TITLE: STANDARDIZATION OF ANTIFUNGAL SENSITIVITY TEST BY FLOW CYTOMETRY COMPARED TO EUCAST

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ABSTRACT:
Antifungal sensitivity tests *in vitro* are important in the prediction and success of the therapeutic response of an antifungal drug. However, there are several factors that limit their use in the laboratory routine. Among them are the long incubation time required, resulting in the delay in obtaining the results, and the difficulties in the standardization of the readings. Thus, this study aims to standardize an antifungal sensibility test using flow cytometry in order to determine MIC (minimum inhibitory concentration) of amphotericin B, fluconazole, and caspofungin. Standardization was done in three strains of *Candida albicans* and one control strain of *Candida kruzei* ATCC 6258, with the initial procedure following the EUCAST (European Committee on Antimicrobial Susceptibility Testing) broth microdilution protocol. After incubation times of 2h, 4h, 7h, and 24h, aliquots were taken and fungal cells were labeled with PI (propidium iodide, 1µg/mL). Samples were analyzed on BD Accuri C6 flow cytometry for a total of 10,000 events and 50% and 90% MICs were determined by percentage growth inhibition, calculated from the number of live (non-PI-labeled) cells compared to growth control. The results obtained were compared with those obtained by the standard EUCAST method. For amphotericin B, the 50% and 90% MICs obtained with 2h incubation by cytometry varied by up to two dilutions between the conventional methods in the two *C. albicans* strains tested. For fluconazole, the 50% and 90% MICs obtained by cytometry with 4h of incubation were 64µg/mL in the control strain, and 16µg/Ml by the conventional method, which was expected for this control strain. For the other strains, the results showed no correlation between the methods. For caspofungin, the MICs obtained with 7h incubation varied by up to two dilutions between the methods in the three strains tested. In the analysis of the results obtained by flow cytometry with 24h incubation, it was observed that the MICs varied in up to two dilutions in the three strains tested for fluconazole and caspofungin. For amphotericin, only one dilution variance was observed for one of the *C. albicans* strains tested, while for the others, no correlation was observed. Thus, it is concluded that flow cytometry is shown as an important tool to assist in obtaining more reliable and rapid results, and further studies are needed to standardize new assays for antifungal activity.

Keywords: antifungal, susceptibility test, flow cytometry

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