TITLE: AZOLES RESISTANCE SCREENING TEST FOR CLINICAL *ASPERGILLUS FUMIGATUS* ISOLATES

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ABSTRACT:
Aspergillus spp. are opportunistic fungi being *A. fumigatus* the main causative agent involved in systemic infections with high mortality rates in immunocompromised patients. Considering their resistance to fluconazole (FCL) and the high toxicity of amphotericin B (AMB), voriconazole (VRC) has been indicated for treatment. However, reports of isolates also resistant to VRC, itraconazole (ITC) and posaconazole (PSC) are increasing. They show mutations in *cyp51A* gene encoding the 14α-demethylase enzyme, a main azole target. At the University of Campinas Hospital (UNICAMP) - a tertiary and teaching hospital, many patients are susceptible to these pathogens (bone marrow transplant recipients, other immunocompromised and cystic fibrosis patients). This study aimed to evaluate a screening test for resistant *Aspergillus* isolates using methodology accessible to microbiology routines. Concentrations of 1 and 2µg/mL of ITC, 2 and 4µg/mL of VRC and 0.5 and 1µg/ml for PSC were incorporated to Mueller Hinton agar (MHA) with 2% dextrose. Several types of plates (96-well microtiter plates; 48 well cell culture plates and Petri dishes) were evaluated. *A. flavus* ATCC 204304 and 130 *A. fumigatus* clinical isolates were evaluated. Inoculum was established between 5.5 x 10^4 and 2 x 10^5 CFU/mL. All microorganisms considered resistant in the screening test and a significant number of the considered susceptible were submitted to the broth microdilution test (Clinical and Laboratory Standards Institute, CLSI M38-A2). *A. flavus* ATCC was susceptible to all concentrations. For ITC, 15 isolates showed resistance at 1ug/mL with 11 susceptible to 2ug/mL and 3 resistant isolates confirmed by microdilution. For VRC 19 isolates were resistant at 2ug/mL with 16 susceptible to 4ug/mL, and 3 resistants confirmed by microdilution. For PSC, 1 isolate was resistant at 0.5 and 1ug/mL, confirmed by the reference test. False-sensitive rate was 0.588% for VRC and 0% for ITC and PSC, that permits to conclude that MHA medium supplemented with 2% dextrose can be employed for screening with a satisfactory correlation with the reference broth microdilution method (CLSI 38-A2). Petri dishes showed better handling, easier to read results and lower cost. Due to the increasing need for surveillance for emergence of resistant *Aspergillus* isolates, this technique, can be indicated as an option for this large-scale screening, always followed by the microdilution test to confirm resistant isolates.

Keywords: *Aspergillus* spp.; *Aspergillus fumigatus*; antifungals; screening; resistantance.

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