

TITLE: SUSCEPTIBILITY OF MONOMICROBIAL AND MIXED BIOFILMS OF *ASPERGILLUS FUMIGATUS* AND *PSEUDOMONAS AERUGINOSA* TO ANTIMICROBIALS

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ABSTRACT:

The association of *Aspergillus fumigatus* and *Pseudomonas aeruginosa* is prevalent in cystic fibrosis patients, people with chronic obstructive pulmonary diseases and lung transplant recipients. Commonly, this interaction occurs with the formation of biofilms of both pathogens, which hinders the action of the immune system and the treatment with antimicrobials. Thus, the aim of this work is to evaluate the effect of antimicrobials on the monomicrobial and polymicrobial biofilms of *A. fumigatus* and *P. aeruginosa*. Biofilms were formed on the 96-well polystyrene microtiter plate in RPMI 1640 medium buffered with 0.16M MOPS. The *A. fumigatus* conidia (ATCC 16913, Af1304 and Af1220 strains) were standardized at 10^5 cfu/ml and incubated at 35°C for 24 h and then *P. aeruginosa* (PA01, Pa151 and Pa481997-A strains) were added to final concentration at 10^6 cfu/ml. After incubation of 1.5 h the antimicrobials [amphotericin B (AMB), caspofungin (CPF), micafungin (MCF) and polymyxin B (PMB)] were added on the wells. In parallel, monomicrobial biofilms were also treated with the same antimicrobials. After 24 h of incubation, violet crystal staining was performed to determine the lowest concentrations that inhibit 50% and 90% of biofilms (BMIC₅₀ and BMIC₉₀, respectively). AMB had a good action on *A. fumigatus* biofilms and also on mixed biofilms presenting BMIC₅₀ of 1-4µg/ml for the fungal biofilms and BMIC₅₀ of ≤1-8µg/ml for the mixed biofilms, except on Af1220+Pa151 and Af1220+Pa481997-A. It is important to note that Af1304 and Af1220 monomicrobial biofilms were inhibited at 8 µg/ml of AMB (BMIC₉₀). CPF also had a relevant action on *A. fumigatus* biofilms with BMIC₅₀ of 16-256µg/ml and for mixed biofilms BMIC₅₀ ranged from 32 to 128µg/ml. In contrast, MCF inhibited only the Pa151 strain at 8µg/ml (BMIC₅₀). The antibacterial PMB had an effective action only against bacterial biofilms with BMIC₅₀ of 2-4µg/ml and BMIC₉₀ of 2-8µg/ml. The action of echinocandins (CPF and MCF) on bacteria has been studied since the discovery of the presence of β- (1,3) D-glucan in the serum of patients infected with *P. aeruginosa*, and the presence in the periplasm and extracellular matrix of biofilms. Therefore, this work has a focus on the use of these antifungals in the combat of the mixed biofilms formed by *A. fumigatus* and *P. aeruginosa* and it has shown promising results; but more studies are still necessary in the future.

Keywords: polymicrobial biofilm; echinocandins; *Pseudomonas aeruginosa*; *Aspergillus fumigatus*.

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