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Abdominal Candidiasis Is a Hidden Reservoir of Echinocandin Resistance

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***FKS* mutant *Candida* isolates were recovered from 24% (6/25) of abdominal candidiasis patients exposed to echinocandin. *Candida glabrata* (29%) and *Candida albicans* (14%) mutants were identified. Multidrug-resistant bacteria were recovered from 83% of *FKS* mutant infections. Mutations were associated with prolonged echinocandin exposure ($P = 0.01$), breakthrough infections ($P = 0.03$), and therapeutic failures despite source control interventions (100%). Abdominal candidiasis is a hidden reservoir for the emergence of echinocandin-resistant *Candida*.**

Echinocandin antifungals are front-line therapy for invasive candidiasis (1). Echinocandin resistance has emerged with increased use of these agents (2, 3). Resistance is mediated by point mutations within hot spots of *FKS* genes, which encode the echinocandin target enzyme β-1,3-D-glucan synthase. In studies from our center and others, prior echinocandin exposure was the major risk factor for emergence of *FKS* mutant *Candida* (2–4). The phenomenon is most common for *Candida glabrata*, a species that is unique for its haploid rather than diploid genome. We demonstrated that *C. glabrata FKS* mutations were predictive of echinocandin therapeutic failure among patients with invasive candidiasis (2). To date, the overwhelming majority of patients reported to be infected with echinocandin-resistant *Candida* have candidemia (2–4). Abdominal candidiasis is at least as common as candidemia (5), but the incidence and clinical impact of echinocandin resistance are unknown. In the present study, we tested the hypothesis that *FKS* mutant *Candida* isolates are common causes of abdominal candidiasis among patients who have received an echinocandin previously.

Patients with abdominal candidiasis and ≥3 days of prior echinocandin exposure were identified at the University of Pittsburgh Medical Center, a tertiary referral center that specializes in critical care medicine and solid-organ transplantation. Abdominal candidiasis was defined by a *Candida*-positive culture obtained from an intra-abdominal site by surgery or sterile procedure. Patients must have exhibited at least one of the following: fever (temperature of ≥38°C), hypotension (systolic blood pressure of ≤90 mm Hg or a decrease of >30 mm Hg from the baseline), local signs and symptoms of inflammation, and/or radiologic findings that suggested abdominal candidiasis. Patients with *Candida* organisms isolated exclusively from indwelling drains or catheters were excluded.

Candida isolates collected at the onset of abdominal candidiasis were selected from our biorepository. Echinocandin MICs were determined in duplicate by Sensititre YeastOne (Trek Diagnostics), which is the echinocandin susceptibility testing method used most commonly in clinical microbiology laboratories (6). YeastOne assays may limit the interlaboratory variability in caspofungin MICs reported with the CLSI broth microdilution method (6, 7). We previously demonstrated a trend toward improved identification of *FKS* mutant *C. glabrata* isolates with YeastOne (8, 9). *Candida krusei* ATCC 6258 and *Candida parapsilosis* ATCC 22019 were used as quality controls. MICs were interpreted according to CLSI susceptibility breakpoints (10); intermediate MICs were considered resistant. Multidrug-resistant (MDR) bacteria were defined by nonsusceptibility to at least three antimicrobial drug classes (11). Hot spots 1 and 2 of *FKS1* (all species) and *FKS2* (*C. glabrata* only) were amplified using PCR (2, 8).

Echinocandin therapeutic failure was defined as breakthrough abdominal candidiasis occurring while the patient had been receiving an echinocandin for ≥3 days or nonbreakthrough disease that failed to respond to an echinocandin and source control interventions (2, 12). In the latter scenario, failure was determined in accordance with consensus definitions for outcomes of antifungal therapy at 14 days (13). Comparisons between groups were made by Wilcoxon rank sum tests for continuous variables and chi-square or Fisher's exact tests for categorical variables. Significance was set at P values of ≤0.05 (two tailed).

Twenty-five patients with abdominal candidiasis who received an echinocandin for a median of 42 days (range, 4 to 438 days) were included (Table 1). Forty percent (10/25) of patients were men; the median age was 54 years (range, 23 to 73 years). All patients had underlying gastrointestinal (GI) diseases, and 92% (23/25) had undergone GI surgery within 30 days preceding the onset of abdominal candidiasis; 44% were solid-organ transplant recipients (multivisceral, 4 patients; small bowel, 4 patients; kidney, 1 patient; liver, 1 patient; and kidney-liver, 1 patient). Disease manifestations included abdominal abscesses ($n = 13$), peritonitis ($n = 8$), both abscesses and peritonitis ($n = 2$), and cholangitis or cholangitis plus peritonitis ($n = 1$ each). In one patient, a blood culture was also positive for the same *Candida* species. Forty percent (10/25) of abdominal candidiasis cases were echinocandin breakthrough infections, which occurred during caspofungin ($n = 9$) or micafungin ($n = 1$) therapy.

Twenty-seven *Candida* isolates were recovered from the 25 pa-

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TABLE 1 Demographics and clinical characteristics of patients with echinocandin breakthrough and nonbreakthrough abdominal candidiasis[a]

Patient	Age (yr)/sex	Underlying disease	No. of days of prior echinocandin therapy	GI surgery[b]	Echinocandin therapy (duration prior to breakthrough[c])	Type(s) of candidiasis	Infecting *Candida* species
Echinocandin breakthrough							
1	27/M	Lupus erythematosus	12	Yes	Caspofungin (6)	Abdominal abscess	*C. albicans*
2	47/M	Chronic pancreatitis	27	Yes	Caspofungin (5)	Abdominal abscess	*C. albicans*
3	43/F	Crohn's disease	28	Yes	Micafungin (14)	Abdominal abscess and peritonitis	*C. glabrata*
4	56/M	Kidney/liver transplant	42	Yes	Caspofungin (16)	Peritonitis	*C. albicans*
5	68/F	Multivisceral transplant	43	Yes	Caspofungin (30)	Peritonitis	*C. glabrata*
6	55/M	Small bowel transplant	68	Yes	Caspofungin (22)	Abdominal abscess	*C. glabrata*
7	66/F	Short gut syndrome	106	Yes	Caspofungin (58)	Abdominal abscess	*C. glabrata*
8	50/M	Crohn's disease	228	Yes	Caspofungin (191)	Abdominal abscess	*C. krusei*
9	37/M	Small bowel transplant	247	Yes	Caspofungin (23)	Peritonitis	*C. glabrata*
10	33/F	Multivisceral transplant	438	Yes	Caspofungin (68)	Peritonitis and candidemia	*C. tropicalis* *C. glabrata*
Nonbreakthrough							
11	54/F	Small bowel transplant	4	Yes		Abdominal abscess	*C. albicans*
12	46/F	Ischemic bowel	12	Yes		Peritonitis	*C. glabrata*
13	30/M	Crohn's disease	13	Yes		Abdominal abscess	*C. albicans*
14	58/F	Metastatic colon cancer	17	Yes		Cholangitis and peritonitis	*C. glabrata* *C. glabrata*
15	23/F	Short gut syndrome	21	Yes		Peritonitis	*C. glabrata*
16	66/F	Diverticulitis	22	Yes		Abdominal abscess and peritonitis	*C. glabrata*
17	64/F	Neutropenic colitis	22	Yes		Abdominal abscess	*C. glabrata*
18	27/F	Kidney transplant	23	Yes		Abdominal abscess	*C. tropicalis*
19	66/M	Liver transplant	28	Yes		Peritonitis	*C. albicans*
20	65/F	Small bowel transplant	58	Yes		Peritonitis	*C. glabrata*
21	56/F	Short gut syndrome	72	Yes		Abdominal abscess	*C. glabrata*
22	51/F	Crohn's disease	74	Yes		Abdominal abscess	*C. glabrata*
23	63/F	Short gut syndrome	78	No		Abdominal abscess	*C. glabrata*
24	31/M	Multivisceral transplant	81	No		Abdominal abscess	*C. glabrata*
25	73/M	Multivisceral transplant	133	Yes		Cholangitis	*C. albicans*

[a] ANF, anidulafungin; BT, breakthrough; CoNS, coagulase-negative *Staphylococcus*; CSP, caspofungin; del, deleted; ESBL, extended-spectrum beta-lactamase; F, female; GI, gastrointestinal; KPC, *Klebsiella pneumoniae* carbapenemase; M, male; MCF, micafungin; MDR, multidrug resistant; PTC, percutaneous transhepatic cholangiography; VRE, vancomycin-resistant *Enterococcus*; WT, wild type.
[b] Within 30 days of disease onset.
[c] Number of consecutive days of echinocandin therapy at the time point of the first positive culture.
[d] In days, from time point of positive culture.

tients. *C. glabrata* ($n = 17$) was the most common, followed by *C. albicans* ($n = 7$), *Candida tropicalis* ($n = 2$), and *C. krusei* ($n = 1$). Two patients had mixed *Candida* infections (*C. albicans-C. glabrata* and *C. glabrata-C. tropicalis*). Eleven *Candida* isolates were recovered from the 10 echinocandin breakthrough infections (*C. glabrata* [$n = 6$], *C. albicans* [$n = 3$], *C. tropicalis* [$n = 1$], and *C. krusei* [$n = 1$]). One patient had breakthrough infections with both *C. glabrata* and *C. tropicalis*. The median anidulafungin, caspofungin, and micafungin MICs were 0.03 μg/ml (range, 0.015 to 4 μg/ml), 0.12 μg/ml (range, 0.06 to 16 μg/ml), and 0.03 μg/ml (range, 0.008 to 4 μg/ml), respectively. The corresponding rates of resistance were 22% (6/27), 30% (8/27), and 19% (5/27), respectively; 19% (5/27) of isolates were resistant to all 3 agents. Twenty-four percent (6/25) of patients were infected with *FKS* mutant *Candida*. Mutations were identified in 22% (6/27) of isolates, including 29% (5/17) of *C. glabrata* and 14% (1/7) of *C. albicans*

TABLE 1 (Continued)

MIC (μg/ml)					Treatment of abdominal candidiasis			
CSP	ANF	MCF	*FKS* genotype	Concomitant bacterial species	Source control	Antifungal (duration[d])	Outcome	Response to echinocandin therapy
0.12	0.015	0.008	WT	MDR *Enterobacter aerogenes*	Surgical debridement	Fluconazole (34)	Failure: persistence (days 16 and 24)	Failure: BT
0.5	0.5	0.25	WT	VRE, CoNS	Surgical debridement	Caspofungin (10)	Cure	Failure: BT
2	1	0.25	*FKS1*-D632Y	CoNS	Surgical debridement	Fluconazole (28)	Cure	Failure: BT
0.06	0.015	0.03	WT	MDR *Enterobacter aerogenes*	Percutaneous drain	Caspofungin (14)	Failure: death (day 14)	Failure: BT and death
0.5	0.5	0.5	*FKS2*-S663P	VRE	Percutaneous drain	Caspofungin (7)	Failure: death (day 8)	Failure: BT and death
0.25	0.015	0.03	WT	VRE	Surgical debridement	Caspofungin Voriconazole (51)	Failure: recurrence (day 46)	Failure: BT and recurrence
16	2	4	*FKS2*-F659del	MDR *Enterobacter cloacae*	Surgical debridement		Cure	Failure: BT
0.25	0.06	0.06	WT	*Escherichia coli*, *Lactobacillus spp.*	Surgical debridement	Caspofungin (56)	Cure	Failure: BT
0.12	0.015	0.015	*FKS1*-R653I	MDR *Escherichia coli*	Percutaneous drain	Caspofungin Fluconazole (7)	Cure	Failure: BT
0.12	0.015	0.06	WT	*Streptococcus viridans*				
4	0.5	0.12	*FKS1*-R636S	VRE	Surgical debridement and enterectomy	Caspofungin Voriconazole (12)	Failure: persistence, candidemia, and death (day 20)	Failure: BT, persistence, and death
0.5	0.12	0.015	WT		Surgical debridement and enterectomy	Caspofungin (35)	Cure	Cure
0.12	0.03	0.06	WT		Surgical debridement	Caspofungin (27) Fluconazole (41)	Cure	Cure
0.12	0.015	0.015	WT	KPC *Klebsiella pneumoniae*	Surgical debridement	Fluconazole (45)	Cure	
0.12	0.015	0.03	WT	VRE				
0.12	0.03	0.015	WT	ESBL-producing *Klebsiella pneumoniae*, ESBL-producing *Escherichia coli*, *Enterococcus faecalis*	Repair of bile duct and placement of PTC catheter	Caspofungin (39)	Failure: persistence (days 7 and 22)	Failure: persistence
0.12	0.06	0.03	WT		Surgical debridement and placement of drain	Caspofungin (28)	Cure	Cure
0.12	0.06	0.03	WT	VRE, CoNS	Percutaneous drain	Caspofungin (33)	Failure: persistence (days 19 and 33)	Failure: persistence
0.06	0.03	0.008	WT		Surgical debridement	Fluconazole (27)	Cure	
0.03	0.015	0.06	WT	VRE	Percutaneous drain	Caspofungin (6)	Cure (de-escalation to fluconazole)	Cure
0.12	0.015	0.015	WT		Percutaneous drain	Caspofungin (26)	Cure	Cure
0.06	0.015	0.015	WT	VRE		Fluconazole (10)	Cure	
0.12	0.06	0.03	WT	MDR *Acinetobacter baumannii*		Fluconazole (35)	Cure	
0.12	0.015	0.015	WT		Surgical debridement	Fluconazole (20)	Failure: persistence (day 19)	
0.12	0.015	0.03	WT	*Klebsiella pneumoniae*, VRE	Percutaneous drain	Fluconazole (23)	Failure: persistence (day 11)	
0.06	0.03	0.06	WT	*Enterococcus gallinarum*, *Proteus mirabilis*	Surgical debridement		Cure	
4	1	4	*FKS1*-S645P	*Morganella morganii*, VRE	Placement of PTC catheter	Caspofungin (22)	Failure: persistence (days 16 and 29)	Failure: persistence

isolates. Median duration of prior echinocandin exposure was longer among patients infected with *FKS* mutant isolates than in those infected with the wild type (119 versus 27 days; $P = 0.01$). Forty-five percent (5/11) of echinocandin breakthrough isolates were *FKS* mutants, compared to 6% (1/16) of nonbreakthrough isolates ($P = 0.03$). All 5 isolates resistant to each echinocandin were *FKS* mutants. The same *FKS* mutant *C. glabrata* isolate was recovered from intra-abdominal and blood cultures in the patient with abdominal candidiasis and candidemia.

Concomitant bacterial pathogens were recovered from 76% (19/25) of patients, including each of the patients infected with *FKS* mutant *Candida*. *Enterococcus* spp. were most common (63% [12/19] patients). Abdominal cultures revealed MDR bacteria in 64% (16/25) of patients. Organisms included vancomycin-resistant *Enterococcus* (VRE; $n = 10$), MDR *Enterobacter* spp. ($n = 3$), extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* ($n = 1$), ESBL-producing *Klebsiella pneumoniae* ($n = 1$), *Klebsiella pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae* ($n = 1$), MDR *Acinetobacter baumannii* ($n = 1$), and MDR *E. coli* ($n = 1$). MDR bacteria were recovered from 83% (5/6) of patients infected with *FKS* mutant *Candida*.

Echinocandin therapy failed in 52% (13/25) of patients. Therapeutic failures included the 10 breakthrough infections and 3 nonbreakthrough infections that were treated with an echinocan-

din and a source control. Echinocandin failure occurred in 100% (6/6) of patients infected with *FKS* mutant *Candida*. Seven patients who had nonbreakthrough abdominal candidiasis due to wild-type *FKS Candida* were treated with an echinocandin and source control; the cure rate was 71% (5/7). In comparison, the abdominal candidiasis cure rates were 67% (6/9) among patients who were treated with fluconazole (including 57% [4/7] of patients treated with a concomitant source control and 100% [2/2] of patients treated with fluconazole alone).

This is the first study of echinocandin-resistant candidiasis consisting exclusively of patients with infections other than candidemia. Echinocandin resistance during abdominal candidiasis shared many features previously reported for bloodstream infections, including a predominance of *C. glabrata* (2–4). Indeed, the incidence of *FKS* mutant *C. glabrata* was virtually identical to those observed in patients with candidemia and prior echinocandin exposure (29% and 32%, respectively) (2, 8). As in candidemia, *FKS* mutations were detected in the setting of extensive prior echinocandin usage (ranging from 28 to 438 days). Moreover, mutations were associated with poor responses to echinocandin therapy, manifested as breakthrough abdominal candidiasis or treatment failures despite concomitant source control. At our center and many others, *Candida* isolates recovered from nonblood sites are not tested routinely for echinocandin susceptibility. Since blood cultures have poor sensitivity for detecting abdominal candidiasis (14), most of the echinocandin-resistant isolates in this study were identified retrospectively rather than at the time of infection. In one patient, abdominal candidiasis was the source of *FKS* mutant *C. glabrata* candidemia. Therefore, our experience suggests that abdominal candidiasis is a hidden reservoir of echinocandin resistance. In an era of widespread echinocandin use, it is plausible that *FKS* mutations are also selected at other unrecognized reservoirs. A limitation of this study is that the overall rate of *FKS* mutations during abdominal candidiasis at our center is unknown. Prospective surveillance studies of echinocandin resistance among *Candida* isolates recovered from abdominal and other, nonblood sites are warranted.

Our most ominous finding was that *FKS* mutant *Candida* isolates were almost always recovered with MDR bacteria. Most cases of abdominal candidiasis are complicated by bacterial coinfections, which are typically due to enteric organisms. The emergence of abdominal infections caused by mixtures of antimicrobial-resistant bacteria and fungi reflects the complexity of patients' underlying medical conditions, shared risk factors, and extensive antibiotic and antifungal usage. Abdominal candidiasis constitutes a spectrum of diseases that typically present as abscesses, cholangitis, or peritonitis (15). Echinocandin pharmacokinetics-pharmacodynamics during abdominal candidiasis have not been evaluated systematically. Data on penetration into intra-abdominal abscesses are absent. Case reports described caspofungin and micafungin concentrations within bile and ascites to be 30% and 15% of serum, respectively (16, 17). In animal studies of caspofungin disposition, 35% of a single dose was present within liver parenchyma after 24 h; however, elimination occurred slowly, with 14.2 and 2.8% of the dose still present at days 5 and 12, respectively (18). The terminal half-life of caspofungin is prolonged when serum and tissue concentrations are comodeled, compared to the half-life in serum alone, supporting a model in which abdominal organs are reservoirs for sustained drug release (19). It is likely that *Candida FKS* mutations emerged within the abdominal cavity, biliary tree, or abscesses in the face of prolonged subinhibitory echinocandin concentrations.

Source control is a key element of treatment for abdominal candidiasis, but surgery alone is inferior to combined surgical-antifungal approaches. If adequate source control is achieved, our data indicate that echinocandins can be used successfully against nonbreakthrough abdominal candidiasis due to *FKS* wild-type isolates. In cases of echinocandin breakthrough abdominal candidiasis, however, our experience suggests that alternative agents are more-judicious options. The challenges of treating echinocandin-resistant abdominal candidiasis, particularly when complicated by MDR bacterial coinfection, attest to the importance of rational antimicrobial stewardship policies and other preventive strategies. Studies are needed to better define the epidemiology and clinical aspects of abdominal candidiasis, which is understudied and poorly understood compared to candidemia.

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