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Predominance of Non-albicans Candida, High Virulence, and Fluconazole Resistance in Clinical Isolates: A Cross-Sectional Study

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Abstract

Background: Invasive and mucocutaneous Candida infections are increasingly attributed to non-*albicans Candida* species, with variable antifungal resistance and virulence traits. This study aimed to assess the distribution, virulence characteristics, and antifungal susceptibility of *Candida* isolates from clinical specimens in a tertiary care setting.

Methods: A cross-sectional study was conducted over six months at a tertiary hospital in Chennai. Fifty-two *Candida* isolates from various clinical specimens were identified to the species level using germ tube testing, CHROMagar, sugar fermentation, and assimilation. Virulence factors (proteinase, phospholipase, and biofilm formation) were assessed phenotypically. Antifungal susceptibility testing to fluconazole and voriconazole was performed using the disc diffusion method according to CLSI guidelines.

Results: Of the 52 isolates, 42.3% were *C. tropicalis*, 34.6% *C. albicans*, 11.5% *C. parapsilosis*, 7.7% *C. glabrata*, and 3.8% *C. krusei*. The majority of isolates (65.4%) were non-*albicans Candida*. Isolates were most frequently obtained from catheterized urine (34.6%), followed by blood and respiratory samples. Virulence factor analysis showed proteinase production in 73% and phospholipase in 46% of isolates. Biofilm formation was common among urinary isolates. All species showed 100% sensitivity to voriconazole, while 28.8% of isolates were resistant to fluconazole, particularly among *C. tropicalis* and *C. glabrata*.

Conclusions: Non-*albicans Candida* species predominate in clinical infections and demonstrate significant virulence traits and fluconazole resistance. Routine species-level identification and antifungal susceptibility testing are essential to guide effective therapy.

Keywords: *Candida*, non-*albicans Candida*, antifungal resistance, virulence factors, proteinase, phospholipase, fluconazole, voriconazole, biofilm, CHROMagar

INTRODUCTION

Candidiasis, caused by yeasts of the genus Candida, remains a major health concern worldwide, presenting with a spectrum of clinical manifestations ranging from superficial infections to life-threatening systemic involvement [1]. Candida albicans is the most frequently isolated species; however, a notable shift toward non-albicans species such as C. tropicalis, C. glabrata, C. krusei, and C. parapsilosis has been observed in recent years, especially in nosocomial environments [2]. This epidemiological trend is driven by an increasing population of immunocompromised individuals, extended hospitalization, use of invasive medical devices, and extensive administration of broad-spectrum antibiotics [3]. Laboratory diagnosis of candidiasis is challenged by non-specific clinical symptoms and limited resources in many healthcare settings [4]. Accurate identification of Candida species and their antifungal susceptibility profiles is essential for guiding effective therapy, particularly in light of emerging resistance to azole agents such as fluconazole [5].Virulence attributes including enzyme production (proteinases, phospholipases), biofilm formation, and phenotypic switching further complicate the clinical picture and contribute to treatment failure [1][2]. Nosocomial candiduria and candidemia,

in particular, have emerged as significant complications among ICU patients [6]. Given these complexities, this study was designed to analyze the distribution of Candida species in clinical isolates, assess their virulence characteristics, and determine antifungal susceptibility patterns, to support improved clinical outcomes through targeted therapy and timely diagnosis.

AIMS AND OBJECTIVES

This study was undertaken to investigate the distribution and characteristics of *Candida* species isolated from clinical samples in a tertiary care hospital. The specific objectives were:

1. To identify and characterize the different species of *Candida* isolated from various clinical specimens.
2. To evaluate the expression of virulence factors such as proteinase and phospholipase among the isolates.
3. To determine the antifungal susceptibility patterns of the isolates to fluconazole and voriconazole.
4. To assess associations between species, virulence traits, and antifungal resistance patterns.

MATERIALS AND METHODS

This cross-sectional study was conducted over a six-month period (May to November 2022) in the Clinical Microbiology Laboratory at Saveetha Medical College and Hospital, Chennai, after obtaining institutional ethical clearance. Clinical samples (urine, blood, BAL, vaginal swabs, wound swabs, and pus) submitted from inpatient departments were processed as per standard microbiological protocols. Only the first culture-positive sample per patient was included; repeat isolates of the same species from a single patient were excluded.

Samples were inoculated on Sabouraud Dextrose Agar (SDA) and incubated at 37°C. Colonies suggestive of yeast were confirmed microscopically using Gram staining and 10% KOH mounts. Further species-level identification employed the Germ Tube Test, growth at 45°C, CHROMagar Candida (based on colony morphology), and sugar fermentation and assimilation profiles using standard biochemical methods.

Virulence factor detection included proteinase activity on bovine serum albumin agar, phospholipase activity on egg yolk agar (quantified by the Pz index), and biofilm formation in glucose-enriched Sabouraud broth with safranin staining. Activities were scored semi-quantitatively based on standard grading criteria.

Antifungal susceptibility testing was performed using the disc diffusion method on Mueller-Hinton agar with glucose and methylene blue, following CLSI M44-A2 guidelines. Fluconazole and voriconazole susceptibility was interpreted based on inhibition zone diameters. Data were analyzed descriptively using Microsoft Excel, and group-level associations were explored between species, virulence traits, and antifungal resistance.

RESULTS

1. Demographics and Sample Distribution

Age Distribution

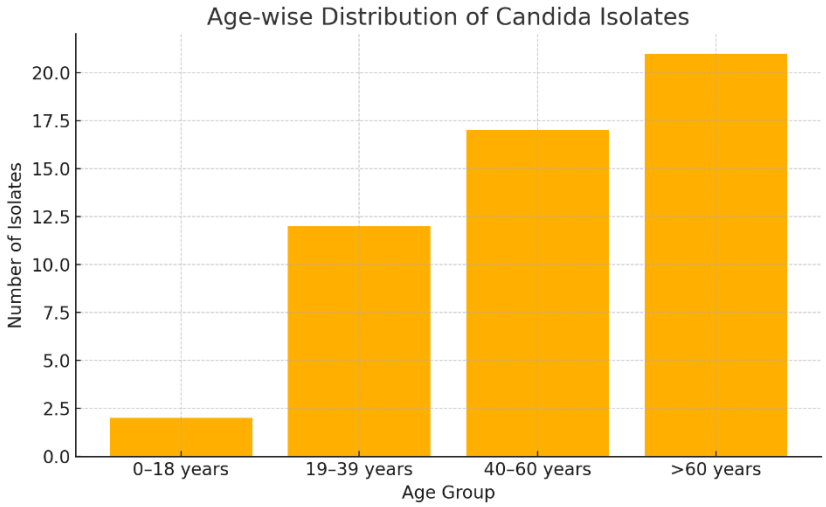

Figure 1. Age-wise distribution of Candida isolates.

The study included a total of 52 Candida isolates from patients aged 2 to 85 years, with a mean age of 51.75 years (SD = 18.17). The age group with the highest number of isolates was those above 60 years (n = 21; 40%), followed by 40–60 years (n = 17; 33%). The youngest age group (0–18 years) showed the least number of cases (n = 2; 4%). This trend aligns with existing evidence suggesting increased vulnerability to fungal infections in older adults.

Table 1. Number of Candida isolates by age group.

Age Group	Number of Isolates
0–18 years	2
19–39 years	12
40–60 years	17
>60 years	21

Gender Distribution

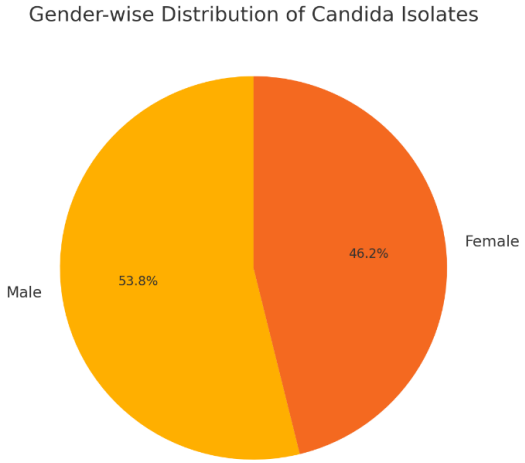

Figure 2. Gender-wise distribution of Candida isolates.

Out of the 52 patients, 28 (54%) were male and 24 (46%) were female. This shows a slight male predominance, although the overall distribution is nearly equal.

Table 2. Gender distribution of Candida isolates.

Gender	Number of Isolates
Male	28
Female	24

Sample Type Distribution

The majority of Candida isolates were recovered from urine samples (n = 39; 75%), highlighting the urinary tract as the most common site of candidiasis in this cohort. This was followed by isolates from blood (n = 5; 10%), bronchoalveolar lavage (n = 3), vaginal swabs (n = 2), wound swabs (n = 2), and pus (n = 1). The predominance in urine samples may reflect the frequency of catheterization or other urinary interventions.

Table 3. Distribution of Candida isolates by clinical sample type.

Sample Type	Number of Isolates
Urine	39
Broncho Alveolar Lavage	3
Blood	5
Pus	1
High Vaginal Swab	2
Wound Swab	2

2. Species Identification

Among the 52 Candida isolates, Candida tropicalis was the most commonly identified species (n = 22; 42%), followed by Candida albicans (n = 18; 35%). Other non-albicans species included Candida parapsilosis (n = 6; 11%), Candida glabrata (n = 4; 8%), and Candida krusei (n = 2; 4%). These results indicate a higher prevalence of non-albicans Candida species (65%) in this cohort, which is of clinical significance due to differing drug resistance patterns and virulence profiles.

Table 4. Species-wise distribution of Candida isolates.

Species	Number of Isolates
C.albicans	18
C.tropicalis	22
C.glabrata	4
C.krusei	2
C.parapsilosis	6

2.1 Distribution by Clinical Samples

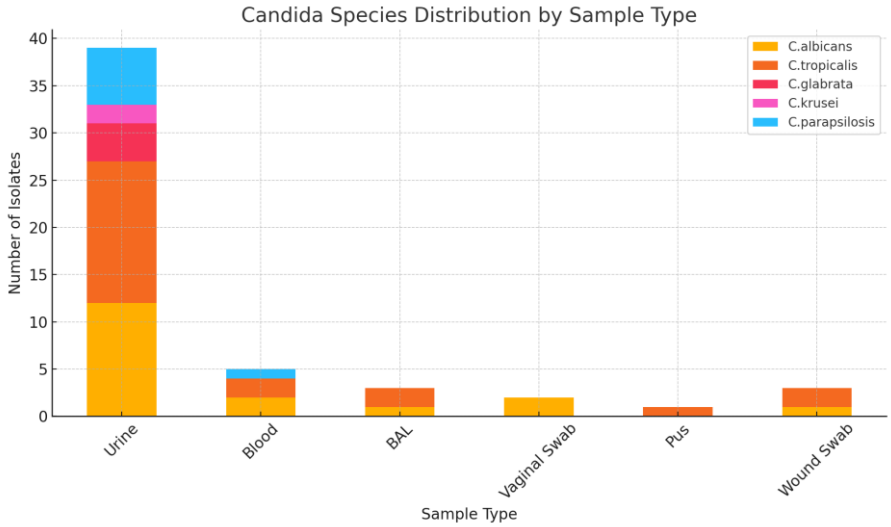

Figure 3. Stacked bar chart showing distribution of Candida species across different clinical sample types.

The distribution of Candida species varied by clinical sample type. Candida tropicalis and Candida albicans were predominantly isolated from urine samples. Candida albicans also showed notable presence in vaginal swabs and blood. Non-albicans Candida such as C. glabrata, C. krusei, and C. parapsilosis were primarily found in urine samples. This visual differentiation underscores the relevance of sample source in Candida speciation.

Table 5. Candida species distribution across clinical sample types.

Sample Type	C.albicans	C.tropicalis	C.glabrata	C.krusei	C.parapsilosis
Urine	12	15	4	2	6
Blood	2	2	0	0	1
BAL	1	2	0	0	0
Vaginal Swab	2	0	0	0	0
Pus	0	1	0	0	0
Wound Swab	1	2	0	0	0

3. Hospital Ward Distribution

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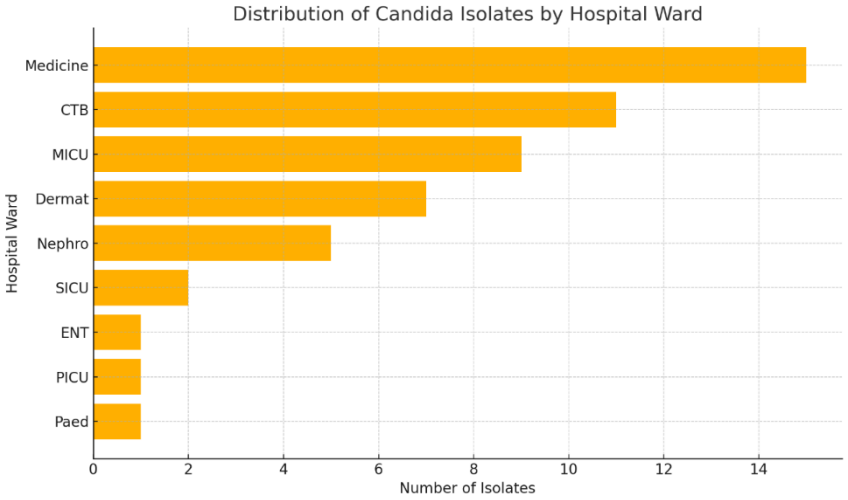

Figure 4. Distribution of Candida isolates across hospital wards.
Candida isolates were predominantly obtained from patients in the Medicine ward (n = 15), followed by Chest and TB (n = 11), MICU (n = 9), and Dermatology (n = 7). Fewer isolates were reported from the Pediatric, PICU, Nephrology, SICU, and ENT wards. This distribution may reflect higher patient load or prevalence of immunocompromised conditions in medical units.

Table 6. Ward-wise distribution of Candida isolates.

Ward	Number of Isolates
Medicine	15
CTB	11
MICU	9
Dermat	7
Paed	1
PICU	1
Nephro	5
SICU	2
ENT	1

4. Virulence Factors
Proteinase Activity

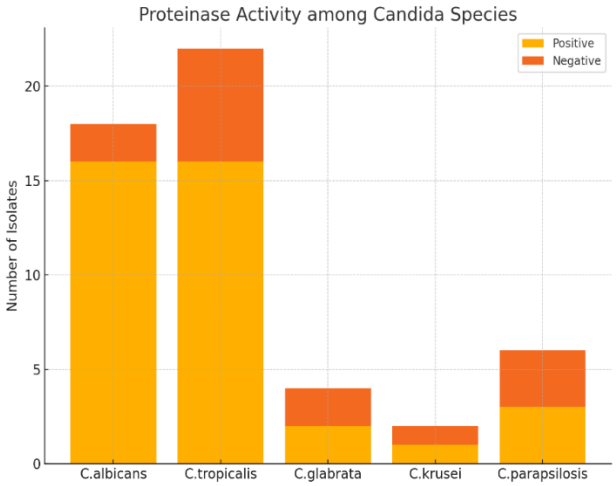

Figure 5. Proteinase activity among Candida species.

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Among the 52 isolates, 38 (73%) exhibited proteinase activity. Candida albicans and Candida tropicalis showed the highest levels of proteinase production, with 16 positive isolates each. Proteinase activity was less common among non-albicans species such as C. glabrata, C. krusei, and C. parapsilosis.

Table 7. Proteinase activity in Candida species.

Species	Proteinase Positive	Proteinase Negative
C.albicans	16	2
C.tropicalis	16	6
C.glabrata	2	2
C.krusei	1	1
C.parapsilosis	3	3

Phospholipase Activity

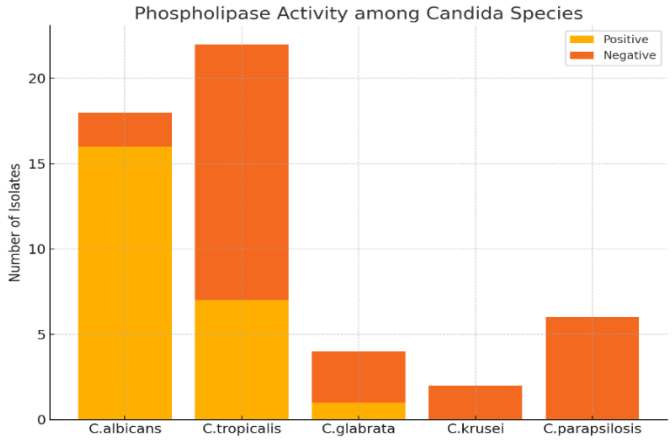

Figure 6. Phospholipase activity among Candida species.

Phospholipase production was observed in 24 (46%) of the 52 isolates. Candida albicans accounted for the majority of phospholipase-positive isolates (16 out of 18). Among non-albicans Candida species, phospholipase production was relatively rare, with C. tropicalis and C. glabrata showing limited activity and none observed in C. krusei or C. parapsilosis.

Table 8. Phospholipase activity in Candida species.

Species	Phospholipase Positive	Phospholipase Negative
C.albicans	16	2
C.tropicalis	7	15
C.glabrata	1	3
C.krusei	0	2
C.parapsilosis	0	6

5. Antifungal Susceptibility

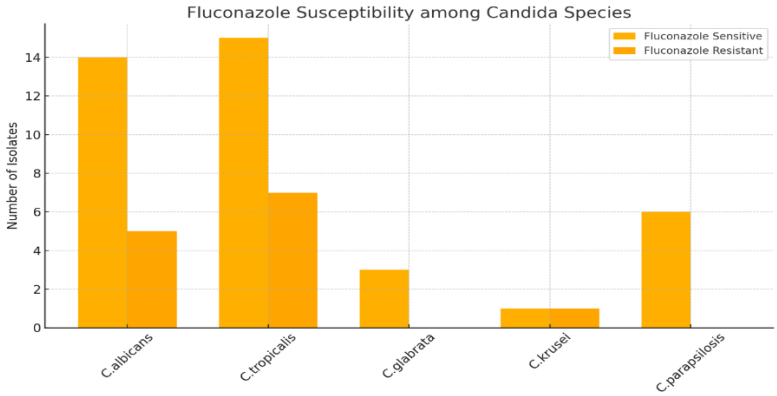

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Figure 7. Susceptibility to Fluconazole among Candida species.

Antifungal susceptibility testing revealed that most Candida species remained sensitive to fluconazole. Candida tropicalis showed 15 sensitive and 7 resistant isolates, while Candida albicans had 14 sensitive and 5 resistant isolates. Resistance was minimal or absent in Candida glabrata, Candida krusei, and Candida parapsilosis. These findings highlight the continuing utility of fluconazole for common Candida infections, though emerging resistance among certain strains necessitates vigilance.

Table 9. Fluconazole susceptibility of Candida species.

Species	Fluconazole Sensitive	Fluconazole Resistant
C.albicans	14	5
C.tropicalis	15	7
C.glabrata	3	0
C.krusei	1	1
C.parapsilosis	6	0

Voriconazole Susceptibility

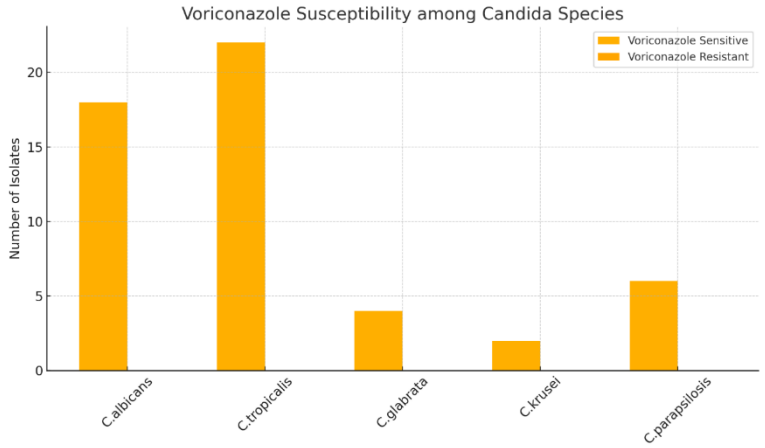

Figure 8. Susceptibility to Voriconazole among Candida species.

All 52 Candida isolates tested were sensitive to voriconazole, highlighting its high efficacy as a second-line antifungal agent. Candida albicans, Candida tropicalis, and all non-albicans species showed complete susceptibility. This uniform sensitivity makes voriconazole a reliable treatment option, particularly in fluconazole-resistant cases.

Table 10. Voriconazole susceptibility of Candida species.

Species	Voriconazole Sensitive	Voriconazole Resistant
C.albicans	18	0
C.tropicalis	22	0
C.glabrata	4	0
C.krusei	2	0
C.parapsilosis	6	0

6. Correlation Summary

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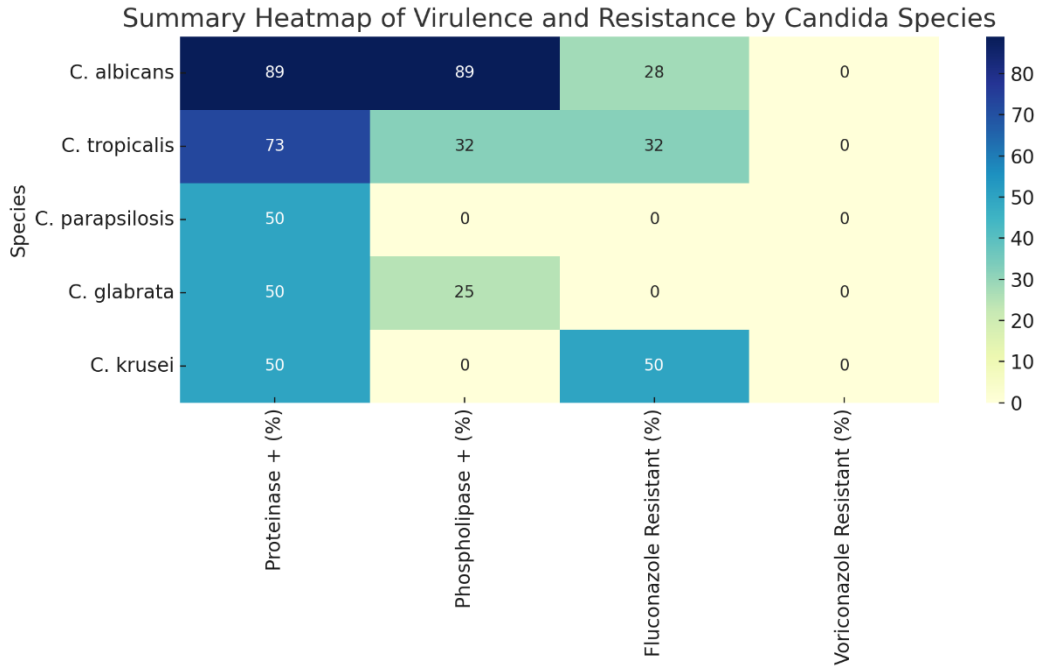

Figure 9. Heatmap showing virulence and antifungal resistance markers across Candida species.
This heatmap summarizes the prevalence of key virulence factors (proteinase and phospholipase) and antifungal resistance patterns (fluconazole and voriconazole) across Candida species in this study. Notably, C. albicans demonstrated the highest expression of both virulence enzymes, while non-albicans species like C. tropicalis showed moderate resistance to fluconazole.

Table 11. Summary matrix of Candida species by virulence and resistance markers.

Species	% of Total	Proteinase + (%)	Phospholipase + (%)	Fluconazole Resistant (%)	Voriconazole Resistant (%)
C. albicans	35.0	89	89	28	0
C. tropicalis	42.0	73	32	32	0
C. parapsilosis	11.5	50	0	0	0
C. glabrata	8.0	50	25	0	0
C. krusei	4.0	50	0	50	0

DISCUSSION

In our study, the mean age of the patients was 51.75 years (SD = 18.17), ranging from 2 to 85 years. Candida infection was observed across all age groups, with the highest percentage (40%) among individuals over 60 years of age, a finding that was statistically significant ($p < 0.01$) [7]. This observation aligns with the findings of Jha et al. [8], and a large European study by Aikaterini Flevari et al. reporting 28% of candidemia in those over 65 years [9]. However, it contrasts with Seshu Kumari et al., who found the 40–60 years age group more affected [10].

Gender distribution in our cohort was nearly equal, with 54% male and 46% female patients. This aligns with findings from Jha et al. [8]. Murray et al. hypothesized that higher male exposure to environmental factors and substance use may explain this trend [11], although Hidalgo et al. reported equal colonization rates between genders [12].

Regarding ward-wise distribution, most Candida isolates originated from the Medicine ward (31%), followed by the Chest and TB ward (21%) and MICU (18%). These results contrast with studies by Fadda et al. [13], who observed higher isolation from Chest and TB wards, and with Ali Zarei and Raminder Sandh, who reported ICU as the major source [14].

Proteinase, a major virulence enzyme, was produced by 73% of isolates in our study. This result supports findings by Kantarcioglu and Yucel, who reported 78.9% proteinase positivity in Candida strains [15]. Specifically, 89% of C. albicans and 73% of C. tropicalis showed proteinase activity, aligning with their known virulence profiles and Yamamoto et al.'s findings of 65–75% positivity among C. tropicalis isolates.

Proteinase production was especially common in vaginal swabs (100%) and catheterized urine (83.3%), consistent with Kantarcioglu et al. (91.3% urogenital isolates) [15] and Livia de Souza et al., who found 81.2% positivity in blood and 78.1% in urine samples [16]. This association was statistically significant ($p < 0.0001$).

Phospholipase, another important virulence factor, was produced by 46% of the Candida isolates. This is close to findings by Deepa et al. who reported 52.6% [17]. C. albicans showed the highest phospholipase activity (89%), corroborating Sachin et al.'s results of 92.3% [18]. However, in our study, among non-albicans species, C. glabrata showed 25%, followed by C. tropicalis (32%) and C. parapsilosis (0%), diverging from Sachin and Thangam's results [18][19].
Phospholipase was most frequent in isolates from pus (55.5%), vaginal swabs (55%), and catheterized urine (44%), though this trend is discordant with Ruchika et al., who observed higher activity from vaginal and urine samples [20]. Interspecies differences in phospholipase activity were statistically significant ($p < 0.0001$).

CONCLUSION

This study provides a comprehensive overview of the species distribution, virulence factors, and antifungal susceptibility patterns of *Candida* isolates in a clinical setting. The findings underscore the predominance of non-*albicans Candida* species, particularly *C. tropicalis*, followed by *C. albicans*. The elderly population showed the highest vulnerability to candidiasis, reflecting both age-related immunosuppression and increased exposure to healthcare interventions.
A significant proportion of isolates exhibited proteinase (73%) and phospholipase (46%) activity, with *C. albicans* demonstrating the highest virulence potential. The analysis also revealed that while fluconazole resistance was present in a moderate proportion of isolates, voriconazole retained full efficacy against all tested species. These findings reinforce the need for routine species-level identification and susceptibility testing in guiding appropriate antifungal therapy.

Limitations

This study had several limitations. The small sample size (n=52) reduced statistical power, particularly for subgroup analyses. Being a single-centre study, the findings may not be generalizable to other populations. The use of aggregate data prevented isolate-level correlation between virulence factors and antifungal resistance. Additionally, while biofilm formation was noted, it was not quantitatively assessed or linked to resistance patterns. Finally, only fluconazole and voriconazole were tested, excluding broader antifungal classes such as echinocandins or amphotericin B.

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