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Abstract (oral session)

Evaluation of the fungicidal activity of micafungin by flow cytometry

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Objectives: Candida spp. is responsible for severe infections contributing to the increase of morbidity and mortality particularly of immunocompromised patients. Micafungin is a recent fungicidal drug by inhibiting the 1,3-beta-D-glucan synthetase. The standard susceptibility testing is time consuming and gives results only after 24 hours. We propose a new approach for micafungin susceptibility evaluation based upon flow cytometric assessment. Methods: Candida spp. susceptible (n=20) and resistant (n=8) strains to micafungin according to the CLSI protocol were assayed. Yeast cells were incubated with several concentrations of micafungin (0, 0.125, 0.25, 0.5, 1, 2, 4 and 8 µg/ml) during different incubation times (1, 2 and 3 hours). Afterwards, cells were stained with different fluorochromes : DiBAC4 (Molecular Probes) 1 µg/ml, a membrane potential dye; FUN-1 (Molecular Probes) 0.5 µM, a metabolism marker dye; Propidium Iodide (PI; Molecular Probes) 1 µg/ml, an acid nucleic dye. Changes on membrane potential of the cells or metabolic disturbance will increase the intensity of fluorescence of yeast cells stained respectively with DIBAC4 or FUN-1, while PI only stains cells with severe membrane lesion i.e. dead cells. A minimum of 20.000 cells were analysed in the flow cytometer and the intensity of fluorescence at FL1 (530nm), FL2 (585nm) and FL3 (650nm) was registered. Results: Regarding susceptible strains, an increase of intensity of fluorescence was evident in a dose-dependent manner soon after 1 hour incubation while in resistant strains, the staining intensity remained constant independently on the antifungal concentration and incubation time. PI staining was evident only in susceptible stains after 3 hours of incubation. Conclusions: Micafungin is a fungicidal drug whose activity can be quickly demonstrated using flow cytometry. It affects initially the metabolism and membrane potential of yeast cells being the membrane lesion demonstrated later on.