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PREVALENCE OF CANDIDA SPECIES AND ANTIFUNGAL SUSCEPTIBILITY TESTING AT A TERTIARY CARE HOSPITAL

Medical Microbiology

Anil Kumar* Tutor, Department of Microbiology, Shri Gorakshnath Medical College Hospital and Research Centre, Mahayogi Gorakhnath University Gorakhpur, Uttar Pradesh, India-273007. *Corresponding Author

Sshrutkirti Gupta Assistant Professor, Department Of Microbiology, Integral Institute Of Medical Science & Research, Dasauli, Kursi Road, Lucknow-226026.

ABSTRACT

Objective: This study aimed to evaluate the study the prevalence of Candida species and antifungal susceptibility testing at a tertiary care hospital. **Methods:** Hospital – based observational prospective study. Over a period of six months, sample received from various clinical departments with suspicion of candidiasis we evaluated 104 clinical sample for culture and antifungal susceptibility testing. **Results:** Among 104 clinically suspected cases, 38 (36.5%) were positive for Candida species, 52 showed bacterial growth, and 14 had no growth. Gender-wise, 47.4% of males and 52.6% of females were infected. Rural participants had higher positivity (24/72) than urban (8/32). Age-wise, the highest prevalence was in 51–60 years (28.9%). Positivity by sample type was highest in sputum (60%), followed by urine (47.4%) and high vaginal swabs (42.9%). Candida albicans accounted for 34.2% of isolates, while non-albicans Candida (65.8%) included C. tropicalis, C. glabrata, C. krusei, C. parapsilosis, and C. dubliniensis. Voriconazole and fluconazole showed the highest efficacy (80–100%), while clotrimazole, nystatin, and itraconazole were less effective (40–75%). **Conclusions:** The study highlights a significant prevalence of non-albicans Candida species, with C. tropicalis and C. glabrata emerging as important pathogens. Voriconazole and fluconazole were found to be the most effective antifungal agents, while reduced susceptibility was noted with clotrimazole, nystatin, and itraconazole. These findings emphasize the need for routine species-level identification and antifungal susceptibility testing to guide effective therapy and prevent resistance.

KEYWORDS

Candida Albicans, Non Candida Albicans, Candidiasis, Antifungal Susceptibility, Fungal Infection.

INTRODUCTION

Candida species are commensal yeasts that typically reside in the environment (soil, water, plants), on human mucous membranes (oral cavity, vagina), and on the gastrointestinal system and skin (R & Rafiq, 2025). They are unicellular, pleomorphic yeasts with an incomplete sexual cycle that are usually ovoid or spherical in shape. In healthy individual lactobacilli protective vaginal flora and the host's immune system control their proliferation. However, illnesses like vulvovaginal candidiasis have been connected to a disturbance of this equilibrium (Chee et al., 2020). Candida species are the most prevalent fungal diseases worldwide. Globally, Candida species are the most frequent fungal pathogens. Although Candida albicans remains the leading cause its prevalence is declining while non-albicans Candida (NAC) species are increasingly reported. Of more than 200 known species, around 20 are pathogenic with six C. albicans, C. tropicalis, C. glabrata, C. parapsilosis, C. krusei, and C. dubliniensis responsible for over 90% of invasive infections (Sharma & Chakrabarti, 2023). Invasive candidiasis, especially in hospitalized patients, accounts for nearly one-fourth of fungal bloodstream infections and has emerged as a major nosocomial infection in both adults and children over the past two decades (Alkharashi et al., 2019). Risk factors for invasive disease include immunosuppressive therapy, transplantation, central venous catheterization, parenteral nutrition, ICU stay, severe illness, diabetes, renal failure, hemodialysis, and broad-spectrum antibiotics (Muskett et al., 2011). NAC species are becoming more and more isolated, with geographic and patient demographic variations, although C. albicans accounts for almost half of invasive infections (Abdel-Hamid et al., 2023a). Candida is another significant cause of UTIs, accounting for 10–15% of nosocomial UTIs, especially in hospitalized or catheterized patients (Rishpana & Kabbin, 2015). Immunosuppression, diabetes, indwelling catheters and long-term antibiotic use are risk factors. Due to the potential for increased morbidity and mortality from inefficient or delayed therapy the advent of drug-resistant NAC species has complicated patient management (Sharifzadeh Kermani et al., 2025). Finding the prevalence of candidiasis in a tertiary care hospital and relating incidence to risk profiles and sociodemographic characteristics would help improve preventative and control measures.

MATERIAL AND METHODS

Study Design and Period: This prospective study received ethical clearance from the Institutional Research Committee and Institutional Ethical Committee on 17 October 2023(IEC/IIMSR/2023/24). It was conducted at the Department of Microbiology, IIMSR, Lucknow, over a period of six months (October 2023 to March 2024).

Data Collection: We evaluated 104 clinical samples for culture and antifungal susceptibility test which were received from various clinical departments with suspicion of candidiasis.

Inclusion Criteria:

1. Sample will be taken from all the clinically suspected patients of Candidiasis.
2. Patients who give consent will be included.

Exclusion Criteria:

1. Those who have taken systemic antifungal drugs during the past week and topical antifungal drugs before one day.
2. Infants will be excluded.

Laboratory Diagnosis:

Sample Collection and Sample Processing: Oral, genital and cutaneous swabs were collected from affected areas using sterile cotton swabs, while skin scrapings were taken with a sterile scalpel. High vaginal swabs (HVS) and other clinical specimens were transported in sterile tubes or transport media to preserve integrity and minimize contamination (Vilstrup et al., 2018). Blood culture bottles were processed promptly in shown (Figure 1).

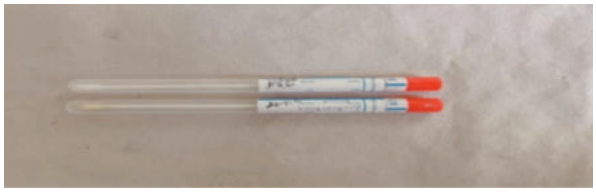

Figure: 1 HVS Swab Sticks

Microscopic Examination- Smears were prepared, heat-fixed, and stained with crystal violet, Gram's iodine, acetone, and safranin. Candida appears as Gram-positive, oval/circular budding yeast, sometimes with pseudohyphae (Tripathi et al., 2025) in shown (Figure 2).

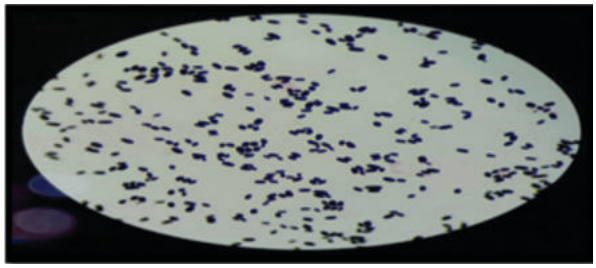

Figure 2: Candida Gram Positive Budding Yeast Cell

Potassium Hydroxide (KOH) Examination: Swabs were immersed in 10% KOH for 10–15 minutes to observe fungal hyphae under low and high-power microscopy (Hasan et al., n.d.) in shown (Figure 3).

Figure 3: KOH Microscopy

Fungal Culture- Samples were inoculated on Sabouraud Dextrose Agar (SDA) with chloramphenicol and cycloheximide and incubated at 25°C for 24–48 h (up to 14 days if no growth). Candida colonies were identified based on morphology, Gram staining and subsequent tests (Das et al., 2010) in shown Figure 4.

Figure 4: SDA Culture Bottles

Identification Tests:

Germ Tube Test: Presumptive identification of C. albicans and C. dubliniensis in shown Figure 5.

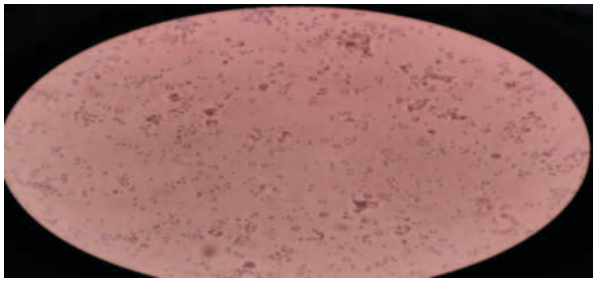

Figure 5: Positive Germ Tube

Chlamydospores Formation: Observed on cornmeal or rice agar to differentiate species.

Biochemical Test: Sugar fermentation and assimilation tests were performed for species-level identification.

CHROM Agar- Color-based differentiation of Candida species after SDA growth (Byadarahally Raju & Rajappa, 2011) in shown Figure 6.

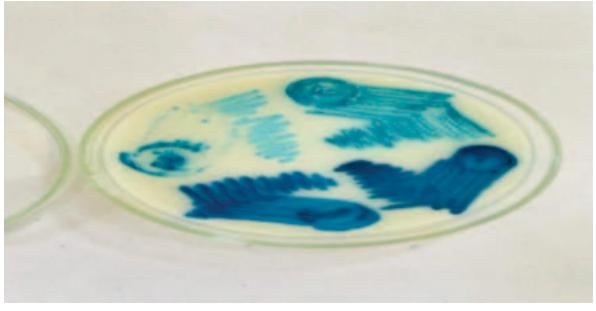

Figure 6: Positive Hichrome

Typing of Candida Strains- Phenotypic (morphotyping, resistance pattern) and genotypic (karyotyping, RFLP, PNA-FISH) methods were used for strain differentiation (Iwata-Otsubo et al., 2016).

Detection of Metabolites: D-mannose and D-arabinitol detection via gas–liquid chromatography (de Repentigny et al., 1983).

Antifungal Susceptibility Testing: Performed by disc diffusion on Mueller-Hinton agar + 2% glucose with methylene blue (0.5 µg/mL). Drugs tested included fluconazole (10 µg), voriconazole (10 µg), clotrimazole (10 µg), nystatin (100 µg), and itraconazole (10 µg) (Pfaller et al., 2004) in Figure 7.

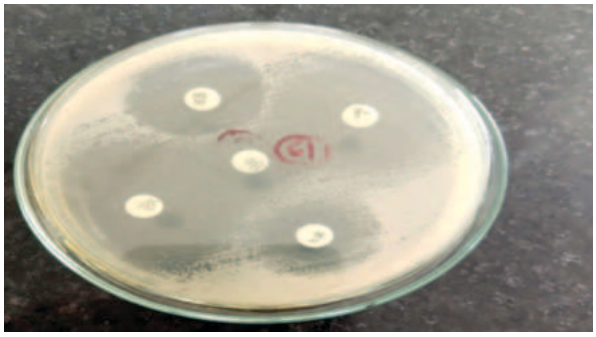

Figure 7: Antifungal Susceptibility Testig

OBSERVATIONS AND RESULTS

❖ 38 positive cases out of 104 sample taken from patients who were clinically suspected with candidiasis. Bacteria showed positivity in 52 sample, whereas remaining 14 samples tested negative in shown figure 8.

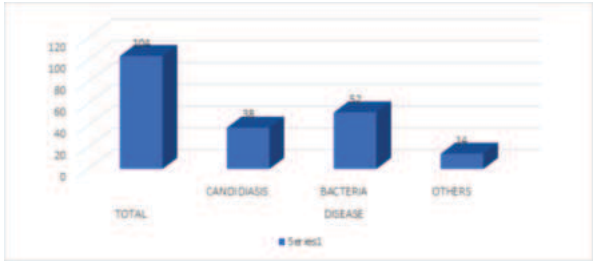

Figure 8: Columns Displaying the Distribution of Infection Among Total Suspected Patient.

❖ Gender-wise Distribution of Candida Infection: Gender-wise distribution showed that 47.4% of males (n=18) and 52.6% of females (n=20) were positive for Candida infection in shown figure 9.

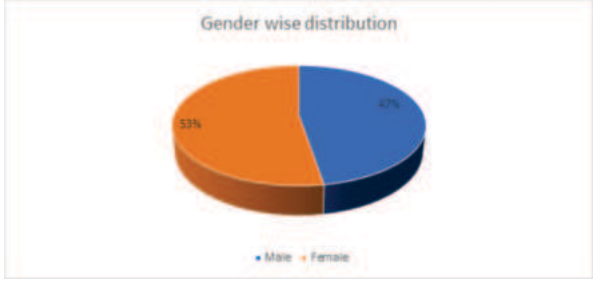

Figure 9: Pie Chart Gender-wise Distribution of Candida Infection

The Sociodemographic Profile Was Thoroughly Examined, and Many Variables Such as Patient Resident Type (rural/urban), Age, Literacy, Hygiene Were Thoroughly Compared. Here are Some of the Outcomes.

❖ **Candidiasis Distribution Based on Area of Residence (Rural/Urban):** The sociodemographic profile was analyzed considering variables such as residence (rural/urban), age, literacy and hygiene. Among rural participants 24 were positive and 48 negative (n=72), while among urban participants 8 were positive and 24 negative (n=32). Overall positivity rate 34.6%

❖ **Candidiasis Distribution According to Age Group:** In shown table 1.

Table 1: Candidiasis Distribution According to Age Group

AGE Group	Total	Disease		Frequency
		Negative	Positive	
<10	104	0	0	0
11-20		10	1	11
21-30		12	5	17
31-40		9	7	16
41-50		8	9	17
51-60		7	11	18
61-70		9	3	12
71-80		11	2	13

- **Candidiasis Distribution Based on Literacy Rate:** There were 70 total samples from the illiterate group, of which 28 were positive and 34 were negative for candidiasis, while there were only 10 positive candidiasis cases and 24 negative instances in the literate section.
- **Candidiasis Distribution Based on Hygiene:** The comparison study based on good and poor hygiene is explained in the table and figure above. Patients who practiced good hygiene were 38, of whom 16 tested positive for candidiasis and 22 tested negative, whereas patients who practiced poor hygiene were more numerous, totalling 66 cases of which 22 tested positive and 44 tested negative.
- A total of 104 samples were analysed. Positivity rates were sputum (60%), urine (47.4%), high vaginal swabs (42.9%), blood (30.8%), swabs (18.2%), pus (9.1%) and endotracheal samples (9.1%) in shown table 2.

Table 2: Candidiasis Distribution According to Specimen

Sample	Positive	Negative	Total Number Of Sample
Sputum	15	10	25
Urine	9	10	19
HVS	6	8	14
Blood	4	9	13
Swab	2	9	11
Pus	1	10	11
Et	1	10	11
Total	38	66	104

- For proper identification, all samples were inoculated on two culture medium: SDA and Candida CHROM agar. Since only one isolate of each species of Candida was detected in each sample, the prevalence of positive Candidiasis was determined to be 36.53%. On the basis of the colour produced in the Candida CHROM agar and Germ tube tests, it was determined that out of 38 positive cases, 25 isolates were Non albicans Candida (NAC) and 13 isolates were of Candida albicans. Candida tropicalis (8), Candida glabrata (7), Candida krusei (5), candida parapsilosis (3) and Candida dubliniensis (2) were the most frequent NAC isolates in shown figure 10.

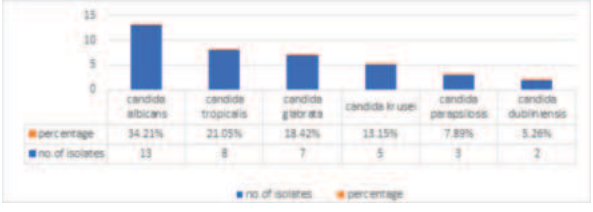

Figure 10: The Distribution of Candida Species in the Candidiasis Instance is Depicted by a Bar Graph

Isolated Candida Species and its Antifungal Susceptibility Pattern:

The antifungal susceptibility testing of isolated Candida species against five commonly used antifungal agents (Fluconazole, Voriconazole, Clotrimazole, Nystatin and Itraconazole) revealed varying sensitivity patterns in shown table 3 and figure 11.

Table 3: Candida Species and its Antifungal Susceptibility Pattern

Candida species	Fluconazole (FLC)	Voriconazole (VRC)	Clotrimazole (CC)	Nystatin (NYS)	Itraconazole (IT)
C. albicans	100%	100%	69%	62%	62%
C. glabrata	71.42%	85.71%	57.14%	42.85%	42.85%
C. krusei	80%	80%	40%	60%	40%
C. parapsilosis	100%	100%	67%	67%	67%
C. tropicalis	75%	87.50%	75%	75%	62.50%
C. dubliniensis	100%	100%	100%	50%	50%

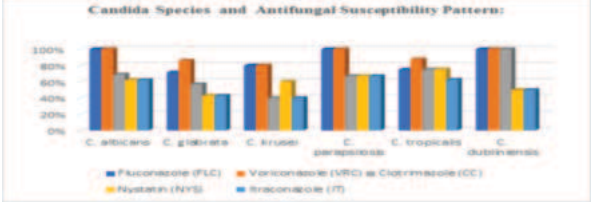

Figure 11: Candida Species and its Antifungal Susceptibility Pattern

C. albicans, C. parapsilosis, and C. dubliniensis showed complete (100%) sensitivity to both fluconazole and voriconazole. C. glabrata exhibited the lowest sensitivity rates, particularly to itraconazole and nystatin (42.85% each). C. krusei demonstrated moderate sensitivity to fluconazole and voriconazole (80%) but lower sensitivity to clotrimazole and itraconazole (40%). C. tropicalis showed high sensitivity to voriconazole (87.5%) and moderate sensitivity to the other agents.

DISCUSSION

Infections continue to be a common issue for hospitalized patients and in recent decades, Candida species have become important pathogens. The prevalence of non-albicans Candida (NAC) emphasizes how crucial a precise laboratory diagnosis is to prompt and efficient treatment (Abdel-Hamid et al., 2023b). 104 clinical samples examined throughout the six-month research (October 2023–March 2024), 38 (36.53%) tested positive for Candida. The highest number of isolates were from sputum (39.47%), followed by urine (23.68%), high vaginal swab (15.78%), blood (10.52%), swab (5.26%), pus (1.63%) and ET fluid (1.63%). Similar distributions have been reported in previous studies by (Ortiz et al., 2022). Candida albicans was the most common species (34.21%), followed by Candida tropicalis (21.05%), Candida glabrata (18.42%), Candida krusei (13.15%), Candida parapsilosis (7.89%), and Candida dubliniensis (5.26%). Although some study suggests that NAC species are becoming more isolated, indicating their emergence as important pathogens findings are in line with previous observations from India (Saeed et al., 2024) and international investigations. C. tropicalis was the most common NAC species is consistent with European data (Acosta-Mosquera et al., 2024) and observations from (Abdel-Hamid et al., 2023c). Using CHROMagar to identify species was quicker, easier, and more accurate than using conventional techniques. It enables the identification of mixed cultures and provides data in 24–48 hours, making it a cost-effective phenotypic alternative to molecular assays in resource-constrained settings. Antifungal susceptibility testing showed significant species to species variation (Nadeem et al., 2010). Fluconazole (FLC) and voriconazole (VRC) exhibited 100% sensitivity in Candida albicans, while clotrimazole (69%), nystatin (62%) and itraconazole (62%) displayed decreased sensitivity. The sensitivity of C. tropicalis was the highest to VRC (87.5%), the lowest to IT (62.5%) and the lowest to FLC, clotrimazole and nystatin (75% each). The sensitivity of C. glabrata to VRC was (85.71%), NYS and IT, was decreased (42.85%). Although C. krusei was only 40% sensitive to CC and IT, it was 80% sensitive to FLC and VRC. Whereas C. dubliniensis showed full sensitivity to FLC, VRC and CC but only 50% to NYS and IT. C. parapsilosis was moderately sensitive (67%) to other agents and entirely sensitive to FLC and VRC. Overall, C. albicans remained the most common isolate. Rapid species-level identification using CHROMagar, combined with susceptibility testing is crucial for guiding antifungal therapy, improving patient outcomes, and reducing morbidity, mortality and treatment costs.

CONCLUSION

This study at a tertiary care hospital highlights a significant prevalence of candidiasis, emphasizing the need for ongoing surveillance and targeted interventions. Among 104 samples analyzed, 38 (35.18%) were positive, with Candida albicans accounting for 13 (34.21%) and non-albicans Candida (NAC) species for 25 (65.78%). The highest infection incidence (61.11%) occurred in patients aged 51–60 years, likely due to age-related immune decline, comorbidities, and medical interventions, making elderly individuals particularly vulnerable. Antifungal susceptibility testing revealed variable resistance among isolates, including reduced sensitivity to fluconazole, underlining the importance of routine testing and judicious antifungal use. The increasing prevalence of NAC species and emerging resistance patterns pose significant challenges, necessitating effective antifungal stewardship and species-level identification to guide therapy. Overall, candidiasis remains a multifaceted health issue, requiring comprehensive strategies to improve outcomes and reduce its impact within healthcare settings.

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