Accurate PCR-based identification of Candida auris infections using oligonucleotides based on auris-specific GPI-protein encoding genes

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Objectives:
Candida auris is an emerging pathogen that is increasingly being identified as cause of life-threatening systemic infections in human societies around the world. Due to its low susceptibility to antifungal compounds, mortality rates of *C. auris* are high and, therefore, rapid and accurate diagnosis is crucial to successfully cure infected patients and to control outbreaks of *C. auris*. With the current methodologies available in most hospitals, achieving accurate species-level diagnosis of *C. auris* infections is complicated and has led to misidentifications with direct consequences for treatment and curing prospective. The aim of our study was to provide a new and easily accessible diagnostic tool for rapid and reliable identification of *Candida auris* infections.

Methods:
By *in silico* predicting GPI proteins in *C. auris* on a genome-wide scale, and subsequently designing oligonucleotides for *auris*-specific GPI protein-encoding genes and then applying these in a rapid colony PCR method, we intent to develop a new diagnostic tool for identification of *C. auris* infections

Results:
We show that *C. auris* isolates are easily and reliably identified by our PCR method. Robustness of the method was verified by blind testing of a library of 142 clinical isolates including 113 *C. auris* isolates (55 blood isolates and 58 isolates from other body sites) from a Spanish hospital (La Fe, Valencia), 27 *C. auris* isolates derived from hospitals in various countries (Spain, India, Colombia, Brazil, Japan, and Korea), and two non-*auris* isolates as negative controls. Specificity of the primers for *C. auris* was also validated using a set of representative *Candida* species including the clinically most important (e.g. *C. albicans*, *C. parapsilosis* and *C. glabrata*) and the phylogenetically more related *C. lusitanae* and *C. haemulonii*. Moreover, we show that isolates that were not or not reliably identified by MALDI-TOF MS identification are correctly identified by our PCR approach.

Conclusion:
We propose that our PCR method based on unique GPI protein-encoding genes may be useful to achieve easy, timely and accurate diagnosis of *C. auris* infections, in particular for hospitals and countries where healthcare budgets do not allow acquisition of expensive MALDI-TOF, qPCR or sequencing technology.

Colony PCR screening of clinical isolates using primers of unique *C. auris*

GPI protein-encoding genes				
CT	Origin	QG37_03410	QG37_05701	MALDI-TOF MS
		positive	positive	identification
S	B	55/55	55/55	44/55
	U	15/15	15/15	12/15
	RE	4/4	4/4	2/4
	PE	13/13	13/13	12/13
	WL	1/1	1/1	1/1
	OE	12/12	12/12	9/12
	BA	1/1	1/1	0/1
	L			
	VE	1/1	1/1	1/1
	AS	6/6	6/6	5/6
	NS	2/2	2/2	2/2
	CT	2/2	2/2	2/2
	Total	112/112	112/112	90/112
C	B	12/12	11/12	nd
	U	1/1	1/1	nd
	S	1/1	0/1	nd
	WL	1/1	1/1	nd
	Total	15/15	13/15	nd
I	B	2/2	2/2	nd
	UK	6/6	6/6	nd
	Tot al	8/8	8/8	nd
B	B	2/2	2/2	nd
J	E	1/1	1/1	nd
K	E	1/1	1/1	nd

CT=Country;S=Spain; C=Colombia; I=India; B=Brazil; J=Japan; K=Korea
B=Blood;U=Urine;RE=Rectal Exudate;PE=Purulent Exudate;WL=Wound Liquid; OE= Oropharyngeal Exudate; BAL= Brocoalveolate Lavage; VE= Valvular Exudate; AS=Axillary Swab; NS=Nose Swab; CT= Catheter Tip; S=Secrete; UK= Unknown; E=Ear.

Figure 1. Specific PCR amplification of *C. auris* DNA using unique GPI protein-encoding genes. (A) Colony PCR verification of the specificity of QG37_05701 (upper panel) and QG37_03410 (middle panel) primers using a set of different *Candida* species and *S. cerevisiae*. Template DNA quality control was provided using generic *Candida*/*Saccharomyces* 5.8S rDNA primers (bottom panel). (B) Testing the specificity of QG37_05701 primers on isolated genomic DNA using a mix of QG37_05701 and 5.8S rDNA primers.

Figure 2. PCR identification of clinical *C. auris* isolates that were collected in a Spanish hospital using QG37_03410 and QG37_05701 primers in a single PCR. (A) Bloodstream isolates. (B) Isolates from other clinical samples. *C. haemulonii* was used as negative control.