Minimal inhibitory concentration of fluconazol for planktonic and biofilm cells of oral *Candida* species

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Non-Candida albicans Candida (NCAC) species are emerging as fungal pathogens causing Candidiasis. Fluconazole is commonly used in antifungal therapy in doses determined according to the susceptibility of Candida albicans grown in suspension. However, it is well known that, in order to colonize and infect, microbial cells form biofilms. Thus, it is of utmost importance to determine the susceptibility of the NCAC species, to antifungal agents, in both planktonic and biofilm forms. Several clinical isolates were obtained from the oral cavity of patients with Candidiasis. The isolates were identified using CHROMagar Candida and some were selected, along with one Candida albicans reference strain, to be used in the determination of the minimal inhibitory concentration (MIC) of fluconazole. It was also added to the assay one Candida albicans reference strain. MIC was determined when 50% of cell death was obtained in both planktonic and biofilm cells. In the former, MIC was measured according to the NCCLS standard (by optical density at 620 nm) and to cellular activity (using XTT). MIC, of 48h biofilms, was determined by the quantification of the total biomass (using crystal violet) and the activity (XTT). Over 50% of the isolates, in a total of 71, presented green colour. Violet, white, blue and pink colonies were also identified, confirming the presence of Candida albicans, Candida glabrata, Candida tropicalis and Candida parapsilosis, respectively, on the oral cavity of infected patients. The 8 clinical isolates selected for MIC determination, with one exception, presented lower MIC values than the reference strain. It was interesting to notice that isolates, belonging to the same species, displayed different MIC values. As it was expected, MIC values obtained for biofilm cells were high.

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