



Utility of CHROMagar™ Candida Plus for presumptive identification of *Candida auris* from surveillance samples

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Abstract *Candida auris* is a nosocomial fungal pathogen of prime importance due to its global emergence and rapid spread in healthcare facilities worldwide. One important concern is that routine, conventional methods fail to identify *C. auris*. While molecular and protein-based assays accurately detect/identify *C. auris*, these methods are time-consuming, expensive, and require expertise. Therefore, the objective of the present study was to assess the potential use of a novel chromogenic medium, CHROMagar™ Candida Plus, as an economical alternative to expensive and laborious diagnostic tests. We compared CHROMagar™ Candida Plus with the standard enrichment (salt Sabouraud Dulcitol broth) medium to test the recovery efficiency of *C. auris* from surveillance samples. We also tested CHROMagar™ Candida Plus for its ability to distinguish *C. auris* from other yeast species. One hundred surveillance samples

were cultured on CHROMagar™ Candida Plus and Dulcitol broth and incubated at 37 °C and 40 °C, respectively. Additionally, 32 *Candida* and yeast species were cultured on CHROMagar™ Candida Plus at 37 °C for three days to rule out any close resemblance to *C. auris*. Of 100 surveillance samples tested, 69 yielded presumptive positive *C. auris* exhibiting creamy pink colonies with a blue halo on CHROMagar™ Candida Plus within three days of incubation, and MALDI-TOF MS confirmed all by day 4. On the other hand, 69 of 100 surveillance samples yielded turbidity in Dulcitol broth by days 3–14 with final MALDI identification by days 5 to 17. Both media failed to identify one sample each, resulting in assay sensitivity and specificity of 99% and 97%, respectively. Of *Candida* and yeast species tested, 75–80% of *C. metapsilosis* and *C. orthospilosis* were misidentified as *C. auris*. However, previous studies indicated that these species are rarely detected in surveillance screening of *C. auris*. *Naganishia diffluens* also resembled *C. auris*, although it required different temperature growth (30 °C). In conclusion, CHROMagar™ Candida Plus provides rapid presumptive identification of *C. auris*. It would be another valuable tool in surveillance efforts to control the spread of *C. auris* in healthcare.

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Introduction

Candida auris is the newly discovered member of the *Candida/Clavispora* clade that presents a serious global health threat. Even though *C. auris* was first identified from ear discharge of a patient in Japan in 2009, a retrospective study revealed that its earliest occurrence dated back to 1996 in South Korea, where it was misidentified as *C. haemulonii* [1, 2]. Whole genome sequencing has clustered *C. auris* isolates into four clades corresponding to the regions of origin and spread. These clades are South Asia (Clade I), East Asia (Clade II), Africa (Clade III) and South America (Clade IV) [3]. Recently, a fifth clade of *C. auris* was discovered in Iran, separated by > 200,000 SNPs from the other four clades [4, 5]. Over the past decade, several healthcare-associated outbreaks of *C. auris* have been a major concern [6–10]. In 2016, the Centers for Disease Control and Prevention (CDC) issued a global alert for *C. auris*, recommending surveillance and infection control protocols. After worldwide alerts from different organizations, *C. auris* cases have been tracked in all continents except Antarctica [11]. *C. auris* is troublesome in clinical settings due to high transmission rates and multi-drug resistance [3, 9, 10, 12]. Prolonged colonization on the skin and indoor surfaces and tolerance to healthcare disinfectants further increases the risks of *C. auris* hospital outbreaks [9, 12]. Colonization not only increases the risk of transmission but also elevates the risk of invasive infection especially in immunocompromised patients [13]. Invasive infections of *C. auris* often have high mortality rates ranging from 40 to 60% [13, 14]. Therefore, the early diagnosis of this “superbug” is crucial to reduce the rate of transmission as well as the progression to invasive infection. Many commercially available phenotype-based methods such as API ID 32 C system (version 4.0 database, Biomerieux), BD Phoenix (BD Diagnostics, Sparks, MD, USA) and RapID Yeast Plus (Remel, Thermo Fisher Scientific, Lenexa, KS, USA) misidentify *C. auris* [10, 12, 15]. Molecular methods like *C. auris* real-time PCR, Sanger sequencing of the Internal Transcribed Spacer (ITS) and D1/D2 regions of the ribosomal gene, protein-based technologies like matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) can identify *C. auris* accurately [12, 15–17]. Although these technologies provide reliable detection of *C.*

auris, the instruments and reagents, along with the maintenance, are expensive for low-complexity laboratories with limited resources. Therefore, low-complexity laboratories depend on culture-based methods such as selective or differential media for the identification of *C. auris* [15]. Welsh et al. have established salt Sabouraud Dulcitol broth as a selective medium for recovery of *C. auris* from clinical and environmental samples [18]. Many high-complexity laboratories, including regional, and state public health laboratories in the USA, have successfully utilized salt Sabouraud Dulcitol broth for recovery of *C. auris* from surveillance samples. However, we observed that this culture method requires two weeks incubation before calling it negative [17]. Chromogenic isolation media provide better detection rates of pathogenic *Candida* spp. in mixed cultures than traditional media due to the production of species-specific color based on enzymatic reactions [19, 20]. However, currently used commercial chromogenic media were designed to target major *Candida* pathogenic spp. prior to *C. auris* outbreaks, and these media do not clearly distinguish *C. auris* from all other *Candida* spp. [21–23]. In this study, we compared CHROMagar™ *Candida* Plus with the salt Sabouraud Dulcitol broth for presumptive identification of *C. auris* from surveillance samples collected from patients from healthcare facilities in northeast region of the United States. We also evaluated the potential of the CHROMagar™ *Candida* Plus (CHROMagar, Paris, France) for presumptive identification of *C. auris* from other *Candida* and yeast species.

Materials and Methods

A total of 100 surveillance samples were collected as swabs from a single colonization site (axilla, groin, or nares) or as a composite swab (axilla/groin or nares/axilla/groin) from patients in health care facilities from the northeast region of the United States [16]. These swabs were submitted to the Wadsworth Center Mycology Laboratory (WCML) within 24 h of sample collection in the BD ESwab Liquid Amies Collection and Transport System (Becton, Dickinson, Franklin Lakes, NJ, USA) at ambient temperature. A total of 32 *Candida* and other yeast species procured from the Wadsworth Center’s Mycology Culture Collection Repository were also part of this investigation. For *C.*

auris recovery from surveillance samples, ESwab containing 1 ml liquid Amies medium were vortexed for 30 s, and 200 µl of the ESwab liquid was inoculated in salt Sabouraud Dulcitol broth (henceforth, Dulcitol broth) and incubated at 40 °C for 2 weeks in a shaking incubator at 150 RPM [18]. CHROMagar™ Candida Plus plates were prepared from the dry powder as per the manufacturer's instructions and stored at 4 °C in the dark with a shelf life of 4 weeks. Approximately 10 µl and 50 µl of surveillance samples were also inoculated on CHROMagar™ Candida Plus medium using spread (Fig. 1A) and streak (Fig. 1B) methods, and plates were incubated at 37 °C for 24 to 72 h. The choice of two volumes of surveillance samples was to ensure isolation of individual colonies of presumptive *C. auris* on CHROMagar™ Candida Plus. Colonies recovered from CHROMagar™ Candida Plus and Dulcitol broth were streaked on Sabouraud dextrose agar, followed by MALDI-TOF MS (Bruker, Bremen, Germany) identification. Since colony color is a critical factor for presumptive identification of *C. auris* on CHROMagar™ Candida Plus, other *Candida* (21) and pathogenic yeast (11) spp. were simultaneously streaked on this medium and incubated at 37 °C for 24–72 h. Colony images were taken from the front and back of the plate using Canon EOS 80D Camera

through Ortery Photosimile software and processed using Photoshop 22.4.2.

Results and Discussion

Of the 100 surveillance swabs tested, 69 yielded positive colonies at 72 h with a creamy pink color with a blue halo on CHROMagar™ Candida Plus, indicating the presence of *C. auris*. According to the manufacturer's instruction manual, *C. auris* yields a light blue color colony with a blue halo on CHROMagar™ Candida Plus at 48 h [24]. However, we observed that an additional day of incubation allowed the development of creamy pink color along with the blue halo, thus helping the distinction of *C. auris* from other *Candida* spp. studied in this investigation. All suspected colonies were confirmed to be *C. auris* by MALDI-TOF MS, providing 100% agreement with the phenotypic results. These results also suggested that the unique creamy pink color with a blue halo produced by *C. auris* on CHROMagar™ Candida Plus can be utilized as presumptive identification of *C. auris* from surveillance samples. The spread and streak plate methods were both helpful in the isolation of single colonies of *C. auris* from surveillance samples with a moderate number of *C. auris*. The streak plate method was useful for the isolation of

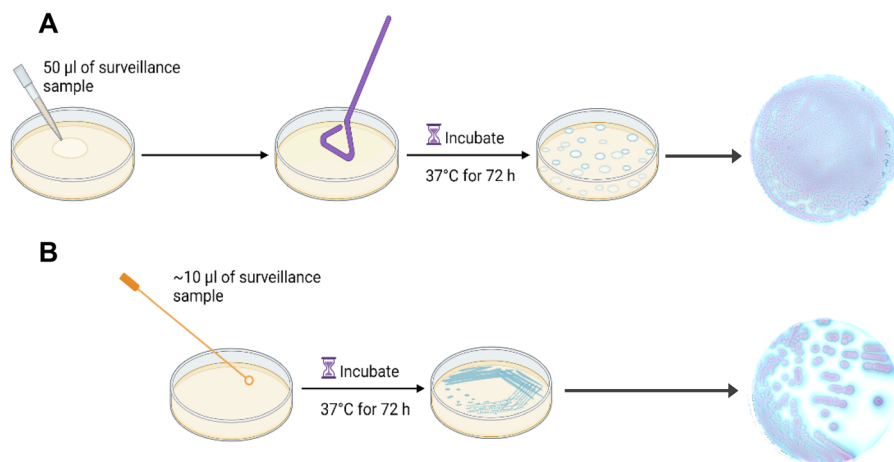


Fig. 1 Workflow of surveillance sample testing using CHROMagar™ Candida Plus medium. **A** 50 µl of surveillance sample was inoculated on a CHROMagar™ Candida Plus plate and spread evenly using a disposable spreader. **B** 10 µl of surveillance sample was also inoculated on another CHROMagar™ Candida Plus plate and streaked with a 10 µl yellow

plastic loop to obtain single colonies. Plates were incubated at 37 °C for 72 h and then imaged using a Canon EOS 80D Camera through Ortery Photosimile software and processed using Photoshop 22.4.2. Workflow template was created with BioRender.com

Table 1 Method comparison for *C. auris* identification (ID) using CHROMagar™ Candida Plus and Dulcitol broth.

	Positive ID on Dulcitol Broth	Negative ID on Dulcitol Broth
Positive ID on CHROMagar™ Candida Plus	a 69	b 1
Negative ID on CHROMagar™ Candida Plus	c 1	d 29

a = true positives

b = false positives

c = false negatives

d = true negatives

Sensitivity of CHROMagar™ Candida Plus = $a/a + c = 99\%$

Specificity of CHROMagar™ Candida Plus = $d/d + b = 97\%$

PPV (Positive Predictive Value) = $a/a + b = 99\%$

NPV (Negative Predictive Value) = $d/c + d = 97\%$

single colony from surveillance samples with high load (> 1000 CFU/50 μ l) of *C. auris*, while the spread plate method was useful for the isolation of a single colony from surveillance samples with a low load (< 10 CFU/50 μ l) of *C. auris*. A combination of spread and streak plate methods yielded 69 of 100 surveillance samples positive for *C. auris* on CHROMagar™ Candida Plus, which were also positive in Dulcitol broth. Both media failed to identify one sample each resulting in assay sensitivity of 99% and specificity of 97% (Table 1).

Next, we compared the time taken to presumptively identify *C. auris* on CHROMagar™ Candida Plus to that of Dulcitol broth. CHROMagar™ Candida Plus yielded results in 3 days for all positive surveillance samples irrespective of the presence of a high (> 1000 CFU/ 50 μ l) or low (< 10 CFU/ 50 μ l) load of *C. auris*. In contrast, it took anywhere from 3 to 14 days for the same surveillance samples to become turbid in Dulcitol broth, with timing dictated by how high or low a load of *C. auris* was present in each surveillance sample. All presumptive positive *C. auris* on CHROMagar™ Candida Plus were confirmed by MALDI-TOF MS by day 4, while the final MALDI-TOF MS identification of turbid surveillance samples from Dulcitol broth ranged from 5 to 17 days (Fig. 2).

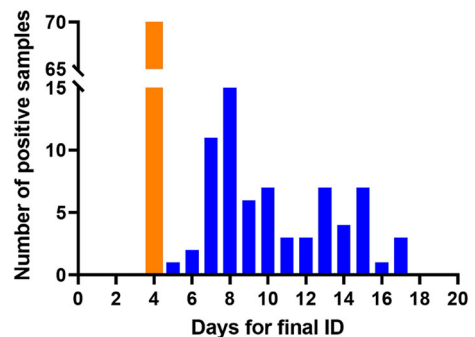


Fig. 2 Comparison of days required for final identification of *C. auris* using CHROMagar™ Candida Plus and Dulcitol broth. Presumptive identification on CHROMagar™ Candida Plus for all *C. auris* positive surveillance samples took 3 days followed by final identification by MALDI-TOF MS on the fourth day (orange bar). Final identification with Dulcitol broth and MALDI-TOF MS had a broad range from day 5 to day 17 (blue bars)

Dulcitol broth relies on the enrichment of *C. auris* at higher temperatures (40 °C), higher salinity (10%), and dulcitol as a carbon source [18]. Additionally, other yeasts are not grown but are viable in Dulcitol broth, hence confirmation by MALDI-TOF MS requires an additional step of streaking 10 μ l of turbid broth onto Sabouraud dextrose agar and incubation at 40 °C for at least 48 h for the isolation of single colonies followed by streaking those single colonies

Table 2 Colony images of *Candida* and other yeast spp. at 72 h on CHROMagar™ Candida Plus

Groups	Yeast ID	CHROMagar™ Candida Plus images at 72 h		
		Front	Back	
C. auris Clades	<i>Candida auris</i> Clade I (South Asia)			
	<i>Candida auris</i> Clade II (East Asia)			
	<i>Candida auris</i> Clade III (Africa)			
	<i>Candida auris</i> Clade IV (South America)			
Closely related to C. auris	<i>Candida duobushaemulonii</i>			
	<i>Candida haemulonii</i>			
	<i>Candida lusitanae</i>			
Prevalent Candida spp.	<i>Candida albicans</i>			
	<i>Candida glabrata</i>			
	<i>Candida tropicalis</i>			
Rare Candida spp.	<i>Candida blankii</i>			
	<i>Candida bracarensis</i>			
	<i>Candida catenulata</i>			
	<i>Candida diddensiae</i> isolate 1			
Groups	Yeast ID	CHROMagar™ Candida Plus images at 72 h		
		Front Back		
		<i>Candida diddensiae</i> isolate 2		
		<i>Candida diddensiae</i> isolate 3		
		<i>Candida diddensiae</i> isolate 4		
		<i>Candida diddensiae</i> isolate 5		
		<i>Candida diddensiae</i> isolate 6		
		<i>Candida dubliniensis</i>		
		<i>Candida famata</i>		
		<i>Candida fermentati</i>		
		<i>Candida guilliermondii</i>		
		<i>Candida kefyr</i>		
		<i>Candida krusei</i>		
		<i>Candida nivariensis</i>		
		<i>Candida viswanathii</i>		
Other rare yeast	<i>Cryptococcus diffluens</i>			
	<i>Cryptococcus gatti</i>			
	<i>Cryptococcus neoformans</i> var. <i>grubii</i>			

Table 2 continued

Groups	Yeast ID	CHROMagar™ Candida Plus images at 72 h	
		Front	Back
	<i>Cryptococcus neoformans</i> var. <i>neoformans</i>		
	<i>Hanseniaspora opuntiae</i>		
	<i>Kodamea ohmeri</i>		
	<i>Lodderomyces elongisporus</i>		
	<i>Pichia farinosa</i>		
	<i>Rhodotorula mucilaginosa</i>		
	<i>Saccharomyces cerevisiae</i>		
	<i>Trichosporon asahii</i>		

on Sabouraud dextrose agar at 30 °C for 24 h before MALDI-TOF MS identification. This additional step increases the processing time for Dulcitol broth cultures. On the other hand, CHROMagar™ Candida Plus not only allows the rapid growth of *Candida* and yeast spp. but can successfully distinguish *C. auris* from other yeasts based on color change within 3 days of incubation. Thus, CHROMagar™ Candida Plus would be a useful tool in the context of hospital outbreaks where faster detection would prove effective in curbing the spread. This is the first publication directly comparing Dulcitol broth, a selective medium for growing *C. auris*, against CHROMagar™ Candida Plus, a differential medium. Our results indicate that CHROMagar™ Candida Plus provides rapid presumptive identification of *C. auris* from surveillance samples. These results are encouraging and provide an additional culture-based method to identify this deadly fungal pathogen from surveillance samples.

Before advocating the use of CHROMagar™ Candida Plus for presumptive identification of *C. auris* from surveillance samples, we wanted to observe the pigmentation from different *Candida* and yeast spp. to rule out any close resemblance to *C. auris*. To achieve this objective, an additional 32 *Candida* and yeast species either recovered from surveillance samples [16], or identified as part of reference testing, were used (Table 2). The majority of *Candida* and yeast species were incubated at 37 °C, except for *Candida famata*, *Naganishia diffluens*, and

Table 3 Colony images of *Candida parapsilosis* species complex on CHROMagar™ Candida Plus

Yeast ID	Strain #	CHROMagar™ Candida Plus images at 72 h	
		Front	Back
<i>C. auris</i>	M5941		
<i>C. metapsilosis</i>	M17110		
	M17233		
	M17035		
	M17051		
	M17025		
<i>C. orthopsilosis</i>	19953-1		
	19953-2		
	M11062		
	M10713		
	M10468		
	M10023		
	M9719		
	M7335		
<i>C. parapsilosis</i>	26186		
	26309		
	M15752		
	M16250		
	26624		

Rhodotorula mucilaginosa, which were incubated at 30 °C. The poor growth of these species at temperatures above 30 °C has been observed previously [25]. *C. auris* colonies were creamy pink with a blue halo post 72 h incubation at 37 °C. Of the 32 comparator species tested, *Naganishia diffluens* most closely resembled *C. auris* (Table 2). However, its inability to grow at 37 °C precludes the probability of confusing one for another. Another important distinction was the texture of colonies; *Naganishia diffluens* colonies were mucoid, unlike the creamy colonies of *C. auris* (Table 2). Recently, while performing surveillance studies, we noted that *C. orthopsilosis* and *C. metapsilosis* resembled *C. auris* on CHROMagar™ Candida Plus. This led us to investigate an extensive panel of *C. parapsilosis* species complex (Table 3). Of the *C. parapsilosis* species complex tested, a high percentage (75–80%) of *C. metapsilosis* and *C. othropsilosis* indeed resembled *C. auris* in color and texture and would have easily been misidentified as *C. auris* (Table 3). However, since these species are rarely the cause of *Candida* infection and colonization [16], the similarity between these species on CHROMagar™ Candida Plus should not hinder the presumptive identification of *C. auris*. Recently, Borman et al. reported that *Candida diddensiae*, a rare yeast that resembled *C. auris* on CHROMagar™ Candida Plus. Therefore, we looked closely at all the *C. diddensiae* isolates available in Mycology Culture Collection Repository, but we found no resemblance to any of the *C. auris* clades tested (Table 2).

In conclusion, our findings indicate that CHROMagar™ Candida Plus is a valuable medium for presumptive identification of *C. auris* from yeast isolates recovered from clinical specimens, and it can also serve as a rapid surveillance screening tool for assessing *C. auris* colonization from patients in the healthcare setting. A CHROMagar™ Candida Plus medium, a valuable culture-based tool, can easily be adapted by both the low- and high-complexity laboratories for the presumptive identification of *C. auris*. The quick identification supported by this tool will boost the effort to curb the spread of *C. auris*, which is a critical and immediate need considering the significant rise of *C. auris* infection/colonization in combination with the emergence of COVID-19 [26–29].

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Author Contributions AM was responsible for surveillance sample testing, *Candida* spp. culture and imaging, data analysis, and draft preparation. YZ helped with *Candida* spp. culture and imaging, review, and editing. VC provided expertise with the study design and critically reviewed the manuscript. SC conceived and designed the study, edited, and critically reviewed the manuscript.

Declarations

Conflict of Interest The authors declare that they have no financial or non-financial conflicts of interest.

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