

TITLE: Molecular identification of *Aspergillus* spp. in clinical settings and *in vitro* antifungal susceptibility testing

AUTHORS: BRAGA, V. F.; BARIÃO, P. H. G.; TONANI, L.; NASCIMENTO, E.; VON ZESKA KRESS, M. R.

INSTITUTION: Faculdade de Ciências Farmacêuticas de Ribeirão Preto – Universidade de São Paulo

ABSTRACT:

Aspergillus is a genus of filamentous fungi that cause a wide variety of infections in humans. This genus is divided into the sections or complex *Fumigati*, *Flavi*, *Terrei*, *Usti*, *Nigri* and *Nidulantes*. The section *Fumigati* is the most relevant to the clinical area which is the main cause of infection and allergy. Currently, many species of this genus demonstrate resistance to antifungals that are used in the treatment, as the azoles. Thus, the correct identification of the species and the determination of the antifungal susceptibility will aid to better understand the evolution of invasive and non-invasive aspergillosis. In this study were studied *Aspergillus* spp. isolated from clinical specimens obtained from Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto-USP. Genomic DNA from these isolates was extracted and used for the molecular identification by PCR amplification of rDNA ITS region and the gene that encodes the calmodulin. The sequences analyzed in ChromasPro program and the polymorphism study was performed using the Molecular Evolutionary Genetics Analysis program-MEGA 6.0 by the method of multiple alignments. The identification of isolates was carried out by agreement of the results obtained in the analysis of morphology (macro and micro) and molecular analysis. The antifungal susceptibility was tested for clinical isolates according to the Protocol M38A of the Clinical and Laboratory Standards Institute (CLSI). Antifungals of the class of polyenes (amphotericin B) and azole (itraconazole and voriconazole) were tested. The molecular identification has shown that out of a total of 23 isolates, 9 belong to the section *Flavi* and 11 belong to the section *Fumigati*. Within the section *Flavi*, a geometric mean (MG) of the minimum inhibitory concentration (MIC) of 1.25 µg/mL to amphotericin B, 0.73 µg/mL to itraconazole and 1.85 µg/mL to voriconazole were observed. The isolates belonging to the section *Fumigati* have shown the values of GM-MIC of 0.88 µg/mL, 1.06 µg/mL and 2.13 µg/mL to amphotericin B, itraconazole and voriconazole, respectively. The data correspond to the breakpoints of antifungal susceptibility described for *A. fumigatus*. In conclusion, species belonging to the sections *Flavi* and *Fumigati* were isolated from the clinical specimens in which all isolates were sensitive to the antifungal agents, except an isolate from the section *Fumigati* (identified as *A. fumigatus*) which showed resistance to itraconazole.

Keyword: *Aspergillus*, section *Fumigati*, section *Flavi*, molecular identification, *in vitro* susceptibility

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