

Original Article

Evaluation of Chromogenic agar media for rapid identification of *Candida* species

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Abstract

Rapid identification of *Candida* isolates to the species level is essential in order to optimize the antifungal treatment. This study aimed to isolate and identify different species of *Candida* from various clinical specimens and to evaluate the use of chromogenic agar media as primary culture media for culture of *Candida* as well as for rapid identification of *Candida* species. A total of 100 different clinical specimens were studied (oral swab 35, high vaginal swab 28, catheterized urine 15, nail 14, bronchoalveolar lavage 04 and peritoneal fluid 04). Isolation of *Candida* species was done by primary culture in Sabouraud Dextrose Agar (SDA). Subsequent identification of species was performed by germ tube test, carbohydrate assimilation test (with commonly used twelve sugars) and subculture in chromogenic agar medium. Out of 64 isolated *Candida*, *C. albicans* 33 (51.53%) was the most predominant *Candida* species followed by *C. tropicalis* 17 (26.56%). The species of *C. glabrata* was 4 (6.25%), *C. parapsilosis* 4 (6.25%), *C. krusei* 3 (4.68%) and *C. guilliermondii* 2 (3.2%). One of the isolated *Candida* species was unidentified. The sensitivity and specificity of chromogenic agar media for *C. albicans* were as 96.97% and 96.87% respectively. The sensitivity and specificity for *C. tropicalis* were 94.12% and 97.87% respectively. *C. krusei* and *C. glabrata* both showed 100% sensitivity and specificity on chromogenic agar media. Efficacy of chromogenic agar media is nearly similar to carbohydrate assimilation method in species identification of *Candida*.

Key words: *Candida albicans*, Chromogenic agar media, Non-albicans *Candida* species.

Introduction:

Candidiasis, the main opportunistic fungal infection has steadily increased over the past few decades. Though *Candida albicans* is the most common cause of candidiasis, the frequency of isolation of non-albicans *Candida* species is increasing gradually¹. The non-albicans *Candida* species encountered are *C. dubliniensis*, *C. tropicalis*, *C. parapsilosis*, *C. glabrata*, *C. lusitaniae*, *C. guilliermondii*, *C. pseudotropicalis* (*C. kefyr*), *C. krusei*, *C. rugosa*, *C. famata*, *C. lipolytica* and *C. zeylanoides* etc². However, more than 90% of invasive infections are caused by *Candida albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis* and *C. krusei*². Non-albicans *Candida* species such as *C. glabrata*, *C. krusei* and *C. tropicalis* are emerging as important opportunistic

pathogens and this transition has had a significant clinical impact due to decreased susceptibility of these non-albicans yeasts to antifungal agents¹. *C. tropicalis* is less susceptible to fluconazole than *C. albicans*. *C. glabrata* is intrinsically more resistant to antifungal agents, particularly to fluconazole³. *C. krusei* is also resistant to fluconazole and infections caused by this species are strongly associated with prior fluconazole prophylaxis and neutropenia⁴. Accurate and rapid identification of *Candida* spp. is therefore crucial for clinical management and for facilitating hospital control measures⁵. There are several methods for identification of different species of *Candida*, such as culture on Sabouraud Dextrose Agar (SDA) media, germ tube test, culture on cornmeal agar, carbohydrate fermentation and carbohydrate assimilation test and culture on chromogenic agar media. In order to facilitate rapid identification, several chromogenic substrate containing culture media has been developed. Chromogenic agar is a selective and differential medium, with the inclusion of chromogenic substrates, allowing identification of yeast isolates by their color and colony characteristics to the species level within 48 hours⁶. These special media yield microbial

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colonies with varying colors secondary to chromogenic substrates that react with enzymes secreted by microorganism. Another potential advantage of chromogenic media is the straight forward identification of mixed yeast infections, which have a significant clinical bearing⁷. So, subculture on chromogenic media was done to evaluate its efficacy over carbohydrate assimilation test which is a gold standard for *Candida* species identification.

Methods:

This cross sectional study was carried out in the department of Microbiology and Immunology, Bangabandhu Sheikh Mujib Medical University (BSMMU) from May, 2013 to April, 2014 and was approved by Institutional review board (IRB) No. BSMMU/2013/7580, Date:16-06-2013. A total of 100 specimens were collected from in patients and out patients of different department of BSMMU, these patients were clinically suspected or diagnosed cases of candidiasis (oral swab 35, high vaginal swab 28, catheterized urine 15, nail 14, bronchoalveolar lavage 04 and peritoneal fluid 04).

All the clinical specimens were collected with proper clinical and laboratory procedures^{8,9}. The specimens were examined microscopically by 20% KOH and Gram staining, primarily cultured in Sabourad dextrose agar (SDA) media with supplement of chloramphenicol and gentamycin in screw capped test tube. The *Candida* species produced a creamy colored pasty round moist colonies having a distinct yeast smell. The budding yeast cells were seen by direct microscopy in wet mount preparation both from clinical specimens and culture. Identification of *Candida* species from growth in SDA media was done by germ tube test, carbohydrate assimilation test and subculture in chromogenic agar media. Germ tube test was performed to identify *Candida albicans* and to differentiate *Candida albicans* from non-*albicans* groups. Lightly touched single yeast colony with a sterile wire loop from the culture plate was inoculated in fresh human pooled serum and incubated at 37° C for 3 hours. In germ tube positive cases, the wet mount preparation showed germ tube which is a hyphal projection without constriction at the point of origin from the yeast cell (Fig.1).

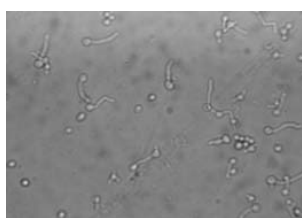


Fig. 1: Germ tube formation in human serum × 40

For carbohydrate assimilation test, 12 sugars are used which are listed in carbohydrate assimilation reaction profile in mycology laboratory manuals⁸. A lawn culture of *Candida*

isolate was made on yeast nitrogen base agar plate with suspension of *Candida* species in distilled water (equivalent to McFarland No.4 standard) and sugar disks dextrose, maltose, galactose, lactose, sucrose, mellibios, cellobios, trehalose, raffinose, xylose. Inositol and dulcitol were put and incubated at room temperature for 48 to 72 hours. Both commercially available sugar disks and laboratory prepared disks were used. Commercially available disks (Hi-Media, India) containing sugar at a concentration of 25mg/disk and the laboratory prepared sugar disks were made by soaking the sterile filter paper disks in 10% solution of each sugar¹⁰. Most of the isolates showed increased growth around the sugars they utilized within 48 hours except for few (dulcitol, melibiose and raffinose) which required incubation up to 5 to 7 days (Fig. 2). The result was noted and tabulated.

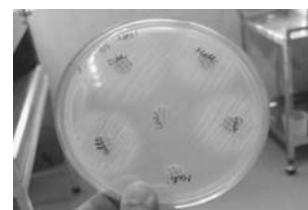


Fig.2. Carbohydrate assimilation by *Candida* spp. (Dextrose, mellibiose, sucrose & cellobiose)

Chromogenic agar medium was prepared according to the manufacturer's instructions (Hi-Media, India). Suspension of isolates was prepared in sterile distilled water (Turbidity was adjusted to 2 McFarland standard) from primary culture in SDA and was inoculated onto Chromogenic Agar medium. The plate was incubated at 37°C for 48 hours. Different color produced by different species of *Candida* are shown in Table 3. The sensitivity and specificity of chromogenic agar media considering carbohydrate assimilation test as gold standard^{8,11}, was calculated as below:

$$\text{Sensitivity} = \frac{(\text{true positive}) \times 100}{(\text{true positive} + \text{false negative}) \times 100}$$

$$\text{Specificity} = \frac{(\text{true negative}) \times 100}{(\text{true negative} + \text{false positive}) \times 100}$$

Result:

This cross sectional study was carried out to identify the species of *Candida* in clinically diagnosed or suspected 100 cases of candidiasis. Different *Candida* species were identified by germ tube test, carbohydrate assimilation test and sub-culture in chromogenic agar medium (Table.I). Considering carbohydrate assimilation test as gold standard^{8,11}, out of 64 isolated *Candida* most of the species were identified as *C. albicans* 33 (51.56%), *C. tropicalis* 17 (26.56%), *C. glabrata* 04 (6.25%), *C. parapsilosis* 04 (6.25%), *C. krusei* 03 (4.68%) and *C. guilliermondii* 02 (3.2%). One of the species was unidentified.

Table 1: Identification of different species of *Candida* by different methods (n = 64).

Species identified	Methods			
	Chromogenic agar media No (%)	Carbohydrate assimilation test No (%)	Germ tube test	
			Positive (%)	Negative (%)
<i>C. albicans</i>	32 (96.97)	33 (100)	33 (100)	
<i>C. tropicalis</i>	16 (94.12)	17 (100)	01 (5.88)	16 (94.11)
<i>C. Krusei</i>	03 (100)	03 (100)	03 (100)	
<i>C. glabrata</i>	04 (100)	03 (100)	04 (100)	
<i>C. parapsilosis</i>	Unidentified	04 (100)	04 (100)	
<i>C. guilliermondii</i>	Unidentified	02 (100)	02 (100)	
Unidentified spp.	01 unidentified	unidentified	01 (100)	

In chromogenic agar media, 32 (96.97%) species were identified as *C. albicans*, 16 (94.12%) were *C. tropicalis*. Number of identified species of *C. glabrata* were 4 (100%) and *C. krusei* were 3 (100%). But chromogenic agar couldn't give the conclusive differentiating color between the species of *C. parapsilosis* and *C. guilliermondii*, further identification was done by carbohydrate assimilation test. Thirty-three isolates of *C. albicans* were identified by germ tube test which were similar to carbohydrate assimilation test. Only 01 isolates of *C. tropicalis* has given positive result in germ tube test, but other identified *Candida* species were negative. Assimilation of different sugars by different species of *Candida* are shown in Table 2. One of the species of *Candida* remained unidentified by carbohydrate assimilation test.

Species	Total No.	Dextrose	Maltose	Sucrose	Lactose	Galactose	Melibiose	Cellobiose	Xylose	Raffinose	Trehalose	Dulcitol	Inositol
<i>C. albicans</i>	33	+	+	+	0	+	0	0	+	0	+	0	0
<i>C. tropicalis</i>	17	+	+	+	0	+	0	+	+	0	+	0	0
<i>C. parapsilosis</i>	04	+	+	+	0	+	0	0	+	0	+	0	0
<i>C. glabrata</i>	04	+	0	0	0	0	0	0	0	0	+	0	0
<i>C. guilliermondii</i>	02	+	+	+	0	+	+	+	+	+	+	+	0
Unidentified spp.	01	+	+	+	0	+	0	+	+	+	+	0	0
Total	64												

The color and colony characteristic of most of the *Candida* species in chromogenic agar media was similar as mentioned by the manufacturer (Table 3).

Table 3 : Color and colony characteristics of *Candida* species in chromogenic agar media (n = 64).

Species	Total No. of Isolates	Colony characteristics on chromogenic agar	Identification by *CHO assimilation test
<i>C. albicans</i>	33	Apple green colony, consistent	Identified as <i>C. albicans</i>
<i>C. tropicalis</i>	17	Dull blue to metallic blue colony with pale pink edges	Identified as <i>C. tropicalis</i>
<i>C. glabrata</i>	04	White large glossy to pale pink colony	Identified as <i>C. glabrata</i>
<i>C. parapsilosis</i>	04	Pink to pinkish purple colony	Identified as <i>C. parapsilosis</i>
<i>C. krusei</i>	03	Large, flat, pale pink colony with matt surfaces	Identified as <i>C. krusei</i>
<i>C. guilliermondii</i>	02	Small pink to purple colony	Identified as <i>C. guilliermondii</i>
Unidentified	01	Pink to pinkish purple	Did not correspond with ** standard chart
Total	64		

*CHO - carbohydrate

**Standard chart - CHO assimilation reaction profile listed in table in mycology laboratory manuals.

C. albicans produced light to medium green coloured colonies, *C. tropicalis* produced dark blue to metallic blue-purple colonies, *C. krusei* produced pink with a whitish bordered rough, dry colonies and *C. glabrata* produced pale pink colonies. *C. parapsilosis* and *C. guilliermondii*, both species produced colonies ranging from slight dark to light pink color (Fig.3).

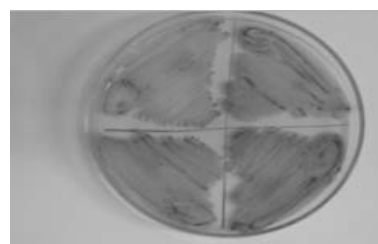


Fig.3. *C. albicans* (light green), *C. krusei* (pinkish purple matt surface), *C. tropicalis* (metallic blue) in chromogenic media

The color of colonies of one species of *C. albicans* and one species of *C. tropicalis* were confusing, further identification was confirmed by sugar assimilation tests. Both the species of *C. krusei* and *C. glabrata* were confirmed with the color and colony characteristics in chromogenic agar media. *C. parapsilosis* and *C. guilliermondii*, both species produced colonies ranging from slight dark to light pink color, hence the interpretation was difficult. They could not be identified with color production in chromogenic agar medium, further identification was done by carbohydrate assimilation test. Unidentified *Candida* species produced pinkish purple colonies. In present study, considering carbohydrate assimilation test as gold standard^{8,11} in species identification of *Candida*, the sensitivity and specificity of chromogenic agar media for *C. albicans* were as 96.97% and 96.87% respectively. The sensitivity and specificity for *C. tropicalis* were 94.12% and 97.87% respectively. *C. krusei* and *C. glabrata* both showed 100% sensitivity and specificity on chromogenic agar media.

Discussion:

Present study revealed that *Candida albicans* were 51.56% and the non-albicans *Candida* species were 48.44%. Among the non-albicans *Candida* species, *C. tropicalis* were 17 (26.56%), *C. glabrata* were 04 (6.25%), *C. parapsilosis* were 04 (6.25%), *C. krusei* were 03 (4.68%) and *C. guilliermondii* were 02 (3.2%). In this study, different *Candida* species were identified by carbohydrate assimilation test (with twelve sugars), subculture in chromogenic agar media and by germ tube test. Considering carbohydrate assimilation test as gold standard in species identification of *Candida*, out of 33 *C.*

albicans, chromogenic media identified 32 (96.97%) *C. albicans*. Out of 17, *C. tropicalis*, chromogenic media identified 16 (94.12%) *C. tropicalis*. Chromogenic media identified both the species of *C. glabrata* and *C. krusei* in 100% cases. In present study, regarding carbohydrate assimilation test as gold standard^{8,11} the sensitivity and specificity of chromogenic agar media was evaluated. The sensitivity and specificity of chromogenic media for *C. albicans* were 96.97% and 96.87% respectively. Baradkar *et al* in 2010 reported 96.55% sensitivity and 96.42% specificity of chromogenic agar media for *C. albicans*⁶. Willinger *et al* reported sensitivity and specificity of chromogenic agar media for *C. albicans* are 98.81% and 100% respectively¹². Findings of the above mentioned studies are nearly similar to our findings. The sensitivity and specificity of chromogenic agar media for *C. tropicalis* were 94.12% and 97.87% respectively in present study. These values are lower than that of the study with Baradkar *et al* which showed 100% sensitivity and 100% specificity of chromogenic agar for *C. tropicalis*⁶. Willinger *et al* showed sensitivity of 66.1% and specificity of 99.8% on chromogenic agar¹².

Rapid identification of *C. glabrata* and *C. krusei* are important because they are less sensitive than other species to antifungal drugs such as ketoconazole and fluconazole. Baradkar *et al* showed sensitivity of 90.90% and specificity of 88.23% for *C. glabrata* and didn't find any species of *C. krusei*⁶. In present study *C. krusei* and *C. glabrata* both showed 100% sensitivity and 100% specificity on chromogenic agar media which is higher than that of above mentioned study. Willinger *et al* reported 98% sensitivity and 95% specificity for *C. glabrata* on chromogenic agar media¹². They found 100% sensitivity and 100% specificity for *C. krusei* on chromogenic agar media which is in consistent with the findings of present study. In present study *C. parapsilosis* and *C. guilliermondii* could not be differentiated from each other on chromogenic media. Though Willinger *et al* have solved this confusion by giving special attention to the evidence of pale edges on pink colonies of *C. glabrata*, but we couldn't appreciate this property for differentiation from other species¹². Thirty three isolates of *C. albicans* were identified by germ tube test which were similar to finding of carbohydrate assimilation test. Only 01 *C. tropicalis* was positive by germ tube test, but other identified *Candida* species were negative. Hazen and Cutler in 1979 also found germ tube production by a strain of *C. tropicalis*¹³. An increase in the predisposing conditions in recent years has resulted in an increasing incidence of *Candida* infections. Therefore, the species level identification of the *Candida* isolates can greatly influence the treatment options for the clinicians and therefore crucial for facilitating hospital control

measures. Though species identification of *Candida* in conventional carbohydrate assimilation method is considered as Gold standard, but it is laborious and time consuming, but in contrast chromogenic agar media is less time consuming and its efficacy is almost similar to carbohydrate assimilation method. From the findings of this study it can be concluded that chromogenic agar media could be used for rapid identification of *Candida* to the species level as a secondary culture media from clinical sample. Further study may be carried out to find the efficacy of chromogenic media as primary culture media to detect mixed culture of yeast in the clinical specimens.

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