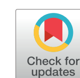
Diagnostic Microbiology and Infectious Disease 98 (2020) 115168

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Evaluation of a novel chromogenic medium for *Candida* spp. identification and comparison with CHROMagar™ Candida for the detection of *Candida auris* in surveillance samples



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ARTICLE INFO

Article history:
Received 29 June 2020
Received in revised form 2 August 2020
Accepted 3 August 2020
Available online xxxx

Keywords:
Candida auris
Yeasts
Outbreak
Multidrug resistant
Chromogenic

ABSTRACT

A shift to *Candida* non-*albicans* infections has been noted during the last years, and the emergence of multiresistant *Candida auris* has complicated their management. The aim of this study was first to compare the performance of the novel chromogenic medium CHROMagar™ Candida Plus (CHROMagar, France) with CHROMagar™ Candida (Becton Dickinson, Germany) for the presumptive identification of *Candida* species; and then, to evaluate its utility in the detection of *C. auris* in surveillance samples. CHROMagar™ Candida Plus showed a good performance compared with the reference medium CHROMagar™ Candida. Sensitivity and specificity were 100% in both media for tested species at 48 h of incubation, except for *Candida glabrata* and *Candida lusitaniae*. Furthermore, the new medium allows a reliable presumptive identification of *C. auris*, as a new specific color for this species is assigned (light blue with a blue halo), obtaining a sensitivity and specificity of 100% at 36 h of incubation.

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1. Introduction

An increase in the prevalence of *Candida* infections has been noted during the last years, becoming an important cause of morbidity and mortality in hospitalized patients. While *Candida albicans* has generally been considered the major pathogen causing candidemia, a shift to *Candida* non-albicans species has been observed, including multidrug resistant species, such as the emerging pathogen *Candida auris* (Enoch et al., 2017; van Schalkwyk et al., 2019).

C. auris was first isolated in 2006, from an auditory canal of a Japanese patient, but it was not identified until 2009, which indicates it could had been misidentified in previous patients (Jeffery-Smith et al., 2018). Since then, it has been isolated from several body sites in 5 continents. The first outbreaks in Europe occurred in 2014 and 2015, which prompted European Centre for Disease Prevention and Control (ECDC) to publish a rapid risk assessment alerting that there was a need for raising awareness in European healthcare facilities, for them to adapt their laboratory testing strategies and implement enhanced control measures early enough to prevent further hospital outbreaks (European Centre for Disease Prevention and Control, 2018). In June 2016, after 13 cases were identified in the United States between 2013 and 2016, Centers for Disease Control and Prevention (CDC) issued a clinical alert describing the global emergence of *C. auris*, and requesting to laboratories the report of *C. auris* cases and the shipment of patients' samples to state and local health departments and (CDC, n.d.-a). The reasons why *C. auris* is considered a growing threat to the global health are: a) the resistance to antifungals, which drastically limit therapeutic options, b) the ease of transmission among patients, especially in intensive care units, where it spreads causing outbreaks, and c) the difficulty of identification in the laboratory (Forsberg et al., 2018). Other organizations, such as the Public Health England (PHE) or the Infection Prevention and Control (IPC) group of International Society for Antimicrobial Chemotherapy (ISAC) have also published recommendations for the diagnosis, management of patients, prevention and control of this new pathogen (Kenters et al., 2019; Shetty et al., 2017). They agree in that there is urgency in using reliable methods to identify *Candida* spp. isolates to the species level, and they encourage hospitals to do screening colonization studies in units that have ongoing cases and in patients coming from other affected hospitals. In 2017, we reported a *C. auris* outbreak in the Intensive Care Unit which led us to adopt different

Abbreviations: CC, Chromagar™ Candida; CC-Plus, Chromagar™ Candida Plus.

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https://doi.org/10.1016/j.diagmicrobio.2020.115168
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control measures, including the active surveillance screening of all patients admitted to the ICU at time of admission and weekly in axillary-rectal and pharyngeal samples (Salvador García et al., 2020).

A large variety of methods for the identification of yeasts have been developed (Freydiere et al., 2001). However, traditional methods are cumbersome and beyond the expertise range available in local laboratories, while recent methods might not be available in resource-limited settings (Nadeem et al., 2010). Chromogenic isolation media are widely used, because they have demonstrated better detection rates of yeasts in mixed cultures than traditional media, and this often results in costs savings from reduced labor time, reagents or fewer confirmation tests (Perry, 2017), and this is especially useful in the context of a hospital outbreak. The aim of this study was first to compare the performance of the novel chromogenic medium CHROMagar™ Candida Plus (CHROMagar, France) with CHROMAgar™ Candida (Becton Dickinson, Germany) for the presumptive identification of *Candida* species; and then, to evaluate its utility in the detection of *C. auris* in surveillance samples.

2. Materials and methods

First, a total of 42 suspensions containing different strains and mixtures of strains of *Candida* spp. were inoculated in CHROMagar™ Candida (reference medium, CC) and CHROMagar™ Candida Plus (CC-Plus) media in order to evaluate their ability to differentiate among variable *Candida* species. Suspensions containing different *Candida* species were prepared and adjusted to 0.5 McFarland. After that, they were diluted to ¼ with physiological saline, and 1 and 10 μL of these suspensions were then inoculated in both media. Twelve suspensions containing 2 different species were prepared by mixing together 100 μL of the previous suspensions, and 1 and 10 μL were inoculated again in both media. The same procedure was followed to prepare 11 suspensions with 3 species, and 1 μL was inoculated. Plates were incubated at 37 °C and were read at 24, 36, and 48 h independently by 2 different qualified laboratory staff.

Secondly, the new medium was evaluated for its intended use in detecting *C. auris* colonization in patients in the context of an outbreak. A total of 23 swabs from surveillance studies (axillary-rectal and pharyngeal samples) submitted to the Microbiology Department of Consorcio Hospital General Universitario de Valencia, Spain, were selected. They were inoculated in CC agar plates following the usual workflow of our laboratory, and in CC-Plus agar plates in parallel. The plates were then incubated at 37 °C and were read at 24, 36 and 48 h independently by 2 different qualified laboratory staff.

The isolated *Candida* species and their expected color according to manufacturers are shown in Table 1. The studied growth characteristics were colony quantity (reaching first, second or third streak), colony size, and colony color. The colonies were presumptively identified by colony color, according to manufacturer's instruction, and the identification was always confirmed using Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF, Bruker-Daltonics, Bremen, Germany). Sensitivity and specificity were calculated for all isolates

Table 1
Color of the colonies on CHROMagar™ Candida Plus and CHROMagar™ Candida, according to manufacturers, at an incubation of 36 h for CHROMagar™ Candida Plus, and 48 h for CHROMagar™ Candida.

Species	Color at 36 h, 37 °C, CHROMagar™ Candida Plus	Color at 48 h, 37 °C, CHROMagar™ Candida
C. albicans	Turquoise blue/green	Green
C. krusei	Pink to purple with white edges	Pink, fuzzy
C. glabrata	Pink to purple	Mauve-brown
C. tropicalis	Metallic blue with pink halo	Metallic blue
C. auris	Light blue with blue halo	White to mauve
C.	*parapsilosis/orthopsilosis*	Light blue
White to mauve		
C. lusitaniae	Pink to purple	White to mauve

from strain suspensions and colonization swabs and, then, specifically for *C. auris* detection in the colonization swabs.

3. Results

A total of 95 isolates were obtained by culturing the colonization swabs and the strain suspensions. Examination of inoculated media with *Candida* spp. suspensions at 24 h showed that this was not sufficient time to correctly differentiate among species, given the small size of colonies and that most of them were colorless. However, *Candida lusitaniae* and *C. auris* seemed to grow better in the CC-Plus medium. At 36 h, color differentiation was possible for most species (colors were as described in Table 1). All *Candida* spp. colonies were generally larger in the CC-Plus medium at 36 h (see Fig. 1), and the greatest difference in size was found for *C. auris, C. lusitaniae* and *Candida krusei*. In the CC medium, at this incubation time, differentiation was difficult between mauve/pink species (*Candida glabrata*, mauve-brown; *Candida krusei*, pink), species without a specific color, growing white to pink (*Candida parapsilosis, Candida orthopsilosis, C. auris, C. lusitaniae)* and colorless species in an early stage of growth (the previously mentioned species plus *Candida tropicalis)*. In CC-Plus, although some species had a similar bluish color (*C. auris*, *C. albicans, C. parapsilosis/orthopsilosis or C. tropicalis*), differentiation was generally possible because they had different tonalities or halos with different colors (see Fig. 2, B and C), always at a minimum of 36 h. *C. tropicalis*, however, was difficult to identify in CC-Plus because not all colonies developed the metallic blue color described by the manufacturer and had a pinkish color similar to *C. glabrata*. *C. lusitaniae* also could be mistaken with *C. glabrata* in both media, being considered as a false positive for this specie.

At 48 h, both media allowed an optimal identification, although CC did not allow differentiating among species having an appearance described by manufacturer as white to mauve (*C. auris, C. parapsilosis, C. orthopsilosis* and *C. lusitaniae*). The difference in the size of the colonies was more significant at this incubation time (Fig. 1C). The degree of concordance between the 2 plate readers was complete for describing the appearance and color of the colonies. There was not any difference between the 2 different concentrations tested. Sensitivity and specificity for all the obtained isolates from strain suspensions and colonization swabs ($n = 95$) are shown in Table 2. The CC-Plus medium displayed good results, comparable to those from CC. CC-Plus also allowed the identification of *C. auris* and *C. parapsilosis/orthopsilosis.*

Respect to the study of *C. auris* colonization samples, 14 surveillance samples were confirmed to be positive for *C. auris*, 5 of which were mixed with at least another *Candida* species (*C. albicans, C. glabrata* or *C. parapsilosis)*. Moreover, one of the samples was positive for *C. auris, C. albicans* and *C. glabrata* (Fig. 2). Nine samples were negative for *C. auris*, 7 of which grew a different *Candida* species (*C. albicans, C. glabrata, C. parapsilosis, C. orthopsilosis, C. tropicalis* and *C. lusitaniae*), while the remaining 2 were totally negative. Sensitivity and specificity for the detection of *C. auris* in the surveillance samples were calculated (Table 3), obtaining excellent results at an incubation time of 36 and 48 h. By contrast, CC had a low specificity for *C. auris*, as expected, since this medium does not differentiate it from other species growing white to mauve. At 24 h, 5 of 14 samples carrying *C. auris* did not grow in reference medium, whereas they did grow in CC-Plus (although they were not colored enough to allow a correct identification). However, for 2 clinical samples, it was necessary to incubate both media at least 36 h to observe growth of *Candida* species including *C. auris.* In this case, colony quantity was similar and *C. auris* colonies appeared light blue with a blue halo on CC-Plus.

4. Discussion

Chromogenic media are widely used in the identification of yeasts, since they can give a presumptive identification of the species and a preliminary antifungal treatment can be administered to the patient

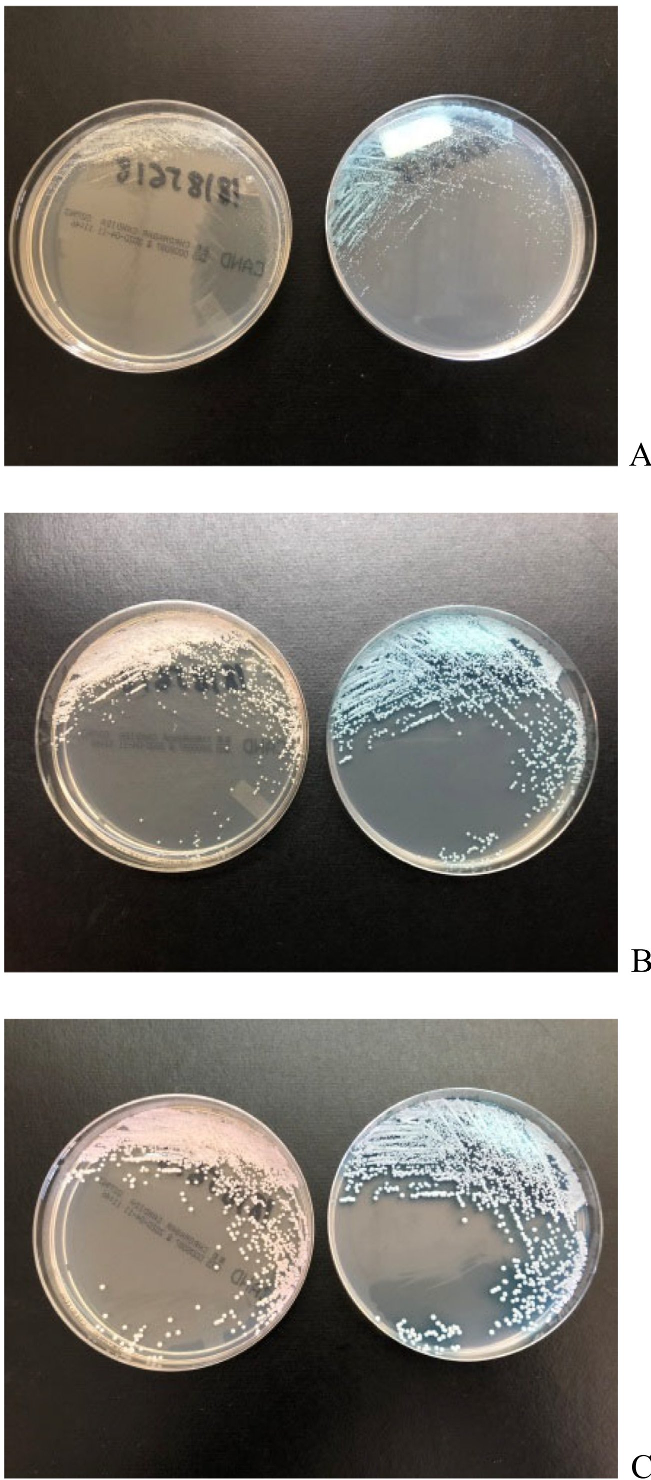

Fig. 1. *C. auris* culture in CHROMagar™ Candida medium (left, colonies white, beige or pink) and CHROMagar™ Candida Plus medium (right, color light blue with blue halo) at 24 (A), 36 (B) and 48 (C) h of incubation. Larger colonies in CHROMagar™ Candida Plus can be observed at 36 and 48 h.

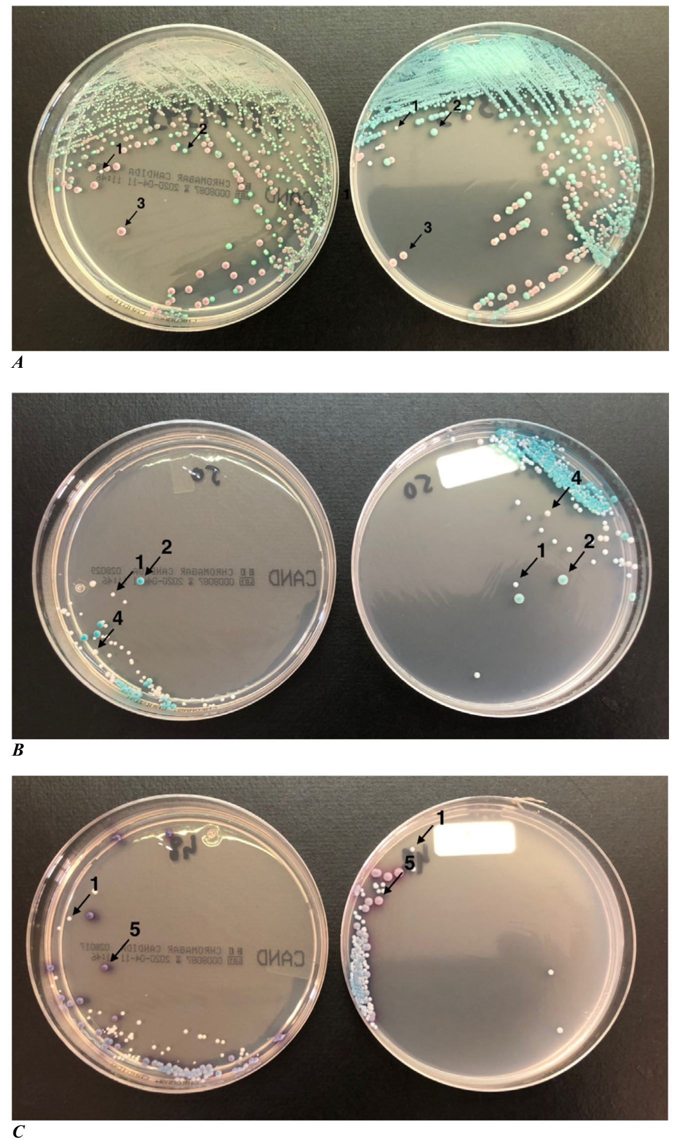

Fig. 2. A) Surveillance sample containing *C. auris* (1; CC: white to mauve; CC-Plus: light blue with a blue halo), *C. albicans* (2; CC: green; CC-Plus: turquoise blue), and *C. glabrata* (3; CC: mauve-brown; CC-Plus: pink to purple) in CC (left) and CC-Plus (right). **B)** Sample containing *C. auris* (1), *C. albicans* (2), and *C. parapsilosis* (4; CC: white to mauve; CC-Plus: light blue) in CC (left) and CC-Plus (right). **C)** Sample containing *C. auris* (1) and *C. tropicalis* (5; CC: metallic blue; CC-Plus: metallic blue with pink halo) in CC (left) and CC-Plus (right).

(Nadeem et al., 2010). The novel medium CHROMagar™ Candida Plus showed a good performance compared with the reference medium CHROMagar™ Candida, obtaining high sensitivity and specificity for the most commonly isolated *Candida* species, even in mixed cultures. Although some species could be presumptively identified at an incubation of 24 or 36 h, the best results in sensitivity and specificity were obtained at 48 h of incubation. Therefore, 48 h incubation is recommended for an optimal performance on all species, for both CC-Plus and CC. Lower results were obtained for the identification of *C. glabrata*, although similar limitations were observed for the reference medium. The main advantage of CC-Plus is that this medium is able to identify the emerging multi-resistant yeast *C. auris*.

In the reference medium, *C. auris* grows in colonies that may seem mauve, beige or pink, as other species do (e.g. *C. glabrata*, *C. parapsilosis*), or colorless at an early stage of its growth. Therefore, the lack of a specific color for *C. auris* species makes mandatory to confirm identification with additional methods such as MALDI-TOF, increasing the time to result. Furthermore, in some cases *C. auris* grows slowly in the reference medium, obliging to incubate the plates 48 h in order to give a result (in fact, colony appearances are described after a 48 h incubation in technical specifications of this medium). Otherwise, the CC-Plus medium could allow a reliable identification of *C. auris* in only 36 h, according to our results. This medium proved to be more specific for *C. auris* detection at all

Table 2
Comparison of sensitivity and specificity for *Candida* species isolated from strain suspensions and colonization swabs in CHROMagar™ Candida (reference medium) and CHROMagar™ Candida Plus.

Candida species	Nr. of tested strains	Incubation time	CHROMagar™ Candida		CHROMagar™ Candida Plus	
			Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
C. albicans	16	24 h	100	100	100	100
		36 h	100	100	100	100
		48 h	100	100	100	100
C. tropicalis	4	24 h	0	100	0	100
		36 h	25	100	25	100
		48 h	100	100	100	100
C. krusei	5	24 h	100	100	100	100
		36 h	100	100	100	100
		48 h	100	100	100	100
C. glabrata	15	24 h	60	90	73,3	93,8
		36 h	93,3	90,2	93,3	90
		48 h	100	86,9	100	90
C. auris[a]	37	24 h	-	-	59,5	89,7
		36 h	-	-	100	100
		48 h	-	-	100	100
C. lusitaniae[a]	8	24 h	-	-	50	81,6
		36 h	-	-	100	80,5
		48 h	-	-	100	82,8
C. parapsilosis/orthopsilosis[a]	10	24 h	-	-	30	70,6
		36 h	-	-	80	100
		48 h	-	-	100	100

[a] For these species, sensitivity and specificity have not been calculated in CC medium, because this medium does not differentiate them and the colonies are all described by manufacturer as white to mauve.

incubation periods (24, 36, and 48 h), as a new specific color for this species is assigned (light blue with a blue halo), although highest sensitivity and specificity results were obtained at 36 and 48 h of incubation.

A correct and rapid identification of *C. auris* in the laboratory is crucial to manage an outbreak caused by this pathogen. Identification was difficult until 2017 because most commercial biochemical identification systems could misidentify it with other species (Lockhart et al., 2017; Mizusawa et al., 2017; Snayd et al., 2018). It was also unidentified by MALDI-TOF if databases lacked of reference spectra (Mizusawa et al., 2017). Nowadays, some automated biochemical systems, such as VITEK 2 (bioMérieux, France), and MALDI-TOF systems, such as MALDI Biotyper (Bruker-Daltonics, Bremen, Germany) and VITEK MS (bioMérieux, France), can correctly identify *C. auris* in most cases. However, most recent software version of VITEK 2 (version 8.01) has been reported to incorrectly identify *C. auris* as *Candida duobushaemulonii*, especially for East Asian and African clades (Ambaraghassi et al., 2019). For MALDI-TOF systems, there can be also variations in the mass spectra among different clades (Kathuria et al., 2015), which might result in a lower resolution. Also, MALDI-TOF identification of yeasts is sometimes difficult and may need a previous extraction method. Sequencing of DNA loci (D1/D2 or ITS regions) can be used to identify and also differentiate between the phylo-geographic clades (Jeffery-Smith et al., 2018), although it is not routinely performed in clinical laboratories. Other available methods for rapid screening of C. auris include culture in selective salt/dulcitol agar and Real-Time PCR (CDC, n.d.-b). However, both MALDI-TOF systems and PCR assays may not be available in all clinical microbiology laboratories.

Table 3
Sensitivity and specificity for the 14 surveillance samples confirmed positive for *C. auris* (pharyngeal and axillary-rectal swabs).

	CHROMagar™ Candida			CHROMagar™ Candida Plus		
	24 h	36 h	48 h	24 h	36 h	48 h
Sensitivity	64,3	76,6	85,8	64,3	100	100
Specificity	44,4	55,6	33,3	77,8	100	100

Some limitations of this study are the relatively small sample size and that other species with which *C. auris* can be misidentified by biochemical identification systems were not tested, such as *C. haemulonii* and *C. duobushaemulonii*. For more information, a recent study compared the growing of *C. auris* in CC-Plus between clades and to other different species (Borman et al., 2020). By contrast, an advantage of this study is that clinical samples from patients suspected of being colonized with *C. auris* were used, from an established *C. auris* outbreak.

5. Conclusions

The novel chromogenic medium CHROMagar™ Candida Plus is therefore a valuable option for *C. auris* detection and identification in the context of a hospital outbreak for colonization studies, as well as for the identification of other yeasts. Although the sample size is relatively small, this novel medium might reduce the time-to-result and complementary tests for detecting *C. auris*. However, it must be taken into account that, as a chromogenic method, there might be a variation in the interpretation, requiring some expertise, so unclear identifications should be confirmed with complementary methods.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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