

TITLE: EFFECT OF ANFOTERICIN B AND MICONAZOLE ON *ASPERGILLUS FLAVUS* AND *ASPERGILLUS FUMIGATUS* BIOFILMS

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ABSTRACT:

Filamentous fungi are adapted to the growth on surfaces forming biofilms. Fungal biofilms are heterogeneous community of microorganisms, consisting of cells and hyphae, developed on an inert substrate or on a tissue, protected by a polymeric extracellular matrix. The presence of *Aspergillus flavus* and *Aspergillus fumigatus* biofilms in medical and industrial environments has been widely reported. The *A. fumigatus* is a pathogenic fungus commonly found in hospital environments, being one of the species of fungi responsible for causing aspergillosis, with mortality rate about 85%, even after administration of antifungals. The *A. flavus* is a fungal species of great medical interest responsible for the direct infection of immunosuppressed patients. In addition, *A. flavus* produces aflatoxin, that is a toxic and hepatocarcinogenic substance. This fact arouses scientific interest, since biofilms are more resistant to antifungal therapies. amphotericin B and miconazole are efficiently used in antifungal therapy against the planktonic and mycelial forms of *A. flavus* and *A. fumigatus*. For evaluation of the effect of these antifungal agents on *A. flavus* and *A. fumigatus* biofilms, pieces of polyethylene (1x1 cm) were used as inert supports for biofilm formation in Khanna medium into a 24 wells microplate for 24 h at 38°C. The fungal biofilms and planktonic cells were treated with different concentrations of antifungal agents (4-0.5 mg/mL amphotericin B and 7.5-0.05 mg/mL Miconazole). After 48 h treatment with these agents, resazurin was added. Resazurin was partially metabolized by *A. flavus* and *A. fumigatus* biofilms treated with antifungal agents. The colorimetric analysis indicates that at high concentrations of amphotericin B and miconazole, the metabolization was lower than the metabolization observed for untreated biofilms, indicating the fungistatic effect of both agents used. The minimum inhibitory concentration (MIC) using amphotericin B was 4 µg/mL for the planktonic cells of *A. flavus* and *A. fumigatus*. The MIC for miconazole was 0.026 µg/mL and 0.71 µg/mL for planktonic cells of *A. flavus* and *A. fumigatus*, respectively. The biofilms of *A. flavus* and *A. fumigatus* were more resistant to the antifungal agents when compared to the planktonic cells.

Keywords: Biofilms, Amphotericin B, Miconazole, *A. flavus*, *A. fumigatus*

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