Master Thesis Abstract

By: Ahmed Osama El-Gendy

"Studying of *Candida albicans* Biofilms and Their Susceptibility to Antifungal Agents"

Biofilms represent the most prevalent type of microbial growth in nature and are crucial to the development of clinical infections. They can serve as a cause for disease and are often associated with high-level antimicrobial resistance of the associated organisms.

The objectives of this work were to study the effect of different antifungal drugs on the preformed *C. albicans* biofilm and to study the antifungal penetration pattern as a mechanism of biofilm resistance.

A total of 152 isolates of Candida isolates were collected from different clinical specimens from Egyptian hospitals. They were identified as *C. albicans* (64); *C. tropicalis* (42); *C. krusei* (35); and *C. glabrata* (11).

The antifungal sensitivity pattern and the antifungal`s minimum inhibitory concentrations (MICs) of the tested planktonic *C. albicans* was determined using the agar dilution method on RPMI-1640 medium. The obtained data showed that nystatin showed activity against all tested isolates followed by amphotericin B and clotrimazole which showed resistance by only 3 isolates.

Determinations of antifungal susceptibilities and degree of adherence of biofilm-grown Candida isolates were done in 96-well microtiter plates using RPMI medium and using the semi-quantitative XTT-reduction assay. For many years, the XTT assay has been the

mainstay for the estimation of biomass in yeast biofilms. The tetrazolium salt XTT is intracellularly reduced to a water soluble formazan, which is colorimetrically determined in the cell supernatant. The obtained results showed that biofilm originated from vaginal isolates were more complicated with high degree of adherence than those from catheters and urinary tract infections. *C. albicans* cells in biofilm conditions display dramatically increased resistance to antifungal agents compared to that of cells in planktonic conditions.

This work moved then to study the different stages and kinetic of biofilm formation on the wells of microtiter plates over 48 hrs. The results showed that the biofilms were highly metabolically active after the first 12 hours but the complexity increased after (24 to 48 hrs). A marked decrease in the complexity and the cellular density of the formed biofilm when exposing *C. albicans* to planktonic subinhibitory levels of antifungal agents and these results may point to approaches for preventive or prophylactic treatment. A severe drop in the finally formed biofilm was obtained when adding nystatin at concentration equal to biofilm sub $SMIC_{50}$ after 3 and 6 hrs. While, there was a paradoxical rise in metabolic activity of mature biofilms when adding nystatin after 12 or 24 hrs.

An investigation and evaluation of the penetration of antifungal agents through 48-h biofilms (as a possible mechanism that may protect microorganisms in biofilms from antibiotics) using polycarbonate membrane filter were done. The results demonstrated that azole antifungal agents permeated all Candida biofilms more rapidly than terbinafine and polyene antifungal agents. Viability of biofilm cells after exposure to antifungal agents for 24 h reveals the failure of penetrated drug to produce complete killing of biofilm cells. Although, amphotericin B was the least penetrant through biofilms but viable count observations revealed that it caused the most damage to the biofilm cells in comparison

to fluconazole. These results indicate that poor drug penetration is not a major resistance mechanism for Candida biofilms.

Scanning electron microscope (SEM) revealed that the fully mature biofilms is produced after incubation for up to 48 hours and it is consisted of a dense network of yeast cells. There was a wrinkled, ruptured, and ballooning effect of the drug on yeast cells after applying nystatin in its inhibitory concentration on 24-h biofilms.

د/ أحمد أسامة مصطفي الجندي

مدرس بقسم الميكروبيولوجيا والمناعة
كلية الصيدلة – جامعة بني سويف