0762 An in vitro Model to Study Antifungal Perfusion in Candida Biofilms

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Candidal infections are recalcitrant, especially in immunosuppressed, partly due to their resistance to antifungals. Objective: As one reason for this may be the incomplete permeation of antifungals through the surface biomass of Candida biofilms an in vitro method to determine antibiotic penetration in biofilms was developed. Method: Standardized Candida biofilms were developed by inoculating 50 µls of 107cells/ml yeast suspension on milipore membrane filters (MMF: diameter, 25 mm; pore size, 12 µm) placed on YNB/ 500mM Galactose/agar media. 2-fold dilutions of the antifungal agent (AA) were prepared and sterile absorbant pads (diameter 47mm; pore size, 0.45 µm; Advantec MFS, Inc. USA) were soaked with 2 mLs of the antibiotic dilutions. The biofilms were then placed on the AA soaked pads, covered with another MMF (diameter, 13mm; pore size, 0.2 µm) with a superficially placed sterile filter paper disc (diameter, 9mm; Macherey-Nagel, Germany) and, incubated at 370C for 4 hrs. Antibiotic penetration through the biofilm was measured by assaying the concentration of AA that diffused through the biofilm to the overlying filter paper disc. To quantitate drug penetration, standard curves of a series of drug dilutions vs areas of gowth inhibition were drawn for the three AAs examined using a standard growth inhibition assay on a lawn of cultured yeasts. Sterile filter paper discs placed on the two membrane system without the biofilm was used to measure the total effective drug penetration through the biofilm. Results: C. albicans biofilms was the most resistant to antifungal penetration in comparison to C. parapsilosis and C. krusei biofilms. Of the three antifungals studied ie., 5-fluorocytosine, fluconazole and amphotericin B, fluconazole was the most penetrant and AmB the least. Conclusion: Our data indicate that the inefficacy of antifungal penetration through biofilms could be a contributory factor for the failure of Candida biofilm-associated infections.

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