

Susceptibility to antimicrobials of *Candida parapsilosis* polymicrobial biofilms with *Staphylococcus aureus* or *Acinetobacter* sp.

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Summary

The clinical importance of biofilms, including those formed by *Candida parapsilosis*, *Staphylococcus aureus* or *Acinetobacter* sp., is based on their resistance to antimicrobial treatments, chemical disinfections, and their association with increased mortality rates in infected patients. This study evaluated the ability of *C. parapsilosis sensu stricto* to interact with *S. aureus* and *Acinetobacter* sp., in two-species polymicrobial biofilms, and their tolerance to treatment with ketoconazole, vancomycin or polymyxin B, respectively. Initially, the susceptibility of these isolates to the antimicrobials was confirmed using microdilution broth assay, according to parameters described in CLSI document. Monomicrobial and polymicrobial biofilms were developed in 96 well-polystyrene plate using RPMI-1640 medium. After 6, 12, 24 and 48 h, biofilms were subjected to treatment with ketoconazole (0.0313 to 16 µg/mL), and mature 48 h biofilms were also treated with vancomycin (1 to 32 µg/mL) or polymyxin B (0.25 to 32 µg/mL), always in triplicate. The plates were incubated for 24 h. After the treatments, the biofilms were subjected to sonication for 10 min, the cell suspensions were submitted to serial dilutions and plated on Sabouraud Dextrose Agar with chloramphenicol (0.05 mg/mL), for yeast growth, and/or Brain Heart Infusion Agar with amphotericin B (0.025 mg/mL), for bacterial growth. The viability of yeast and bacterial cells forming the biofilms was assessed by counting the colonies and estimating the CFU/mL values. From these data, it was observed that *C. parapsilosis sensu stricto*, *S. aureus* and *Acinetobacter* sp., in planktonic condition, were susceptible for their respective antimicrobials. *C. parapsilosis sensu stricto* cells from mono- and polymicrobial biofilms showed an increased tolerance to ketoconazole, compared to their planktonic condition. A similar result was observed regarding *S. aureus* and *Acinetobacter* sp. biofilms treated with vancomycin and polymyxin B, respectively. Moreover, in *C. parapsilosis sensu stricto* polymicrobial biofilms, both bacteria presented an enhanced tolerance to their respective antimicrobials, compared to their monomicrobial biofilms. These results revealed that *C. parapsilosis* was able to form polymicrobial biofilms with *S. aureus* and *Acinetobacter* sp., and that, in this condition, there was an increase in the tolerance of bacterial cells to the antimicrobials tested.

Keywords: *Acinetobacter* sp., Antimicrobial drugs, *Candida parapsilosis*, *Staphylococcus aureus*.

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