International Journal of Research in Medical Sciences
*Kanna BV et al. Int J Res Med Sci. 2017 Aug;5(8):3520-3522*
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20173554

**Original Research Article**

# Isolation and identification of candida species from various clinical samples in a tertiary care hospital

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**Received:** 24 April 2017
**Accepted:** 22 May 2017

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## ABSTRACT

**Background:** *Candida spp* is a member of the normal flora of the skin, mucous membrane and gastrointestinal tract. They are endogenous opportunists which cause secondary infection in individuals with underlying immunocompromised conditions. Candidiasis is a common fungal disease in humans. An increase in the prevalence of non-albicans species has been noted during the last decades because of increasing use of azoles. This study aims to *Spectate Candida* using chromogenic medium.
**Methods:** A total of 50 *Candida* isolates from various clinical samples were included in the study. These isolates were subjected to gram's stain, germ tube test and inoculation on commercially available CHROM agar (HiMedia India).
**Results:** In current study majority of isolates were from high vaginal swab (34%) followed by sputum (28%), urine (18%), pus from surgical sites and others constituted to 20%. *Candida albicans* (51%) was the most common *candida* species, followed by *C. tropicalis* (25%), *C. krusei* (16%), *C. glabrata* (6%) and *C. dubliniensis* (1%).
**Conclusions:** Along with *Candida albicans*, non-albicans *candida spp* like *C. tropicalis*, *C. krusei*, *C. glabrata*, and *C. dubliniensis* are increasingly being isolated from clinical samples. CHROM agar is a simple, rapid and inexpensive method for identification of such species. Characterization to species level helps to identify species which might be intrinsically resistant to commonly used antifungal agents.

**Keywords:** *Candida*, CHROM agar, Non- albicans *candida*

## INTRODUCTION

*Candida* is a yeast like fungus and ubiquitous human commensal. They become pathogens and cause infections when the host's resistance to infection is lowered either locally or systemically.[1] *Candida albicans* is the most common cause of candidiasis accounting for about 60-80% of infections. An increase in prevalence of non-albicans species has been noted during last decades.[2,3] Characterization to species level helps to identify those strains which might be intrinsically resistant to some antifungal agents.[3,4] Speciation of *Candida* isolates is conventionally done by germ tube test, sugar assimilation and fermentation tests.

Newer methods include CHOM agar, API system, Vitek 2 ID system and molecular methods.[5] Since API system, Vitek 2ID system and molecular techniques are expensive, use of CHROM agar for species differentiation would be of benefit for easy and rapid speciation.[6] They contain chromogenic substrates that react with enzymes secreted by microorganisms

producing colonies with various pigmentation. These enzymes are species specific, allowing organisms to be identified to the species level by their colour and colony characteristics.[7] It is necessary to identify *Candida* to species level as non albicans candida species are showing drug resistance. The present study was undertaken to evaluate the advantages of CHROM agar over conventional methods for speciation of *Candida* isolates.

## METHODS

This is a prospective study conducted in the department of microbiology during August 2015 to January 2016. A total of 50 consecutive and non-repetitive *Candida* isolates from various clinical specimens like high vaginal swab, urine, blood, sputum, pus, catheter tip, ear swab and stool sample from patients with antibiotic associated diarrhea were included in the study.

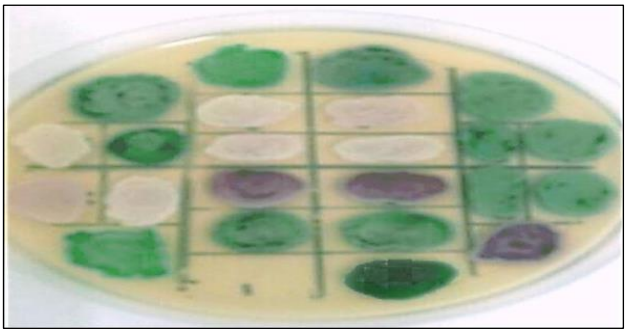

**Figure 1: CHROM agar showing various species of *Candida*.**

Gram's stain was performed from direct samples and inoculated on Sabouraud dextrose agar, incubated at 37°C for 24 hours. Germ tube test was done and the positives were identified as either *C. albicans* or *C. dubliniensis*. *C. albicans* was further identified by growth at 45°C and chlamydospore formation on cornmeal agar. All the isolates were subjected to sugar assimilation test for final confirmation of species. Simultaneously the *Candida spp*. were inoculated on CHROM agar (Hi-media, India) and incubated at 37°C for 24 hours and the species were identified by type and colour of the colonies on CHROM agar media as per manufacturer's instruction. (Figure 1 and Table 1).

**Table 1: Colour of various *Candida spp*. on CHROM agar for identification.[10]**

| Name | Colour on CHROM agar |
|---|---|
| *C. albicans* | Light green |
| *C. tropicalis* | Metallic blue |
| *C. krusei* | Rose pink |
| *C. glabrata* | White |
| *C. parapsilosis* | Pale cream |
| *C. dubliniensis* | Dark green |

## RESULTS

A total of 50 *Candida spp*. was isolated from various clinical samples. Distribution of samples of *Candida* isolates were mentioned in Table 2. *Candida albicans* (51%) was the most common species isolated.

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

| Sample | No. of *Candida* isolates | *C. albicans* | *C. tropicalis* | *C. krusei* | *C. glabarata* | *C. dubliniensis* |
|---|---|---|---|---|---|---|
| Vaginal swab | 17 | 13 | 2 | 1 | 1 | 0 |
| Urine | 9 | 4 | 2 | 2 | 0 | 1 |
| Sputum | 14 | 3 | 7 | 3 | 1 | 0 |
| Ear swab | 5 | 3 | 1 | 0 | 1 | 0 |
| Stool | 2 | 1 | 1 | 0 | 0 | 0 |
| Pus | 2 | 0 | 0 | 2 | 0 | 0 |
| Catheter tip | 1 | 1 | 0 | 0 | 0 | 0 |
| Total | 50 | 25 | 13 | 8 | 3 | 1 |

**Table 3: Sensitivity and specificity of CHROM agar for speciation 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 |
|---|---|---|---|---|
| *C. albicans* | 26 | 25 | 100% | 94% |
| *C. tropicalis* | 13 | 13 | 100% | 100% |
| *C. krusei* | 8 | 8 | 100% | 100% |
| *C. glabarata* | 3 | 3 | 75% | 100% |
| *C. dubliniensis* | 0 | 1 | 96% | 100% |

Among the non-albicans *Candida*, *C. tropicalis* (26%), *C. krusei* (16%), *C. glabarata* (6%) and *C. dubliniensis* (1%). Sensitivity and specificity of CHROM agar was 100% for *C. tropicalis* and *C. krusei*. Sensitivity and specificity for *C. albicans* was 100% and 94% respectively. Sensitivity and specificity for *C. glabarata* was 75% and 100%, for *C. dubliniensis* was 96% and 100% (Table 3).

## DISCUSSION

Potential clinical importance of species level identification has been recognized as *Candida* species differ in the expression of virulence factors and antifungal susceptibility.[8] Candida species also have a direct impact on the choice of empirical antifungal therapy and clinical outcome. Non-albicans candida species are on the rise due to increasing immunocompromised condition. In the present study, *C. albicans* is predominant (51%). Predominance of *C. albicans* was also seen in a study by Manjunath et al.[9] However, higher incidence of non-albicans candida ranging from 54-74% have been seen in numerous studies.[4,10,11] Among the non-albicans candida, *C. tropicalis* is reported to be the most predominant species. In this study, also *C. tropicalis* was the most common non-albicans species.

Conventional speciation of Candida isolates were performed by germ tube test, chlamydospore formation, sugar fermentation and assimilation tests. They are laborious and time consuming. CHROM agar is a rapid method to spectate the various candida species. It facilitates the detection and identification of *Candida* species from mixed culture and provides results in 24-48 hours. In this study, sensitivity and specificity of CHROM agar for *Candida albicans* were 96% and 100%, *C. tropicalis* were 100% and 100%, *C. krusei* were 100% and 100%, *C. glabarata* were 100% and 100%, *C. dubliniensis* were 100% and 100% respectively. A sensitivity of 80% for *C. tropicalis* and 89% for *C. albicans* has been reported in a study.[5] In this study, there were difficulties in identifying *C. dubliniensis* by conventional methods. CHROM agar helped us in this regard with the added advantages of being technically simple, rapid, and cost effective as compared to the conventional methods. Present study had its own limitations like 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.

## CONCLUSION

Characterization of Candida to species level helps in identifying the intrinsically resistant species. Along with *Candida albicans*, non-albicans *candida spp* like *C. tropicalis*, *C. krusei*, *C. glabarata*, *C. dubliniensis* 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.

*Funding: No funding sources*
*Conflict of interest: None declared*
*Ethical approval: The study was approved by the Institutional Ethics Committee*

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**Cite this article as:** Kanna BV, Kumar GA, Swapna M, Easow JM. Isolation and identification of candida species from various clinical samples in a tertiary care hospital. Int J Res Med Sci 2017;5:3520-2.