We herein describe a cost-effective multiplex array platform for rapid detection and differentiation of major clinically relevant Candida species and the recently emerged multidrug resistant species, C. auris, directly from blood cultures. This approach utilizes a novel polymer-mediated signal amplification process targeting the ribosomal RNA to exploit phenotypic differences for unambiguous species identity. Fungal bloodstream infections are a significant nosocomial infection in U.S. with an attributable mortality rate of up to 40%. Early diagnosis to direct appropriate therapy has been shown to be critical to reduction of mortality rates. Conventional phenotypic methods for fungal detection take several days, which in often too late to impact outcomes. This novel probe-based assay could detect and differentiate seven major pathogenic Candida species (C. albicans, C. glabrata, C. parapsilosis, C. tropicalis, C. lusitaniae, and C. auris) simultaneously to provide clinicians with species-level information that is critical for proper treatments with different anti-fungal drugs in less than 80 minutes with the limits of detection at 2.5×10^3 CFU/mL, or as low as 100 CFU per assay. We have verified the described assay with 67 clinical samples (including mixed multiple-species infections) with 100% agreement compared with the mass spectrometry based reference results.

**INTRODUCTION**

Candida is one of the major nosocomial bloodstream infections in U.S. and the worldwide as well. The main causative agent for this infection is the fungal Candida species and the seven most prevalent species are C. albicans, C. glabrata, C. parapsilosis, C. tropicalis, C. lusitaniae and C. auris, which account for more than 90% of the candidiasis cases globally. This infection is opportunistic and could be life-threatening for those immunocompromised and critically ill patients, such as those with cancer, organ transplant, chemotherapy, broad-spectrum antibiotics treatment, and as those with organ transplant and other undergoing large surgeries, particularly the use of central venous and arterial catheters.

In addition to those major Candida species, C. auris is a newly emerged pathogenic species, which is multiresistant and has been associated with recent outbreaks across the world. Further investigation has been recently performed to those isolated C. auris strains belonging to different clades by whole-genome sequencing and epidemiological analysis, and have emerged simultaneously on three continents. The level of resistance and high mortality associated with this Candida species, the U.S. government agent CDC (Center for Disease Control and Prevention) has recently issued a clinical alert to U.S. healthcare facilities. Furthermore, all the non-ribosomal based array platforms currently available on the market for C. auris identification either would misidentify C. auris or other species in the 10% of cases or detect all at 4%. To fulfill healthcare associated outbreaks, accurate molecular identification assay for C. auris is also urgently needed.

Here, we report the development of a rapid and cost-efficient molecular assay based on our intellectual chip-array and novel AMPED amplification technologies targeting ribosomal RNA for detecting and differentiating C. auris and other major clinically relevant Candida species simultaneously with high sensitivity and specificity to provide a rapid and accurate approach for clinicians to identify Candida bloodstream infection with valuable species-level information.