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CLINICAL MICROBIOLOGY - SHORT COMMUNICATION

Lack of efficacy of echinocandins against high metabolic activity biofilms of *Candida parapsilosis* clinical isolates

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Abstract

Candida parapsilosis produces biofilm, which colonizes catheters and other invasive medical devices that are manipulated by health care workers. In previous studies, *C. parapsilosis* in vitro biofilms have exhibited high resistance rates against conventional antifungals, but susceptibility to both echinocandins and lipid formulations of amphotericin B (lipid complex and liposomal). However, a recent study showed good activity of amphotericin B deoxycholate on the biomass of *C. parapsilosis* biofilms. Although moderate activity of echinocandins has been demonstrated against low metabolic activity biofilms of *C. parapsilosis*, few studies have analyzed the action of these drugs on high metabolic activity biofilms. Moreover, high biofilm-forming isolates have been associated with central venous catheter-related fungemia outbreaks and higher mortality rates. Therefore, it is relevant to verify the activity of the main antifungal drugs against high metabolic activity biofilms of *C. parapsilosis*. Our study aimed to evaluate the in vitro activity of amphotericin B deoxycholate, anidulafungin, caspofungin, and micafungin against high biofilm-forming and high metabolic activity clinical isolates of *C. parapsilosis*. Our results showed good activity of amphotericin B against *C. parapsilosis* biofilms, but none of the echinocandin drugs was effective. This suggests that amphotericin B deoxycholate may be a better choice than echinocandins for the treatment of biofilm-associated infections by *C. parapsilosis*, mainly in countries with insufficient health care resources to purchase lipid formulations of amphotericin B. These results warn of the possibility of persistent catheter-related candidemia caused by high biofilm-forming *C. parapsilosis* strains when treated with echinocandin drugs.

Keywords *Candida parapsilosis* · Biofilm · XTT · Amphotericin B · Echinocandins · Antifungal resistance

Introduction

Candida spp. are among the main agents of bloodstream infections worldwide, not only due to implementation of high immunosuppressive therapies (e.g., chemotherapy, transplants) but also due to the increasing use of invasive devices such as central venous catheters (CVCs) [1], leading to mortality rates of 25–40% [2]. *Candida* spp., the third most common pathogen after coagulase-negative staphylococci and *Staphylococcus aureus*, are responsible for approximately 8% of catheter-associated infections [3].

Although *C. albicans* remains the most frequently isolated species worldwide, its incidence is decreasing, whereas *C. parapsilosis* infections are emerging related to the increased use of intravascular devices [4, 5]. *C. parapsilosis* is the major non-*C. albicans* species causing vascular catheter candidemia, primarily in pediatric patients [6], with the largest increase in incidence since 1990 [7]. Among the *Candida* spp., *C. parapsilosis* is one of the major biofilm-forming species, colonizing catheters and other invasive medical devices that are manipulated by health care workers [7–9].

Biofilms are microbial communities embedded in an extracellular matrix, irreversibly attached to the surface of inert

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materials or living tissues, and exhibit lower growth rates and higher resistance to antibiotics. They can develop on the surface of many hospital devices, such as cardioverter defibrillators, prostheses, and catheters, hindering the eradication of *Candida* from the hospital environment [10].

The phenotype of *Candida* biofilms has a high resistance rate against conventional antifungals (azoles and polyenes) due to a complex and multifactorial process that includes extracellular matrix production, overexpression of sterols and efflux pumps, the presence of persistent cells, among others [11]. However, biofilm resistance to amphotericin B (AMB) is less reported than that for azole drugs [12].

Echinocandins have fungicidal activity against *Candida* spp. and are the recommended first-line treatment for candidemia in the main clinical guidelines [13, 14]. This antifungal class decreases extracellular matrix production by inhibiting 1,3-β-D-glucan synthesis [9] and it has been considered the first choice for treatment of biofilm-associated invasive candidiasis [15]. The development of biofilm resistance to echinocandins is relatively slow [16]. Furthermore, previous reports demonstrated the effectiveness of these drugs against catheter infections in vivo and in vitro, indicating their use as a potential anti-*Candida* biofilm therapy [17, 18].

On the other hand, high biofilm-forming (HBF) isolates of *Candida parapsilosis* have been associated with outbreaks [19] and higher mortality rates [8]. HBF strains of this pathogen have been associated with CVC-related fungemia and death within 30 days from the onset of the episode [20].

Recently, an investigation involving cultures of clinical isolates obtained from blood and non-sterile sites showed that *C. parapsilosis* (66.7%) had the highest biofilm production rate followed by *C. tropicalis* (44.7%) and *C. albicans* (20.8%). Furthermore, *C. parapsilosis* strains showed the highest metabolic activity and biofilm biomass [21].

Therefore, to better address the issue of *C. parapsilosis* nosocomial infections, it is important to identify the most suitable antifungal drugs for treatment of *C. parapsilosis* biofilm-associated infections caused by HBF isolates and in those presenting high metabolic activity (HMA). The purpose of this study was to evaluate the in vitro activity of AMB deoxycholate (d-AMB), anidulafungin (ANF), caspofungin (CAF), and micafungin (MIF) against sessile cells of HBF and HMA *C. parapsilosis* clinical isolates.

Materials and methods

First, we determined the biofilm formation profile of 38 *C. parapsilosis* clinical isolates obtained from cases of invasive candidiasis. Biofilm biomass and metabolic activity were measured by crystal violet staining [22] and 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) reduction assay [23], respectively. The results were interpreted according to a previous published protocol [24]. Each biofilm experiment was performed at least three times on different days and *C. albicans* SC5314 was used as a quality control strain.

Isolates classified as HBF and HMA were selected for antifungal susceptibility testing. The sessile minimum inhibitory concentrations (SMICs) for each isolate were calculated from the measurement of biofilm metabolic activity by the XTT reduction assay after antifungal treatment with AMB (Sigma-Aldrich, St. Louis, MO, USA), ANF (Pfizer, New York, NY, USA), CAF (Sigma-Aldrich, St. Louis, MO, USA), or MIF (Astellas Pharma, Tokyo, Japan). $SMIC_{50}$ and $SMIC_{80}$ were defined as the antifungal concentration at which a 50% or 80% decrease in absorption was detected in comparison with the untreated biofilm, respectively [23]. Each experiment was performed at least three times on different days and *C. albicans* SC5314 was used as a quality control strain. The minimal inhibitory concentrations (MICs) of d-AMB, ANF, CAF, and MIF for planktonic cells of the isolates were determined by the European Committee for Antimicrobial Susceptibility Testing (EUCAST) microdilution assay [25] and correlated with the SMICs of the respective biofilms. Each MIC experiment was performed at least three times on

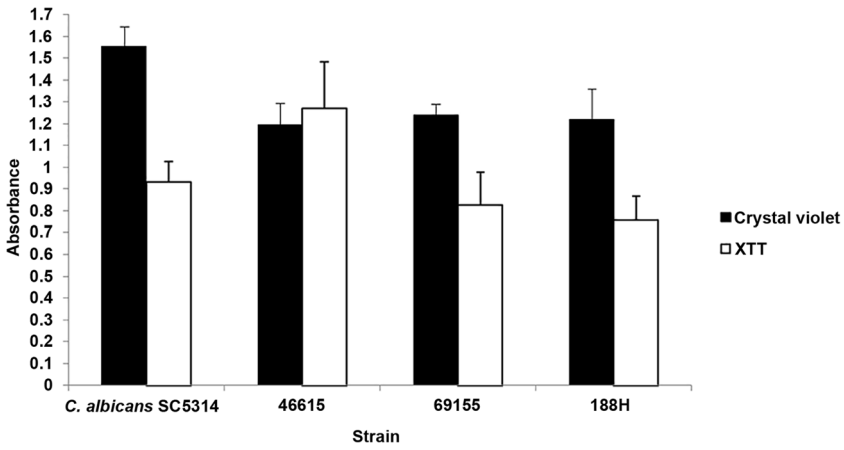

Fig. 1 Biofilm quantification of the *Candida parapsilosis* clinical isolates and *Candida albicans* SC5314 reference strain by crystal violet staining and XTT reduction assay. Error bars represent the standard deviation

Table 1 Activity of amphotericin B and echinocandins against biofilms with high metabolic activity from *Candida parapsilosis* clinical isolates and *Candida albicans* SC5314

Isolate	Amphotericin B (mg/L)		Anidulafungin (mg/L)		Caspofungin (mg/L)		Micafungin (mg/L)	
	$SMIC_{50}$	$SMIC_{80}$	$SMIC_{50}$	$SMIC_{80}$	$SMIC_{50}$	$SMIC_{80}$	$SMIC_{50}$	$SMIC_{80}$
46615	0.25	0.5	> 16	> 16	> 16	> 16	> 16	> 16
69155	0.25	0.5	> 16	> 16	> 16	> 16	> 16	> 16
188H	0.25	0.5	> 16	> 16	> 16	> 16	> 16	> 16
SC5314	0.5	1	0.03	0.25	0.03	0.06	0.03	0.25

$SMIC_{50}$, $SMIC_{80}$, 50%, and 80% reduction, respectively, in the metabolic activity of the biofilm treated with the antifungal compared with the control

different days and *C. parapsilosis* ATCC22019 and *C. krusei* ATCC6258 were used as quality control strains.

Results and discussion

All 38 isolates were positive for biofilm formation capability, although the level of biofilm production was highly variable; most of the isolates showed a low level of biofilm formation (71.1%). Most biofilms presented low metabolic activity (71.1%), and biofilm metabolic activity did not correlate with the amount of biofilm biomass (data not shown). The three isolates classified as HBF and HMA (numbers 46615, 69155, and 188H, Fig. 1) were submitted to antifungal susceptibility testing.

The planktonic cells of all isolates were susceptible to d-AMB (0.125–0.5 mg/L) and intermediate to ANF (0.25–1 mg/L), CAF (1 mg/L), and MIF (1 mg/L), according to the EUCAST Antifungal Clinical Breakpoints Table v.9.0 [26]. The echinocandins did not show activity against sessile cells of the isolates at the highest concentration tested (16 mg/L) (Table 1).

Limited therapeutic options make biofilm formation a significant clinical problem for critically ill patients [16], and it is important to investigate whether HBF and HMA isolates are a complicating factor in *C. parapsilosis* infections. In this study, we found that d-AMB presented activity against both planktonic and sessile cells of *C. parapsilosis* clinical isolates. Moreover, we demonstrated the lack of efficacy of the three echinocandin agents against sessile cells of HBF and HMA *C. parapsilosis* clinical isolates.

The main antifungal drugs available have been found to have minimal activity against *Candida* spp. biofilms [12]. *C. parapsilosis* planktonic cells demonstrate innately high MICs for echinocandins [27], and some studies have demonstrated activity of these antifungal agents against biofilms of this species [28–32]. However, only moderate susceptibility to echinocandins was reported in low metabolic activity biofilms of *C. parapsilosis* [31, 32].

Taking into account the variable susceptibility of *C. albicans* biofilms to MIF, depending on biomass production or metabolic activity, and the fact that isolates with HBF or HMA were more susceptible to this antifungal agent [33], we evaluated echinocandins activity against *C. parapsilosis* biofilms. In contrast with *C. albicans*, our results did not show any activity of MIF against sessile cells of HBF and HMA *C. parapsilosis* isolates, in accordance with previous observations [34]. Interestingly, recent in vivo findings suggest that lock therapy with MIF may promote *C. parapsilosis* biofilm dispersal rather than biofilm-cidal activity [35].

Biofilm formation by *C. parapsilosis* has shown a high degree of variability among isolates [5, 36] and the anti-biofilm activity of d-AMB has been reported to be species and strain dependent [37, 38]. An earlier study showed that *C. parapsilosis* biofilms were resistant to d-AMB and susceptible to lipid formulations of AMB (both lipid complex and liposomal) [28]. Conversely, our results are in agreement with recently published data demonstrating good activity of d-AMB on the biomass reduction of *C. parapsilosis* biofilms [38, 39]. Moreover, a previous study showed higher activity of d-AMB than ANF against *C. parapsilosis* biofilms [39], in agreement with our results, which extend this observation to CAF and MIF.

In conclusion, *C. parapsilosis* sessile cells showing HBF and HMA were susceptible only to d-AMB, indicating that this agent as a better choice than echinocandins for the treatment of biofilm-related infections by this species, mainly in countries with insufficient health care resources to purchase lipid formulations of AMB. A larger cohort of isolates from multiple centers and in vivo models should confirm our results. However, these data may alert physicians who empirically prescribe echinocandins as therapy for catheter-related candidemia regarding the possible persistence of infections caused by *C. parapsilosis*.

Author contributions All authors contributed to the conception and design of the study. Material preparation, data collection, and analysis were performed by DYT and GDN. The first draft of the manuscript was written by DYT, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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