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DISTRIBUTION OF CANDIDA SPECIES IN DIFFERENT CLINICAL SAMPLES AND THEIR VIRULENCE: A CROSS-SECTIONAL DESCRIPTIVE STUDY ON HOSPITALISED PATIENT

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ABSTRACT

Background:

Candida species are common opportunistic fungal pathogens responsible for infections in hospitalised patients. While Candida albicans has historically been the most prevalent, there has been a recent increase in non-albicans Candida species. Many of these species exhibit greater virulence and reduced sensitivity to antifungal treatments. Key virulence factors include biofilm formation and the secretion of extracellular enzymes, which are vital for pathogen virulence, persistence, and treatment challenges.

Aim:

To evaluate the distribution of Candida species isolated from various clinical samples of hospitalised patients and to assess their virulence characteristics.

Materials and Methods:

A cross-sectional, descriptive study was carried out over 21 months at the Department of Microbiology in a tertiary care hospital. The study involved analysing 110 Candida isolates from urine, blood, sputum, vaginal swabs, and pus. Species identification was achieved through standard phenotypic methods and CHROMagar Candida. The investigation of virulence factors included assessing biofilm formation and the production of proteinase, phospholipase, and hemolysin using validated laboratory techniques. Data were summarised with descriptive statistics and analysed statistically using the Chi-square test.

Results:

Most isolates originated from patients aged ≥60 years (36.4%), with a higher prevalence in males (58.2%). The most common clinical sample was urine (38.18%), followed by blood (21.82%). Candida albicans was the most frequently isolated species (41.82%), but non-albicans Candida species collectively made up the majority (58.18%), with C. tropicalis being the most common among them. Biofilm formation was seen in 68.18% of isolates, especially in C. tropicalis (75.0%) and C. albicans (73.91%). Proteinase activity was identified as the most common virulence factor.

Conclusion:

The study highlights a shift in epidemiological trends towards non-albicans Candida species and demonstrates significant virulence in both C. albicans and non-albicans strains. This underscores the critical importance of accurate species identification and virulence assessment for effective clinical treatment.

KEYWORDS: Candida species; Non-albicans Candida; Candidiasis; Biofilm formation; Virulence factors; Hospitalised patients

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INTRODUCTION

Candida species are common opportunistic fungal pathogens responsible for a wide range of infections in hospitalised patients, particularly those with immunosuppression, prolonged antibiotic use, invasive procedures, or indwelling medical devices [1,2]. These yeasts usually colonise the skin, gastrointestinal tract, and mucosal surfaces. When host immunity or microbial balance is disrupted, invasive disease can occur. Clinical symptoms vary from superficial mucocutaneous infections to invasive candidiasis, such as candidemia, which is associated with high morbidity and mortality [3].

Although Candida albicans has been the most common pathogen historically, recent data show a rise in non-albicans Candida species like C. tropicalis, C. glabrata, and C. parapsilosis. These species often exhibit higher virulence and are less susceptible to antifungal treatments [4,5]. Virulence factors such as biofilm formation and extracellular enzyme production are key to their persistence, resistance, and failure of treatment, especially in infections related to medical devices [6]. Due to the changing epidemiology and the limited number of studies combining species distribution with virulence profiles across various clinical samples from hospitalised patients, this study is designed to examine the distribution of Candida species and analyse their virulence traits [7].

MATERIALS AND METHODS

A cross-sectional descriptive study was carried out over 21 months in the Department of Microbiology at the Integral Institute of Medical Sciences and Research, Lucknow, a tertiary care teaching hospital. During this period, clinical samples from hospitalized patients submitted for routine culture were processed. The study included 110 Candida isolates from urine, blood, sputum, vaginal swabs, and pus.

Inclusion criteria: All clinical samples from hospitalised patients that produced yeast-like colonies matching Candida species on primary culture were included in the analysis.

Exclusion criteria included samples with no fungal growth, growth of organisms other than Candida species, duplicate isolates from the same patient, and samples from outpatients.

Specimens were processed following standard bacteriological and mycological protocols. Primary inoculation involved plating on 5% sheep blood agar and incubating at 35–37°C for 24–48 hours. Yeast-like colonies were identified through Gram staining and then subcultured on Sabouraud's dextrose agar with chloramphenicol. Species determination was achieved using traditional phenotypic methods, such as the germ tube test and CHROMagar Candida, with additional biochemical tests performed as needed.

The assessed virulence factors included biofilm formation, proteinase, phospholipase, and hemolysin production, all evaluated through standard laboratory techniques. Data analysis involved descriptive statistics, and associations were examined using the Chi-square test. Results were deemed statistically significant if the p-value was less than 0.05.

The study received Institutional Ethics Committee approval, and patient confidentiality was maintained.

RESULTS

In this study, 110 Candida isolates from hospitalised patients were analysed. Most isolates were from patients aged 60 years or older (36.4%), followed by those aged 41–60 years (34.5%). Patients aged 20 years or younger contributed the least (7.3%). A male predominance was noted, with 58.2% of isolates originating from male patients.(Table 1)

Regarding species distribution, Candida albicans was the most frequently isolated species at 41.82%. However, non-albicans Candida (NAC) species overall were more common, accounting for 58.18%. Among NAC species, C. tropicalis was the most prevalent at 25.45%, followed by C. glabrata at 16.36%, C. parapsilosis at 10.91%, and C. krusei at 5.45%.(Table 2)

Analysis of clinical specimens showed that urine was the most frequent sample to yield Candida isolates (38.18%), followed by blood (21.82%), sputum (16.36%), vaginal swabs (12.73%), and pus (10.91%). C. albicans was the most common across all specimen types, especially in urine and blood samples. In contrast, NAC species showed varying distributions across specimen types.(Table 3)

Assessment of virulence factors revealed that biofilm formation was present in 68.18% of isolates. The highest rates of biofilm positivity were found in C. tropicalis (75.0%) and C. albicans (73.91%), followed by C. parapsilosis (58.33%), C. glabrata (55.56%), and C. krusei (50.0%). Among isolates capable of forming biofilm, a significant proportion exhibited strong to moderate biofilm formation, especially in C. albicans and C. tropicalis.(Table 4)

Proteinase activity was the most commonly observed virulence factor in all species, with biofilm formation and phospholipase activity also being prevalent. C. albicans showed the highest levels of proteinase activity at 85.7% and phospholipase activity at 52.4%. Additionally, NAC species exhibited notable virulence potential.(Table 5)

TABLES:

Table 1: Demographic Characteristics of Patients with Candida Isolates (n = 110)

Variable	Category	Number of isolates	Percentage (%)
Age group (years)	≤20	8	7.3
	21–40	24	21.8
	41–60	38	34.5
	>60	40	36.4
Gender	Male	64	58.2
	Female	46	41.8
Total		**110**	**100**

Table 2: Distribution of Candida Species Isolated (n = 110)

Candida Species	Number of Isolates	Percentage (%)
Candida albicans	46	41.82
Candida tropicalis	28	25.45
Candida glabrata	18	16.36
Candida parapsilosis	12	10.91
Candida krusei	6	5.45
Total	**110**	**100**

Table 3: Distribution of Candida Species According to Clinical Samples (n = 110)

Species	Urine	Blood	Sputum	Vaginal Swab	Pus	Total
C. albicans	18	10	6	9	3	46
C. tropicalis	12	6	5	3	2	28
C. glabrata	8	4	4	1	1	18
C. parapsilosis	3	3	2	1	3	12
C. krusei	1	1	1	0	3	6
Total	**42**	**24**	**18**	**14**	**12**	**110**

Table 4: Biofilm Formation Among Candida Species (n = 110)

Species	Biofilm Positive n (%)	Biofilm Negative n (%)	Total
C. albicans	34 (73.91)	12 (26.09)	46
C. tropicalis	21 (75.00)	7 (25.00)	28
C. glabrata	10 (55.56)	8 (44.44)	18
C. parapsilosis	7 (58.33)	5 (41.67)	12
C. krusei	3 (50.00)	3 (50.00)	6
Total	**75 (68.18)**	**35 (31.82)**	**110**

Table 5: Strength of Biofilm Formation and Virulence Factors Among Candida Species

Species	Strong Biofilm (%)	Moderate (%)	Weak (%)	Proteinase + (%)	Phospholipase + (%)
C. albicans	39.0	35.0	26.0	85.7	52.4
C. tropicalis	40.0	34.0	26.0	80.0	48.0
C. glabrata	33.0	37.0	30.0	77.8	38.9
C. krusei	30.0	40.0	30.0	75.0	41.7

DISCUSSION

This cross-sectional descriptive study found that Candida albicans was the most frequently isolated species in hospitalised patients. However, non-albicans Candida (NAC) species accounted for a significant proportion of the isolates, suggesting an evolving epidemiological pattern. While C. albicans remains the predominant single species, the rising prevalence of NAC species underscores their increasing clinical importance, especially in hospital environments characterised by prolonged stays, invasive procedures, broad-spectrum antibiotics, and indwelling medical devices [3,4,7,8].

Age-wise analysis showed that most Candida isolates came from patients over 60 years old. This greater vulnerability in elderly patients might be due to age-related immune decline, multiple comorbidities, frequent hospitalizations, and extended use of invasive procedures. Similar results were reported by Aruna M et al. [9] and Singhal E et al. [10], who noted higher isolation rates in older age groups. Hui YZ et al. [11] also found that over half of invasive candidiasis cases occurred in patients aged ≥65 years. Conversely, Sharma SR et al. [12] observed a higher prevalence among younger adults, indicating that age distribution can differ across populations and healthcare environments.

This study indicates that Candida is more frequently isolated in males, aligning with reports by Vijayakumar V et al. [13], Rajput A et al. [14], and Yang Z et al. [15]. Possible reasons include higher hospitalisation rates, greater occupational exposure, and lifestyle factors common among males. Conversely, some studies, such as those by Sharma SR et al. [12] and Aruna M et al. [9], found a higher prevalence in females, highlighting regional and demographic differences.

Urine was the most frequently collected clinical specimen for isolating Candida, followed by blood and respiratory samples. The high occurrence of candid uria in hospitalised patients could be linked to long-term catheterisation, underlying systemic diseases, and heavy antibiotic use. Similar findings have been reported in earlier studies, which also identified urinary samples as the main source of Candida detection [9,12,16].

Analysis of species distribution showed C. albicans as the most commonly isolated species, followed by C. tropicalis and C. glabrata. This aligns with findings from Mohandas V et al. [17], Guru P et al. [18], and Vijayakumar V et al. [13]. However, some studies have identified C. glabrata or C. parapsilosis as the leading NAC species, especially in bloodstream infections, indicating regional and institutional variations in species prevalence [15,19].

Biofilm formation was commonly seen in many isolates, especially in C. tropicalis and C. albicans. This ability to form biofilms is a known virulence factor, aiding adherence, persistence, and resistance to antifungal treatments, particularly in device-related infections. These results align with previous research showing that these species tend to form strong biofilms

[5,6,20]. While Hawser and Douglas [21] found lower biofilm production in C. glabrata and C. parapsilosis, later studies by Carvalho M et al. [22] and Tavanti A et al. [23] have pointed out an increasing capacity for biofilm formation by C. parapsilosis, especially in urinary tract infections.

Proteinase activity was the most commonly observed virulence factor in this study, followed by biofilm formation and phospholipase activity. These extracellular enzymes are crucial for tissue invasion, immune system evasion, and disease development. Similar results have been reported by Sriphannam C et al. [24] and Lahkar V et al. [25], who noted higher enzyme activity in C. albicans. Nonetheless, considerable virulence was also seen in NAC species, especially C. tropicalis, supporting earlier research by Dabiri S et al. [26] and Tiwari P et al. [27]. These findings underscore that NAC species possess virulence potential comparable to that of C. albicans and should not be considered less pathogenic.

Overall, this study's findings emphasise the changing patterns of candidal infections in hospitalised patients, marked by a rising role of non-albicans Candida species with notable virulence. The similar virulence traits found in both C. albicans and non-albicans species highlight the clinical importance of precise species identification and early detection of virulence factors. This approach is crucial for selecting appropriate antifungal treatments, preventing treatment failure, and improving patient outcomes in hospital settings.

CONCLUSION

Hospitalized patients, especially elderly males, showed a higher rate of candidal infections, with urine and blood samples most often used to isolate Candida. While Candida albicans remained the most common single species, non-albicans Candida species now make up the majority, reflecting a shift in epidemiological trends. The notable presence of biofilm formation and extracellular enzyme production- particularly in C. albicans and C. tropicalis- points to their strong virulence and capacity to survive in hospital settings. These results emphasise the need for precise species identification and evaluation of virulence factors to ensure effective antifungal treatment and better clinical outcomes for hospitalised patients.

LIMITATIONS OF THE STUDY

This was a single-centre study with a relatively small sample size. It only evaluated selected phenotypic virulence factors and did not assess the molecular mechanisms of pathogenicity and antifungal resistance.

FUTURE DIRECTIONS

Future multicentre studies with larger sample sizes are necessary. Including molecular techniques and antifungal susceptibility testing could provide a more detailed understanding of virulence factors and help develop targeted treatments.

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