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EMERGING TREND OF NON-ALBICANS CANDIDA SPECIES: CLINICO-MICROBIOLOGICAL PROFILE AND ANTIFUNGAL SUSCEPTIBILITY PATTERN AMONG CLINICAL ISOLATES FROM A TERT6IARY CARE HOSPITAL OF RAJASTHAN, INDIA

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Abstract

Background: Clinical Candida infections continue to pose a significant healthcare burden worldwide, especially among hospitalized patients and individuals with compromised immunity. In recent years, a notable shift in the epidemiology of candidiasis has been observed, characterized by an increasing prevalence of non-albicans Candida species and the emergence of antifungal resistance, which have created additional challenges in patient management.[21, 24]

Aim: To evaluate the emerging trend of non-albicans Candida species, their Clinicomicrobiological profile, and antifungal susceptibility pattern among Candida isolates recovered from various clinical specimens in a tertiary care hospital.

Methodology: A cross - sectional study was conducted in the Department of Microbiology, Ananta Institute of Medical Science and Research Centre, Rajasthan, India. A total of 180 Candida isolates recovered from different clinical specimens were included in the study. Identification of Candida species was performed using standard mycological methods including Gram staining, KOH mount, culture on Sabouraud Dextrose agar (SDA), germ tube test, sugar assimilation test and sugar fermentation tests and CHROMagar Candida medium. Antifungal susceptibility testing was carried out by disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines.[20]

Results: Out of 6317 clinical specimens received for culture and sensitivity testing during the study period, 180 Candida isolates were recovered, with an overall prevalence of 2.84%. Non - albicans Candida species 118 (65.56%) were isolated more frequently than Candida albicans 62 (34.44%). Candida tropicalis was the predominant non-albicans species followed by Candida krusei, Candida parapsilosis, and Candida glabrata. Most isolates demonstrated good susceptibility to Amphotericin B, whereas reduced susceptibility to azole antifungal agents was observed among some non-albicans Candida isolates.

Conclusion: The study demonstrated an increasing predominance of non-albicans Candida species with variable antifungal susceptibility patterns. Routine species identification and antifungal susceptibility testing are essential for effective management of candidiasis and surveillance of emerging antifungal resistance.

INTRODUCTION

Fungal infections have emerged as a significant cause of morbidity and mortality worldwide, particularly among hospitalized patients, critically ill individuals, and those with impaired immune function[24,25]. Among the various fungal pathogens, Candida species are frequently associated with a broad spectrum of infections ranging from superficial mucocutaneous lesions to invasive and life - threatening systemic diseases[3,7].

In recent years, a notable shift in the epidemiology of candidiasis has been

observed, characterized by an increasing prevalence of non-albicans Candida species and the emergence of antifungal resistance[10,21]. Although Candida albicans remains important pathogens, species such as Candida tropicalis, Candida krusei, Candida parapsilosis, and Candida glabrata are being isolated with increasing frequency from clinical specimens[10,21]. This changing species distribution has important clinical implications because several non-albicans Candida species exhibit reduced susceptibility or intrinsic resistance to commonly used antifungal agents[4,21].

Candida species are normal commensals of the skin, gastrointestinal tract, and genitourinary tract; however, disruption of host defenses or alterations in the normal microbial flora may facilitate their transition from colonization to infection[7,28]. Several factors, including diabetes mellitus, prolonged hospitalization, intensive care unit admission, urinary catheterization, mechanical ventilation, broad - spectrum antibiotic use, and other invasive procedures, have been identified as important risk factors for candidiasis[6,8,24].

Accurate species identification and antifungal susceptibility testing are essential for effective patient management because the distribution of Candida species and their susceptibility patterns may vary across different healthcare settings[21,27]. Continuous monitoring of local epidemiological trends is therefore necessary to support appropriate antifungal therapy and to address the growing challenge of antifungal resistance[21,27].

Therefore, the present study was undertaken to evaluate the prevalence, Clinicomicrobiological profile, species distribution, and antifungal susceptibility pattern of Candida isolates recovered from various clinical specimens in a tertiary care hospital in Rajasthan, India.

MATERIALS AND METHODS

A cross-sectional study was conducted in the Department of Microbiology, Ananta Institute of Medical Sciences and Research centre, Rajasthan, India, after obtaining institutional ethical approval. The study included Candida isolates recovered from various clinical specimens received in the microbiology laboratory from patients with suspected fungal infections. The study was undertaken as a part of the doctoral research work at PIMSR, Parul University, Vadodara during the study period from April 2025 to February 2026.

Inclusion criteria:

- Patients of all age groups with clinically suspected fungal infection
- Clinical specimens received for fungal culture and identification.

Exclusion Criteria:

- Patients receiving antifungal therapy before sample collection.
- Specimens showing only bacterial growth.
- Specimens with polymicrobial growth or mixed fungal and bacterial growth.

Sample Collection

Clinical specimens including urine, blood, sputum, pus, endotracheal secretion, Swab (Vaginal swab, Wound swab, Ear swab etc), Fluid (Pleural, CSF, BAL Fluid), were collected under aseptic precautions.

All specimens were transported promptly to the laboratory and processed as early as possible. Well-mixed specimens were inoculated onto nutrient agar, MacConkey agar and inoculated plates were examined after 24 hours. Growth obtained on Nutrient agar was subcultured onto Sabouraud's Dextrose Agar (SDA).

Colony Morphology

On nutrient agar, Candida colonies appeared creamy, white and pasty after incubation at 37°C for 24-48 hours. On SDA, Candida produced creamy, moist colonies after 24-48 hours of incubation at 37°C. Candida isolates were identified by colony morphology, Gram's staining, KOH mount, germ tube test, and color production on CHROMagar Candida medium, sugar assimilation test, sugar fermentation test and urease test[2,21].

Subculturing on Hi-Crome Candida Differential Agar

Further speciation of Candida isolates was done by sub-culturing on chromogenic medium (HiCrome Candida; HiMedia, Mumbai, India) and incubated at 37°C for 48 hours. Presumptive identification of Candida species was done by noting the color of the colonies as per the manufacturer's instructions in HiCrome medium (Candida albicans - apple green, Candida tropicalis – blue, Candida krusei - pink colonies, Candida parapsilosis - cream to pale pink)[2,7].

Further identification and speciation of Candida isolates were performed using germ tube test, sugar fermentation test, and sugar assimilation test according to standard microbiological methods. The germ tube test was used for presumptive identification of Candida albicans, while sugar fermentation and sugar assimilation

tests were used for differentiation and speciation of Candida isolates[2,29].

Antifungal susceptibility testing: Antifungal susceptibility testing of Candida isolates was performed by the disc diffusion method in accordance with Clinical and Laboratory Standards Institute (CLSI) M44 guidelines[20]. Mueller-Hinton agar (MHA) supplemented with 2% glucose and 0.5µg/ml methylene blue was used as the test medium to enhance fungal growth and improve readability of inhibition zones. A standardized inoculum equivalent to 0.5 McFarland standards was prepared and uniformly swabbed over the agar surface to obtain a confluent lawn culture. Antifungal discs of Amphotericin B (20µg), Ketoconazole (10µg), Itraconazole (10µg) Fluconazole (25 µg) and Voriconazole (1 µg) were placed on the inoculated plates at appropriate distances. Plates were incubated at 35 ± 2℃ for 24 hours. After incubation, zone diameters for Fluconazole and Voriconazole were interpreted according to the applicable CLSI M60 criteria[27]. For Amphotericin B, Ketoconazole, Itraconazole interpretive criteria were considered based in previously published literature[24,30], as CLSI does not provide standardized breakpoints for these agents against Candida species.

RESULTS

During the study period, a total of 6317 clinical specimens were received in the microbiology laboratory for culture and sensitivity testing. Among these, 1419 samples showed positive microbial growth. Candida species were isolated from 180 samples, representing an overall prevalence of 2.84% among total specimens and 12.68 % among culture - positive isolates.

Table 1: Prevalence of Candida isolates

Total Clinical samples processed	Candida isolates recovered	Prevalence (%)
6317	180	2.84 %

Table 2: Distribution of Candida isolates according to clinical specimens

Clinical specimen	**Number of Isolates (n=180)**	**Percentage (%)**
Urine	67	37.2%
Vaginal swab/discharge	34	18.9%

Sputum	30	16.7%
ET secretion	18	10.0%
Blood	17	09.4%
Pus	7	3.9%
BAL Fluid	4	2.2%
Others	3	1.7%

In the present study, Candida isolates were obtained from various clinical specimens. The highest number of isolates was recovered from urine samples (37.2 %), followed by vaginal swabs/discharge (18.9%), sputum (16.7%), endotracheal secretion (10.0%), and blood samples (9.4%). Lowest isolation rates were observed in pus (3.9%), BAL fluid (2.2%), other specimens (1.7%). Among the 67 urine isolates, 17 (25.4%) showed colony count of $< 10^5$ CFU/mL, whereas 50 (74.6%) showed colony counts of $>10^5$ CFU/mL. Although the former may represent colonization, all urine samples included in the study were obtained from patients with relevant clinical symptoms or associated risk factors[19,31].

Among the 34 vaginal isolates, 27 (79.4%) were recovered from patients presenting with vaginal discharge and related symptoms, suggesting clinically significant vulvovaginal candidiasis, while remaining 7 (20.6%) isolates may represent colonization[31].

In the present study, Candida species were isolated from 30 sputum samples. According to standard microbiology references, Candida recovered from respiratory specimen is frequently considered a colonizer rather than a true pathogen. However, the majority of patients in the present study had respiratory symptoms and associated risk factors[31].

Table 3: Gender - wise and Age-wise distribution of Candida isolates

Gender	**Number of Isolates (n=180)**	**Percentage (%)**
Male	79	43.89 %
Female	101	56.11 %

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Variables (Age group) years	Number of Patients (n=180)	Percentage (%)
0-20	19	10.55%
21-40	59	32.78%
41-60	41	22.78%
61-80	52	28.89%
>80	9	5.00 %

Out of 180 isolates, 101 (56.11%) were from female patients and 79 (43.89%) from male patients. Age-wise distribution showed isolates across all age groups, with 21–40 years (32.78 %) and 61–80 years (28.89%) contributing a major proportion.

Table 4: Distribution of Isolates according to IPD and OPD cases

Category	**Number of Isolates (n=180)**	**Percentage (%)**
IPD	157	87.2 %
OPD	23	12.8 %

In the present study, the majority of Candida isolates were obtained from inpatient department (IPD) cases, accounting for 87.2%, whereas 12.8% isolates were from outpatient department (OPD) cases. The higher proportion of isolates from IPD patient suggests a greater burden of infection among hospitalized individuals.

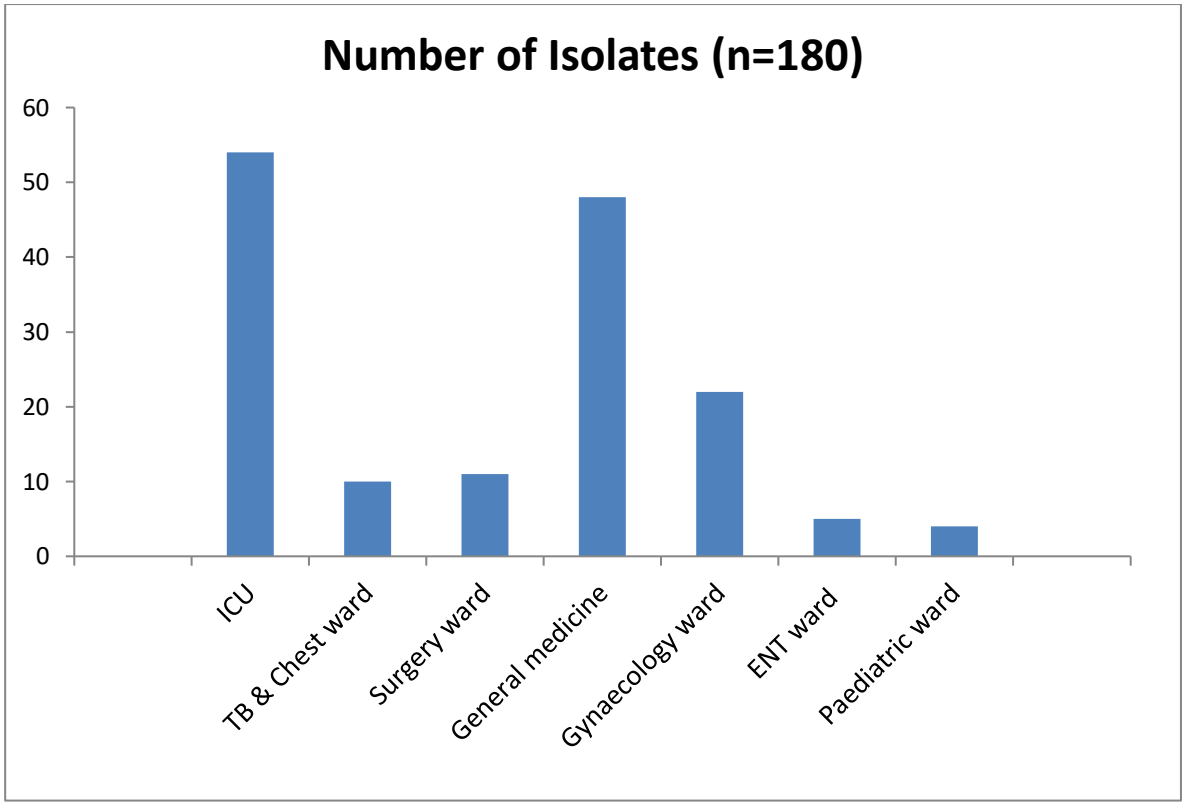

Table 5: Ward-wise Distribution of Candida isolates

Ward	Number of Isolates (n=180)	Percentage (%)
ICU	54	34.4 %
General medicine	48	30.6 %
Gynaecology ward	22	14.0 %
Surgery ward	11	7.0 %
TB & Chest ward	10	6.4 %
ENT ward	5	3.2 %
Paediatric ward	4	2.5 %
Urology ward	3	1.9 %

In the present study, Among the IPD wards, the highest number of Candida isolates form ICU (34.4%), followed by General medicine (30.6 %), and Gynaecology ward (14.0 %). Lower isolation rates were observed in Surgery ward (7.0%), TB ward (6.4 %), ENT (3.2%), Paediatric ward (2.5%), and Urology (1.9%).

Table 6: Clinical Risk Factors Associated with Candida Infection

Clinical Risk Factors	Number (n=180)	Percentage (%)
Diabetes mellitus	42	23.3 %
Hypertension	28	15.6 %
ICU admission	54	30.0 %
Ventilator support	19	10.6 %
Tuberculosis	10	5.6 %
Shock/critical illness	14	7.8 %
Vaginal discharge/leucorrhea	20	11.1 %
Urinary catheterization	31	17.2 %
Broad spectrum antibiotic use	67	37.2 %

Among the clinical risk factors, broad - spectrum antibiotic use was the most common, observed in 67 (37.2%) patients, followed by ICU stay 54 (30.0%), diabetes mellitus 42 (23.3%), hypertension in 28 (15.6%), and vaginal infection/associated conditions in 20 (11.1%) patients.

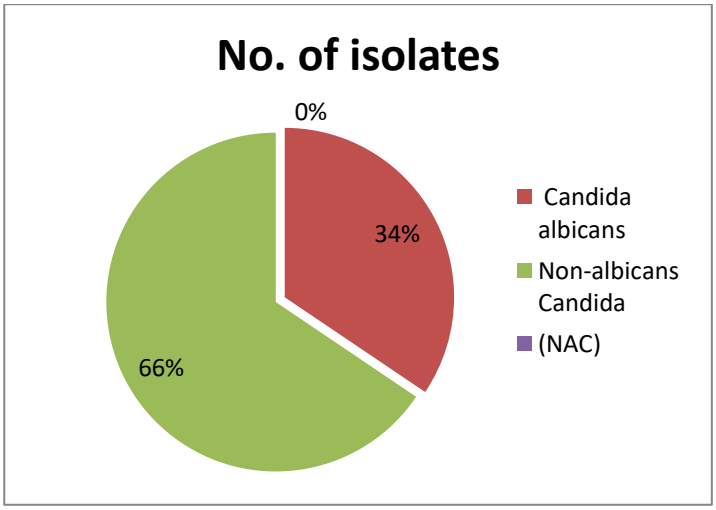

Table 7: Comparison between Candida albicans and Non-albicans Candida

Total	No. of isolates (n = 180)	Percentage (%)
Candida albicans	62	34.4 %
Non-albicans Candida (NAC)	118	65.6 %

"Out of 180 Candida isolates, 62 (34.4%) were identified as Candida albicans and 118 (65.6%) as non-albicans Candida species, indicating a predominance of non-albicans Candida (NAC) isolates".

Table 8: Species distribution of Candida isolates

Candida species	Number of isolates (n = 180)	Percentage (%)
Candida albicans	62	34.44%
Candida tropicalis	54	30.0 %
Candida krusei	35	19.4%
Candida parapsilosis	18	10.0 %
Candida glabrata	11	6.11%

In the present study, Candida albicans was the most common species (34.4%), followed by Candida tropicalis (30.0%), Candida krusei (19.4%), and Candida parapsilosis (10.0%), and Candida glabrata (6.11%).

Table 9: Number of Candida isolates susceptible to antifungal agents

Candida species	Number of isolates (n=180)	Amphotericin B	Fluconazole	Itraconazole	Voriconazole	Ketoconazole
Candida albicans	62	60	54	56	58	59
Candida tropicalis	54	49	37	43	46	48
Candida parapsilosis	18	17	14	15	16	17

Candida glabrata	11	9	4	5	6	8
Candida krusei	35	32	Intrinsic resistance	22	26	28

We found that Amphotericin B demonstrated the highest antifungal activity against Candida isolates, whereas Fluconazole showed the highest resistance rate. Candida krusei exhibited intrinsic resistance to Fluconazole

Table 10: Overall Antifungal Susceptibility profile of Clinical isolates

Antifungal agent	Sensitive (%)	Resistant (%)
Amphotericin B	167 (92.8%)	13 (7.2%)
Fluconazole	109 (60.6%)	71 (39.4%)
Itraconazole	141 (78.3%)	39 (21.7%)
Voriconazole	152 (84.4%)	28 (15.6%)
Ketoconazole	160 (88.9%)	20 (11.1%)

The study found that an overall antifungal susceptibility profile showed that all isolates were classified as either susceptible or resistant, and no susceptible dose – dependent (SDD) interpretation was observed".

DISCUSSION

The present study evaluated the prevalence, species distribution, associated risk factors, and antifungal susceptibility profile of Candida isolates obtained from different clinical specimens in a tertiary care hospital. Among 6317 specimens processed during the study period, 180 Candida isolates were recovered, resulting in an overall prevalence of 2.84%. The finding indicates that Candida continues to be an important opportunistic pathogen in clinical practice, particularly among hospitalized patients and individuals with underlying predisposing conditions. The prevalence observed in the present study is comparable to that reported in other tertiary care-based studies by Warghade et al.[10] and Rajput et al.,[23]. Variations in prevalence rates across different studies may be attributed to differences in study population, geographical region, healthcare settings, and specimen selection criteria.

In the present study, urine was the most common specimen yielding Candida

isolates (37.2%) followed by vaginal swab/discharge (18.9%), sputum (16.7%), endotracheal secretion (10.0%), blood (9.4%) and other clinical specimens. The predominance of candiduria may be associated with factors such as urinary catheterization, prolonged hospitalization, diabetes mellitus, advanced age, and prior exposure to broad - spectrum antibiotics, all of which facilitate Candida colonization and infection of the urinary tract. The high recovery rate of Candida from urine specimens observed in this study is consistent with finding reported by Patel VA et al.[21] who also identified urine as the leading source of Candida isolation. Differences in the proportion of urinary isolates among various studies may be attributed to variations in patient characteristics, healthcare settings, catheter utilization practices, and inclusion criteria.

Sputum and endotracheal secretion samples also accounted for a considerable proportion of Candida isolates, contributing 16.7% and 10.0% of the total isolates, respectively. The majorities of these isolates were recovered from patients with respiratory symptoms and associated risk factors such as ICU admission, tuberculosis, and broad - spectrum antibiotic use. However, Candida isolated from respiratory specimens should be interpreted cautiously, as it may represent colonization rather than true infection. A comparable distribution of Candida isolates in respiratory specimens has been documented by Subba et al.[26]. However, the isolation of Candida from sputum and endotracheal secretions should be interpreted cautiously, as these organisms often represent colonization of the respiratory tract rather than true invasive infection[19]. Therefore, microbiological findings should always be correlated with the patient's clinical presentation, radiological findings, and underlying risk factors before establishing a diagnosis of Candida respiratory infection.

Candida isolated from blood samples remains clinically important because bloodstream infections are associated with significant morbidity and may complicate the management of hospitalized patients. A comparable observation was reported by Mistry et al.[17], who also documented the occurrence of candidemia in a tertiary care hospital setting. The presence of Candida in blood cultures emphasizes the need for prompt laboratory diagnosis and appropriate antifungal management.

We found that vaginal specimens represented an important source of Candida isolation in the present study. This observation highlights the role of Candida species in vulvovaginal infections among women. Comparable findings have

been reported by Tripathi M et al.[9] and Siddiqui R et al.[6]. The occurrence of Candida in vaginal specimens may be associated with factors such as hormonal factors, pregnancy, diabetes mellitus, antibiotic use, and poor genital hygiene, which predispose women to Candida colonization and symptomatic infection.

Female patients accounted for 56.11% of isolates in the present study. Similar findings were reported in previous studies,[9] which documented a higher frequency of Candida infections among females, particularly due to vulvovaginal candidiasis.

Non - albicans Candida species (65.56%) predominated over Candida albicans (34.44%) in the present study. Similar findings were reported by Patel VA et al.[21] and Ahir et al.,[14] who also documented a higher prevalence of non-albicans Candida species. Among the Candida species isolated, Candida tropicalis (30.00%) was the predominant non-albicans Candida species. Similar species distribution was reported by Warghade et al.[10].

Broad - spectrum antibiotic use, ICU admission, diabetes mellitus, and urinary catheterization were the most common risk factors observed in the present study. Similar risk factors have been reported by Siddiqui et al.[6] and Sharma et al.[8]. Amphotericin B showed the highest susceptibility (92.8%) in the present study. Comparable findings were reported by Rajput et al.[23] and Patel VA et al.,[21] who also observed high susceptibility of Candida isolates to Amphotericin B. Fluconazole showed the lowest susceptibility (60.6%) in the present study. Similar findings were reported by Patel VA et al.[21] and Barot et al.,[22] who documented increasing azole resistance among Candida species.

Overall, the present study highlights the increasing predominance of non-albicans Candida species and the emerging challenge of antifungal resistance. Routine species identification, antifungal susceptibility testing, and continuous surveillance are essential and improved patient care.

CONCLUSION

The present investigation demonstrated a clear predominance of non-albicans Candida species over Candida albicans among clinical isolates recovered from patients attending in a tertiary care hospital. Candida tropicalis emerged as the leading non-albicans species, highlighting a changing epidemiological pattern of candidiasis. The high frequency of Candida isolation among hospitalized

patients particularly those with diabetes, urinary catheterization, ICU admission, and prior antibiotic exposure emphasizes the contribution of these risk factors to the development of Candida infections.

The antifungal susceptibility profile revealed that Amphotericin B remained highly effective against most isolates, whereas reduced susceptibility to azole antifungal agents was observed among certain Candida species. These findings underscore the growing importance of species – level identification and antifungal susceptibility testing for appropriate patient management and selection of effective antifungal therapy.

Furthermore, the increasing predominance of non-albicans Candida species and the emergence of antifungal resistance highlight the need for continuous epidemiological surveillance. Regular monitoring of species distribution and antifungal susceptibility patterns can assist clinicians in making evidence-based treatment decisions and contribute to improved patient outcomes. Continuous local surveillance remains essential for guiding empirical therapy, strengthening antifungal stewardship practices, and monitoring emerging resistance trends.

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