

Isolation and Identification of Various *Candida* Species from Clinical Samples at a Tertiary Care Hospital of Southern Rajasthan

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ABSTRACT

Introduction: *Candida* species are yeast-like fungi that normally inhabit humans as harmless commensals but can become opportunistic pathogens when host resistance is compromised. Among the *Candida* species, *Candida albicans* is typically regarded as the main pathogen. Over the past few decades, there has been a noticeable rise in the prevalence of non-*albicans* species. Species-level identification of *Candida* spp. is of clinical significance due to the variability in virulence and antifungal resistance among them. **Objective:** The objective of this research is to isolate and identify the species of *Candida* from diverse clinical samples within a tertiary care hospital, to ascertain the range of *Candida* infections present in clinical specimens, and to characterize the *Candida* species that have been isolated. **Methods:** *Candida* isolates from various clinical samples like urine, sputum, tracheal swab, vaginal swab, and endotracheal secretions were included in this study. After initial identification of *Candida* species by conventional methods, *Candida* spp. were inoculated onto CHROM agar and incubated at 37°C for 48 to 72 hours, with species identification based on the type and colour of colonies on the CHROM agar medium. **Results:** In this present study, a total of 45 *Candida* species were isolated from various clinical specimens, 20 from urine, 12 from sputum, 6 from tracheal swab, 6 from vaginal swab and 1 from endotracheal secretions as shown in Table 2. Among 45 *Candida* isolates, 25 (55.56%) were of *Candida albicans*, 13 (28.89%) of *Candida tropicalis*, 6 (13.33%) of *Candida galbrata* and 1 (2.22%) of *Candida krusei*. **Conclusion:** In addition to *Candida albicans*, non-*albicans* *Candida* species such as *C. tropicalis*, *C. krusei*, *C. galbrata*, and *C. dubliniensis* are being more frequently obtained from clinical specimens. Accurate species identification of *Candida* is crucial for

effective treatment, as not all species respond uniformly to therapy, and there is a growing concern over antifungal resistance.

Keywords: *Candida*; Chrome agar; *C. Tropicalis*; *C. Glabrata*

INTRODUCTION

Candida species are yeast-like fungi that typically exist as harmless commensals on mucosal surfaces of the human body, particularly in the gastrointestinal and genitourinary tracts. However, under certain conditions such as immunosuppression, invasive procedures, or antibiotic use, they can become opportunistic pathogens, causing a spectrum of infections ranging from superficial candidiasis to invasive, life-threatening disease. [1–3] The genus *Candida* comprises over 170 species, of which more than 17 are known to cause human infections. [1] *Candida albicans* is traditionally the most common species implicated in candidiasis. However, in recent decades, there has been a notable shift toward non-*albicans* *Candida* (NAC) species, such as *C. tropicalis*, *C. parapsilosis*, *C. glabrata*, and *C. krusei*. [4–7] Among these, *C. tropicalis* is frequently isolated in clinical settings in India. [1, 8] Risk factors for candidiasis include prolonged hospitalisation, the use of indwelling medical devices, immunosuppressive therapy (e.g., corticosteroids, chemotherapy), broad-spectrum antibiotic use, and HIV/AIDS. [1, 9] *C. krusei* is intrinsically resistant to fluconazole, while *C. glabrata* exhibits reduced susceptibility. *C. tropicalis* is noted for its biofilm-forming ability on catheters and prosthetic devices, contributing to its resistance profile. Resistance to azoles, particularly fluconazole, has been increasingly reported in both *C. albicans* and *C. tropicalis*. [1, 10] Species-level identification is crucial due to the varying antifungal resis-

tance patterns and virulence among *Candida* species. [11] Additionally, distinguishing between colonisation and true infection—especially in non-sterile specimens such as sputum or endotracheal secretions—is essential for accurate diagnosis and effective treatment. The present study aims to isolate and identify *Candida* species from a range of clinical specimens in a tertiary care hospital, assess their pathogenic potential, and correlate findings from sugar assimilation testing with CHROMagar identification.

MATERIALS AND METHODS

Study Design and Setting

A retrospective study was conducted in the Department of Microbiology, GBH Hospital, Udaipur, from 16th September 2024 to 16th November 2024, after approval from the Institutional Ethics Committee [AIIMSUDR/2025/6392].

Sample Collection: In the present study, a total of 45 *Candida* isolates were recovered from various clinical specimens. The majority were obtained from urine samples (n=20), followed by sputum (n=12), tracheal swabs (n=6), vaginal swabs (n=6), and endotracheal secretions (n=1).

To address the concern regarding sample quality: Bartlett's grading was performed for sputum samples to ensure specimen adequacy. Endotracheal secretions were evaluated alongside clinical signs, duration of intubation, and radiological findings to differentiate colonization from infection. Although tracheal swabs are not routinely recommended, they were included in this retrospective analysis and are reported with limitations.

Isolation and Culture: Clinical specimens were first examined by Gram stain. Samples were inoculated onto Sabouraud Dextrose Agar (SDA) and incubated at 37°C for 48–72 hours, following standard guidelines for fungal culture to ensure adequate recovery of yeast colonies.

Identification of *Candida* Species

Germ tube test was conducted on all isolates. Germ tube-positive isolates were presumptively identified as *C. albicans* or *C. dubliniensis*.

Cornmeal Agar (CMA) with Tween 80 was used to observe chlamydospores formation, hyphae/Pseudohyphae and colony morphology, which aided in species-level differentiation. Growth at 45°C was used to differentiate *C. albicans* (positive) from *C. dubliniensis* (negative).

Pathogenic vs. Commensal Assessment

The clinical importance of each isolate was evaluated by correlating:

- The site of isolation
- Clinical diagnoses and symptom presentations

- Pertinent laboratory parameters (e.g., pyuria in urine specimens)
- The application of invasive devices (e.g., Foley catheters, central lines) This helped distinguish pathogenic isolates from colonizers or commensals.

Statistical Analysis

Descriptive statistics were used to calculate frequencies and percentages of each *Candida* species by specimen type. Species distribution and association with sample types were evaluated. The correlation between sugar assimilation tests and CHROMagar colony morphology was analysed for accuracy. All data were compiled in Microsoft Excel and analysed using SPSS version 26.0.

Name of <i>Candida</i> species	Colour on CHROM agar
<i>Candida albicans</i>	Light green
<i>Candida tropicalis</i>	Metallic blue
<i>Candida krusei</i>	Rose pink
<i>Candida glabrata</i>	White
<i>Candida parapsilosis</i>	Pale cream
<i>Candida dubliniensis</i>	Dark green

Table 1: Colour characteristics of various *Candida* species on CHROM agar

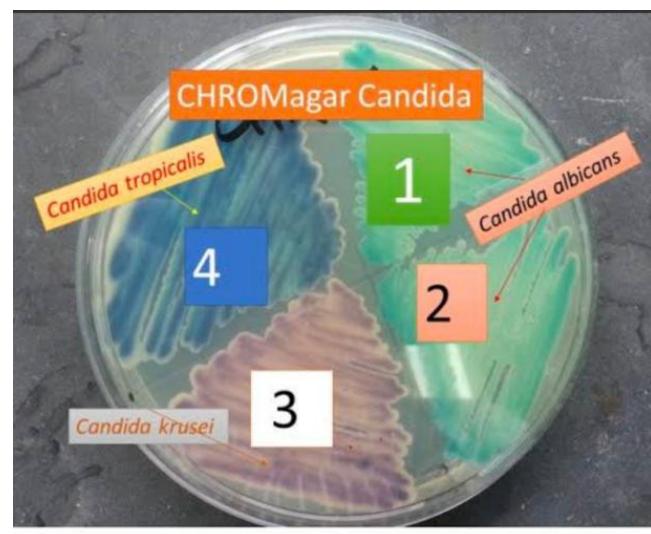


Figure 1: CHROM agar showing growth of different colonies of *Candida* species

RESULTS

C. albicans remained the predominant species, but a significant proportion (44.44%) of isolates were non-*albicans*

Candida species, reflecting an emerging epidemiological trend. The distribution of isolates was as follows: 20 from urine, 12 from sputum, 6 from tracheal swabs, 6 from vaginal swabs, and 1 from endotracheal secretions, as shown in Table 2. All specimens were incubated for 48–72 hours at 37°C as per the recommended guidelines for *Candida* isolation.” Among the 45 *Candida* isolates, 25 (55.56%) were identified as *Candida albicans*, 13 (28.89%) as *Candida tropicalis*, 6 (13.33%) as *Candida glabrata*, and 1 (2.22%) as *Candida krusei*, as shown in Table 3. Gender-wise distribution revealed a higher prevalence in females (n=32) compared to males (n=13). Quality control of sputum specimens was done using Bartlett’s criteria. Isolates from endotracheal secretions and tracheal swabs were interpreted cautiously, with clinical correlation and available patient data considered to differentiate colonisation from infection.

Species identification was primarily performed using CHROMagar *Candida*. However, confirmatory identification by sugar assimilation test was done for representative isolates and correlated well with CHROMagar results, showing good concordance.

Clinical Specimen	Total <i>Candida</i> Isolates (n=45)
Urine	20
Sputum	12
Tracheal Swab	6
Vaginal Swab	6
Endotracheal Secretions	1

Table 2: Number of *Candida* isolates from various clinical specimens

<i>Candida</i> Species	No. of Isolates (%)	Male	Female
<i>Candida albicans</i>	25 (55.56%)	7	18
<i>Candida tropicalis</i>	13 (28.89%)	3	10
<i>Candida glabrata</i>	6 (13.33%)	2	4
<i>Candida krusei</i>	1 (2.22%)	1	0
Total	45	13	32

Table 3: Distribution of different *Candida* species and gender-wise distribution

DISCUSSION

The present study confirms the predominance of *Candida albicans* among clinical isolates, although a significant proportion (44.44%) consisted of non-*albicans* *Candida* (NAC) species, including *C. tropicalis*, *C. glabrata*, and *C. krusei*. This pattern aligns with several recent studies indicating an ongoing epidemiological shift.

A study by Kaup et al. [12] reported 50% NAC isolates, with *C. tropicalis* accounting for 27.08%, closely matching our finding of 28.89%. Previous research by Manjunath et al. (2012) [13], Baradkar et al. (2010) [14], and Usharani et al. (2011) [15] also supports our observation that *C. albicans* remains the most frequently isolated species. However, reports by Istalingam et al. [4] and Jayalakshmi L et al. [16] demonstrated higher NAC isolation rates (56% and 68.57%, respectively), suggesting regional variation and an increasing prevalence of NAC species.

The presence of *Candida* in specimens such as sputum and endotracheal secretions must be interpreted cautiously. In our study, specimen quality was ensured using Bartlett’s scoring for sputum samples, and clinical history, radiological findings, and immune status were considered for invasive samples to distinguish colonisers from true pathogens.

CHROMagar *Candida* proved to be a useful preliminary tool for rapid species identification. However, the absence of Cornmeal Agar (CMA) use is a limitation. Sugar assimilation tests were performed on a subset of isolates and showed concordance with CHROMagar-based identification, supporting its reliability in our setting.

CONCLUSION

This study highlights a growing trend of non-*albicans* *Candida* species in clinical infections, although *Candida albicans* remains predominant. Rapid presumptive identification using CHROMagar *Candida* provides a practical approach, particularly in resource-limited settings. However, incorporating confirmatory methods such as sugar assimilation tests or CMA would enhance diagnostic accuracy.

Accurate species-level identification is essential for guiding antifungal therapy, especially given that some NAC species exhibit intrinsic resistance to commonly used antifungal agents. Furthermore, clinical correlation and appropriate specimen validation are crucial to distinguish colonisation from true infection, particularly in respiratory and urinary samples.

Future studies should include a larger sample size, comprehensive statistical analysis, and clinical outcome data to better understand the pathogenic role of various *Candida* species and their antifungal susceptibility patterns.

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