The number of infections caused by Candida species has greatly increased in the past ten years. This has been attributed to an increase in the number of AIDS patients, the elderly population and immunocompromised patients. Moreover, the increased use of indwelling medical devices has also been implicated with the rise of Candida infections. Most candidiasis have been attributed to Candida albicans, however, recently, new non-Candida albicans Candida (NCAC) species have been identified as common pathogens, namely Candida tropicalis. Formation of C. tropicalis biofilms has important clinical repercussions because of their increased resistance to antifungal therapy and the ability of cells within biofilms to withstand host immune defenses. Thus, the aim of this study was to compare biofilms formed by different clinical isolates of C. tropicalis. A total of 6 C. tropicalis strains isolated from the vagina (n=2), urinary (n=2) and oral tract (n=2) were used. A reference strain, C. tropicalis ATCC 750, was also assayed. Biofilms were formed in 96-well microtiter plates, in Sabouraud dextrose broth at 37°C (agitated at 130 rpm). The ability of biofilm formation was assessed after 48h through total biomass quantification by crystal violet staining and cellular activity by the reduction of a tetrazolium salt (XTT). Moreover, the number of viable C. tropicalis cells in biofilms was determined by Colony Forming Units (CFUs). Matrix material was extracted from biofilms by sonication and their protein and total carbohydrate contents were determined by the Lowry and Dubois methods, respectively. The ultrastructure of the C. tropicalis strains biofilms was observed by Scanning Electron Microscopy (SEM). The results showed that all clinical isolates of C. tropicalis were able to form biofilms, although there were differences on biomass and biofilm activity depending on strains. Furthermore, comparison of biofilm biomass with cell activity did not reveal any correlation. Matrix recovered from C. tropicalis biofilms present an high amount of proteins and small amounts of carbohydrates per gram of biofilm cell dry weight. C. tropicalis biofilms revealed a multilayer structure that consists of a dense network of yeast, hyphae and pseudohyphae. As a general conclusion, it was possible to infer that clinical isolates of C. tropicalis present different behaviors in terms of biofilm formation, structure and chemical composition.

A271 ZOONOTIC ASPECT OF CANDIDIASIS IN GHARBIA GOVERNORATE, EGYPT

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Abstract: In this study the following samples were examined for detection of candida species: - 180 vulvovaginal swabs of pregnant and non pregnant women claiming for vulvovaginitis; - 100 skin swabs from the diaper area in infants suffering from dermatitis in the diaper area; - 200 chicken comb swabs from chicken farms and chicken homes; - 90 air samples were collected from the chicken farms that have comb candidiasis, human hospitals in which their patients infected with vulvovaginal or diaper candidiasis and patient’s homes; - 90 swabs from equipments and instruments were collected from the same places in which the air samples were collected. All samples located in Gharbia governorate, Egypt.

The result indicates that the infection rate of vulvovaginal candidiasis in the pregnant women was 53.3%, in non pregnant women 23.3% and in the pregnant women administrated the Yoghurt (Probiotic) during the pregnancy was 28.3%. The rate of infection by diaper candidiasis in infants used the cloth diapers was 44% and 14% in infants used superabsorbant disposable diapers. In comb candidiasis the infection rate in chicken administrated antibiotics was 37% and 6% in chicken without antibiotics use. The rate of candida pollution in the air of chicken farms was 23.3% and in the air of human hospitals and patient’s homes were 16.6% and 13.3% respectively. Equipments and instruments contamination in the chicken farms was 23.3% ; in the human hospitals and patient’s homes were 26.6% and 10% respectively. The public health importance of candidiasis was discussed as well as suggestive control measures.

C270 CANDIDA TROPICALIS CLINICAL ISOLATES: BIOFILM COMPOSITION AND ARCHITECTURE

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In this study the following samples were examined for detection of Candida species: -180 vulvovaginal swabs of pregnant and non pregnant women, -100 skin swabs from the diaper area in infants suffering from dermatitis in the diaper area. - 90 swabs from equipments and instruments in Gharbia governorate, Egypt.

The number of infections caused by Candida species has greatly increased in the past ten years. This has been attributed to an increase in the number of AIDS patients, the elderly population and immunocompromised patients. Moreover, the increased use of indwelling medical devices has also been implicated with the rise of Candida infections. Most candidiasis have been attributed to Candida albicans, however, recently, new non-Candida albicans Candida (NCAC) species have been identified as common pathogens, namely Candida tropicalis. Formation of C. tropicalis biofilms has important clinical repercussions because of their increased resistance to antifungal therapy and the ability of cells within biofilms to withstand host immune defenses. Thus, the aim of this study was to compare biofilms formed by different clinical isolates of C. tropicalis. A total of 6 C. tropicalis strains isolated from the vagina (n=2), urinary (n=2) and oral tract (n=2) were used. A reference strain, C. tropicalis ATCC 750, was also assayed. Biofilms were formed in 96-well microtiter plates, in Sabouraud dextrose broth at 37°C (agitated at 130 rpm). The ability of biofilm formation was assessed after 48h through total biomass quantification by crystal violet staining and cellular activity by the reduction of a tetrazolium salt (XTT). Moreover, the number of viable C. tropicalis cells in biofilms was determined by Colony Forming Units (CFUs). Matrix material was extracted from biofilms by sonication and their protein and total carbohydrate contents were determined by the Lowry and Dubois methods, respectively. The ultrastructure of the C. tropicalis strains biofilms was observed by Scanning Electron Microscopy (SEM). The results showed that all clinical isolates of C. tropicalis were able to form biofilms, although there were differences on biomass and biofilm activity depending on strains. Furthermore, comparison of biofilm biomass with cell activity did not reveal any correlation. Matrix recovered from C. tropicalis biofilms present an high amount of proteins and small amounts of carbohydrates per gram of biofilm cell dry weight. C. tropicalis biofilms revealed a multilayer structure that consists of a dense network of yeast, hyphae and pseudohyphae. As a general conclusion, it was possible to infer that clinical isolates of C. tropicalis present different behaviors in terms of biofilm formation, structure and chemical composition.

B272 P-113 BINDING TO THE C. ALBICANS CELL WALL IS SALT SENSITIVE AND IS REQUIRED FOR SUBSEQUENT ENERGY-DEPENDENT CYTOSOLIC TRANSLLOCATION

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P-113 is a 12 amino acid active fragment of Histatin 5 (Hst 5) that retains complete candidacidal activity of the parent peptide against Candida albicans. P-113 toxicity is initiated intracellularly, thus cell wall binding and cytosolic translocation are prerequisites for peptide activity. Incubation of cells in 100 mM extracellular salt or with inhibitors of the energy metabolism (sodium azide and carbonyl cyanide m-chlorophenylhydrazone (CCCP)) renders C. albicans cells resistant to the killing action of P-113. Objective: To identify whether salt, sodium azide and CCCP inhibit binding of P-113 with the yeast cell wall, or if subsequent translocation and/or cytosolic functions are affected. Methods: Antifungal activities of P-113 and its inactive mutant P-113Q.10 were tested against C. albicans by using standard microdilution plate assays. P-113 localization within C. albicans was examined using two sequential cellular fractionation steps consisting of β-mercaptoethanol (β-ME) cell wall extraction, followed by cytosolic fractionation. P-113 was quantified in each cellular compartment by Western blotting. Results: P-113 had potent killing activity against C. albicans cells in 10 mM NaPB, but was completely inactivated in the presence of 100 mM NaPB, 10 mM sodium azide or 500 μM CCCP. Similarly, P-113 was translocated to the C. albicans cytosol within 5 min under low salt conditions; while P-113 was absent in cytosolic extracts from cells incubated in the presence of 100 mM NaPB, azide or CCCP. However, in cell wall binding assays, P-113 was detected in cell wall extracts within 5 min in cells suspended in 10 mM NaPB, azide or CCCP, whereas little to no P-113 was found in the cell wall of C. albicans cells incubated in 100 mM NaPB. The translocation incompetent derivative P-113Q.10 remained bound at saturated levels with the cell wall, showing that peptide association with the wall is a transitional step prior to internalization, and that translocation is dependent upon the primary structure of the substrate peptide. Conclusions: Initial binding of P-113 with the C. albicans cell wall requires low salt conditions, suggesting that binding is predominantly through electrostatic interactions rather than by binding with specific peptide motifs. Translocation of P-113 to the cytosolic compartment first requires initial cell wall binding and is significantly reduced in cells energy depleted by sodium azide or CCCP. Therefore, intracellular transport of P-113 likely occurs via energy dependent permeases or other energy-dependent processes such as endocytosis which recognize specific peptide sequences.