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Letter to the Editor

Infectious endocarditis caused by *Candida glabrata*: evidence of *in vivo* development of echinocandin resistance

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Sir,

Candida glabrata is a major agent of invasive candidiasis and its incidence is on the rise [1]. Overall rates of echinocandin resistance among *C. glabrata* blood isolates vary according to region, ranging from <1% in Europe to over 10 % in some hospitals located in the United States of America [1]. These isolates usually harbor mutations in hot spot regions of the *FKS* genes, which confer resistance after long-term exposure to echinocandins [1, 2]. Moreover, there is an additional concern regarding *C. glabrata* strains because of their capacity of rapidly acquiring antifungal resistance during treatment [3].

Candida endocarditis is an infrequent entity, being *C. glabrata* specifically responsible for about 0.2% of all cases of infectious endocarditis [4]. Despite its low prevalence, *Candida* endocarditis remains a difficult-to-treat infection, entailing a poor prognosis for most patients [5]. Furthermore, infections by strains with *FKS* mutations are independently associated with treatment failure and even higher mortality rates [3]. To the best of our knowledge, this is the first report of endocarditis caused by a strain of *C. glabrata* that acquired resistance to echinocandins during treatment.

An 80-year-old male with a prosthetic aortic valve replacement in 2011 was admitted in December 2015 due to a community acquired pneumonia. He responded well to treatment but remained hospitalized because of recurrent episodes of gastrointestinal bleeding. By March, he presented with fever, diarrhea, and positive blood cultures for *Clostridium perfringens* and *Candida glabrata*. Antibiotics and intravenous fluconazole 400mg/day were started adjusted to renal function, which recovered shortly. Abdominal perforation was excluded and the imaging screening for infection source was unremarkable. The patient had a favorable clinical response, being discharged after two weeks of fluconazole at the same dose.

In June, he was re-admitted with fever and hyporexia for over a month. Blood cultures were drawn, yielding a recurrent *C. glabrata*. Micafungin 100 mg/day was initiated, and follow-up blood cultures were sterile after five days. Nevertheless, the transesophageal echocardiogram (TEE) showed prosthetic aortic valve endocarditis and aortic abscess. The dose of micafungin was increased to 150 mg/day and combined with fluconazole 400 mg/day. Although surgical debridement was recommended, conservative management was preferred due to the patient´s high operative risk.

Within a month of antifungals, he presented clinical signs of treatment failure with severe aortic valve deterioration, requiring urgent valve replacement. Heart valve cultures yielded two morphologically different strains of *C. glabrata*, being one of them phenotypically resistant to echinocandins. Treatment was switched to liposomal amphotericin B (3 mg/kg/day) combined with fluconazole 800mg/day. Unfortunately, the patient acquired a ventilator-associated pneumonia due to *Pseudomonas aeruginosa* a week after surgery and died of refractory septic shock.

The antifungal susceptibility results of all *Candida* isolates were performed according to EUCAST EDef 7.3 [6] and are summarized in table 1. Hot spots 1 and 2 of the *FKS1* and *FKS2* genes were sequenced and the isolate showing resistance to echinocandins harbored a point mutation in the *FKS2* gene. There were two *C. glabrata* strains from blood culture and two morphologically different strains from the heart valve culture. Nevertheless, all of them proved to be identical after microsatellite genotyping [7], which suggests secondary acquisition of resistance during treatment. Also, all *C. glabrata* isolates were tested in a *Galleria mellonella* model [7] and showed no differences in terms of growth kinetics or virulence (data not shown).

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Table 1 **Antifungal susceptibility and analysis of *FKS2* gene mutations of *Candida* isolates from a patient with recurrent candidemia and infectious endocarditis by *Candida glabrata***

Sample	Date	Species	Amphotericin B	Fluconazole	Voriconazole	Posaconazole	Micafungin	Anidulafungin	FKS2
Blood culture	2016 Mar 22	*C. glabrata*	0.25	2	0.25	0.5	≤0.015	0.03	WT
Blood culture	2016 Jun 06	*C. glabrata*	0.125	4	0.125	0.5	≤0.015	0.03	WT
Heart valve culture	2016 Jul 05	*C. glabrata*	0.125	8	0.25	0.5	≤0.015	0.03	WT
			0.125	2	0.125	0.25	2[a]	1[a]	S663P

Antifungal susceptibility testing was performed according to EUCAST EDef 7.3 [6] The minimal inhibitory concentrations are expressed in mg/L. All isolates were susceptible to amphotericin B, voriconazole, posaconazole, and susceptible dose-dependent to fluconazole. [a]Antifungal resistance according to EUCAST EDef 7.3 [6] breakpoints for micafungin and anidulafungin.

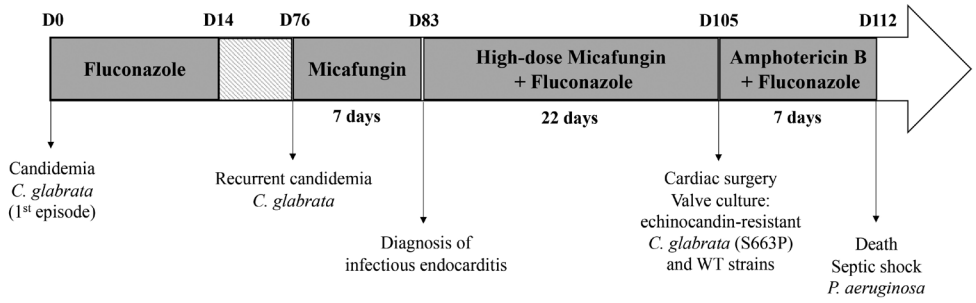

Figure 1 **Disease progression of recurrent candidemia by *C. glabrata* complicated with endocarditis and development of echinocandin resistance during antifungal treatment**

Relevant aspects may have potentially contributed to infection recurrence. First, the patient might have received a lower dose of fluconazole than the one recommended for *C. glabrata* in current guidelines [8]. He presented a very unstable renal function, which presumably required more frequent dose adjustments. Although not routinely recommended, fluconazole therapeutic drug monitoring (TDM) could have been useful, especially for microorganisms with higher minimal inhibitory concentration (MIC) values [9]. Second, our patient had diarrhea and intermittent gastrointestinal bleeding, facilitating *Candida* gastrointestinal translocation. In fact, the gastrointestinal tract is known to be a reservoir for *Candida* and a potential source of antifungal resistance due to irregular drug penetration [1,10]. Noteworthy, the most common source of late recurrent candidemia is intra-abdominal, followed by endocarditis [11].

To the best of our knowledge, this is the first report of IE with an echinocandin-resistant *Candida glabrata* isolate, though the incidence of such event is probably underestimated for several reasons. First, not all patients undergo surgery, so valve cultures are not routinely available. Second, susceptibility testing is hardly ever performed in strains other than those isolated from blood. Testing several isolates would increase the chances to point out the resistant strain in cases caused by both susceptible and resistant *Candida*. Thus, we believe that complex biofilm-related infections, such as *Candida* endocarditis, should have antifungal susceptibility testing of all invasive isolates to promptly detect resistant strains.

Even though echinocandins are highly active against *Candida* biofilms, infections with a high microbial burden in sites of poor drug penetration may contribute to the emergence of resistant strains [1]. In addition, biofilm formation creates an ideal environment to harbor resistant mutants [12]. This could explain why one of the isolates from the heart valve acquired an *FKS2* mutation, whereas those from blood – where the concentration of micafungin is much higher – remained fully susceptible to antifungals. In this context, high-dose echinocandin combined with liposomal amphotericin B could have been more effective against biofilm formation, potentially avoiding the development of echinocandin resistance [13]. Furthermore, *C. glabrata* strains with *FKS* mutation seem to have a fitness cost and may regain full susceptibility to echinocandins after treatment with echinocandins is suspended [14]. Thus, improving antifungal prescribing practices may contribute to prevent the development and the spread of resistant clones [14].

In conclusion, we describe a case of recurrent candidemia by *C. glabrata* complicated with endocarditis by a strain that developed resistance to echinocandins during treatment. This report highlights the importance of performing antifungal susceptibility testing of all invasive isolates to pursue the

diagnosis of resistant mutants. Moreover, antifungal stewardship programs are warranted to optimize therapeutic management and thus, prevent the development of resistant strains.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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