

**TITLE:** REDUCTION OF BIOMASS AND CELL VIABILITY OF MIXED BIOFILMS OF *Aspergillus fumigatus* AND *Pseudomonas aeruginosa* TREATED WITH ANTIMICROBIALS

**AUTHORS:** NAKANISHI, B.; ISHIDA, K.

**INSTITUTION:** LABORATÓRIO DE QUIMIOTERAPIA ANTIFÚNGICA, INSTITUTO DE CIÊNCIAS BIOMÉDICAS, UNIVERSIDADE DE SÃO PAULO (RUA PROFESSOR LINEU PRESTES 1374, 05508-000, SÃO PAULO/SP, BRASIL)

**ABSTRACT:**

The coexistence of *Aspergillus fumigatus* and *Pseudomonas aeruginosa* in cystic fibrosis and immunocompromised patients may result in a mixed infection with biofilm formation, increased antimicrobial resistance, worsening prognosis and constant reinfections. Therefore, it is necessary to study therapies to control this mixed infection. This work aims to evaluate the activity of antimicrobials on polymicrobial biofilms of *A. fumigatus* and *P. aeruginosa*. Biofilms were formed on a 96-well polystyrene microplate in RPMI 1640 buffered with 0.16M MOPS. Firstly,  $10^5$  cfu/ml of *A. fumigatus* conidia (Af1304) were incubated at 35°C for 24 hours and then  $10^6$  cfu/ml of *P. aeruginosa* (PAO1 or Pa151 strains) were added and incubated together for 1.5 hours. Then, polymyxin B (PMB, 2-8µg/ml), amphotericin B (AMB, 4-32µg/ml), or caspofungin (CAS, 64-256µg/ml) were added to the mixed biofilms. After 24 hours of incubation at 35°C, the total biomass was determined by crystal violet assay and the cell viability was obtained by colony forming unit (CFU) count. It was not possible to determine CFU of Af1304 in the biofilm mixed with Pa151, because it is an MDR strain bacterium, including chloramphenicol. PMB significantly reduced the biomass and bacterial viable cells at lowest tested concentration of 2µg/ml in both mixed biofilms. AMB reduced the biomass of mixed biofilms at concentrations  $\geq 4$  µg/ml (Af1304+Pa151) and  $\geq 8$  µg/ml (Af1304+PAO1). Furthermore, in the mixed biofilm Af1304+PAO1, AMB reduced viable *A. fumigatus* cells since 8 µg/ml and higher reduction was obtained at 32 µg/ml, but had no action on bacteria. CAS reduced 50% of the biomass from both mixed biofilms at 64µg/ml, as well as viable fungal cells in the mixed biofilm with PAO1. Additionally, CAS reduced the Pa151viability with 64 µg/ml and reached the greatest reduction at concentrations up to 256 µg/ml but it showed no results against PAO1. Therefore, this work reveals promising results of antimicrobials on the mixed biofilms of *A. fumigatus* and *P. aeruginosa* and more studies will be conducted in the future to better understand this subject.

**Keywords:** biofilms; *Aspergillus fumigatus*; *Pseudomonas aeruginosa*; polymicrobial infection

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