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Short communication

β-Lactam antibiotics and vancomycin inhibit the growth of planktonic and biofilm *Candida* spp.: An additional benefit of antibiotic-lock therapy?

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ABSTRACT

The aim of this study was to evaluate the effects of cefepime, meropenem, piperacillin/tazobactam (TZP) and vancomycin on strains of *Candida albicans* and *Candida tropicalis* in planktonic and biofilm forms. Twenty azole-derivative-resistant strains of *C. albicans* ($n = 10$) and *C. tropicalis* ($n = 10$) were tested. The susceptibility of planktonic *Candida* spp. to the antibacterial agents was investigated by broth microdilution. The XTT reduction assay was performed to evaluate the viability of growing and mature biofilms following exposure to these drugs. Minimum inhibitory concentrations (MICs) ranged from 0.5 mg/mL to 2 mg/mL for cefepime, TZP and vancomycin and from 0.5 mg/mL to 1 mg/mL for meropenem and the drugs also caused statistically significant reductions in biofilm cellular activity both in growing and mature biofilm. Since all of the tested drugs are commonly used in patients with hospital-acquired infections and in those with catheter-related infections under antibiotic-lock therapy, it may be possible to obtain an additional benefit from antibiotic-lock therapy with these drugs, namely the control of *Candida* biofilm formation.

1. Introduction

Candida spp. are the fourth and third leading causes of hospital-acquired bloodstream and urinary tract infections, respectively, and most of these infections are associated with implanted medical devices such as central venous and bladder catheters owing to biofilm formation within these materials [1]. A relevant characteristic of *Candida* biofilms is resistance to antifungal agents, which can be intrinsic or acquired by transfer of genetic material between biofilm cells [2]. Biofilm-associated *Candida* infections are usually difficult to diagnose, causing delayed therapy and high lethality rates in hospitalised patients worldwide [3], and the ability of *Candida* spp. to form drug-resistant biofilms is an important contributing factor to human diseases [1].

In systemic infections, biofilms can also be polymicrobial, formed by *Candida* spp. and bacteria [1]. Patients with confirmed or strongly suspected hospital-acquired infections primarily receive antibacterial therapy, including cefepime, meropenem, piperacillin/tazobactam (TZP) and vancomycin [4], which, on the other hand, predisposes them to the occurrence of *Candida* infections because it decreases microbial competition within the host's microbiome [1,3]. It has already been shown that antibacterial drugs can affect *Candida* biofilm formation. Tigecycline, for instance, is highly active against growing and mature biofilms of *Candida albicans* [5], whilst rifampicin can induce biofilm formation by this *Candida* species [6]. Thus, this study aimed to evaluate the effects of β-lactams (cefepime, meropenem and TZP) and vancomycin on strains of *C. albicans* and *Candida tropicalis* in planktonic and biofilm forms.

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2. Materials and methods

2.1. Fungal strains

Azole-derivative-resistant strains of *Candida* spp. from human cases of candidaemia (four *C. albicans* and six *C. tropicalis*) and healthy animals (six *C. albicans* and four *C. tropicalis*) were included in this study. The strains came from the culture collection of the Specialized Medical Mycology Center of Federal University of Ceará (Fortaleza, Brazil) and were selected based on their antifungal resistance [7]. The identity of the strains was confirmed as previously described [7].

2.2. Antimicrobial agents and antifungal susceptibility of Candida planktonic cells

A stock solution of amphotericin B (AmB) (Sigma-Aldrich, St Louis, MO) at 1 mg/mL was prepared according to Clinical and Laboratory Standards Institute (CLSI) guidelines [8] and was used as a control. The antibacterial drugs cefepime (Novafarma, Anápolis, GO, Brazil), meropenem (AstraZeneca, Cotia, SP, Brazil), TZP (Novafarma) and vancomycin (AstraZeneca) were diluted with sterile distilled water as recommended by the manufacturers. A stock solution of vancomycin was prepared with distilled water at 50 mg/mL as previously described [5] and, based on this research [5], the other antibacterial drugs were diluted to the same concentration. Serial two-fold dilutions of each drug were prepared in RPMI 1640 medium with L-glutamine and without sodium bicarbonate (Sigma-Aldrich, St Louis, MO), buffered to pH 7.0 with 0.165 M MOPS (3-[*N*-morpholino]propane sulfonic acid) (Sigma-Aldrich, St Louis, MO).

The susceptibility of *Candida* spp. to the antibacterial agents was investigated by broth microdilution according to CLSI guidelines [8]. The final inoculum was diluted with RPMI to reach a concentration of 0.5–2.5 × 10^3 cells/mL. AmB was also tested as a control drug. To determine the susceptibility of planktonic cells, the tested concentration ranges were 0.0039–4 mg/mL for cefepime, meropenem, TZP and vancomycin and 0.03125–16 μg/mL for AmB. All isolates were tested in duplicate. For the antibacterial drugs, the minimum inhibitory concentration (MIC) was defined as the lowest drug concentration capable of inhibiting 50% of fungal growth [5] compared with the control well, whilst for AmB the MIC was defined as the lowest drug concentration capable of inhibiting 100% of planktonic fungal growth [8]. *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Candida parapsilosis* ATCC 22019 were included as quality control strains for each test [8].

2.3. Biofilm formation

For biofilm testing, inocula were prepared as previously described [9] with some modifications. Strains of *C. albicans* ($n = 10$) and *C. tropicalis* ($n = 10$) were grown in Sabouraud dextrose broth (Himedia, Mumbai, India) at 30 °C for 24 h in a rotary shaker at 150 rpm. After this period, cells were collected by centrifugation (3000 rpm, 10 min) and the pellet was washed twice with phosphate-buffered saline (PBS). Suspensions were adjusted to 1 × 10^6 cells/mL in RPMI medium and then 100 μL aliquots of inoculum were transferred to flat wells of 96-well polystyrene plates (TPP, Trasadingen, Switzerland). The plates were incubated at 37 °C for 48 h and the wells were washed three times with 0.05% Tween 20 (Sigma-Aldrich, São Paulo, SP, Brazil) in Tris-buffered solution (Sigma-Aldrich, São Paulo, SP, Brazil) to remove non-adherent cells. Biofilm viability was monitored through the use of 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carbonyl]-2*H*-tetrazolium hydroxide (XTT) (Sigma-Aldrich, St Louis, MO) as described previously [9].

2.4. Effect of antibacterial drugs on growing and mature Candida biofilms

The ability of the tested drugs to inhibit formation of *C. albicans* and *C. tropicalis* biofilms was evaluated as described previously [9]. During the plate inoculation step, 100 μL aliquots of the antibacterial solutions were added to each well. Each drug was tested at five different concentrations (MIC/50, MIC/10, MIC, 10× MIC and 50× MIC). Biofilm formation was then performed as previously described. Following incubation at 37 °C for 48 h, the effect of the antimicrobial drugs on growing biofilms was evaluated based on biofilm cell activity using the XTT reduction assay with 75 μL of XTT salt solution (1 mg/mL in PBS), 6 μL of menadione solution (1 mM in acetone) (Sigma-Aldrich, St Louis, MO) and 50 μL of sterile PBS, which were added to each well, followed by incubation at 36 °C for 5 h. The metabolic activity of biofilm cells was measured with a microplate reader (Epoch; Bio-Tek, Winooski, VT) at 492 nm.

The inhibitory activity of the tested drugs against mature biofilms of *C. albicans* and *C. tropicalis* was evaluated as previously described [9]. The antibacterial drugs were tested at five different concentrations (MIC/50, MIC/10, MIC, 10× MIC and 50× MIC). For this purpose, aliquots of 200 μL of each drug were added to viable 48-h-old biofilms grown in flat wells of 96-well polystyrene plates, followed by incubation at 35 °C for 48 h. After this period, inhibition of biofilm metabolic activity was monitored by XTT reduction [9] as described above.

All biofilm experiments were performed in duplicate and were repeated at three independent moments. Controls were grown in medium without antimicrobials, and AmB was used as the control drug for biofilm inhibition.

2.5. Statistical analysis

In order to verify differences in absorbance values to evaluate the effects of antibacterial drugs on biofilm cell activity, Student's *t*-test for paired samples was used. For all of the analyses, a significance level lower than 5% indicated statistically significant findings ($P < 0.05$).

3. Results

The antibacterial MICs against the 20 tested *Candida* strains ranged from 0.5 mg/mL to 2 mg/mL for cefepime and TZP and from 0.5 mg/mL to 1 mg/mL for meropenem. The MICs for vancomycin ranged from 0.5 mg/mL to 1 mg/mL against *C. albicans* and from 0.5 mg/mL to 2 mg/mL against *C. tropicalis*. For AmB, the MICs ranged from 0.5 μg/mL to 2 μg/mL against *C. albicans* and from 0.5 μg/mL to 4 μg/mL for *C. tropicalis*.

Regarding the effects of antibacterial drugs on growing *Candida* spp. biofilms, cefepime, TZP and vancomycin caused statistically significant reductions in biofilm cellular activity at MIC/10 ($P < 0.001$), MIC ($P < 0.0001$), 10× MIC ($P < 0.0001$) and 50× MIC ($P < 0.0001$), whilst meropenem only caused significant reductions at the MIC and higher concentrations ($P < 0.05$). AmB significantly inhibited growing biofilm cellular activity at all tested concentrations ($P < 0.01$) (Fig. 1).

Regarding mature *Candida* biofilms, cefepime, meropenem, TZP and vancomycin caused statistically significant reductions in biofilm cellular activity at MIC/10 ($P < 0.05$), MIC ($P < 0.01$), 10× MIC ($P < 0.0001$) and 50× MIC ($P < 0.0001$), but not at MIC/50 (Fig. 1) compared with the control growth. AmB significantly

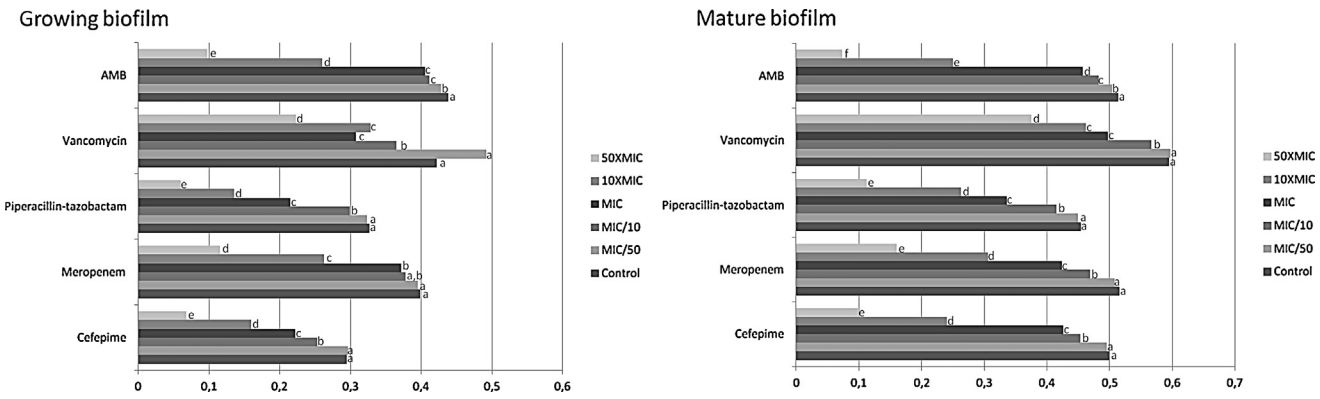

Fig. 1. Effect of different concentrations of cefepime, meropenem, piperacillin/tazobactam, vancomycin and amphotericin B (AMB) on the metabolic activity of growing and mature biofilms of *Candida* spp. analysed by the XTT reduction assay. The minimum inhibitory concentration (MIC) is that obtained against planktonic growth of *Candida* spp. Letters indicate statistically significant differences compared with the control and with growth at different antibacterial concentrations ($P < 0.05$). The tested antimicrobial concentrations are presented in the following order top-down: 50× MIC, 10× MIC, MIC, MIC/10, MIC/50 and control.

inhibited mature biofilm cellular activity at all tested concentrations ($P < 0.05$) (Fig. 1).

4. Discussion

The capacity of *Candida* to form biofilms on abiotic and biotic surfaces is an important virulence factor for the establishment of recurring candidiasis [10]. It is well known that the use of systemic antibiotics predisposes the proliferation of *Candida* spp., hence the occurrence of candidiasis [1,3]. However, little is known about the effects of antibiotics on growing and mature biofilms of *Candida* spp. within medical devices, which are continuously exposed to antibacterial drugs when patients are under treatment. This lack of knowledge was a motivation to investigate the in vitro effects of cefepime, meropenem, TZP and vancomycin, which are commonly used against hospital-acquired infections [4], on *Candida* biofilms and, considering that these drugs predispose the occurrence of *Candida* infections, it was initially hypothesised that they could induce biofilm formation or the maintenance of mature biofilms.

Initially, the antimicrobials were tested against azole-derivative-resistant *C. albicans* and *C. tropicalis* strains and all of them effectively inhibited the growth of planktonic *Candida* spp., with MICs ranging from 0.5 mg/mL to 2 mg/mL. Although all tested antibacterial drugs were able to inhibit the in vitro growth of planktonic *Candida*, the MICs surpassed the desirable therapeutic blood concentrations for all of these antibacterial drugs [11–14]. Hence, they cannot be used to effectively treat *Candida* infections.

The mechanisms through which these antibacterial drugs inhibited fungal growth remain unknown. Only a few studies have been performed tackling the effects of antibacterial drugs on *Candida* biofilms and none of them have addressed possible mechanisms of action [5,6]. However, it can be suggested that these drugs act through unspecific mechanisms, without specific target molecules, since extremely high concentrations of the four tested drugs were required to cause this growth inhibition. Thus, considering this new potential use of cefepime, meropenem, TZP and vancomycin, it is necessary to design specific experimental protocols to elucidate the mechanisms behind the observed antifungal effect.

On the other hand, the results demonstrate that antibacterial concentrations as low as MIC/10 were capable of significantly reducing the biofilm cellular metabolic activity both of growing and mature *Candida* biofilms. The most interesting finding of this study was that antibacterial concentrations lower than the MICs against planktonic cells were able to significantly decrease the in vitro viability of growing and mature *Candida* biofilms.

Clinically, these findings may be ground-breaking since all of the tested antibacterial drugs (cefepime, meropenem, TZP and vancomycin) are commonly used in patients with hospital-acquired infections [4] and are also used in antibiotic-lock therapy [15]. This technique involves the prolonged instillation of a solution containing extremely high concentrations of antimicrobial or antiseptic agents, 100–1000-fold higher than those used systemically, within an infected intravascular catheter as in an attempt to sterilise the interior of the catheter and control bloodstream infections [5,15]. This kind of treatment appears to be a viable alternative in those special cases in which the salvage of the catheter is desirable, although removing the device is the treatment of choice for persistent or complicated bacteraemia or fungaemia related to its use [15].

It is important to emphasise that the use of systemic broad-spectrum antibiotics is an important risk factor for developing candidaemia and that biofilms play a major role in maintaining bloodstream *Candida* infections [10]. Therefore, considering the low minimum antibacterial concentrations (MIC/10; 50–200 μg/mL) required to significantly decrease the viability of *Candida* biofilm cells, we believe that the use of cefepime, meropenem, TZP and vancomycin in the antimicrobial-lock solution might not only aid the patient in the management of bacterial infection associated with indwelling catheters, but also control the formation of *Candida* biofilms within these medical devices during antibacterial therapy. However, more studies will be necessary to establish the effects of this therapy on *Candida* biofilms inside the patient during treatment.

5. Conclusion

The antibacterial drugs cefepime, meropenem, TZP and vancomycin are able to decrease the in vitro viability of growing and mature *Candida* biofilms. These results may bring the perspective of obtaining an additional benefit with antibiotic-lock therapy, namely the control of fungal biofilms when using these drugs in hospitalised patients.

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Competing interests

None declared.

Ethical approval

Not required.

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