

Performance of chromogenic media for *Candida* in rapid presumptive identification of *Candida* species from clinical materials

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ABSTRACT

Background: In perspective of the worldwide increase in a number of immunocompromised patients, the need for identification of *Candida* species has become a major concern. The development of chromogenic differential media, introduced recently, facilitate rapid speciation. However, it can be employed for routine mycology workup only after an exhaustive evaluation of its benefit and cost effectiveness. This study was undertaken to evaluate the benefit and cost effectiveness of chromogenic media for speciation of *Candida* clinical isolates. **Materials and Methods:** Sputum samples of 382 patients were screened for the presence of *Candida* spp. by Gram stain and culture on sabouraud dextrose agar. *Candida* species were identified using Gram stain morphology, germ tube formation, cornmeal agar with Tween-80, sugar fermentation tests and morphology on HiCrome *Candida* differential agar. All the *Candida* isolates were inoculated on HiCrome *Candida* agar (HiMedia, Mumbai, India). **Results:** The sensitivity and specificity of HiCrome agar for identification of *Candida albicans* were 90% and 96.42%, respectively whereas sensitivity and specificity of carbohydrate fermentation test were 86.67% and 74.07%, respectively. Sensitivity and specificity values of HiCrome agar for detection of *C. albicans*, *Candida parapsilosis* and *Candida glabrata* were above 90%. **Conclusions:** We found HiCrome agar has high sensitivity and specificity comparable to that of the conventional method. In addition, use of this differential media could significantly cut down the turnaround time as well as cost of sample processing.

Key words: *Candida*, Chromogenic differential agar, Presumptive identification

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INTRODUCTION

Opportunistic fungal infections are emerging challenge in perspective of the worldwide increase in a number of immunocompromised patients.^[1] While *Candida albicans* remains the leading pathogen, other *Candida* species have established their pathogenic role with the development of antifungal resistance.^[2] Consequently, identification of *Candida* to species level has become a routine practice. The conventional speciation scheme is based on phenotypic characteristics. Microscopic morphology of yeast cells in Gram stained smear, growth on cornmeal agar, demonstration of germ tube and colony morphology on

sabouraud dextrose agar (SDA) are essential components of conventional identification scheme.^[3,4] Likewise, carbohydrate metabolism in terms of both carbohydrate assimilation and fermentation has been employed for *Candida* species identification.^[4] Except germ tube test, which is used to provisionally identify *C. albicans* and *Candida dubliniensis*, none of these tests provides quick interpretation. Molecular methods for *Candida* species differentiation are considered gold standard with high sensitivity, specificity and short turnaround time.^[5] However, these tests require proficient personnel and expensive instruments, which are often not affordable in resource poor setups. Several chromogenic differential media have been introduced recently to facilitate rapid speciation.^[6-8] Several authors have utilized it for simultaneous isolation and identification of *Candida* species directly from clinical samples.^[9,10] These are particularly helpful in cases where multiple yeast species are present in the sample.^[11] However, discrepancies in

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interpretation may arise owing to subjective variation that is likely to compromise quality of reports as well as patient care. Therefore, it can be employed for routine mycology workup only after an exhaustive evaluation of its benefit and cost effectiveness.

MATERIALS AND METHODS

This study was carried out in a tertiary care hospital in South India over a period of 9 months (from June, 2010 to March, 2011). Sputum samples of 382 in-patients and out-patients were screened for the presence of *Candida* spp. by Gram stain and culture on SDA. Yeast like colonies on SDA after 24 and 48 h of incubation at 37°C was subjected to Grams stain, germ tube test, urease test, fermentation test for 2% glucose, maltose, lactose and sucrose along with demonstration of chlamydospore, blastospores and pseudohyphae on cornmeal agar to differentiate *Candida* from other yeasts and to identify them up to species level.

All the *Candida* isolates were inoculated on HiCrome *Candida* agar (HiMedia, Mumbai, India), a commercially available chromogenic agar for *Candida* (CAC). HiCrome agar colony characteristics were interpreted as per manufacturer's specifications by two personnel to avoid subjective variation. Light green colonies were identified as *C. albicans* isolates, whereas *C. dubliniensis*, *Candida tropicalis*, *Candida krusei* were considered in case of dark green, blue to purple and purple colonies respectively. *Candida parapsilosis* and *Candida glabrata* were identified by cream to white colonies on HiCrome agar.

On cornmeal agar, pseudohyphae with plenty of spherical blastoconidia at the constriction sites and thick walled large round terminal single chlamydospores were identified as *C. albicans* and isolates displaying pseudohyphae with clusters of terminal chlamydospores were considered as *C. dubliniensis*.^[4] *C. parapsilosis* strains were recognized by Christmas tree appearance due to the presence of branched pseudohyphae, which gradually become smaller distally. Isolates showing long branching abundant pseudohyphae with narrow sterile apex and ellipsoid balastoconidia were labelled as *C. tropicalis*, whereas, clusters of small, budding blastospores without pseudohyphae was very typical of *C. glabrata*.^[4]

Candida albicans ATCC 611098, *C. tropicalis* ATCC 13803 and *C. parapsilosis* ATCC 22019 strains were used for quality control. Results of HiCrome agar and carbohydrate fermentation were compared with conventional species identification based on Grams stain, germ tube and cornmeal agar test. All findings were entered in MS Excel datasheet and on completion of the study data were

statistically analyzed for sensitivity, specificity, positive and negative *P* value.

RESULTS

A total of 87 strains of *Candida* was recovered from 382 sputum samples during the study period. *C. albicans* (68.96%) was most common species, followed by *C. tropicalis* (17.24%), *C. glabrata* (6.89%), *C. parapsilosis* (4.59%), *C. krusei* (1.15%) and *C. dubliniensis* (1.15%). The distribution of *Candida* species is listed in Table 1.

In this present study, both SDA and HiCrome agar were employed as primary plating medium for sputum samples. There were no major discrepancies between SDA and HiCrome agar in growth rate or colony size for primary isolation of *Candida*. Both media supported growth of all 87 *Candida* isolates. After 18–24 h of incubation, colonies of most *Candida* isolates on HiCrome agar started developing characteristic colors, which became more prominent after 48 h. However, false positive and false negative results were also seen. Different colony colors displayed by *Candida* isolates on HiCrome agar is given in Table 2. Results of HiCrome agar and carbohydrate fermentation are compared with conventional identification in Tables 3 and 4 respectively.

DISCUSSION

Until recent years, laboratory processing and identification of non-*albicans Candida* (NAC) from clinical specimen was in a dilemma. In most laboratories, NAC isolates were reported based on germ tube test only, without species

Table 1: Distribution of *Candida* species

<i>Candida</i> species	Number of isolates (n=87)	Percentage
<i>C. albicans</i>	60	68.96
<i>C. tropicalis</i>	15	17.24
<i>C. parapsilosis</i>	4	4.59
<i>C. krusei</i>	1	1.15
<i>C. glabrata</i>	6	6.89
<i>C. dubliniensis</i>	1	1.15

Table 2: Colors of *Candida* colonies on HiCrome agar

<i>Candida</i> species	Light green	Dark green	Blue	Purple	Cream to white
<i>C. albicans</i> (n=60)	54	2	3	1	0
<i>C. tropicalis</i> (n=15)	2	1	12	0	0
<i>C. parapsilosis</i> (n=4)	0	0	0	0	4
<i>C. glabrata</i> (n=6)	0	0	0	0	6
<i>C. krusei</i> (n=1)	0	0	0	1	0
<i>C. dubliniensis</i> (n=1)	0	1	0	0	0

Table 3: Performance of HiCrome agar for identification of different *Candida* species

<i>Candida</i> species	Sensitivity %	Specificity %	PPV %	NPV %
<i>C. albicans</i>	90	96.42	94.73	80.64
<i>C. tropicalis</i>	80.00	100	100	96
<i>C. parapsilosis</i>	100	100	100	100
<i>C. glabrata</i>	100	100	100	100

PPV=Positive predictive value; NPV=Negative predictive value

Table 4: Performance of carbohydrate fermentation test for identification of different *Candida* species

<i>Candida</i> species	Sensitivity %	Specificity %	PPV %	NPV %
<i>C. albicans</i>	86.67	74.07	88.13	71.42
<i>C. tropicalis</i>	93.33	94.44	77.78	98.55
<i>C. parapsilosis</i>	75	100	100	98.80
<i>C. glabrata</i>	66.67	100	100	97.60

PPV=Positive predictive value; NPV=Negative predictive value

level identification and antifungal susceptibility test.^[10] However, NAC has emerged as an opportunistic pathogen with potential to cause life-threatening, invasive infections that are often not amenable to anti-fungal therapy.^[12] Not only HIV, the development of advanced therapeutic modalities for the treatment of cancers, infectious diseases and immunosuppressive drugs has caused a major shift in the epidemiology of opportunistic fungal infections.^[13] There are frequent reports of multi-species yeast infections and azole resistance among NAC.^[2,11] Although *C. albicans* remains sensitive to most conventional and newer anti-fungal agents, the emergence of resistance to these agents in NAC is likely to limit the treatment options.^[14-17] *Candida* species is known to colonize respiratory tract, especially in patients with pulmonary tuberculosis and in patients receiving immunosuppressive or long-standing anti-microbial therapy.^[18,19] Although *Candida* species are frequently isolated from sputum, their significance as a possible pulmonary pathogen is often doubtful. We found that *C. albicans* ($n = 60$) is the most common isolate followed by *C. tropicalis* ($n = 15$), *C. glabrata* ($n = 6$) and *C. parapsilosis* ($n = 4$) [Table 1]. This is in accordance with other Indian studies. Latha *et al.* found *C. albicans* was the predominant (45.01%) respiratory isolates of *Candida* and *C. tropicalis*, *C. glabrata*, *C. parapsilopsi*s and *C. krusei* constituted 19.95%, 16.54%, 13.14%, and 5.10% respectively.^[20] In another study, *C. tropicalis* (9.1%), *C. pseudotropicalis* (6.06%), and *C. krusei* (6.06%) have been reported.^[19]

With respect to increasing clinical significance of NAC, isolation of *Candida* from clinical specimen warrants rapid identification to species level. The concept of developing chromogenic differential media for *Candida* came from the observation that *C. albicans* produces an

enzyme N-acetyl-galactosaminidase, which can break down chromogenic hexosaminidase substrates incorporated in a medium.^[21] Although chromogenic differential media for *Candida* were commercially available since 1990s,^[21] these were not included in routine mycology practice and were not considered a substitute of convention speciation procedures. It has limitations like subjective variation in interpretation, alteration of color with incubation and inability to differentiate certain *Candida* species. Authors have reported difficulties in differentiation of *C. albicans* from *C. dubliniensis*.^[22] *C. dubliniensis* is closely related to *C. albicans*. Both species produce the germ tube, chlamydospores and grow on CAC as green colonies. Although *C. dubliniensis* colonies are dark green unlike light green colonies of *C. albicans*, this difference may be inadequate for primary identification and often lost after serial subculture.^[23,24] Hospenthal *et al.* reported all *C. dubliniensis* strains ($n = 17$) in their study had colonies identical to *C. albicans* on CAC. In this study, we isolated one *C. dubliniensis*, which had typical dark green colonies. However, two *C. albicans* and one *C. tropicalis* strain also displayed similar colony morphology on HiCrome agar. Sahand *et al.* suggested use of CAC supplemented with Pal's medium for better species discrimination.^[22] Likewise, *Candida kefyr*, *C. parapsilosis* and *C. glabrata* produce identical cream to white growth on CAC, which mandates the use of other tests for identification. Cornmeal agar has a vital role in this scenario. *C. glabrata* can be easily differentiated from other two species by small, budding blastospores and absence of pseudohyphae, whereas *C. kefyr* has elongated, cylindrical blastospores in log jam appearance and *C. parapsilosis* displays Christmas tree appearance due to presence of branched pseudohyphae.^[4] Bishop *et al.* found that *Candida rugosa*, *Candida firmetaria* and *C. inconspicua* might be confused with *C. krusei* and apart from these three common species, *C. bracarensis*, *C. nivariensis* and *C. norvegensis* also produced white colonies on CAC, which required molecular methods to differentiate them.^[25] In our study, 10 isolates displayed white to cream colonies. Among these, six isolates were *C. glabrata* and four *C. parapsilosis*. Interestingly, no false positive and false negative result seen in white colony producing isolates.

We noticed HiCrome agar had high sensitivity and specificity compared to carbohydrate fermentation for all four common *Candida* species. *C. parapsilosis* and *C. glabrata*, in particular, had more false negative results in fermentation tests, resulting in lower sensitivity. In case of *C. albicans*, the sensitivity and specificity of HiCrome agar were 90% and 96.42%, respectively whereas sensitivity and specificity of carbohydrate fermentation test were 86.67% and 74.07%, respectively. This finding is in accordance with other studies. Yucesoy

et al. evaluated performance of CAC and BIGGY agar and found CAC was superior to BIGGY agar, displaying above 90% sensitivity and 100% specificity for *C. albicans*, *C. tropicalis*, *C. glabrata* and *C. krusei* isolates.^[26] In another study, sensitivity of CAC for *C. albicans*, *C. tropicalis* and *C. krusei* was 99%, 98% and 100%, respectively.^[10] CAC was found by several authors as a simple method, which offers rapid, reliable identification of commonly isolated *Candida* species based on colony characteristics after 48 h incubation. However, color development may be unstable and incomplete within 24 h. Willinger *et al.* reported *Candida* ID medium had much higher sensitivity (96.8%) compared to CAC (49.6%), especially for rapid identification of *C. albicans* after 24 h of incubation.^[8] In comparison with CAC, sugar fermentation test has several drawbacks. Prolonged incubation and a high number of false positive results from easy contamination of sugar media are common limitations. Broths containing 2% glucose, maltose, lactose and sucrose have been used in this study. In fermentation test, *C. tropicalis* had the highest sensitivity (96.8%) followed by *C. albicans*, *C. parapsilosis* and *C. glabrata*.

Although, the cost of 1 l of CAC is almost five times more than the cost of SDA,^[27] use of CAC for primary isolation may preclude the cost of SDA, cornmeal agar, sugar media and other reagents. If samples are inoculated on either half of CAC plate, it will be further reduced the cost of sample processing. CAC is particularly important in multi-species yeast infections, where subculture on plating medium may be required to isolate different *Candida* strains in pure form for identification.^[11] In this scenario, CAC offers rapid, direct identification after 48 h incubation, without the need to subculture. The main drawback of this study was a limited number of samples. Therefore, conclusions may not be representative and may require further studies for confirmation.

CONCLUSION

HiCrome agar showed no major discrepancies in growth potential, colony characters and growth rates for primary isolation from sputum samples as compared to SDA. With few exceptions, the colony colors developed on HiCrome agar were stable and distinct for common *Candida* species. However, stable color development may take 48 h. We found results sugar fermentation tests were variable and had poor sensitivity, especially for detection of *C. parapsilosis* and *C. glabrata*. Based on these findings, we recommend routine use of HiCrome agar in mycology laboratories. Use of this differential media can significantly cut down the turnaround time as well as cost of sample processing.

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