Friday – 468
June 2, 2017

Rapid Detection and Differentiation of the Recently Emerged Multidrug Resistant *Candida auris* Species and Other Major Clinically Relevant *Candida* Species Simultaneously and Accurately from Blood Cultures Using A Novel Amplification Process

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ABSTRACT

We herein describe a cost-effective multiplex assay platform for rapid detection and differentiation of major clinically relevant *Candida* specie 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 phylogenetic differences for unambiguous species identity. Fungal blood-stream 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 is 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. krusei, C. lusitaniae* and *C. guilliermondii*) 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 1-10 x 10^3 CFU/mL or as few as 100 CFU per assay. We have verified the described assay with 67 clinical samples (including mixed multiple-species infections as well) with 100% agreement compared with the mass spectrometry based reference results.

INTRODUCTION

Candidemia 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. krusei, C. lusitaniae* and *C. guilliermondii*, which account for more than 95% of the candidemia cases globally. This infection is opportunistic and could be life-threatening for those immunocompromised and critically ill patients, such as those with cancers, ongoing chemotherapy, broad-spectrum antibiotics treatment, and as well as those with organ transplant and other undergoing large surgeries, particularlly 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 multidrug resistant and has been associated with recent outbreaks across the world. More recently, it has been confirmed that these isolated *C. auris* strains belong to different clades by whole-genome sequencing and epidemiological analysis, and have emerged simultaneously on these continents. Due to the high mortality rate and multidrug-resistance associated with this *Candida* species, the U.S. government agent CDC (Centers for Disease Control and Prevention) has recently issued a clinical alert to U.S. healthcare facilities. Furthermore, all the non-molecule based assay platforms currently available on the market for *C. auris* identification either would misidentify *C. auris* as other species or cannot identify it at all. To prevent healthcare associated outbreaks, an 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.

Figure 1: AMPED Amplification.

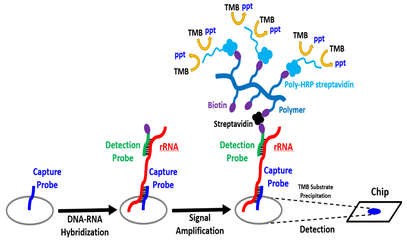
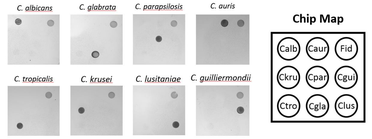


Figure 2: Reactivity Panel of AMPED *Candida* ID



Chip Map (Calb: *C. albicans*, Ckru: *C. krusei*, Cpar: *C. parapsilosis*, Ctro: *C. tropicalis*; Cgui: *C. guilliermondii*,; Cgla: *C. glabrata*; Clus: *C. lusitaniae*; Fid: fiducial).

Figure 3 : LOD of *C. auris*

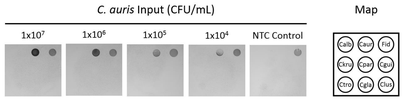


Figure 4: Sequence Alignment of *C. auris* Probe Region with Other Fungal Species

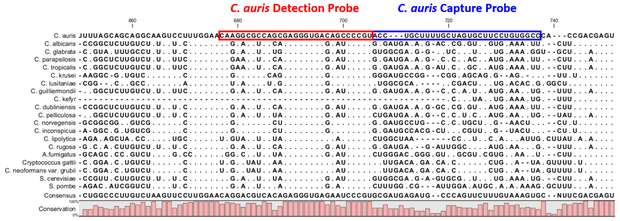


Table 1: LOD of Target *Candida* Species.

Species	Strain #	Limit of Detection
C. albicans	ARUP# 1	1.0 x 10^3 CFU/mL
C. glabrata	ATCC# 2001	5.0 x 10^4 CFU/mL
C. tropicalis	ATCC# 750	5.0 x 10^3 CFU/mL
C. parapsilosis	ATCC# 22019	1.0 x 10^4 CFU/mL
C. krusei	ATCC# 24210	1.0 x 10^3 CFU/mL
C. guilliermondii	ARUP# 2	1.0 x 10^4CFU/mL
C. lusitaniae	ATCC# 60247	1.0 x 10^4CFU/mL
C. auris	CDC# 0832	1.0 x 10^4CFU/mL

Table 3: Specificity Panel

Species	Strain source	Input titer (CFU/mL)	Reactivity
C. dubliniensis	ATCC #: MYA-646	~ 1.0E+6	None
C. utilis	ATCC#: 9905	1.81E+07	None
C. norgegensis	ATCC#: 96301	1.61E+06	None
C. viswanathii	ATCC#: 28269	1.87E+07	None
C. kefyr	Microbiol 2512	2.75E+05	None
C. haemulonii	ATCC#: 22991	4.60E+06	None
C. metapsilosis	ATCC#: 14054	8.65E+06	*C. parapsilosis* probe
C. orthopsilosis	ATCC#: 20503	1.35E+07	*C. parapsilosis* probe
C. rugosa	ATCC#: 20263	3.30E+06	None
C. duobushaemulonii	CDC# 0391	~ 1.0E+7	None
Saccharomuces cerevisiae	ATCC#: MYA-796	~ 1.0E+6	None
Schizosaccharomyces pombe	ATCC#: 10667	~ 1.0E+6	None
Fusarium proliferatum	ATCC# 201904	1.46E+06	None
***Staphylococcus aureus* (MRSA)**	ATCC# 700699	3.70E+07	None
Staphylococcus epidermidis	ATCC#: 700576	4.70E+07	None
Entericoccus facecalis	ATCC#: 29212	7.80E+07	None
Acinetobacter baumannii	ATCC #19606	6.15E+07	None
Bacterorides fragilis	ATCC#: 23745	5.00E+06	None
Enterobactger cloacae	ATCC #13047	1.86E+09	None
Klebsiella oxytoca	ATCC #49134	1.71E+09	None
Klebsiella pneumoniae	ATCC #13883	8.95E+07	None
Morganella morganii	ATCC#: 25829	4.30E+07	None
Serratia mcarcescens	ATCC#: 13880	5.00E+06	None

Figure 5: Sequence Alignment of *C. auris* Probe Region with Closely Related *C. haemulonii* Complex Strains.

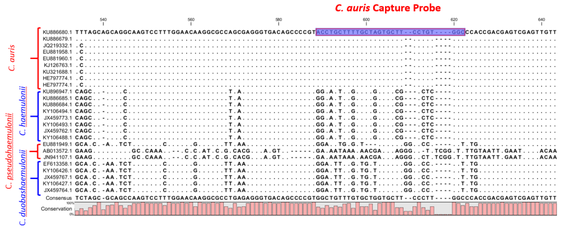


Table 2: AMPED ID of *C. auris.*

CDC Isolate #	MALDI Result	AMPED Result	AMPED LoD (CFU/mL)	Other Methods
0381	*C. auris*	*C. auris*	1.56x 10^4	Often misidentified as: *R. glutinis* *C. harmulonii* *C. catenulata* *C. parapsilosis* *C. famata* *C. lusitaniae* *C. guilliermondii* By API 20C AUX, BD Phoenix, Vitek-2 and MicroScan
0382	*C. auris*	*C. auris*	2.50x 10^4	
0383	*C. auris*	*C. auris*	1.18x 10^4	
0384	*C. auris*	*C. auris*	≤4.57x 10^4	
0385	*C. auris*	*C. auris*	1.36x 10^4	
0386	*C. auris*	*C. auris*	2.60x 10^4	
0387	*C. auris*	*C. auris*	≤5.30x 10^4	
0388	*C. auris*	*C. auris*	2.78x 10^4	
0389	*C. auris*	*C. auris*	≤3.11x 10^4	
0390	*C. auris*	*C. auris*	1.01x 10^4	
0391	*C. haemulonii*	*Negative*	N/A	N/A
0393	*C. duobushaemulonii*	*Negative*	N/A	N/A

Table 4: Clinical Specimen Data Summary.

Reference ID Species (MALDI-TOF MS)	# Positive of MALDI ID	# Positive of AMPED ID	Note
Candida albicans	22	22/22	
Candida glabrata	25	25/25	
Candida parapsilosis	7	7/7	
Candida tropicalis	3	3/3	
Candida krusei	3	3/3	
Candida lusitaniae	0	0/0	
Candida guilliermondii	0	0/0	
Candida auris	0	0/0	
Mixed Infection			
(C. abicans & C. dubliniensis)	1	1/1	*C. dubliniensis* is not detected in this study as it is not a target species.
(C. abicans & C. glabrata)	1	1/1	Both *C. albicans* and *C. glabrata* detected.
Non - *Candida* infection			
Histoplasma capsulatum	1	Negative	
Malassezia pachydermatis	2	Negative	
Rhodotorula species	1	Negative	
No yeast growth. Gram-positive rods resembling Bacillus	1	Negative	

RESULTS AND CONCLUSIONS

We have developed a rapid and cost-effective multiplex platform for unambiguous identification and differentiation of seven major pathogenic *Candida* species and the newly emerged multidrug resistant *C. auris* directly from positive blood culture bottles with high sensitivity and specificity to provide specie-level information for clinicians. In a retrospective comparison for detection of *Candida* species in frozen blood culture aliquots, this novel AMPED ID data is in 100% agreement with the mass spectrometry based MALDI reference ID method. Particularlly for *C. auris*, which is often misdiagnosed as other species using the current ID methods on the market, we could definitively identify all the CDC isolates from geographically different clades with limits of detection below 1x10^5 CFU/mL.

ACKNOWLEDGEMENTS

We thank CDC (Dr. Brendan Jackson, AR Bank) for the *C. auris* panel strain isolates.