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Isolate and Speciate Candida from Various Clinical Samples Using Hicrom Agar and to Detect the Antifungal Susceptibility of the Isolates: A Systematic Review

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Abstract:
Background: Candidiasis has emerged as a leading cause of opportunistic infections globally, particularly affecting immunocompromised patients. The epidemiological shift toward non-albicans Candida (NAC) species, combined with rising antifungal resistance, presents a significant clinical challenge. Accurate species identification and susceptibility profiling are critical for optimising therapeutic outcomes.
Objectives: To isolate and speciate Candida from diverse clinical specimens using Hicrome differential agar and to determine antifungal susceptibility profiles of all isolates against six agents.
Methods: A prospective, observational study was conducted over six months (August 2025–January 2026) at the Mycology Laboratory, Bhandari Hospital and Research Centre, Gurgaon. A total of 179 Candida isolates recovered from 179 patients were speciated using Hicrome agar and the germ tube test. Antifungal susceptibility was evaluated via disk diffusion per CLSI M44-A2 guidelines for fluconazole, itraconazole, amphotericin B, clotrimazole, ketoconazole, and voriconazole.
Results: Female patients accounted for 53% of cases. The predominant age group affected was 61–70 years (19.5%). C. albicans constituted 42% of isolates, while NAC species totalled 58% — C. glabrata (23%), C. krusei (18%), and C. tropicalis (16%). Urine was the most frequent specimen source (66%), followed by sputum (12%) and blood (11%). The Intensive Care Unit contributed 49.1% of isolates. C. albicans demonstrated 100% susceptibility to voriconazole, amphotericin B, and itraconazole. All isolates were fully susceptible to amphotericin B; the lowest susceptibility was observed for fluconazole (89.9% overall).
Conclusion: NAC species have surpassed C. albicans as predominant pathogens. Hicrome agar offers a rapid, cost-effective speciation method. Amphotericin B and voriconazole retain excellent activity. Routine susceptibility testing is indispensable for guiding empirical therapy.
Keywords: Candidiasis; Hicrome agar; non-albicans Candida; antifungal susceptibility; disk diffusion; CLSI; immunocompromised host; ICU.

1. INTRODUCTION

Candida species are dimorphic fungi that colonise the gastrointestinal tract, oropharynx, vagina, and skin of healthy individuals as commensals. Under conditions of altered host immunity, physiological disruption, or prolonged medical intervention, these fungi transition to an opportunistic pathogenic state, causing a spectrum of clinical syndromes ranging from superficial mucosal infections to life-threatening systemic disease. [1,2]

The genus Candida, classified within the kingdom Fungi (phylum Ascomycota, family Saccharomycetaceae), encompasses over 200 recognised species, of which fewer than ten are of primary clinical significance. Historically, Candida albicans was responsible for the majority of human candidiasis; however, epidemiological surveillance data accumulated over the past three decades document a pronounced shift toward non-albicans Candida (NAC) species, including C. glabrata, C. tropicalis, C. krusei, and C. parapsilosis. [3,4]

Several factors underpin this epidemiological transition. The expanding population of immunocompromised patients — resulting from HIV/AIDS, haematological malignancies, solid organ transplantation, and prolonged corticosteroid therapy — provides an expanded reservoir of susceptible hosts. Simultaneously, the widespread prophylactic and empirical use of azole antifungals, particularly fluconazole, has exerted selective pressure that favours intrinsically resistant or acquired-resistance species. [5,6]

Intensive care units (ICUs) have emerged as the primary clinical environment for nosocomial candidaemia. Central venous catheters, mechanical ventilation, broad-spectrum antibacterial therapy, parenteral nutrition, and prolonged hospitalisation collectively constitute a convergent risk milieu. Candidaemia in ICU patients carries a crude mortality rate of 30–60%, and inappropriate initial antifungal therapy is independently associated with increased mortality. [7,8]

Candida albicans expresses several well-characterised virulence determinants, including germ tube and hyphal formation, secreted aspartyl proteinases, phospholipases, phenotypic switching, and multi-layered biofilm architecture. Biofilm formation is of particular clinical relevance because it confers a 100- to 1000-fold reduction in antifungal susceptibility relative to planktonic cells and provides protection against host immune effectors. NAC species exhibit variable biofilm-forming capacity and distinct virulence profiles that complicate treatment decisions. [9,10]

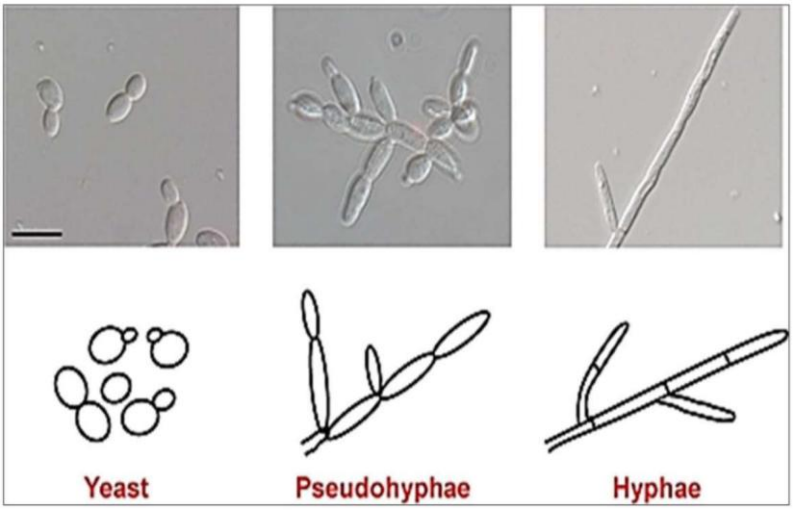

Figure 1: Major Morphological Forms of Candida spp. — Yeast (blastoconidia), Pseudohyphae, and True Hyphae (adapted from original thesis)

Traditional Candida identification relied on morphological observation, the germ tube test, sugar assimilation and fermentation profiles, and chromogenic agar. Modern chromogenic media such as HiCrome Candida Differential Agar leverage species-specific enzymatic activities to produce colourimetrically distinct colony phenotypes, facilitating presumptive species-level identification within 48 hours without specialised equipment. This represents a practical advantage over molecular methods in resource-limited laboratory settings. [11,12]

Antifungal susceptibility testing (AFST) has assumed greater clinical importance as resistance patterns diversify. The Clinical and Laboratory Standards Institute (CLSI) M44-A2 disk diffusion methodology provides a validated, reproducible, and affordable approach to AFST for yeast isolates, enabling categorisation of isolates as susceptible, susceptible dose-dependent, or resistant. Tracking institutional susceptibility data informs empirical prescribing guidelines and antimicrobial stewardship programmes. [13]

MSW MANAGEMENT -Multidisciplinary, Scientific Work and Management Journal
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The present study was undertaken to determine the prevalence and species distribution of Candida isolates recovered from diverse clinical specimens at a tertiary care centre, to evaluate the utility of HiCrome agar for speciation, and to generate a comprehensive antifungal susceptibility dataset that can inform local treatment protocols.

2. MATERIALS AND METHODS

2.1 Study Design and Setting: A prospective, descriptive, observational study was conducted over six consecutive months, from August 2025 to January 2026, at the Mycology Laboratory, Department of Microbiology, Bhandari Hospital and Research Centre, Gurgaon, India. Ethical approval was obtained from the Institutional Ethics Committee, and written informed consent was secured from all participants before enrolment.

2.2 Sample Size Determination: The sample size was calculated using the formula for estimating a proportion with a finite population correction, incorporating a sensitivity estimate of 96% for amphotericin B against Candida isolates [14], a margin of error of 5%, and a 95% confidence interval, yielding a minimum requirement of 179 samples. The formula applied was:

$$n = [Z^2\alpha/2 \times Se \times (1 - Se)] / [d^2 \times (1 - p)] = 178.81 \approx 179$$

Where Zα/2 = 1.96, Se = 0.96, d = 0.05, and p = 0.67.

2.3 Specimen Collection and Processing: Specimens were collected under strict aseptic conditions from patients with clinical suspicion of candidiasis attending outpatient, inpatient, and critical care settings. Specimen types included midstream urine, venous blood, sputum (induced or expectorated), high vaginal swabs, pus from surgical sites, endotracheal (ET) secretions, tracheal aspirates, and wound swabs. All samples were transported promptly to the laboratory and processed within two hours of collection, or refrigerated at 4°C if delay was unavoidable.

2.4 Direct Microscopy: Each specimen was subjected to wet mount examination using 10% potassium hydroxide (KOH) to visualise budding yeast cells with or without pseudohyphae. Gram-stained smears were prepared to identify Gram-positive budding yeast cells and to assess pus cell counts and epithelial cell contamination.

2.5 Culture and Isolation: All specimens were inoculated onto Sabouraud Dextrose Agar (SDA) supplemented with chloramphenicol (0.05 g/L) to suppress bacterial overgrowth. Plates were incubated aerobically at both 25°C and 37°C and examined daily for up to 21 days. Creamy, smooth, convex, off-white colonies appearing within 24–72 hours were provisionally identified as Candida species. Blood cultures were processed using the BD BACTEC™ FX40 automated system; positive alerts triggered subculture onto SDA with chloramphenicol and cycloheximide.

2.6 Species Identification

2.6.1 Germ Tube Test: A suspension of each yeast isolate was prepared in 0.5 mL of human or bovine serum and incubated at 37°C for 2–3 hours. A drop was examined microscopically for germ tube formation, which was used to provisionally differentiate C. albicans and C. dubliniensis from other Candida species. True germ tubes appear as lateral projections with no constriction at the point of attachment to the parent yeast cell. [15]

2.6.2 HiCrome Candida Differential Agar: All isolates were subcultured onto HiCrome™ Candida Differential Agar (HiMedia Laboratories, Mumbai) and incubated at 37°C for 24–48 hours per the manufacturer's instructions. Colony identification was based on characteristic colourimetric reactions: C. albicans — light green smooth colonies; C. dubliniensis — dark green smooth colonies; C. tropicalis — metallic blue raised colonies; C. glabrata — cream to white smooth colonies; C. krusei — purple fuzzy colonies; C. guilliermondii — light pink to pink colonies; C. parapsilosis — light pink colonies. The chromogenic principle relies on substrate-specific hexosaminidase enzymes incorporated into the medium. [16]

2.7 Antifungal Susceptibility Testing: Disk diffusion susceptibility testing was performed in accordance with CLSI document M44-A2 guidelines for yeasts. [13] Mueller-Hinton agar supplemented with 2% glucose and 0.5 µg/mL methylene blue was prepared, poured to a uniform depth of 4 mm, and stored at 2–8°C until use. An inoculum suspension equivalent to 0.5 McFarland turbidity standard (1×10^6 to 5×10^6 cells/mL) was prepared from 24-hour-old colonies in 0.85% sterile saline and inoculated onto supplemented Mueller-Hinton agar plates by uniform swabbing in three directions.

Antifungal disks were applied within 15 minutes of inoculation and incubated at 35°C ± 2°C for 20–24 hours. Zone diameters were measured to the nearest whole millimetre under reflected light against a black background. Interpretation criteria applied were: fluconazole (10 µg) — R ≤14, S-DD 15–18, S ≥19 mm; itraconazole (10 µg) — R ≤9, S-DD 10–15, S ≥16 mm; amphotericin B (20 µg) — R ≤9, S-DD 10–14, S ≥15 mm; clotrimazole (10 µg) — R ≤11, S-DD 12–19, S ≥20 mm; ketoconazole (10 µg) — R ≤22, S-DD 23–29, S ≥30 mm; voriconazole (1 µg) — R ≤13, S-DD 14–16, S ≥17 mm.

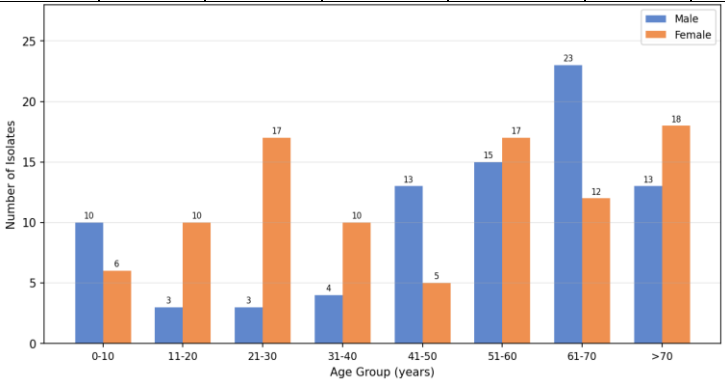
2.8 Statistical Analysis: Data were entered into Microsoft Excel 2019 and analysed using SPSS version 26.0 (IBM Corporation, Armonk, NY). Descriptive statistics — frequency, percentage, mean, and standard deviation — were calculated for all categorical and continuous variables. Proportions between groups were compared using the two-proportion z-test. A p-value of <0.05 was considered statistically significant for all comparisons.

3. RESULTS

3.1 Demographic Characteristics: A total of 179 Candida isolates were recovered and analysed during the study period. Female patients contributed a greater proportion of isolates (94/179; 53%) compared with male patients (85/179; 47%), consistent with the higher susceptibility of women to urogenital candidiasis (Table 1, Graph 1). Infection burden was highest in the elderly population: the 61–70-year age group accounted for 35 isolates (19.5%), followed by patients above 70 years (31 isolates; 17.3%) and the 51–60-year cohort (32 isolates; 17.8%). The lowest case burden was observed in the 11–20-year group (13 isolates; 7.2%). The mean age of affected patients was 49.8 ± 21.3 years.

Table 1: Age and Gender Distribution of Candida Isolates (n=179)

Age Group (yrs)	Male (n)	Male (%)	Female (n)	Female (%)	Total (n)	Total (%)
0–10	10	5.58	6	3.35	16	8.9
11–20	3	1.67	10	5.58	13	7.2
21–30	3	1.67	17	9.49	20	11.1
31–40	4	2.23	10	5.58	14	7.8
41–50	13	7.26	5	2.79	18	10.0
51–60	15	8.37	17	9.49	32	17.8
61–70	23	12.84	12	6.70	35	19.5
>70	13	7.26	18	10.05	31	17.3
Total	**84**	**46.93**	**95**	**53.07**	**179**	**100**



Graph 1: Age and Gender Distribution of Candida Isolates (n=179)

MSW MANAGEMENT -Multidisciplinary, Scientific Work and Management Journal
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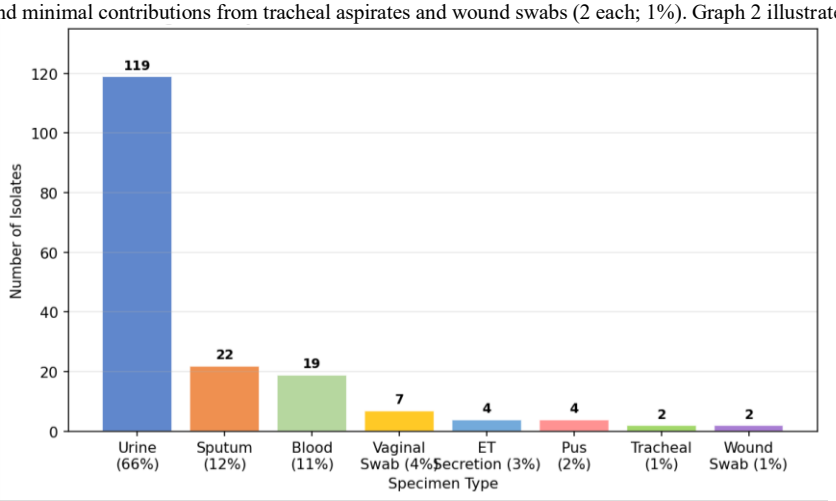
Vol. 36 Issue 1s, 2026, Pages: 4231-4235

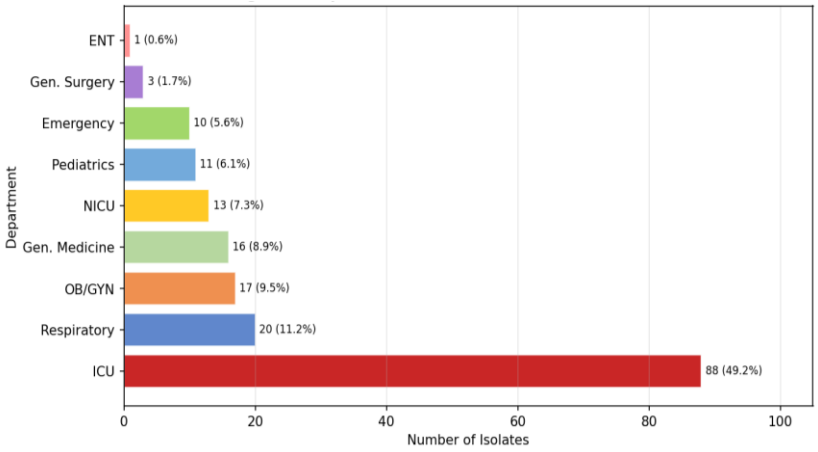
3.2 Inpatient versus Outpatient Distribution: The vast majority of isolates originated from inpatient settings (172/179; 96%), emphasising the strong association between hospitalisation and Candida colonisation or infection. Outpatient sources contributed only 7 isolates (4%), reflecting the predominantly nosocomial nature of this pathogen in clinical practice.

3.3 Specimen-wise Distribution: Urine constituted the dominant specimen type (119/179; 66%), consistent with the high prevalence of candiduria in catheterised and hospitalised patients. Sputum was the second most frequent source (22/179; 12%), followed by blood (19/179; 11%), vaginal swabs (7/179; 4%), ET secretions (4/179; 3%), pus (4/179; 2%), and minimal contributions from tracheal aspirates and wound swabs (2 each; 1%). Graph 2 illustrates the specimen distribution.



Graph 2: Specimen-wise Distribution of Candida Isolates (n=179)

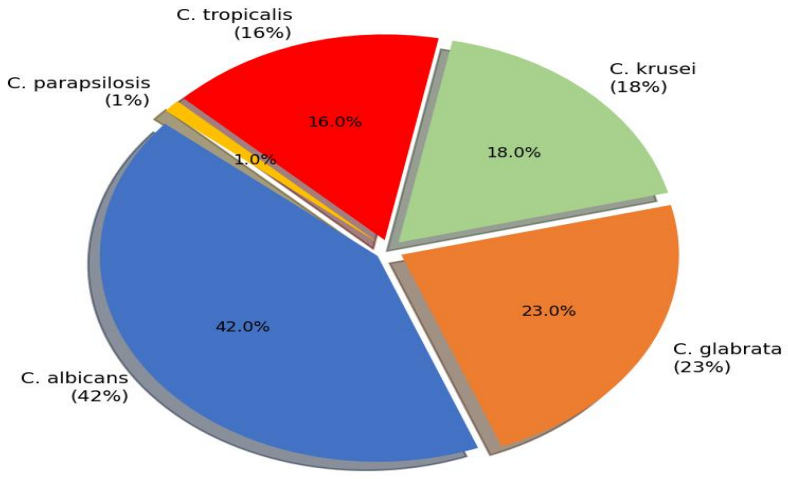
3.4 Department-wise Distribution: The ICU was the largest source of isolates (88/179; 49.1%), underscoring the association between critical illness and fungal superinfection. Respiratory wards contributed 20 isolates (11.1%), the Obstetrics and Gynaecology department provided 17 (9.4%), and the General Medicine ward yielded 16 (8.9%). Further contributions included the NICU (13; 7.2%), Paediatrics (11; 6.1%), Emergency (10; 5.5%), General Surgery (3; 1.6%), and ENT (1; 0.5%). Graph 3 presents department-wise distribution.



Graph 3: Department-wise Distribution of Candida Isolates (n=179)

3.5 Distribution of Candida Species

Among the 179 isolates, C. albicans was the most frequently recovered species (76/179; 42.5%). NAC species collectively predominated at 57.5% (103/179). Within the NAC group, C. glabrata was the most prevalent (41/179; 22.9%), followed by C. krusei (32/179; 17.9%) and C. tropicalis (29/179; 16.2%). C. parapsilosis was recovered in a single case (0.6%). Graph 4 displays the species distribution, and Table 2 summarises isolate counts.



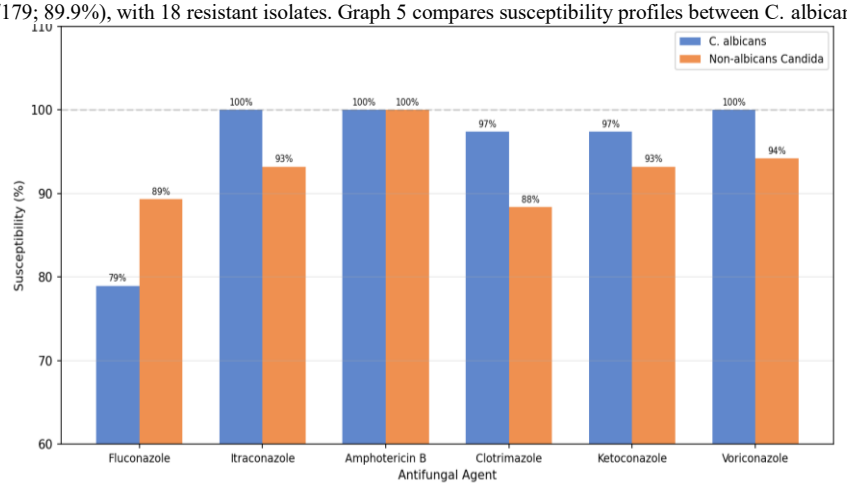
Graph 4: Distribution of Candida Species by Percentage (n=179)

MSW MANAGEMENT -Multidisciplinary, Scientific Work and Management Journal
ISSN: 1053-7899
Vol. 36 Issue 1s, 2026, Pages: 4231-4235

Table 2: Distribution of Candida Species (n=179)

Species	Number of Isolates	Percentage (%)
C. albicans	76	42.5
C. glabrata	41	22.9
C. krusei	32	17.9
C. tropicalis	29	16.2
C. parapsilosis	1	0.6
Total	**179**	**100**

3.6 Antifungal Susceptibility Profile: Table 3 presents the overall antifungal susceptibility data. Amphotericin B demonstrated complete activity across all 179 isolates, with 100% susceptibility for every species tested. Voriconazole achieved the second-highest overall susceptibility (173/179; 96.6%), followed by itraconazole (172/179; 96.1%) and ketoconazole (170/179; 94.9%). Clotrimazole susceptibility was 92.2% (165/179), while fluconazole exhibited the lowest overall susceptibility rate (161/179; 89.9%), with 18 resistant isolates. Graph 5 compares susceptibility profiles between C. albicans and NAC species.



Graph 5: Antifungal Susceptibility Comparison – C. albicans vs Non-albicans Candida

Table 3: Antifungal Susceptibility Patterns of Candida Species (n=179)

Antifungal Agent	Total S	Total S%	Total R	Total R%	CA S%	CA R%	NAC S%	NAC R%	p-value
Fluconazole	161	89.9	18	10.1	78.9	9.2	89.3	10.7	0.074
Itraconazole	172	96.1	7	3.9	100	0.0	93.2	6.8	0.003*
Amphotericin B	179	100.0	0	0.0	100	0.0	100	0.0	—
Clotrimazole	165	92.2	14	7.8	97.4	2.6	88.3	11.6	0.007*
Ketoconazole	170	94.9	9	5.0	97.4	2.6	93.2	6.8	0.089
Voriconazole	173	96.6	6	3.4	100	0.0	94.2	5.8	0.004*

*CA = C. albicans (n=76); NAC = Non-albicans Candida (n=103); S = Sensitive; R = Resistant; * p < 0.05 (statistically significant)*

3.7 Comparative Analysis of C. albicans versus NAC Species

Statistical comparison of susceptibility rates between C. albicans and NAC species using the two-proportion z-test revealed significant differences for itraconazole (p=0.003), clotrimazole (p=0.007), and voriconazole (p=0.004). C. albicans showed superior susceptibility to itraconazole (100% vs 93.2%), clotrimazole (97.4% vs 88.3%), and voriconazole (100% vs 94.2%). For fluconazole and ketoconazole, the differences between C. albicans and NAC isolates were not statistically significant (p>0.05).

4. DISCUSSION

The results of this study underscore a well-documented global trend: NAC species now collectively surpass C. albicans as the predominant causative agents of clinical candidiasis at tertiary care institutions. In this cohort, NAC species constituted 58% of all isolates, a finding congruent with contemporary surveillance data from India and internationally. [17,18]

The female predominance observed in this study (53%) is attributable to the inherently greater susceptibility of women to urogenital candidiasis, stemming from anatomical, hormonal, and microbiological factors. Elevated oestrogen levels during reproductive years increase vaginal glycogen content, promoting Candida colonisation, while pregnancy further amplifies this risk through progesterone-mediated immunomodulation. [5,19]

The concentration of isolates in the 61–70-year age group (19.5%) reflects the convergence of multiple risk factors in elderly patients: declining cell-mediated immunity, higher prevalence of comorbidities (diabetes mellitus, malignancy), frequent exposure to broad-spectrum antibacterials, and greater utilisation of invasive medical devices. This observation aligns with data reported by Athokpam et al., [20], who found the highest Candida burden in patients above 70 years, and by Mukhia et al. [21] who similarly identified elderly age as a major risk determinant.

The ICU accounted for 49.1% of all isolates, reinforcing its status as the primary ecological niche for nosocomial candidiasis. Candidemia in ICU patients is driven by the interplay of immune suppression, disrupted mucosal barriers, invasive devices, and selective antibiotic pressure. These findings parallel those of Azad et al. [22] and Kaur et al. [23], who reported ICU predominance in their respective institutional surveys.

Urine was the most productive specimen source (66%), consistent with the high prevalence of candiduria associated with urinary catheterisation, urinary tract abnormalities, and antibiotic-mediated suppression of normal bacterial flora. This finding is supported by multiple Indian studies demonstrating urine as the leading Candida source, with rates ranging from 45% to 66%. [24,25]

The species profile observed — C. albicans (42%), C. glabrata (23%), C. krusei (18%), and C. tropicalis (16%) — differs modestly from some published series in which C. tropicalis is the most common NAC species. [17,26] The relatively high prevalence of C. glabrata in our cohort may reflect patient demographic factors (elderly population) and antifungal exposure history at this institution. C. glabrata and C. krusei are of particular clinical concern because both species exhibit intrinsic or acquired reduced susceptibility to azoles. [27]

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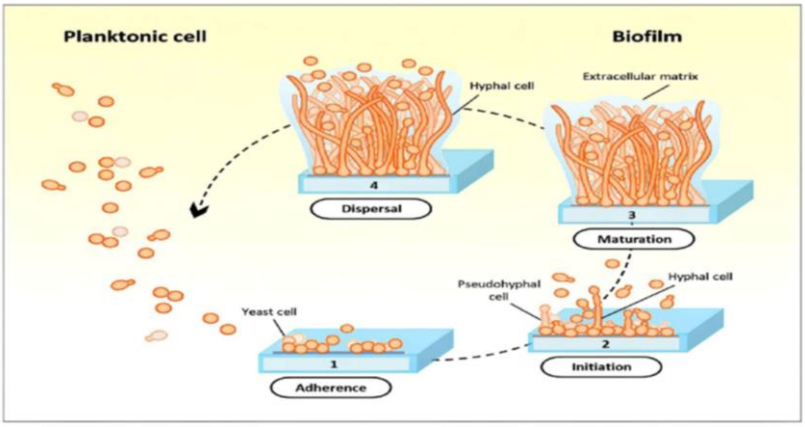

Figure 2: Four-Stage Biofilm Development Cycle of C. albicans — (1) Adherence of yeast cells to surface, (2) Initiation with pseudohyphal cell emergence, (3) Maturation into hyphal biofilm with extracellular matrix, (4) Dispersal of planktonic cells (adapted from original thesis)

The antifungal susceptibility results demonstrate that amphotericin B retains complete efficacy against all tested isolates, consistent with its longstanding role as a gold-standard antifungal with a polyene mechanism of action that targets ergosterol in the fungal cell membrane. No clinically significant resistance to amphotericin B was detected, echoing findings from Gade and Neral [28] (99% susceptibility) and Anita and Kumar [17] (100% susceptibility).

Voriconazole demonstrated excellent overall susceptibility (96.6%) and complete activity against C. albicans, making it a viable option for the management of azole-susceptible candidemia. The statistically significant difference in voriconazole susceptibility between C. albicans and NAC species (100% vs 94.2%; p=0.004) underlines the importance of species-level identification before initiating triazole therapy. Fluconazole exhibited the lowest overall susceptibility rate (89.9%) in this study. The resistance rate of 10.1% encompasses intrinsic resistance in C. krusei and acquired resistance in some C. glabrata and C. tropicalis isolates. These findings are consistent with global trends attributing declining fluconazole susceptibility to long-term prophylactic use in immunocompromised patients. [29,30]. HiCrome Candida Differential Agar provided reliable presumptive species identification within 24–48 hours, with colour-based colony differentiation correlating well with confirmatory germ tube results and conventional biochemical profiling. Its cost-effectiveness and ease of interpretation without specialised equipment render it particularly appropriate for resource-limited laboratory settings. [11,31]. Several limitations of this study merit acknowledgement. The single-centre design limits generalisability. Molecular identification (ITS sequencing) was not performed to confirm rare or atypical colony appearances. Minimum inhibitory concentration (MIC) determination by broth microdilution was not undertaken, precluding definitive quantification of resistance levels. Larger multicentre prospective cohort studies incorporating molecular diagnostics and MIC data are warranted to establish updated national susceptibility benchmarks.

5. CONCLUSION

This prospective study at a tertiary care centre in northern India confirms the epidemiological ascendancy of non-albicans Candida species, which collectively accounted for 58% of clinical isolates. C. glabrata and C. krusei — species with well-characterised azole resistance profiles — emerged as the two most prevalent NAC pathogens. The findings highlight the indispensability of routine Candida speciation and antifungal susceptibility testing in clinical practice. Amphotericin B and voriconazole retained exceptional pan-species efficacy, with 100% and 96.6% susceptibility, respectively. Fluconazole resistance at 10.1% serves as a sentinel indicator necessitating empirical therapy re-evaluation at the institutional level. HiCrome agar proved to be a practical, economical, and reliable tool for species identification without requiring specialised infrastructure. These data provide actionable intelligence for antimicrobial stewardship programmes, advocating for pre-treatment species identification and susceptibility-guided antifungal selection to improve patient outcomes and curtail the proliferation of resistant fungal pathogens in healthcare environments.

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