TITLE: REAL-TIME PCR IN THE DETECTION AND DISCRIMINATION OF *Aspergillus fumigatus*, *Fusarium solani* and *Rhizopus arrhizus* IN BIOPSIES TAKEN FROM MURINE MODELS OF INVASIVE INFECTION

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ABSTRACT

Increase of invasive fungal infections by opportunistic agents, such as *Aspergillus* sp., *Fusarium* sp. and fungi from the order Mucorales, in immunocompromised patients has been reported in the last decades. Molecular methods, including amplifications of nucleic acids by PCR in fresh and paraffined tissues, have been more frequently applied to improve the detection and identification of these pathogens. The real-time PCR (qPCR) technique has the advantage of providing fast results with high sensitivity and specificity. This study aimed to evaluate the effectiveness of this technique for detection and discrimination of (the) fungal pathogens in tissues taken from BALB/c mice intravenously inoculated with *Aspergillus fumigatus*, *Rhizopus arrhizus* and *Fusarium solani*. The organs were removed from animals and analysed by histopathology and qPCR. The fresh and paraffined tissues were submitted to DNA extractions, and samples were evaluated by qPCR with genus-specific primers for *Aspergillus* and *Fusarium*, and order-specific primers for mucoraceous. In the histopathological examinations presence of typical fungal structures and alterations in tissue morphology were observed, confirming the dissemination of infection in the three experimental models. Both PCR assays employing *Aspergillus* and mucoraceous primers demonstrated 100% specificity. On the other hand, molecular tests using at least six different *Fusarium* primers showed cross reactivity, mainly with *Aspergillus* and *Rhizopus* DNA samples. The limit of detection of qPCR for *Aspergillus* and *Rhizopus* was 20 femtograms of DNA, and $2x10^2$ plasmids/µl. Both molecular methods detected *Aspergillus* and *Rhizopus* DNA in 100% of fresh and paraffined tissue samples. The results of the study demonstrated that qPCR assay was able to detect and differentiate *Aspergillus* and *Rhizopus* in fresh and paraffined tissues, with 100% of specificity and high analytical sensitivity. However, the results obtained with several *Fusarium* primers in the PCR assays were inconsistent regarding specificity of the amplifications. Real-time PCR assay is a fast and accurate method to diagnose aspergillosis and mucormycosis in tissues, and it could be implemented as an alternative tool for diagnostic routine in pathology or microbiology laboratories. In contrast, the technique showed low specificity with the *Fusarium* primers selected in this study. Other gene sequences should be evaluated by PCR assays techniques to achieve accurate results.

Keywords: invasive fungal infections; *Aspergillus*; *Fusarium*; Mucorales; biopsy; pathology; real-time polymerase chain reaction.

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