Proof-of-concept of duplex MNAzyme assay for simultaneous detection of Candida auris and Candida spp. using synthetic DNA targets

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Abstract

The global emergence of Candida auris as a multidrug-resistant fungal pathogen presents significant challenges to healthcare systems, particularly in hospital settings. Rapid and accurate diagnostics are crucial for managing C. auris infections. This study presents a preliminary analytical evaluation of a duplex Multicomponent Nucleic Acid Enzyme (MNAzyme) assay designed to simultaneously detect C. auris and genus-wide Candida species using synthetic DNA targets. Target sequences were selected from the conserved D1/D2 region of the LSU rDNA locus, and melting temperature analysis confirmed distinct probe hybridisation profiles, with Substrate 2:PCO1 yielding a Tm of 45°C and Substrate 3:PCO4 at 26°C. The assay demonstrated specificity by differentiating C. auris from other Candida species through melting curve analysis. In reactions containing synthetic

targets, probe– target binding disrupted probe–PCO hybrids, resulting in a marked reduction in melt peaks compared to no DNA controls (NDC). The duplex format allows multiplex detection within a single fluorescence channel, offering a potential advantage over conventional single-target assays. This proof-of-concept establishes a rapid, low-cost diagnostic platform with promising potential for future clinical application. Further validation using clinical and environmental samples is needed to support its broader utility in detecting fungal pathogens.

Author keywords

C. auris; Candidozyma; melting curve; MNAzymes; multiplex; One Health

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