DEVELOPMENT OF SIMPLE NUCLEIC ACID DIAGNOSTIC TOOLS FOR RAPID AND RELIABLE DIAGNOSIS OF *CANDIDA* SPP. INFECTIONS.

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ABSTRACT

Invasive candidiasis is the most common fungal infection among hospitalised patients. Its high morbidity and mortality rates have been attributed to inadequate diagnosis and the delayed implementation of effective antifungal treatment. Traditional diagnostic methods are time-consuming, cumbersome and lack specificity. Therefore, the need for a novel diagnostic tool with high sensitivity, specificity and rapidity have arisen. Here, two different biomarker detection platforms were explored, namely, Multi-component Nucleic Acid Enzymes (MNAzymes) and Multiplex Probe Amplification (MPA), as simple nucleic acid biosensors for the diagnosis of invasive candidiasis. *Candida* DNA biomarkers were used to identify pathogenic *Candida* species (pan-*Candida*) and well-known drug-resistant species *Candida krusei*, *Candida glabrata*, *Candida auris*. The biomarkers were designed from the ribosomal DNA locus to be used in each DNA detection technique. The first technique, MNAzymes, is based on a modified DNA enzyme that consists of multiple, nucleic acid oligonucleotides which are catalytically active in the presence of the target DNA sequence. The catalytic activity of an isothermal MNAzyme assay was determined at 1.0×10^{8} min^{-1} M^{-1} for detection of amplified *Candida albicans* DNA. Secondly, MPA relies on five DNA probes designed with specific melting temperatures for *Candida* spp., the well-known drug-resistant *C. krusei*, *C. glabrata* and *C. auris*, and internal control. All five biomarkers are detected using only two real-time PCR channels (FAM and HEX). In the presence of the target, the corresponding probe is cleaved during amplification, and the signals can be compared with the positive control that can be seen in the post-amplification melting profiles. The assay exhibit high sensitivity, demonstrating a limit of detection ranging from 10-100 copies of genomic DNA. Developing effective diagnostic tests for fungal infections could save thousands of lives through accurate antifungal therapy and deliver substantial cost-cutting benefits to health care budgets worldwide.

Keywords: *Candida* spp., Nosocomial infections, Multiplex Probe Amplification, Multi-component Nucleic Acid Enzymes.

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