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# Speciation of Candida Species Isolated From Clinical Specimens by Using Chrom Agar and Conventional Methods

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***Abstract*-** Candida spp especially non albicans Candida are increasingly being isolated from clinical specimens. The conventional methods of identification are time consuming and difficult to perform. The study was done to evaluate the performance of conventional identification method (phenotypic and biochemical) and commercially available chromogenic Candida speciation media (CHROM agar) for the identification of medically important yeast and yeast-like organisms in a routine clinical microbiology laboratory. A total of 60 yeast strains were included during the one and half years study period. The conventional methods used for speciation of yeast isolates were germ tube test, chlamydospore formation test on corn meal agar, sugar fermentation test and sugar assimilation test and were compared against chromogenic agar medium (CHROM agar). *Candida albicans* (51.6%) was the most common *Candida* species, followed by *C. tropicalis* (25%), *C. krusei* (16.6%) and *C. glabrata* (6.6%). Agreement between the conventional method and chromogenic methods was 96% for *C. albicans*, 100% for *C. tropicalis*, 100% for *C. krusei* and 100% *C. glabrata*. *C. albicans* was the most common single species isolated. However, species other than *C. albicans* are gaining clinical significance (48% of all isolates in the present study). CHROM agar is a convenient and rapid method of identification of Candida species even in resource poor settings.

***Index Terms*-** Candida, CHROM agar, identification, sugar assimilation test

## I. INTRODUCTION

*Candida* spp. are the members of the normal flora of the skin, mucous membranes, and gastrointestinal tract. They are endogenous opportunists which cause secondary infection in individuals with some underlying immunocompromised conditions. Candidiasis is a common fungal disease found in humans affecting mucosa, skin, nails and internal organs of the body.

*Candida albicans* is generally considered the major pathogen among the *Candida* species. An increase in the prevalence of non-albicans species has been noted during the last decades.[1,2] There is growing evidence of the increasing use of azoles causing this epidemiological shift. Characterization to species level helps to identify those strains which might be intrinsically resistant to some of the antifungal agents.[3,4] Speciation of Candida isolates is conventionally done by germ tube test, sugar assimilation and sugar fermentation tests. Newer methods include CHROM agar, API systems, Vitek 2 ID system and molecular methods.[5,6,7] Germ tube test is a rapid method to differentiate *C. albicans* and *C. dubliniensis* from other *Candida* spp. For further speciation chlamydospore formation test, sugar fermentation test and sugar assimilation test can be done. But these tests are time consuming and labour intensive. Among the newer tests, CHROM agar is rapid and cost effective as compared to other expensive systems like API systems, Vitek 2 ID system and molecular methods.

In the present study, we speciated *Candida* isolates using germ tube test, chlamydospore formation test, sugar fermentation test, sugar assimilation test and also evaluated the performance of commercially available chromogenic *Candida* speciation media i.e, CHROM agar.

## II. MATERIALS AND METHODS

The present study was carried out between October 2011 and April 2013 in the Department of Microbiology, SGT Medical College, Budhera, Gurgaon. A total of 60 consecutive *Candida* isolates from various clinical specimens like high vaginal swab, urine, blood, sputum, pus, catheter tip, ear swab from patients with candidiasis and stool sample from patients with antibiotic associated diarrhoea were included in the study. These specimens were processed for the isolation of *Candida* spp. using standard Mycology methods.[5] Gram staining was performed from direct specimen and the specimens were inoculated on Sabouraud's dextrose agar slants, incubated at 37°C for 24 hrs. Germ tube test was done and the positives identified were either *C. albicans* or *C. dubliniensis*. *C. albicans* were further identified by growth at 45°C and Chlamydospore formation on corn meal agar.[7] All the 60 isolates were subjected to Sugar fermentation test and Sugar assimilation test for final confirmation of species.

Simultaneously the *Candida* spp. were inoculated on CHROM agar and incubated at 37°C for 24 hrs and the species were identified by type and colour of the colonies on CHROM agar media as per manufacturer's instructions. (Table 1.)

## III. RESULT

A total of 60 *Candida* spp. were isolated from various clinical specimens .Table 2 gives the distribution and sources of *Candida* spp. identified by the gold standard conventional method. *Candida albicans* (52%) was the most common species isolated. Among the non albicans candida, *C. tropicalis* (25%) was the commonest followed by *C. krusei* (17%) and *C. glabrata* (7%). (Table 2).

These 60 strains were also subjected to identification using CHROM agar. The results along with sensitivity and specificity of Chrome agar for various species are given in table 3. There was an agreement in identification by CHROM agar method in 59 (98%) strains. Only one strain which was identified as *C. glabrata* by the sugar assimilation test was identified as *C. albicans* by chrome agar method. The sensitivity and specificity of CHROM agar was 100% for C. Tropicalis and C. Krusei, for C. albicans the sensitivity was 100% and specificity was 96% and for C. glabrata the sensitivity was 75% and specificity was 100%. (Table 3).

## IV. DISCUSSION

The potential clinical importance of species-level identification has been recognized as *Candida* species differ in the expression of virulence factors and antifungal susceptibility. Non albicans candida are on the rise due to increasing immunocompromised states. Non albicans Candida are more resistant to fluconazole, therefore species level identification has a direct impact on choice of empirical antifungal treatment. Also there may be geographic variation in the species isolated which necessitates that we have data on the distribution of candida species in different geographic regions. In the present study C. albicans predominated i.e, 52%. Predominance of C. albicans was also seen in a study.[8] However higher incidence of non albicans candida ranging from 54 - 74% have been seen in various studies.[4,9,10] Among the non albicans species, Candida tropicalis is reported to be the most predominat species as discussed elsewhere. In our study also C. tropicalis was the most common non albicans species.

For differentiation between different species of candida conventionally Germ tube test, chlamydospore formation, sugar fermentation and assimilation tests are being used which are laborious and time consuming. CHROM AGAR is a rapid method to differentiate between different candida species. It facilitates the detection and identification of candida species from mixed culture and provides result in 24-48 hours. In our study, sensitivity and specificity of CHROM agar for Candida albicans were 100% and 96%, C. tropicalis were 100% and 100%, C. krusei were 100% and 100% and C. glabrata 75% and 100% respectively. A sensitivity of 80% for C. tropicalis, and 89% for C. albicans has been reported in a study who also reported that misidentification of C. tropicalis was seen due to difficulty in interpretation of green color.[6] We, however, did not face any such difficulty and our results were consistent with the results of conventional methods. CHROM agar has the advantage of being technically simple, rapid and cost effective as compared to the conventional methods.

Being a rural hospital and medical college, our study had its own limitations of small sample size, inability to perform antifungal susceptibility tests. However CHROM agar has proved to be a valuable method for identification of Candida species even in resource poor settings.

## V. CONCLUSION

Along with Candida albicans, non albicans Candida spp like C. tropicalis, C, krusei and C. glabrata are increasingly being isolated from clinical specimens. CHROM agar is a simple, rapid and inexpensive method with good sensitivity and specificity for identification of such species.

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**Table 1: Colour of various Candida spp. on CHROM agar for identification**

| | Name | Colour on Chrom agar |
|---|---|---|
| 1. | *C. albicans* | Light green |
| 2. | *C. tropicalis* | Metallic blue |
| 3. | *C. krusei* | Rose pink |
| 4. | *C. glabrata* | White |

**Table 2: Isolation of Candida spp. from clinical specimens**

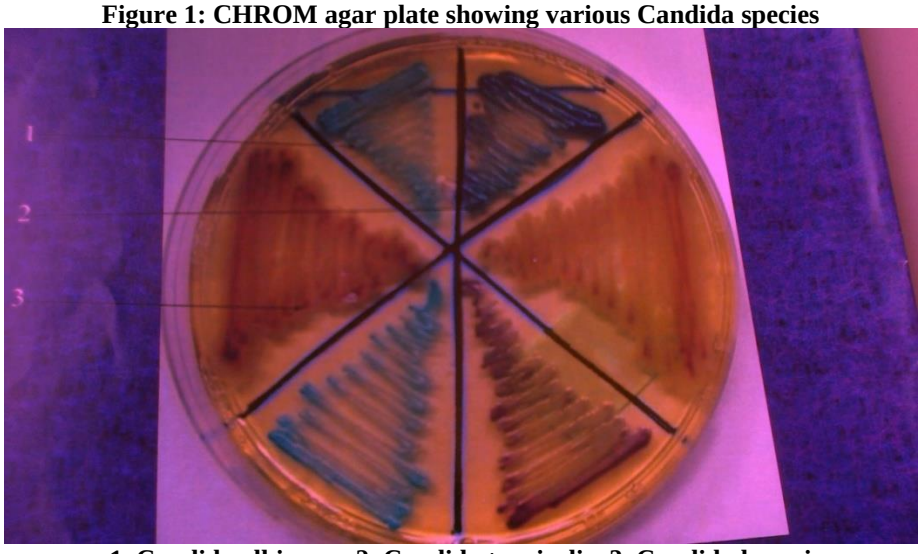
| Nature of specimen | Number of *Candida* spp. | *Candida* spp. identified | | | |
|---|---|---|---|---|---|
| | | *C.albicans* | *C.tropicalis* | *C.krusei* | *C.glabrata* |
| 1. High vaginal swab | 20 | 15 | 2 | 0 | 3 |
| 2. Urine | 12 | 4 | 6 | 2 | 0 |
| 3. Stool | 10 | 5 | 2 | 2 | 1 |
| 4. Blood | 8 | 1 | 4 | 3 | 0 |
| 5. Sputum | 3 | 3 | 0 | 0 | 0 |
| 6. Pus | 3 | 0 | 1 | 2 | 0 |
| 7. Catheter tip | 2 | 2 | 0 | 0 | 0 |
| 8. Ear swab | 2 | 1 | 0 | 1 | 0 |
| Total | 60 | 31 | 15 | 10 | 4 |

**Table 3: Sensitivity and specificity of CHROM agar for identification of various species of Candida**

| Candida spp. | No.of *Candida* spp. identified by conventional method | No.of *Candida* spp. identified using CHROM agar | Sensitivity of CHROM agar | Specificity of CHROM agar |
|---|---|---|---|---|
| *1. C. albicans* | 31 | 32 | 100% | 96% |
| *2. C. tropicalis* | 15 | 15 | 100% | 100% |
| *3. C. krusei* | 10 | 10 | 100% | 100% |
| *4. C. glabrata* | 4 | 3 | 75% | 100% |

FIGURE

**Figure 1: CHROM agar plate showing various Candida species**



**1. Candida albicans; 2. Candida tropicalis; 3. Candida krusei**

**TABLES**

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| | | *C.albicans* | *C.tropicalis* | *C.krusei* | *C.glabrata* |
| **9. High vaginal swab** | 20 | 15 | 2 | 0 | 3 |
| **10. Urine** | 12 | 4 | 6 | 2 | 0 |
| **11. Stool** | 10 | 5 | 2 | 2 | 1 |
| **12. Blood** | 8 | 1 | 4 | 3 | 0 |
| **13. Sputum** | 3 | 3 | 0 | 0 | 0 |
| **14. Pus** | 3 | 0 | 1 | 2 | 0 |
| **15. Catheter tip** | 2 | 2 | 0 | 0 | 0 |
| **16. Ear swab** | 2 | 1 | 0 | 1 | 0 |
| **Total** | 60 | 31 | 15 | 10 | 4 |

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|---|---|---|---|---|
| ***5. C. albicans*** | 31 | 32 | 100% | 96% |
| ***6. C. tropicalis*** | 15 | 15 | 100% | 100% |
| ***7. C. krusei*** | 10 | 10 | 100% | 100% |
| ***8. C. glabrata*** | 4 | 3 | 75% | 100% |

FIGURE

**Figure 1: CHROMagar plate showing various Candida species**


1. Candoida albicans
2. Candida tropicalis
3. Candida krusei