

Study of Different Species of Candida Using CHROM Agar in Various Clinical Samples

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Abstract

Background: Candidiasis has emerged as a major opportunistic fungal infection associated with significant morbidity and mortality, particularly among hospitalized and immunocompromised patients. In recent years, there has been a notable shift from *Candida albicans* to non-*albicans* *Candida* species, many of which exhibit reduced susceptibility to commonly used antifungal agents. Rapid and accurate species-level identification is therefore essential for timely initiation of appropriate antifungal therapy. Chromogenic media such as CHROM agar offer a simple and rapid alternative to conventional identification methods.

Objectives: To determine the species distribution of *Candida* isolates obtained from various clinical samples and to evaluate the diagnostic utility of CHROM agar for rapid speciation in comparison with conventional methods.

Materials and Methods: This hospital-based observational analytical study was conducted in the Department of Microbiology, RNT Medical College, Udaipur, over a period of six months (August 2023 to January 2024). A total of 150 non-repetitive *Candida* isolates obtained from 7,800 clinical samples were included. Samples were processed using standard mycological techniques including direct microscopy, culture on Sabouraud dextrose agar, germ tube test, corn meal agar morphology, and CHROM agar. Species identification was based on colony morphology and characteristic color reactions. Corn meal agar was considered the reference standard. Data were analyzed using descriptive statistics, and the sensitivity and specificity of CHROM agar were calculated.

Results: The overall isolation rate of *Candida* species was 1.9%. The highest number of infections occurred in the 0–20-year age group, with a male predominance. *Candida albicans* was the most common species (57.3%), while non-*albicans* *Candida* species accounted for 42.7% of isolates. Urine (42%) and blood (36.7%) were the most frequent clinical specimens. CHROM agar demonstrated high sensitivity and specificity for the identification of major *Candida* species, particularly *C. albicans* and *C. tropicalis*, with results available within 24–48 hours.

Conclusion: CHROM agar is a reliable, rapid, and cost-effective method for presumptive identification of clinically important *Candida* species and is well suited for routine use in clinical microbiology laboratories.

Keywords: *Candida*; CHROM agar; Non-*albicans* *Candida*; Candidiasis; Speciation; Clinical samples.

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Introduction

Opportunistic yeasts belonging to the genus *Candida* are increasingly recognized as important human pathogens and are associated with significant morbidity and mortality worldwide. These organisms are capable of producing a wide spectrum of clinical manifestations, ranging from superficial infections involving the skin, nails, and mucous membranes to severe invasive and disseminated candidiasis affecting multiple organ systems. *Candida* species rank among the leading causes of nosocomial infections and have emerged as major contributors to bloodstream infections in

hospitalized patients. They are currently reported as the fifth most common cause of bloodstream infections globally [1] and the fourth most common cause of hospital-acquired bloodstream infections. In routine clinical microbiology practice, *Candida* species are frequently isolated from fungal cultures of throat, urine, and genital (TUG) specimens. Over the past four decades, the incidence of *Candida* infections has shown a steady and alarming increase. This rise is particularly evident in healthcare settings and is largely attributed to advances in medical care that have led to prolonged survival of critically ill

patients, increased use of invasive medical devices, broad-spectrum antibiotics, immunosuppressive therapies, and intensive care interventions. Importantly, there has been a notable epidemiological shift in the spectrum of *Candida* infections, with non-albicans *Candida* species constituting an increasing proportion of nosocomial infections. The growing burden of fungal infections is of particular concern in the context of the expanding population of immunocompromised individuals, including patients with malignancies, transplant recipients, individuals with HIV/AIDS, and those receiving long-term corticosteroids or immunosuppressive drugs [2].

Candidiasis, also known as moniliasis, refers to infections caused by *Candida* species, with *Candida albicans* being the most commonly implicated pathogen. These infections can occur at virtually any anatomical site but are most frequently observed in warm and moist areas such as skin folds, digital web spaces, genital regions, cuticles, and the oral mucosa. The clinical presentation of candidiasis varies widely depending on the site of infection and the host's immune status. *Candida* species are ubiquitous yeasts that normally colonize the skin and mucosal surfaces of healthy individuals without causing disease. However, under favorable conditions such as increased moisture, heat, and impaired local or systemic host defenses, these commensal organisms can transform into opportunistic pathogens and cause clinical infection.

Several predisposing factors have been identified that increase the risk of developing candidiasis. These include hot and humid weather conditions, restrictive or occlusive clothing, poor personal hygiene, and infrequent diaper or undergarment changes, particularly in children and elderly individuals. Disruption of normal microbial flora following prolonged or inappropriate antibiotic therapy also plays a significant role in facilitating *Candida* overgrowth. Inflammatory skin conditions such as psoriasis, especially when involving intertriginous areas, further predispose individuals to candidal infections. Additionally, a wide range of systemic factors contribute to susceptibility, including immunosuppression resulting from corticosteroids and other immunosuppressive drugs, pregnancy, diabetes mellitus, endocrine disorders such as Cushing's disease, hypoadrenalism, and hypothyroidism, hematological disorders, and defects in T-cell-mediated immunity [3]. Depending on the immune status of the host and the presence of underlying risk factors, *Candida* species can produce cutaneous, mucocutaneous, and systemic infections of varying severity.

Historically, *Candida albicans* was responsible for more than 80% of all *Candida* isolates recovered from nosocomial yeast infections during the 1980s. Even in more recent reviews, *C. albicans* continues

to account for a substantial proportion of *Candida* bloodstream isolates. However, the emergence of *Candida* species other than *C. albicans* has become a matter of significant concern across several major healthcare institutions [3–6]. Non-albicans *Candida* species such as *Candida krusei*, *Candida glabrata*, and *Candida tropicalis* are being isolated with increasing frequency worldwide. These species are particularly important from a clinical perspective because many of them exhibit reduced susceptibility or intrinsic resistance to commonly used antifungal agents, thereby complicating therapeutic management.

The changing epidemiology of candidiasis, coupled with the availability of newer antifungal drugs with differing antifungal spectra, has underscored the importance of accurate and timely species-level identification of *Candida* isolates. Reliance on broad identification of fungi as “yeasts” is no longer sufficient for guiding effective antifungal therapy. Conventional methods for yeast identification, which are primarily based on carbohydrate assimilation and fermentation profiles, are often labor-intensive, time-consuming, and require technical expertise that may not be readily available in routine or resource-limited laboratory settings. Delays in species identification can result in inappropriate antifungal therapy, leading to poor clinical outcomes, especially in critically ill or immunocompromised patients.

Effective management of candidiasis depends on early diagnosis and prompt initiation of appropriate antifungal treatment. In response to the limitations of traditional identification methods, a variety of newer isolation and identification media have been developed that allow for more rapid detection of fungal pathogens, with turnaround times ranging from 4 to 72 hours depending on the system used. Among these, chromogenic media have gained widespread acceptance for the rapid presumptive identification of yeast species. These media contain chromogenic substrates that react with specific enzymes produced by different microorganisms, resulting in colonies with distinct colors and morphological characteristics. Such species-specific reactions enable direct identification of organisms to the species level based on colony color and appearance.

The utility of selective and differential media for the isolation and identification of *Candida* species has been well documented [7–8]. CHROM agar is a selective chromogenic medium designed specifically for the isolation and differentiation of clinically important *Candida* species. On this medium, yeast cells produce species-specific enzymes that interact with chromogenic substrates, leading to the formation of colonies with characteristic colors. According to the manufacturer and multiple supporting studies, CHROM agar can

reliably differentiate *C. albicans*, which produces light to medium green colonies; *C. tropicalis*, which forms steel blue colonies often accompanied by a purple pigment diffusing into the surrounding agar; and *C. krusei*, which appears as large, fuzzy, rose-colored colonies with white edges after incubation at 37°C for 48 hours. Other yeast species typically produce cream-colored or light- to dark-pink colonies. An additional advantage of CHROM agar is its ability to facilitate the detection of multiple *Candida* species from polymicrobial specimens, thereby allowing rapid and direct identification without the need for extensive subculturing. This significantly reduces the time required to obtain diagnostic results and supports earlier initiation of targeted antifungal therapy. The use of chromogenic media in clinical microbiology laboratories has been shown to be technically simple, less time-consuming, and cost-effective, making it particularly suitable for routine diagnostic use [9].

In light of the increasing clinical importance of candidiasis, the rising prevalence of non-*albicans* *Candida* species, and the need for rapid and reliable diagnostic methods, the present study was undertaken to determine the species-level distribution of *Candida* isolates obtained from various clinical samples using CHROM agar. The findings of this study are expected to provide valuable insights for clinicians and microbiologists, facilitating improved diagnosis, appropriate antifungal therapy, and better patient outcomes.

Materials and Methods

Study Design and Setting: This was a hospital-based observational analytical study conducted in the Department of Microbiology, RNT Medical College, Udaipur, Rajasthan, over a period of six months from August 2023 to January 2024.

Study Population: The study included patients of all age groups and both sexes attending outpatient and inpatient departments of RNT Medical College, Udaipur, with clinical suspicion of candidiasis.

Inclusion Criteria

- All clinical samples yielding *Candida* isolates received in the Department of Microbiology during the study period.
- Samples obtained from patients with suspected candidiasis.

Exclusion Criteria

- Samples yielding bacterial isolates.
- Yeast isolates other than *Candida* species.

Sample Size: A total of 150 non-repetitive clinical isolates of *Candida* were included in the study. The sample size was calculated based on an assumed isolation rate of approximately 10% from various clinical samples, with a 90% confidence interval and

5% absolute error, yielding a calculated sample size of 138. To compensate for possible sample loss or contamination, the sample size was rounded up to 150.

Types of Clinical Samples: The clinical specimens included Urine, Blood, Sputum, Wound swab/pus, Vaginal swab, Tracheal fluid Peritoneal fluid, Ascitic fluid, Pleural fluid, Endotracheal aspirate, Gastric lavage.

Specimen Collection and Transport: All clinical specimens were collected under strict aseptic precautions following standard protocols. Swab samples were obtained by firmly swabbing the lesion site using sterile cotton swabs. Liquid specimens such as urine, blood, and body fluids were collected in sterile containers. All samples were transported immediately to the Microbiology laboratory and processed without delay.

Laboratory Processing of Samples

Direct Microscopic Examination: Each specimen was subjected to preliminary microscopic examination using

- Gram staining to demonstrate budding yeast cells and pseudohyphae
- 10–20% potassium hydroxide (KOH) mount, with or without dimethyl sulfoxide (DMSO), for rapid detection of fungal elements

The presence of budding yeast cells and pseudohyphae was considered suggestive of candidiasis.

Culture and Isolation: All samples were inoculated onto Sabouraud Dextrose Agar (SDA) supplemented with chloramphenicol to inhibit bacterial growth. The inoculated media were incubated at 37°C for 24–48 hours and observed for colony growth. Yeast-like colonies were further processed for species identification.

Identification of *Candida* Species

Germ Tube Test: Presumptive identification of *Candida albicans* was performed using the germ tube test. A small portion of a yeast colony was inoculated into human or horse serum and incubated at 37°C for 2–4 hours. A wet mount was prepared and examined microscopically for the presence of germ tubes. Isolates showing germ tube formation were considered presumptive *Candida albicans* or *Candida dubliniensis*.

Corn Meal Agar (CMA) Morphology: Further species identification was performed using corn meal agar with Tween 80 by the Dalmau plate technique. The inoculated plates were incubated at 25–30°C for 48–72 hours and examined microscopically for the presence of pseudohyphae, blastoconidia, and chlamydospores. Species

identification was made based on characteristic morphological features.

CHROM Agar: All confirmed *Candida* isolates were subcultured onto CHROM agar plates and incubated at 37°C for 24–48 hours. Species identification was performed based on the distinctive color and morphology of colonies:

- *Candida albicans* – light green colonies
- *Candida tropicalis* – blue to metallic blue colonies
- *Candida krusei* – purple, fuzzy colonies
- *Candida glabrata* – cream to white colonies

CHROM agar was used as a rapid and presumptive method for speciation of *Candida* isolates.

Quality Control: Standard laboratory procedures were followed throughout the study. Known reference strains of *Candida* species were used periodically to ensure the accuracy and reliability of culture and identification methods.

Statistical Analysis: Data were entered into Microsoft Excel and analyzed using appropriate

statistical methods. Results were expressed as frequencies and percentages. Corn meal agar morphology was considered the reference standard for comparison. The sensitivity and specificity of CHROM agar for identification of major *Candida* species were calculated.

Ethical Considerations: The study was conducted after obtaining approval from the institutional authorities. Patient confidentiality was maintained, and no personal identifiers were recorded. As the study was laboratory-based and involved routine diagnostic samples, informed consent was waived.

Results

During the study period, a total of 7,800 clinical samples were processed, from which 150 non-repetitive *Candida* isolates were obtained, giving an overall isolation rate of 1.9%. *Candida* species constituted 1.9% of all processed samples, reflecting their clinically important role as opportunistic pathogens in hospital settings. Table 1 shows the overall isolation rate of *Candida* species.

Table 1: Overall Isolation Rate of Candida Species

Parameter	Number	Percentage (%)
Total samples processed	7,800	100
Total <i>Candida</i> isolates	150	1.9
Non- <i>Candida</i> samples	7,650	98.1

The highest number of *Candida* infections occurred in the 0–20 year age group (43.3%), followed by the 21–40 year group (32.7%). Overall, male predominance (54%) was observed. Table 2 shows the age and sex distribution of patients with *Candida* infection.

Table 2: Age and Sex Distribution of Patients with Candida Infection (N = 150)

Age group (years)	Male n (%)	Female n (%)	Total n (%)
0–20	31 (20.7)	34 (22.6)	65 (43.3)
21–40	33 (22.0)	16 (10.7)	49 (32.7)
41–60	13 (8.7)	15 (10.0)	28 (18.7)
>60	4 (2.7)	4 (2.7)	8 (5.3)
Total	81 (54.0)	69 (46.0)	150 (100)

Candida albicans was the predominant isolate (57.3%), while non-*albicans* *Candida* species collectively accounted for 42.7%, indicating a significant epidemiological shift. Table 3 shows the species distribution of *Candida* isolates by different identification methods.

Table 3: Species Distribution of Candida Isolates by Different Identification Methods (N = 150)

<i>Candida</i> species	Germ tube test n (%)	Corn meal agar n (%)	CHROM agar n (%)
<i>C. albicans</i>	86 (57.3)	77 (51.3)	86 (57.3)
<i>C. tropicalis</i>	—	33 (22.0)	29 (19.3)
<i>C. glabrata</i>	—	26 (17.3)	24 (16.0)
<i>C. krusei</i>	—	14 (9.4)	11 (7.4)
Total	150 (100)	150 (100)	150 (100)

All *Candida* species were most frequently isolated from patients aged 0–20 years, with *C. albicans* predominating across all age groups. Table 4 shows the age-wise distribution of *Candida* species.

Table 4: Age-wise Distribution of Candida Species (N = 150)

Species	0–20 yrs n (%)	21–40 yrs n (%)	41–60 yrs n (%)	>60 yrs n (%)	Total n (%)
<i>C. albicans</i>	34 (22.7)	29 (19.3)	19 (12.7)	4 (2.7)	86 (57.3)
<i>C. tropicalis</i>	13 (8.7)	9 (6.0)	4 (2.7)	3 (2.0)	29 (19.3)
<i>C. glabrata</i>	14 (9.3)	7 (4.7)	3 (2.0)	0 (0)	24 (16.0)
<i>C. krusei</i>	4 (2.7)	4 (2.7)	2 (1.3)	1 (0.7)	11 (7.4)
Total	65 (43.3)	49 (32.7)	28 (18.7)	8 (5.3)	150 (100)

The most common clinical specimen was urine (42%), followed by blood (36.7%), highlighting the importance of candiduria and candidemia in hospitalized patients. Table 5 shows the distribution of candida species in various clinical samples.

Table 5: Distribution of Candida Species in Various Clinical Samples (N = 150)

Clinical sample	<i>C. albicans</i> n (%)	<i>C. tropicalis</i> n (%)	<i>C. glabrata</i> n (%)	<i>C. krusei</i> n (%)	Total n (%)
Urine	37 (24.7)	12 (8.0)	10 (6.7)	4 (2.6)	63 (42.0)
Blood	26 (17.3)	13 (8.7)	12 (8.0)	4 (2.7)	55 (36.7)
Sputum	13 (8.7)	1 (0.7)	0 (0)	2 (1.3)	16 (10.7)
Peritoneal fluid	2 (1.3)	0 (0)	1 (0.7)	0 (0)	3 (2.0)
Tracheal fluid	3 (2.0)	0 (0)	0 (0)	0 (0)	3 (2.0)
Endotracheal aspirate	1 (0.7)	2 (1.3)	0 (0)	0 (0)	3 (2.0)
Pus	1 (0.7)	0 (0)	0 (0)	1 (0.7)	2 (1.3)
Others*	3 (2.0)	1 (0.7)	1 (0.7)	0 (0)	5 (3.3)
Total	86 (57.3)	29 (19.3)	24 (16.0)	11 (7.4)	150 (100)

*Others include vaginal swab, ascitic fluid, pleural fluid, and gastric lavage.

CHROM agar demonstrated high sensitivity and excellent specificity, particularly for *C. albicans* and *C. tropicalis*, supporting its usefulness as a rapid and reliable diagnostic tool. Table 6 shows the diagnostic performance of CHROM agar compared with corn meal agar.

Table 6: Diagnostic Performance of CHROM Agar Compared with Corn Meal Agar (Reference Standard)

Species	CMA identified n	CHROM agar identified n	Sensitivity (%)	Specificity (%)
<i>C. albicans</i>	77	86	100.0	87.0
<i>C. tropicalis</i>	33	29	87.9	100.0
<i>C. glabrata</i>	26	24	92.3	100.0
<i>C. krusei</i>	14	11	78.6	100.0

Discussion

The present study was undertaken to evaluate the distribution of Candida species isolated from various clinical samples and to assess the utility of CHROM agar for rapid species identification in a tertiary care hospital setting. In the present study, Candida species were isolated in 1.9% of the total 7,800 clinical samples processed. This isolation rate is comparable to earlier hospital-based studies conducted in similar tertiary care settings, where Candida isolation rates ranged from 1% to 3% [10,11]. Although the overall percentage appears low, the clinical significance of Candida infections is substantial due to their association with severe morbidity, prolonged hospital stay, and increased mortality, particularly among immunocompromised and critically ill patients [12]. The findings reinforce the role of Candida species as important opportunistic pathogens in hospitalized populations.

The present study demonstrated a male predominance (54%), which is consistent with observations reported by Deorukhkar et al. and

Falguni et al., who also documented higher isolation rates among male patients [13,14]. In contrast, some studies have reported female predominance, particularly in genital and urinary tract candidiasis [15]. Age-wise analysis revealed that the 0–20 year age group (43.3%) constituted the largest proportion of cases, followed by the 21–40 year age group. Similar age distribution patterns have been reported by Arora et al., where pediatric and young adult populations showed higher susceptibility to candidiasis [16]. Increased vulnerability in younger age groups may be attributed to immature immune responses in children and higher exposure to risk factors such as antibiotic use and hospitalization.

In the present study, *Candida albicans* was the predominant species (57.3%), while non-*albicans* Candida (NAC) species collectively accounted for 42.7% of isolates. This finding is in agreement with several Indian studies reporting *C. albicans* as the most common isolate, followed by *C. tropicalis* and *C. glabrata* [11,17].

However, the substantial proportion of NAC species observed is clinically significant and reflects a changing epidemiological trend. Increasing isolation of NAC species has been attributed to widespread antifungal use, prolonged hospitalization, and selective pressure favoring intrinsically resistant species such as *C. glabrata* and *C. krusei* [18]. This shift underscores the importance of species-level identification rather than treating *Candida* as a single entity.

Analysis of age-wise distribution showed that all major *Candida* species, including *C. albicans* and NAC species, were most frequently isolated in the 0–20 year age group. *C. albicans* predominated across all age groups, a finding consistent with previous studies by Rajkumari et al. and Nazir et al. [15,19]. The notable presence of NAC species such as *C. tropicalis* and *C. glabrata* in younger patients highlights the need for early identification and appropriate antifungal selection, as these species may exhibit reduced susceptibility to commonly used azoles.

In the present study, urine (42%) was the most common clinical specimen yielding *Candida* isolates, followed by blood (36.7%). These findings are consistent with multiple studies reporting urine and blood as the predominant sample types for *Candida* isolation in hospital settings [13,14,16].

High isolation rates from urine samples may reflect increased use of urinary catheters, diabetes mellitus, and prolonged antibiotic therapy. Similarly, the significant proportion of blood isolates indicates the rising incidence of candidemia, which is associated with high mortality if not promptly diagnosed and treated [12]. The distribution of species across various samples further emphasizes the invasive potential of both *C. albicans* and NAC species. The diagnostic performance of CHROM agar in the present study showed high sensitivity and specificity, particularly for *C. albicans*, *C. tropicalis*, and *C. glabrata*. Comparable results have been reported by Baradkar et al. and Manisha et al., who demonstrated sensitivity and specificity values exceeding 90% for major *Candida* species using chromogenic media [20,21].

CHROM agar provided rapid presumptive identification within 24–48 hours, significantly reducing turnaround time compared to conventional methods such as corn meal agar morphology and biochemical testing. The minor discrepancies observed may be attributed to variations in enzyme expression and overlapping colony colors among less common species. Nonetheless, CHROM agar remains a simple, cost-effective, and reliable tool for routine laboratory use, especially in resource-limited settings.

Conclusion

The present study highlights the growing clinical importance of candidiasis and the changing epidemiological trends in *Candida* infections in a tertiary care hospital setting. *Candida albicans* remained the predominant species; however, a substantial proportion of infections were caused by non-*albicans Candida* species, underscoring an important epidemiological shift.

Urine and blood were the most common clinical specimens yielding *Candida* isolates, reflecting the rising burden of candiduria and candidemia among hospitalized patients. CHROM agar proved to be a rapid, simple, and cost-effective method for presumptive species-level identification of common *Candida* species, demonstrating high sensitivity and specificity when compared with corn meal agar.

Early and accurate speciation is essential for guiding appropriate antifungal therapy, especially in view of the increasing antifungal resistance among non-*albicans Candida*. Routine use of CHROM agar in clinical microbiology laboratories can significantly improve diagnostic efficiency and patient management.

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