A comparative study of identification systems of clinically relevant Candida species: phenotypic versus molecular methods

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Fungal infections, namely candidiasis, are a major public health problem with high morbidity rates. The aim of this work was to compare the identification of 450 clinical samples, mainly from vaginal infections, using conventional (ID32C) and molecular methods. The molecular method chosen was a PCR multiplex strategy based on the simultaneous amplification of two fragments from the ITS1 and ITS2 regions by the combination of two yeast-specific and eight species-specific primers in a single PCR reaction. The biochemical method resulted in approximately 7% of discrepant identifications when compared to the multiplex PCR. These results were later confirmed using DNA fingerprinting which confirmed the results from the multiplex PCR. In this study, C. albicans was the most frequently isolated species (79.9%), followed by C. glabrata (8.9%), C. parapsilosis (4.7%), Saccharomyces cerevisiae (2.3%), C. tropicalis (1.3%), C. krusei (1.0%), and C. lusitaneae (0.5%). Results show differences between the conventional and molecular methods used, with several misidentifications occurring when using the biochemical method. The PCR multiplex showed again to be a simple, quick and trustworthy technique, allowing an easy interpretation. The great advantage is that it obeys the majority of the necessary requisites for the application in clinical laboratories and allows a quicker response (in the same day) comparing to the phenotypic methodologies ID32C (24 to 48 hours).


Histopathological diagnosis of onychomycosis using fluorescent stains

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Onychomycosis is a fungal infection of human nails that occurs worldwide and represents a major cause of abnormal nails. Although dermatophytes are the most commonly isolated microorganisms other filamentous fungi and yeasts may be involved. Histological evaluation of nail plate specimens, using routine stains, has been recommended for cases in which there is strong clinical evidence for onychomycosis, but false-negative results can be produced from fresh specimen preparations and fungal cultures. The Periodic-Acid-Schiff (PAS) stain is the most common method used in the histological evaluation of mycotic nail infections and was used in this study as “gold standard”; even so this staining procedure and its subsequent washing steps enhance the loss of specimen from the slides. In order to overcome the aforementioned problems formaldehyde-fixed paraffin-embedded nails were stained with Congo Red or Calcofluor White Stain, in a single-step staining for the rapid examination and detection of fungi. Considering all positive results obtained by PAS staining, the histopathological diagnosis provided by Congo Red was about 40% being particularly successful in the detection of yeasts and saprophytes. Fungi filaments were easily detected by Calcofluor White Stain in all positive samples. The combined use of fluorochromes and histology provided a fast and simple single-step procedure for fungi detection in cases with clinical suspicion of an onychomycosis.