



EFFECTIVENESS OF CHROM AGAR CANDIDA, A DIFFERENTIAL ISOLATION MEDIUM FOR RAPID IDENTIFICATION OF CLINICALLY IMPORTANT CANDIDA SPECIES

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Abstract-

Background & Objective- Rapid identification of yeast infections is helpful in prompt appropriate antifungal therapy. Chromogenic medium is also helpful in identifying "multi-species" yeast. CHROM agar Candida is a differential culture medium that is claimed to facilitate the isolation and rapid identification of some clinically important yeast species.

Material and Method- A Total of 100 yeast isolates were included in this study. All isolates were identified by inoculation on CHROM agar and compared to the results of Sabouraud's dextrose agar inoculation, followed by *dalmau* plate (cornmeal agar) morphology and other standard identification techniques.

Result- Out of 100 isolates, 29 produced green colonies of *Candida albicans*, 65 non-albicans Candida were 36 produced blue colonies of *C. tropicalis*, 13 produced rose pink colonies of *C. krusei*, 9 produced off white to pale pink colony of *C. parapsilosis*, 7 produced purple colony of *C. guilliermondii* and 6 were *C. glabrata* with pink colonies.

Conclusion- The use of chromogenic medium Candida agar is an easy and reliable method for the rapid identification of most commonly isolated Candida species, with typical color shown by Candida species, in less time & early identification of inherent azole resistant species of Candida which require early antifungal therapy.

Keywords- Candida, Sabouraud's dextrose agar, Germ tube test, CHROM agar, Dalmau culture

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Introduction

Candidal infections are the most common yeast infection occurring in any health care facility. Over the last decade Candida infection due to *Candida albicans* have declined. Now yeast infections other than those caused by *Candida albicans* have been increasingly reported worldwide [1-7]. Other non-albicans Candida species (NAC) are now responsible for about half of candidemia and other deep infections by Candida [8,9].

The emergence of such infections has been attributed to the advancement in medical management and increasing load of severely serious debilitated patients. In obtaining Identification by conventional methods delays the diagnosis as well as the close relationship between species and difference in azole susceptibility, early identification of isolated species Candida can directly effect therapeutic decisions regarding empirical antifungal therapy [10].

Most of the diagnostic laboratories uses Sabouraud's Dextrose Agar (SDA), for the isolation of all yeasts from clinical specimens. Most of the yeast species grow on SDA within 48 hours, although few may require longer incubation period for growth. Moreover,

identification of multiple Candida species infections is delayed during routine isolation procedures, which can have serious effect on selection of antifungal therapy. Germ tube test in serum is used by many diagnostic laboratory as an initial test to differentiate *C. albicans* from other yeast species, which may be a subjective test [11]. This is then followed by more time-consuming methods including microscopic identification based on morphology of growth on corn meal-tween 80 agar (CMA) and battery of biochemical tests. Yeast identification by automated techniques, has been increasingly employed [12,13]. Full identification of yeast may take up to 72 hrs. or more for primary isolation of the organism.

CHROM agar Candida (CaC) is increasingly being reported as a medium used to differentiate *Candida albicans* from Non-albicans Candida (NAC) species. Early identification of NAC can helps the treating consultants in selecting appropriate antifungal therapy.

Material and Methods

A total of 100 yeasts isolated from various clinical specimens received in Microbiology laboratory of B.J. Medical College, Ahmedabad in a period of June 2012 to December 2012. All yeast isolates

cultured on chromogenic medium (HiChrom agar; HiMedia,) compared to the results of Saboraud's dextrose agar inoculation, followed by dalmau plate (cornmeal agar) morphology and other standard identification techniques.

Yeast isolates sub-cultured on chromogenic medium were incubated overnight at 35°C. All yeast isolates grew well and developed distinctive coloured colonies after 24 hrs. of incubation. The better color development was observed after 48 hours of incubation. Identification was made by colour and morphology of the colonies. These isolates were also further identified by Tetrazolium Reduction Medium (TRM) and on the basis of microscopic morphological features of the growth obtained through Dalmau plate - Corn Meal Agar (CMA).

Briefly, pure distinctive colonies obtained from chromogenic medium were inoculated through sterile straight wire on the margins of the 1 cm x 1 cm block of corn meal agar placed on a glass slide which is prior sterilized. The agar block was then covered with a sterile coverslip and incubated in a sterile petridish having moistened filter paper. The assembly was then incubated at 25°C. The slide cultures were observed after overnight incubation and after 48 hrs. of incubation.

Result

Patient Profile of Isolated Candida spp. & Details of its Clinical Specimens

A total 100 yeast isolates were recovered from different, clinical specimens, these yeast isolates were identified by colony colour on the chromogenic medium [Table-1], [Table-2].

Species Identification of Isolated Candida

The isolated Candida spp. were *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. krusei*, *C. guilliermondii* & *C. glabrata* which were identified by their colour using chromogenic medium [Fig-1] & [Table-3].

Table 1- Patient profile of isolated Candida spp.

| Patient Profile | No. of Isolates |
|------------------------|-----------------|
| Neonate | 26 |
| Diabetic | 23 |
| HIV reactive | 10 |
| Female | 23 |
| Age more than 65 years | 18 |

Table 2- Details of clinical specimens of isolated Candida spp.

| Type of specimen | No. of isolates |
|------------------|-----------------|
| Urine | 43 |
| Blood | 37 |
| Oral | 7 |
| Vaginal swab | 13 |

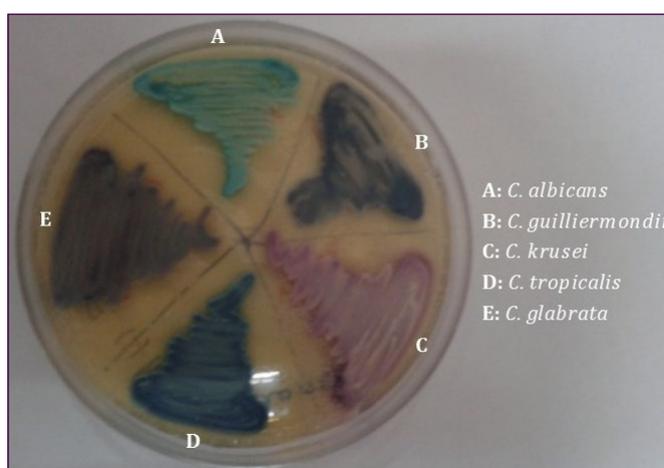


Fig. 1- Various candida species in CHROM agar Candida

Table 3- Species identification of isolated Candida

| Species | Isolates | GTT | Color on chromagar | Color on TRM | Morphology on Cornmeal Agar |
|--------------------------|----------|----------|------------------------|--------------|--|
| <i>C. tropicalis</i> | 36 | Negative | Blue | Maroon | Blastospores singly or in small groups |
| <i>C. albicans</i> | 29 | Positive | Green colonies | Pale pink | Pseudohyphae in clusters, large, terminal chlamydoconidia |
| <i>C. krusei</i> | 13 | Negative | Fuzzy, rose colored | Pink & dry | Pseudohyphae with blastoconidia forming cross-matchstick appearance. |
| <i>C. parapsilosis</i> | 9 | Negative | Off white to pale pink | Rose pink | Short, pencil-like pseudohyphae with blastoconidia arranged singly along pseudohyphae. |
| <i>C. guilliermondii</i> | 7 | Negative | Pink to lavender | Pink & pasty | Pseudohyphae and Blastospores |
| <i>C. glabrata</i> | 6 | Negative | Dark violet | Pale pink | Yeast cells only |

To determine how effective is chrom agar media for rapid diagnosis, all isolates were also processed for Germ Tube Test (GTT), inoculated on TRM and CMA blocks for Dalmau plate culture. Detection of growth patterns on cornmeal agar take 48-72 hrs. and sugar assimilation tests may take 72 hrs. to two weeks. These procedures are labour intensive and take longer time to determine the diagnosis. Identification by observing microscopic morphology on CMA blocks was successful for these isolates that were easily identified by colony colour on the chromogenic medium. All the isolated Candida produced characteristic morphology of particular species and which were confirmed by characteristic colour in chromogenic medium. Identification of yeast in clinical specimens, allowing early initiation of appropriate therapy.

Discussion

Because of the increasing complexity in the management and predisposing condition of patients, there has been an increase in infec-

tions by NAC. Over the past decade, there has been an alarming increase in the number of reports of systemic and deep Candida infections with NAC [3,14,15]. Infections with these yeast species also have a direct impact on the choice of empiric antifungal therapy and clinical outcome. The clinical importance of species-level identification has been increased as Candida species differ in the expression of its virulence factors and antifungal susceptibility [6, 16]. Rapid identification of yeast species also guides early appropriate antifungal therapy. Thus, it has become important to identify all yeast isolates up to the species level in diagnostic microbiology laboratories. Newer techniques like real-time PCR, matrix-assisted laser desorption ionization-time of flight mass spectrometry and multiplex-tandem PCR are being increasingly employed for the identification of yeast species [1,17-19]. However, such methods are much more costly, many of them cannot be afforded by diagnostic microbiology laboratory in Indian settings.

In the present study chromogenic medium helped in the rapid identification of *C. albicans*, *C. tropicalis*, *C. krusei* and *C. parapsilosis*, which was comparable with other studies [20-23]. The chromogenic medium facilitates presumptive identification of yeast isolates to the species level within 24 hrs. of incubation.

Given the fact that many clinical microbiology laboratories do not perform identification beyond a Germ Tube Test, the use of chromogenic medium provides rapid and accurate identification of commonly isolated yeast infections up to species level, as it is a cost effective and simple method. Rapid identification followed by confirmation of yeast species is a useful in initiating appropriate antifungal therapy at early stage, reducing morbidity and mortality in patients.

Conclusion

In our study CHROM agar Candida shows good potential as a differential medium for rapid identification of Candida species. Combined with confirmation of speciation by standard methods and knowledge of the local antifungal susceptibility patterns of these species, CHROM agar Candida may be a useful identification medium for use in the diagnostic microbiology laboratory. This medium can be recommended for use to isolate & direct identify Candida species from direct of clinical specimens. With typical color shown by Candida species, this medium can be used in place of conventional method for identification, which is time consuming.

Clinical microbiologists will be able to save time and costs for the diagnosis of fungi from clinical specimens specially blood cultures in which case early identification may requires change of antifungal agents as *C. glabrata* and *C. krusei* are inherently resistant to azoles.

Conflicts of Interest: None declared.

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