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Breakthrough Candidemia Due to Multidrug-Resistant *Candida glabrata* during Prophylaxis with a Low Dose of Micafungin

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We identified a case of breakthrough candidemia in a 25-year-old patient receiving micafungin prophylaxis (50 mg/day). Five *Candida glabrata* isolates were obtained from blood cultures and were classified as multidrug-resistant isolates, since all of them exhibited high MICs for echinocandin and azole drugs. A mutation (S663F) in hot spot 1 of the *FKS2* gene was found in all five isolates. This mutation yielded a 1,3-β-D-glucan synthase enzyme with highly reduced sensitivities to echinocandin drugs.

In recent years, the epidemiology of candidemia in Latin American hospitals has been changing, with a trend toward an increase in the incidence of candidemia due to *Candida glabrata*, as documented in recently published multicenter studies (1, 2, 3). *C. glabrata* is naturally less susceptible to azoles than other *Candida* species (4), and this has led to the expanded use of echinocandins for fungemia treatment caused by this *Candida* species (5, 6, 7). This class of drug is now reported as the first-line therapy for candidemia in several guidelines of medical societies (5, 6, 7).

Acquired echinocandin resistance among *Candida* spp. is largely infrequent. Yet, episodes of invasive infection due to echinocandin-resistant *Candida* isolates are increasingly being reported in U.S. and European medical centers (8, 9, 10, 11). We describe one case of *C. glabrata* breakthrough candidemia documented in a patient with Burkitt lymphoma and prolonged neutropenia who received antifungal prophylaxis with low doses of micafungin (50 mg/day) in a tertiary care hospital in Curitiba, Paraná, Brazil. This is the first documented case of multidrug resistance among clinical strains of *C. glabrata* from a Latin American medical center.

Case. A 25-year-old man was diagnosed with stage IV adult sporadic Burkitt lymphoma in November 2011, and due to profound neutropenia, he received fluconazole prophylactically at doses of 200 mg/day for 20 days (27 November to 16 December), followed by micafungin at doses of 50 mg/day for 12 days (17 December to 28 December). In January 2012 (3 January), the patient was admitted for a third round of chemotherapy treatment with a cyclophosphamide, vincristine, doxorubicin, dexamethasone (hyper-CVAD) regimen, having presented with profound neutropenia since December 2011. Levofloxacin and sulfamethoxazole-trimethoprim were initiated as prophylaxis therapy. Due to prolonged neutropenia, antifungal prophylaxis was restarted with micafungin at doses of 50 mg/day (posaconazole is not available in Brazil). At day 12 of hospitalization, the patient developed fever during a neutropenic period, without a defined focus of infection, and cefepime treatment was initiated empirically. A blood culture was collected and revealed growth of *Candida* (isolate 1), despite 24 days of exposure to micafungin. Initially, the decision was made to increase the micafungin dose to 100 mg/day. The patient continued to have a fever for 6 days after initiation of the micafungin treatment (100 mg/day), with sequential blood cultures positive for *Candida* (isolates 2 to 5). Possible infectious foci for persistent fungemia (endocarditis, hepatosplenic candidiasis, and fungal meningitis) were ruled out by clinical, imaging, and laboratory tests. Echinocandin resistance was suspected, and a conventional formulation of amphotericin B was initiated with good clinical response. Blood cultures were negative after 5 days of treatment.

A total of five clinical *Candida* isolates were obtained from sequential blood cultures collected during breakthrough candidemia that occurred during antifungal prophylaxis with micafungin (50 mg/day). All isolates tested were identified as *Candida glabrata* by internal transcribed spacer (ITS) sequencing, as previously described by our group (12, 13).

Antifungal susceptibility testing was performed using the broth microdilution method according to Clinical and Laboratory Standards Institute (CLSI) document M27-A3 (14), using current CLSI MIC interpretative criteria (CLSI, M27-S4) (15). The antifungal compounds were kindly provided as pure powders by their manufacturers. All five *C. glabrata* bloodstream isolates tested exhibited resistance to fluconazole (FLC), voriconazole (VRC), and all echinocandins. The MIC values for echinocandins were 16- to 33-fold higher than those for the control strain (ATCC 90030). Table 1 summarizes the MIC values obtained for the five *C. glabrata* isolates for the six antifungal agents tested.

DNA sequencing analyses of the two hot spot (HS) regions of the drug target genes *FKS1* and *FKS2* were performed on the five *C. glabrata* clinical isolates, as previously described (16). The two HS regions of the *FKS1* gene corresponded to nucleotides (nt) 1873 to 1902 (amino acids [aa] 625 to 633) and nt 4018 to 4041 (aa 1340 to 1347) of the published strain *C. glabrata* ATCC 90030 (DNA Data Bank of Japan [DDBJ] accession no. HM366440.1), and the two HS regions of the *FKS2* gene corresponded to nt 1975 to 2001 (aa 659 to 667) and nt 4122 to 4143 (aa 1374 to 1381) of the published strain *C. glabrata* ATCC 90030 (DDBJ accession no. HM366442.1). The sequencing of the HS regions of the *FKS* genes

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TABLE 1 *In vitro* activities of six antifungal agents and GS inhibition profiles for echinocandin drugs against five *C. glabrata* isolates harboring *FKS* mutations

Strain/mutation	*FKS2* hot spot genotype	MIC (mg/liter) for:						IC_{50} (ng/ml) for:		
		AMB	FLC	VRC	ANF	CSF	MCF	ANF	CSF	MCF
ATCC 90030	*FKS1*/*FKS1* (wild type)	0.25	4	0.03	0.06	0.03	0.03	46.1	29.7	15.29
8622 A	*fks1*/*fks1* (S663F)	0.25	>64.0	4.0	1.0	1.0	0.5	2,655	1,263	61,760
8622 B	*fks1*/*fks1* (S663F)	0.25	>64.0	4.0	1.0	1.0	0.5	ND[a]	ND	ND
8622 C	*fks1*/*fks1* (S663F)	0.25	>64.0	4.0	1.0	1.0	0.5	ND	ND	ND
8622 D	*fks1*/*fks1* (S663F)	0.25	>64.0	4.0	1.0	1.0	0.5	ND	ND	ND
8622 E	*fks1*/*fks1* (S663F)	0.25	>64.0	4.0	1.0	1.0	0.5	ND	ND	ND

[a] ND, not determined.

revealed only a nonsynonymous mutation in HS1 in the *FKS2* gene, which led to an S663F amino acid substitution (Table 1).

We performed an *in vitro* glucan synthase inhibition assay, as previously described (17, 18, 19). The echinocandin inhibition parameter 50% inhibitory concentration (IC_{50}) was determined for the wild-type strain (*C. glabrata* ATCC 90030) and the 8622A clinical isolate (Table 1). The *fks* mutant enzyme extracted from the clinical isolate showed significantly higher IC_{50} values than did the corresponding enzyme isolated from the wild-type strain (58-, 43-, and >4,000-fold on average for anidulafungin [ANF], caspofungin [CSF], and micafungin [MCF], respectively) (Table 1 and Fig. 1). These results confirm that the point mutation S663F encoded in the *FKS2* gene of *C. glabrata* yielded a 1,3-β-D-glucan synthase enzyme with highly reduced sensitivities to echinocandin drugs, resulting in elevated MICs with a strong potential for clinical failure. In the present investigation, a patient with Burkitt lymphoma and severe neutropenia that was expected to persist for a longer period of time was subjected to antifungal prophylaxis with micafungin at a dosage of 50 mg daily. The patient developed *C. glabrata* breakthrough candidemia after 19 and 24 days of exposure to fluconazole and micafungin, respectively. *In vitro* antifungal susceptibility testing confirmed the resistance to fluconazole, voriconazole, and all echinocandins.

The epidemiologic impact of using an echinocandin for antifungal prophylaxis in neutropenic patients in terms of promoting the development of multidrug-resistant *Candida* isolates remains unclear, but it is concerning. Prominent *C. glabrata* echinocandin resistance has recently been reported by Alexander et al. (8) and Pfaller et al. (9), whose studies suggested significant emergence of multidrug resistance over time to both azoles and echinocandins in *C. glabrata* isolates.

As echinocandins are more frequently used for the treatment of invasive candidiasis, sporadic cases of echinocandin breakthrough candidemia associated with nonsusceptible *Candida* isolates and treatment failure have been reported (10, 11, 19). In the present study, all five *C. glabrata* isolates recovered from sequential blood cultures collected during prophylaxis therapy with micafungin showed increases in the echinocandin MIC values, and all of them showed a point mutation in HS1 in the *FKS2* gene that led to a S663F amino acid substitution, which has been previously described as being involved in treatment failure and echinocandin resistance in *C. glabrata* isolates (20). Notably, other studies have shown that a point mutation in the same position of the *FKS2* gene, though with a different amino acid substitution (S663P), is the most frequently observed mutation related to echinocandin resistance in *C. glabrata* isolates (21, 22). It is important to note that in our case, in accordance with previous publications, the long-term echinocandin exposure (24 days) resulted in the development of *FKS* gene mutations and echinocandin resistance (10). On the other hand, one case of rapid echinocandin resistance in *C. glabrata* was reported by Lewis et al. (23) in a patient without previous or prolonged echinocandin exposure (8 days of treatment).

Our findings emphasize that the widespread use of echinocandins may increase the occurrence of echinocandin resistance, especially when using low doses, as in the present case in which the neutropenic patient was exposed to 50 mg daily. In this scenario, the use of echinocandin as prophylactic therapy should be recommended cautiously in patients with a high risk of candidemia development, now that previous exposure to echinocandins has been clearly recognized as a risk factor for the development of resistance.

Nucleotide sequence accession numbers. The sequences generated in this study have been deposited in the GenBank (NCBI)

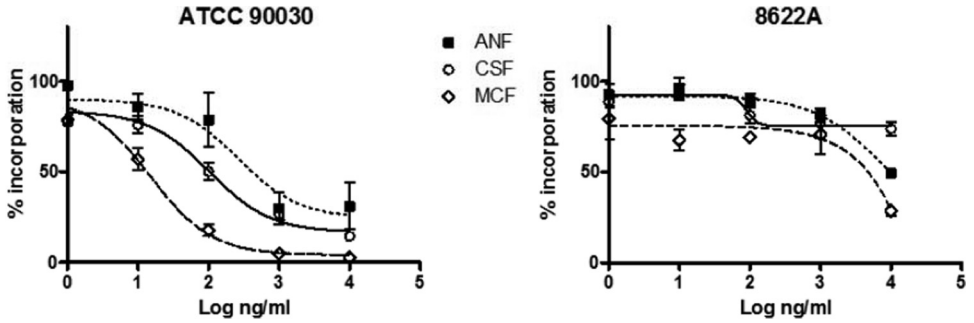

FIG 1 Echinocandin inhibition profiles for product-entrapped 1,3-β-D-glucan synthase enzyme complexes assessed by the incorporation of [^{3}H]glucose into radiolabeled product. Titration curves are shown for anidulafungin (ANF), caspofungin (CSF), and micafungin (MCF) for the wild type and clinical isolate 8622A of *C. glabrata*.

database under accession numbers KF211452, KF211447, KF211442, KF211437, KF211456, KF211448, KF211438, KF211443, KF211455, KF211449, KF211444, KF211439, KF211454, KF211450, KF211445, KF211440, KF211453, KF211451, KF211446, KF211441, KF305827, KF305828, KF305829, KF305830, and KF305831.

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