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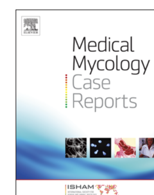

Two echinocandin-resistant *Candida glabrata FKS* mutants from South Africa

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ABSTRACT

Echinocandins are recommended as first-line agents to treat invasive infections caused by *Candida glabrata* since this organism is inherently less susceptible to azoles. However, resistance to echinocandins has been described in *C. glabrata* due to amino acid changes in the hotspot regions of the *FKS1* and *FKS2* genes. In this report, we describe the first two South African *C. glabrata* isolates with echinocandin resistance mediated by mutations in the *FKS2* gene. Both isolates were cultured from urine specimens from private-sector patients.
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1. Introduction

In resource-rich settings, echinocandins are the antifungal agents of choice for invasive infections caused by *Candida glabrata* due to the relative resistance of this species to azole antifungals [1]. Anidulafungin, caspofungin and micafungin disrupt the synthesis of β-1,3-D-glucan, an essential fungal cell wall component, by inhibiting β-1,3-D-glucan synthase. β-1,3-D-glucan synthase is an enzyme complex comprised of two subunits: Fksp, a catalytic subunit and Rho1p, a regulatory subunit [1]. It is thought that the echinocandins bind to Fksp, which is encoded by three related genes: *FKS1*, *FKS2* and *FKS3*. Reduced susceptibility to echinocandins is associated with amino acid substitutions in two highly-conserved hot-spot regions of the *FKS* genes. For *C. glabrata*, a variety of amino acid changes in the hotspot regions of *FKS1* and *FKS2* confer resistance to the echinocandins [2,3]. Here we describe the first two South African *C. glabrata* isolates with echinocandin resistance mediated by mutations in the *FKS2* gene. Both isolates were cultured from urine specimens of patients treated in the private health sector.

2. Case

The first patient was a 29-year-old female with osteogenesis imperfecta who was re-admitted in October 2014 to the intensive care unit (ICU) of a private hospital in Johannesburg in septic shock (Day 0). The patient was admitted one week after a prior prolonged ICU admission for severe pneumonia and was treated with empiric antibiotics and fluids. Information regarding prior antifungal exposure could not be obtained. She required mechanical ventilation, inotropic support and hemodialysis. *Klebsiella pneumoniae* and *Enterococcus faecalis* were isolated from re-admission blood cultures. *C. glabrata* was cultured from a urine specimen on day +46 and this isolate was submitted to a reference laboratory for confirmation of species-level identification and antifungal susceptibility testing. Despite an initial improvement in her condition, the patient deteriorated and she died approximately two months after re-admission (Day +60). At the diagnostic laboratory, the isolate was initially identified as *C. glabrata* using the MALDI-TOF MS instrument (bioMérieux, Marcy l'Etoile, France) and minimum inhibitory concentrations (MICs) were determined using pre-prepared Sensititre YeastOne microbroth dilution panels (Thermo Fisher Scientific, Cleveland, Ohio, USA): 8 μg/ml for fluconazole, 0.5 μg/ml for voriconazole, 0.25 μg/ml for caspofungin and 0.5 μg/ml for anidulafungin. Identification of the isolate was confirmed by sequencing of the internal

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Table 1
Antifungal susceptibility test results for the first-isolated *C. glabrata FKS* mutant.

Antifungal agent	MIC (μg/ml) range[a]:		Category[b]	Wild type[c]
	Broth microdilution	Etest		
Anidulafungin	0.5–1.0	0.5–0.75	Resistant	No
Micafungin	0.25	0.19–0.25	Resistant	No
Caspofungin	0.5–1.0	1.0–1.5	Resistant	No
Flucytosine	< 0.06	0.032–0.064	No breakpoint	Yes
Posaconazole	1.0	1.0–3.0	No breakpoint	Yes
Voriconazole	0.25	0.047–0.25	No breakpoint	Yes
Itraconazole	0.5	0.75–4.0	No breakpoint	Yes
Fluconazole	16.0	3.0–8.0	Susceptible dose-dependent	Yes
Amphotericin B	0.5	0.002–0.5	No breakpoint	Yes

[a] Ranges for minimum inhibitory concentrations (MICs) as determined by 3 independent readers performing the tests in duplicate;
[b] Based on Clinical and Laboratory Standards Institute (CLSI) M27-S4 interpretive breakpoints;
[c] Based on published epidemiologic cut-off values (ECVs), as determined by multiple laboratories, for the Sensititre YeastOne method: anidulafungin (0.12 μg/ml), micafungin (0.03 μg/ml), caspofungin (0.25 μg/ml), flucytosine (0.25 μg/ml), posaconazole (2 μg/ml), voriconazole (4 μg/ml), itraconazole (4 μg/ml), fluconazole (128 μg/ml), amphotericin B (2 μg/ml).

transcribed spacer (ITS) region of the ribosomal RNA gene at the reference laboratory [4]. MICs were then confirmed using the same microbroth dilution panels (Thermo Fisher Scientific, Cleveland, Ohio, USA) and by Etest (bioMérieux, Marcy l'Etoile, France) on RPMI 1640 plates containing 2% glucose (Diagnostic Media Products, NHLS, South Africa), as recommended by the manufacturer. Echinocandin MICs generated by the Sensititre YeastOne method are comparable to those generated using the Clinical and Laboratory Standards Institute (CLSI) M27-A3 method [5].Three reference laboratory personnel performed susceptibility tests independently and in duplicate. The quality control (QC) strains, *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258 were run simultaneously and MICs were within expected QC ranges. The broth microdilution MIC ranges were 0.5–1 μg/ml (CLSI M27-S4 resistance breakpoint ≥ 0.5 μg/ml) for anidulafungin, 0.25 μg/ml for micafungin (CLSI resistance breakpoint ≥ 0.25 μg/ml) and 16 μg/ml for fluconazole (CLSI resistance breakpoint ≥ 64 μg/ml) (Table 1). The Etest MIC ranges were 0.5–0.75 μg/ml and 0.19–0.25 μg/ml for anidulafungin and micafungin respectively and 3 – 8 μg/ml for fluconazole. Published epidemiologic cut-off values (ECVs) determined by multiple laboratories for the Sensititre YeastOne method were also applied to the MIC of each tested antifungal agent to determine whether the isolate was wild type or a mutant (Table 1) [6–8]. A mutation was detected in the hotspot 1 region of the *FKS2* gene where serine was replaced by phenylalanine at position 663 (S663F). In September 2015, a second *C. glabrata* strain from another patient was submitted to the reference laboratory for confirmatory tests (private-sector patient; urine specimen; clinical details unavailable). The anidulafungin and micafungin MICs were both 2 μg/ml by the Sensititre YeastOne method and a change from arginine to lysine at amino acid position 1377 (R1377K) was identified by sequencing the *FKS2* hotspot 2 region.

3. Discussion

This is the first report of echinocandin-resistant *C. glabrata* isolates from South Africa, mediated by *FKS2* gene mutations, and isolated from the urine of two patients in the private sector. The echinocandins have increasingly been used for first-line management of candidaemia, especially in the private sector, since 2007 when caspofungin was registered. Anidulafungin and micafungin have since also been registered. In a national surveillance study conducted in South Africa in 2009 and 2010, only ten of 1252 (0.8%) bloodstream *Candida* isolates had resistant/ intermediate micafungin or anidulafungin MIC values and were considered to be non-susceptible to the echinocandins [9]. However, *FKS* gene sequencing of the ten isolates, including three *C. glabrata* isolates, yielded no mutations in the hotspot regions. As a result of poor *in-vivo* glomerular filtration or tubular secretion, echinocandins achieve very low concentrations in the urinary tract [10]. We hypothesize that exposure of the *C. glabrata* strains to subtherapeutic echinocandin concentrations may have led to emergence of resistance in the two cases that we report here. A travel history was not available for either patient; therefore, it is not known if these mutant strains were acquired. The S663F mutation has not been described as often as the serine to proline replacement at the same amino acid position. In a US population-based surveillance study, of 490 bloodstream *C. glabrata* isolates, 16 (3%) had elevated echinocandin MICs and an *FKS* mutation. Of 11 isolates with mutations in FKS2 hotspot 1 at amino acid position 663, only one had the serine to phenylalanine replacement that we describe here [11]. In another US study, 77 of 1032 (7%) isolates had echinocandin MICs in the intermediate or resistant categories, 51 of which had *FKS* mutations; two isolates had the S663F mutation [12]. Previously, a nonsense mutation at FKS 2 hotspot 2 has been described where arginine was replaced with a stop codon at amino acid position 1377 [2]. Caspofungin MICs are an unreliable indicator of echinocandin resistance owing to inter-laboratory variation of MIC readings especially for *C. glabrata*; instead, anidulafungin or micafungin MICs have been recommended to predict susceptibility to the class [13,14]. In conclusion, we have described the first two cases of echinocandin-resistant *C. glabrata* isolates in South Africa. Systematic active laboratory surveys, including isolates from cases of non-invasive *Candida* infection, are needed to determine if echinocandin resistance has become more widespread in South Africa.

Conflict of interest

For unrelated work, Nelesh P. Govender has received speaker honoraria from Pfizer, Astellas and MSD (Pty) Ltd, has received travel grants from MSD (Pty) Ltd, has provided educational materials for TerraNova and has acted as a temporary consultant for Fujifilm Pharmaceuticals. For the remaining authors, none were declared.

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