	4	>16	4	2: S663P
9	*C. parapsilosis*	Blood	4	1	4	None
10	*C. albicans*[h]	Pleural fluid				
11	*C. dubliniensis*	Pleural fluid	0.06	0.25	0.06	1: L635V + T655A[d]
	C. tropicalis[f]	Blood	4	8	2	1: S80S/P[g]
	C. tropicalis[f]	Pleural fluid	2	4	2	1: S80S/P[g]
12	*C. tropicalis*	Blood	0.12	0.25	0.06	None

[a] ANF, anidulafungin; CAS, caspofungin; MCF, micafungin.
[b] Susceptibility testing was performed using the M27-A3 broth microdilution method (9).
[c] The number preceding the colon has the following meanings: 1 denotes *FKS1*, and 2 denotes *FKS2.* The first letter and following 3-digit number represent the wild-type amino acid for that position in the protein; the last letter denotes the resultant amino acid change from the gene mutation. In cases of diploid organisms, heterozygous mutations are annotated by 2 letters (example, S/P).
[d] Outside the "hot-spot" regions.
[e] Patient 8 *C. glabrata* breakthrough isolates obtained 14 days after the initial *C. parapsilosis* breakthrough infection; the patient was receiving liposomal amphotericin B, 5 mg/kg of body weight/day, and FLU, 400 mg/day, at the time of recovery. See Table 1, footnote *d*, and the primary text for further details.
[f] Patient 11 *C. tropicalis* was presumptively the same strain isolated from different sites (same *FKS* gene sequence and MICs to azoles and echinocandins within one dilution, and identical, unusual MLST sequence type [ST6]).
[g] For Fks1p, the *C. tropicalis* S80S/P amino acid substitution is the *C. albicans* S645S/P amino acid substitution equivalent.
[h] Breakthrough isolate not available.

mained micafungin susceptible. However, all 6 *C. parapsilosis* isolates remained caspofungin susceptible.

FKS gene mutations were detected in 9 isolates (*C. glabrata* [5 isolates], *C. tropicalis* [2 isolates], *C. dubliniensis* [1 isolate], and *C. krusei* [1 isolate]). All mutations detected in *C. glabrata* and *C. tropicalis* were in hot-spot regions; mutations detected in *C. dubliniensis* and *C. krusei* were outside the hot-spot regions and did not confer echinocandin nonsusceptibility. No mutations other than the naturally occurring polymorphism at the 3′ end of hot spot 1 were detected among the *C. parapsilosis* isolates.

Among the 5 *C. glabrata* isolates with hot-spot mutations, all were nonsusceptible to all 3 echinocandins. The *C. tropicalis* heterozygous *FKS* mutants demonstrated mixed susceptibility (MIC range, 2 to 8 μg/ml). Caspofungin *in vitro* testing perfectly separated *FKS* hot-spot mutants (caspofungin MIC $>$ 2 μg/ml) from strains carrying the wild-type *FKS* gene (caspofungin MIC $\leq$ 2 μg/ml). Six additional isolates of interest were also tested. *Candida* sp. isolates recovered from patients prior to micafungin breakthrough infection (n = 3) were echinocandin susceptible and without *FKS* gene rearrangements. Three *C. glabrata* isolates recovered after the initial breakthrough IC episode retained their respective *FKS* gene mutations and nonsusceptible MICs.

Treatment success of breakthrough IC was described in 7/12 (58%) cases; two patients died before receipt of $\geq$2 doses of alternate antifungal therapy, and three patients failed to clear their infection. Treatment regimens included both monotherapy (n = 8) and combination therapy (n = 4). The most common (n = 6) and successful (5/6; 83%) approach was single-agent lipid amphotericin B.

DISCUSSION

The frequency of breakthrough IC during echinocandin therapy varies depending on the indication for which the echinocandin is being used. Based on clinical trial data, rates of breakthrough IC range from 2.9% in patients receiving echinocandins empirically during febrile neutropenia to 0.2% in patients receiving echinocandin therapy for documented IC (7, 16, 25, 30, 32, 35, 38, 44, 48, 50). In case reports of breakthrough infection in the literature, the vast majority of patients were severely immunocompromised, and the indication for echinocandin therapy included febrile neutropenia (n = 4), hematologic malignancy prophylaxis (n = 5), and primary treatment for IC (n = 3) (6, 13, 21, 24, 39, 49). Given the varied indications for micafungin administration in our patients, the 1.8% rate of breakthrough IC appears consistent with previous reports.

The explanation for echinocandin breakthrough may be either clinical factors in the host and/or drug resistance in the pathogen (23). Our data suggest that both host and microbiologic factors may be contributing to echinocandin failure. Among isolates tested and reported in the literature, high MICs obtained *in vitro* have been associated with hot-spot mutations of the *FKS* genes (Table 3). Conversely, global sampling of *Candida* spp. with low (wild-type) echinocandin MICs has demonstrated the absence of such mutations (53). The

TABLE 3. Reports of echinocandin breakthrough or echinocandin-resistant invasive candidiasis[a]

Case report	Reference(s)	Host underlying disease/ indication[b]	Site of culture	Drug used	Drug exposure (days)	*Candida* species	MIC (μg/ml)[c]			Fksp amino acid substitution[d]
							ANF	CAS	MCF	
Breakthrough on echinocandin therapy	Wagner et al., 2005 (49)	HSCT/prophylaxis	Blood	CAS	11	*C. famata*				NR
	Park et al., 2005 (39)	FN	Blood	CAS	14	*C. albicans*		>8		1: S645F
		FN	Mouth		28	*C. albicans*		4		1: S645F
		FN	Stool		17	*C. krusei*		32		1: R1361G
	Cheung et al., 2006 (6)	Abdominal abscess/ treatment	Blood	CAS	12	*C. parapsilosis*		0.25		NR
	Krogh-Madsen et al., 2006 (24)	OLT/treatment	Biliary	CAS	135	*C. glabrata*		>8		NR
	Kabbara et al., 2008 (21)	HSCT/prophylaxis	Blood	CAS	41	*C. parapsilosis*	2	1	8	NR
		HSCT/prophylaxis	Blood	CAS	50	*C. parapsilosis*	2	1	8	NR
		HSCT/prophylaxis	Blood	CAS	26	*C. guilliermondii*	1	0.5	2	NR
	Garcia-Effron et al., 2008 (13)	FN	Blood	CAS	15	*C. tropicalis*	1	4	2	1: S80S/P[e]
		HSCT/prophylaxis	Blood	CAS	44	*C. tropicalis*	2	4	2	1: S80S/P[e]
		Malignancy/treatment	CVC	CAS	21	*C. tropicalis*	0.5	1	0.5	1: F76L[e]
Echinocandin failure with *in vitro* resistance	Hernandez et al., 2004 (17)	HIV	Throat	CAS	90	*C. albicans*		>64		NR
	Moudgal et al., 2005 (33)	IE	Blood	CAS	42	*C. parapsilosis*	2	>16	>16	NR
	Pelletier et al., 2005 (40)	Abdominal abscess	Blood	CAS	13	*C. krusei*		2		NR
	Laverdiere et al., 2006 (27)	HIV	Throat	CAS → MCF	~250	*C. albicans*	1	2	2	1: S645P; 1: R1361H
	Miller et al., 2006 (31)	HIV	Throat	CAS	~450	*C. albicans*		8		1: S645P
	Hakki et al., 2006 (15), and Kahn et al., 2007 (22)	AML	Throat	CAS	17	*C. krusei*	4	8	4	1: P655C/P
	Baixench et al., 2007 (3)	HIV	Throat	CAS	21	*C. albicans*		2	1	1: F641S
	Thompson et al., 2008 (47)	OLT	Peritoneal	CAS	40	*C. glabrata*	0.5	2	0.25	2: F659V
	Cleary et al., 2008 (8)	MOF	Blood	CAS	~28	*C. glabrata*	>2	>2	>2	1: D632E

[a] ANF, anidulafungin; AML, acute myelogenous leukemia; BOLT, bilateral orthotopic lung transplant; CAS, caspofungin; FN, febrile neutropenia; HD, hemodialysis; HIV, human immunodeficiency virus; HSCT, hematopoietic stem cell transplant; MCF, micafungin; MOF, multiorgan failure; NR, not reported; OLT, orthotopic liver transplant.

[b] Treatment is defined as therapy of prior documented invasive candidiasis.

[c] MIC determination was done by the M27-A3 method except for that of Baixench et al. (2007) (EUCAST); MICs not reported are left blank.

[d] The number preceding the colon denotes either 1 for Fks1p or 2 for Fks2p. After the colon, the first letter and following 3-digit number represent the wild-type amino acid for that position in the protein; the last letter denotes the resultant amino acid change from the gene mutation. In cases of diploid organisms, heterozygous mutations are annotated by 2 letters (example, S/P).

[e] For Fks1p, the *C. tropicalis* amino acid position numbers 76 and 80 are the equivalents of *C. albicans* amino acid numbers 641 and 645, respectively.

majority of our breakthrough *C. glabrata* isolates possessed *FKS* hot-spot mutations and nonsusceptible echinocandin MICs. Wild-type *C. glabrata* is inherently susceptible to echinocandins; in global surveillance studies, the MIC at which 90% of isolates were inhibited (MIC_{90}) by micafungin was 0.015 μg/ml, firmly placing it on the susceptible end of the species distribution (43). This wild-type susceptibility finding coupled with broadening azole resistance has driven the use of echinocandins for *C. glabrata* treatment, generating selection pressure for resistant organisms. Unlike that of other common *Candida* spp., the *C. glabrata* genome is haploid, requiring only a single *FKS* gene hot-spot mutation for "homozygosity." In addition, mutations in either *FKS1* or *FKS2* are sufficient to confer resistance. However, even in diploid *Candida* spp., heterozygous *FKS* hot-spot mutations typically result in either a resistant or mixed phenotype, and prior breakthrough case reports do not share this overrepresentation of *C. glabrata* (Table 3) (4, 14).

In contrast, *C. parapsilosis*, our most common breakthrough *Candida* spp., has not been associated with characteristic hot-spot mutations. Instead, a naturally occurring polymorphism in the *FKS* gene is thought to confer higher echinocandin MICs. Among 759 *C. parapsilosis* isolates recovered in global surveillance, the MIC_{90} of micafungin was 2 μg/ml, although no organism had an MIC of >2 μg/ml (42). The amino acid substitution of proline for alanine (P660A) encoded in the *FKS1* hot-spot region of *C. parapsilosis* appears to be responsible for the intrinsically higher MICs (12). In our *C. parapsilosis* breakthrough isolates, none had characteristic hot-spot mutations but all contained the naturally occurring P660A substitution and mixed echinocandin MICs. Eighty-three percent (5/6) of our *C. parapsilosis* isolates had nonsusceptible micafungin MICs (range, 4 to 8 μg/ml), a finding which clearly differs from the global surveillance data (42). These six isolates all had caspofungin MICs of 0.5 to 1 μg/ml. Whether this difference in MICs actually predicts clinical failure with micafungin versus caspofungin is not known. Kabbara et al. reported two cases of *C. parapsilosis* infection that broke through caspofungin treatment, and the 2 isolates had MIC distributions similar to those of our isolates; micafungin and caspofungin MICs in that study were 8 and 1 μg/ml, respectively (21). For *C. parapsilosis*, we are not aware of any animal study investigating the impact of differential echinocandin MICs (i.e., higher micafungin or anidulafungin than caspofungin MICs) on response to echinocandin treatment. The mechanisms responsible for and clinical impact of the mixed echinocandin MICs in *C. parapsilosis* are therefore unclear. Perhaps an unidentified secondary resistance mechanism is at play in these isolates and caspofungin testing *in vitro* is unable to detect its presence. Alternatively, caspofungin may have greater activity than the other echinocandins against these *C. parapsilosis* isolates. Additional investigation in this area is needed.

The apparent difference in echinocandin MICs among *FKS* gene mutants also merits comment. This relationship was previously explored in detail with *C. albicans* (14). In that study, all strains with caspofungin MICs of ≥2 μg/ml had both hot-spot gene rearrangements and a >50-fold decrease in glucan synthase sensitivity to echinocandins, indicating direct drug resistance. Interestingly, the micafungin and anidulafungin MICs of those same mutants were lower (range, 0.16 to 4 μg/ml); most were in the "susceptible" range. However, these differences in drug potency were neutralized and *in vitro* echinocandin cross-resistance became apparent when the culture medium was mixed with 50% human serum, a finding which has been replicated elsewhere (34, 36). In our case series, caspofungin testing by current CLSI guidelines, which does not include the addition of serum to the test medium, detected all 7 isolates with hot-spot mutations, while anidulafungin and micafungin would have missed 1 and 2 of the *C. tropicalis* isolates with heterozygous FKS mutations, respectively. Combining our results with other reports in the literature and based on *FKS* kinetic studies, caspofungin appears to be the most reliable of the echinocandins for detecting *FKS* gene mutations *in vitro* using the currently approved M27-A3 susceptibility testing method for yeasts.

It is important to emphasize that prolonged echinocandin exposure may play a role in the development of *FKS* mutations. Our patients had substantial micafungin exposure, and prior case reports of breakthrough infections describe similar long exposures (median, 24 days). Although the durations of micafungin exposure were not significantly different between species with and without *in vitro* micafungin susceptibility or between yeasts with and without *FKS* gene mutations, this lack of statistical correlation may have been due to the low total number of cases.

There may be a fitness cost to the yeasts from an altered Fks protein. Emerging data from both fly and mouse models comparing wild-type and *FKS* mutant *C. albicans* suggest attenuated virulence in the hot-spot-mutated strain (5). Of our 5 patients infected with hot-spot mutants, 2 were symptomatic with fever while 3 were septic and critically ill with several acute problems in addition to the IC. Thus, it is difficult to assess the clinical consequence, if any, of potential attenuated fitness of the *FKS* gene-mutated strains in our cohort.

In addition to microbiologic resistance, clinical factors clearly played a role in our echinocandin breakthrough infections. Five breakthrough isolates tested susceptible to all echinocandins *in vitro* and negative for hot-spot mutations yet were responsible for invasive disease during micafungin treatment. Microbial resistance was probably not the explanation. Although drug exposure was not formally assessed in our patients, all patients were receiving 100 mg of micafungin by vein daily, the recommended dose for IC (2). The patients in our study were very sick, with prolonged hospitalizations, and all but one either were receiving exogenous immunosuppression or were neutropenic: 11 died within 6 months of breakthrough IC diagnosis. The single long-term survivor was relatively healthy and experienced only temporary single-organ dysfunction at the time of breakthrough IC. The data support the hypothesis that some breakthrough yeast infections are markers for uncontrolled underlying disease rather than inappropriate antifungal therapy. Emerging mechanistic data may provide further insights. For example, echinocandins unmask the β-glucan of fungi enabling increased host macrophage and neutrophil activity *in vitro* (18, 26, 51). A variety of host responses were presumably blunted in our immune-suppressed patients.

Among clinical factors contributing to breakthrough infection, a protected site or persistent nidus of infection appeared

to play a significant role. In all 7 cases in which the CVC was implicated as the source of infection, removal of the catheter was necessary for treatment success. Biofilms on CVCs can protect the pathogen from antimicrobial killing and provide an ideal site for resistance to develop. Although echinocandins do have *in vitro* and *in vivo* activity in biofilm models, this did not seem to be protective in our patients (19, 28). The 3 liver transplant recipients all suffered from dysfunctional grafts which served as functionally irremovable reservoirs of infection. In a similar fashion, an infected pleural space likely served as the primary source of infection for two breakthroughs involving lung transplant recipients. As a class, the echinocandins, including micafungin, have performed well in intra-abdominal infection (25, 32, 54), but data on outcomes with echinocandins in *Candida* empyema are lacking. Distribution of micafungin in humans is limited, but data from animal models suggest adequate levels are achieved in lung, liver, spleen, and kidney tissue (20). Taken together, a protected site and persistent nidus yielded clinical resistance and may also have promoted microbiological resistance with the gradual selection of *FKS* mutant strains.

The echinocandins have emerged as a first-line therapy for IC and neutropenic fever, as well as effective agents for prophylaxis during the preengraftment phase of HSCT. In this report, we describe 12 cases of breakthrough IC occurring in patients receiving micafungin for a variety of indications. While the series is not large enough to declare definitive conclusions regarding the reasons for breakthrough infection, these cases likely involve a combination of microbiological and host factors. The majority of cases were due to either *C. glabrata* with *FKS* hot-spot mutations or wild-type *C. parapsilosis* with a naturally occurring polymorphism (P660A) encoded in the *FKS1* gene hot-spot region. Prolonged micafungin exposure may predispose to echinocandin resistance in *C. glabrata*, and caspofungin appears to be the most reliable surrogate for the echinocandin class for detecting *FKS* hot-spot mutations *in vitro*. Whether the naturally occurring polymorphism within the *C. parapsilosis FKS1* gene responsible for the bimodal wild-type MIC distribution is sufficient for micafungin "resistance" and clinical breakthrough or an alternative mechanism contributes to the nonsusceptible echinocandin MICs requires further study.

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