TITLE: SUSCEPTIBILITY TO FLUCONAZOLE AND MICROMORPHOLOGICAL ANALYSIS OF MICROCOSM BIOFILMS FORMED FROM SAMPLES OF PATIENTS WITH VULVOVAGINAL CANDIDIASIS.

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The genus Candida comprises species commensals of the human microbiota that, in situations of dysbiosis in the host, express several virulence factors, such as biofilms microbial communities characterized by high resistance to antimicrobials and host immunity. In the mucosal, biofilms of Candida spp. are related in infections of great clinical and epidemiological relevance, such as Vulvovaginal Candidiasis (CVV). The present study aimed to evaluate an in vitro Microcosm Biofilm model (BMi) - formed from samples of patients with CVV – regarding fluconazole susceptibility (FLC), and to analyze BMi micromorphology by scanning electron microscopy and confocal microscopy. Therefore, five samples of vaginal secretion from patients with CVV were tested under experimental conditions listed as the most favorable to microbial growth for BMi formation. Furthermore, species of *Candida* spp. were also isolated for formation of Monospecies Biofilm (BMo). The two types of biofilms were formed in Vaginal Fluid Simulator Medium at 35°C for 48h, in microaerophilic. The susceptibility of BMi and BMo to FLC was evaluated at concentration of 512 µg/mL. The BMi's morphological analysis by MEV was carried out in a scanning electron microscope FEI Inspect S50, with previous fixation, processing and coverage in gold. In confocal microscopy, the BMi formed on Thermanox[®] slides were observed in the Nikon C2 Confocal Microscope, using Live/Dead[®], dyes used to check cell viability. The etiological agents implicated in the CVV of studied samples were C. albicans (4/5) and C. glabrata (1/5). BMi cultures showed tolerance to FLC at 512 μ g/mL (3/5). In the BMo model, the metabolic activity was reduced in up to 50% at concentration of 512 µg/mL of FLC (4/5). The biofilm morphology revealed the predominance of *Candida* sp. in BMi, even though the samples had mixed microbiota. Morover, was observed adhesion of fungic cells to the epithelial cells contained in biofilm. Given the above results, was observed that Microcosm Biofilm model has a higher tolerance to antifungal action, and the presented micromorphology is distinct from the Monospecies Biofilm. The proposed model for the study of sessile communities in microcosms can serve as a platform for in vitro studies because it is close to the conditions found in the vaginal environment and may better explain the microbiological aspects of CVV.

KEYWORDS: Vulvovaginitis, *Candida* spp., biofilms.

DEVELOPMENT AGENCY: CAPES. FUNCAP, CNPQ