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

INTRODUCTION

Opportunistic fungal infections are emerging challenge in perspective of the worldwide increase in a number of immunocompromised patients. While Candida albicans remains the leading pathogen, other Candida species have established their pathogenic role with the development of antifungal resistance. 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. Likewise, carbohydrate metabolism in terms of both carbohydrate assimilation and fermentation has been employed for Candida species identification. 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. 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. Several authors have utilized it for simultaneous isolation and identification of Candida species directly from clinical samples. These are particularly helpful in cases where multiple yeast species are present in the sample. However, discrepancies in
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 h of incubation were identified by cream to white colonies on HiCrome agar. All findings were entered in MS Excel for statistical analysis and results 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 chlamydoospores 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.

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 differentiation 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.

### Table 1: Distribution of Candida species

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

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

| Candida species | Light green | Dark green | Blue | Purple | Cream to white |
|----------------|-------------|------------|------|--------|----------------|
| C. albicans (n=60) | 54          | 2          | 3    | 1      | 0              |
| C. tropicalis (n=15) | 2           | 1          | 12   | 0      | 0              |
| C. parapsilosis (n=4) | 0           | 0          | 0    | 0      | 4              |
| C. glabrata (n=6)    | 0           | 0          | 0    | 0      | 6              |
| C. krusei (n=1)      | 0           | 0          | 0    | 1      | 0              |
| C. dubliniensis (n=1)| 0           | 1          | 0    | 0      | 0              |
Table 3: Performance of HiCrome agar for identification of different Candida species

| Candida species | Sensitivity % | Specificity % | PPV % | NPV % |
|-----------------|---------------|---------------|-------|-------|
| C. albicans     | 90            | 96.42         | 94.73 | 80.64 |
| C. tropicalis   | 80.00         | 100           | 100   | 96    |
| C. parapsilosis | 100           | 100           | 100   | 100   |
| C. glabrata     | 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

| Candida species | Sensitivity % | Specificity % | PPV % | NPV % |
|-----------------|---------------|---------------|-------|-------|
| C. albicans     | 86.67         | 74.07         | 88.13 | 71.42 |
| C. tropicalis   | 93.33         | 94.44         | 77.78 | 98.55 |
| C. parapsilosis | 75            | 100           | 100   | 98.80 |
| C. glabrata     | 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 andazole 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. parapsilosis and C. krusei constituted 19.95%, 16.54%, 13.14%, and 5.10% respectively.[28] 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 conversion 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.[25] 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 pseudo hyphae, whereas C. kefyr has elongated, cylindrical blastospores in log jam appearance and C. parapsilosis displays Christmas tree appearance due to presence of branched pseudo hyphae.[4] Bishop et al. found that Candida rugosa, Candida firnearia and C. inonisciu might be confused with C. krusei and apart from these three common species, C. brauniens, C. nivariensis and C. norvegicus also produced white colonies on CAC, which required molecular methods to differentiate them.[29] 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.

**REFERENCES**

1. Kirkpatrick WR, Revankar SG, Mcatee RK, Lopez-Ribot JL, Fothergill AW, McCarthy DI, et al. Detection of Candida dubliniensis in oropharyngeal samples from human immunodeficiency virus-infected patients in North America by primary CHROMagar Candida screening and susceptibility testing of isolates. J Clin Microbiol 1998;36:3007-12.
2. Challenger R, Pinsent S, Ekanayaka R. A case series of the management of symptomatic azole-resistant Candida. Int J STD AIDS 2012;23:375-6.
3. Sood P, Mishra B, Dogra V, Mandal A. Comparison of Vitek Yeast Biochemical Card with conventional methods for speciation of Candida. Indian J Pathol Microbiol 2000;43:143-5.
4. Chander J. Textbook of Medical Mycology. 2nd ed. New Delhi: Mehta Publishers; 2002. p. 428.
5. Jordan JA, Durso MB. Rapid speciation of the five most medically relevant Candida species using PCR amplification and a microtiter plate-based detection system. Mol Diagn 1996;1:51-58.
6. Raut SH, Varaiya A. Differentiation of Candida dubliniensis on chrom agar and Pal’s agar. Indian J Med Microbiol 2009;27:55-8.
7. Sahand IH, Maza JL, Eraso E, Montejo M, Moragues MD, Aguirre JM, et al. Evaluation of CHROM-Pal medium for the isolation and direct identification of Candida dubliniensis in primary cultures from the oral cavity. J Med Microbiol 2009;58:1437-42.
8. Willinger B, Hillowoth C, Selitsch B, Manafi M. Performance of Candida ID, a new chromogenic medium for presumptive identification of Candida species, in comparison to CHROMagar Candida. J Clin Microbiol 2001;39:3793-5.
9. Horvath LL, Hopsenthal DR, Murray CK, Dooley DP. Direct isolation of Candida spp. from blood cultures on the chromogenic medium CHROMagar Candida. J Clin Microbiol 2003;41:2629-32.
10. Nadeem SG, Hakim ST, Kazmi SU. Use of CHROMagar Candida for the presumptive identification of Candida species directly from clinical specimens in resource-limited settings. Libyan J Med 2010;5:1-6. DOI: 10.3402/ljm.v5i0.2144.
11. Agarwal S, Manchanda V, Verma N, Bhatta P. Yeast identification in routine clinical microbiology laboratory and its clinical relevance. Indian J Med Microbiol 2011;29:172-7.
12. Vergheese SL, Padmaja P, Sutha P, Mathew T, Johni ES. Rapid identification of fluconazole resistance using Chrom agar Candida. Indian J Pathol Microbiol 2001;44:305-7.
13. Shigemura K, Osawa K, Jikimoto T, Yoshida H, Hayama B, Ohji G, et al. Comparison of the clinical risk factors between Candida albicans and Candida non-albicans species for bloodstream infection. J Antibi (Tokyo) 2014;67:311-4.
14. Brito SA, Rodrigues FF, Campos AR, da Costa JG. Evaluation of the antifungal activity and modulation between C. albicans and C. tropicalis using agar diffusion, minimum inhibitory concentration and MIC. Pharmacogn Mag 2012;8:103-6.
15. Otang WM, Grierson DS, Ndip RN. Antifungal activity of Arctotis arctoides (L.f.) O. Hoffm. and Gasteria bicolor Haw. against opportunistic fungi associated with human immunodeficiency virus/acquired immunodeficiency syndrome. Pharmacogn Mag 2012;8:135-40.
16. Bag A, Chattopadhyay R, Bhattacharyya S. Medicinal plants and urinary tract infections: An update. Pharmacogn Rev 2008;2:277-83.
17. Jain A, Jain S, Rawat S. Recent herbal trends against fungal infections: A review. Pharmacogn Rev 2008;2:257-65.
18. Kali A, Charles MP, Noyal MJ, Sivaraman U, Kumar S, Easow JM. Prevalence of Candida co-infection in patients with pulmonary tuberculosis. Australas Med J 2013;6:387-91.
19. Jain SK, Agrawal RL, Sharma DA, Agrawal M. Candida in pulmonary tuberculosis. J Postgrad Med 1982;28:24-9.
20. Latha R, Sasikala R, Muruganandam N, Venkatesh Babu R. Study on the shifting patterns of non-Candida albicans Candida in lower respiratory tract infections and evaluation of the CHROMagar in identification of the Candida species. J Microbiol Biotechnol Res 2011;1:114-9.
21. Odds FC, Bernaerts R. CHROMagar Candida, a new differential isolation medium for presumptive identification of clinically important Candida species. J Clin Microbiol 1994;32:1923-9.
22. Sahand IH, Moragues MD, Eraso E, Villar-Vidal M, Quindós G, Pontón J. Supplementation of CHROMagar Candida medium with Pal’s medium for rapid identification of Candida dubliniensis. J Clin Microbiol 2005;43:5768-70.
23. Sullivan D, Coleman D. Candida dubliniensis: Characteristics and identification. J Clin Microbiol 1998;36:329-34.
24. Hospenthal DR, Beckius ML, Floyd KL, Horvath LL, Murray CK. Presumptive identification of Candida species other than C. albicans, C. krusei, and C. tropicalis with the chromogenic medium CHROMagar Candida. Ann Clin Microbiol Antimicrob 2006;5:1.
25. Bishop JA, Chase N, Lee R, Kurtzman CP, Merz WG. Production of white colonies on CHROMagar Candida medium by members of the Candida glabrata clade and other species with overlapping phenotypic traits. J Clin Microbiol 2008;46:3498-500.
26. Yücesoy M, Marol S. Performance of CHROMAGAR Candida and BiGGY agar for identification of yeast species. Ann Clin Microbiol Antimicrob 2003;2:8.
27. HiMedia Price List 2011-12. Mumbai, India: HiMedia Laboratories Pvt. Limited; 2011. p. 1154.