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Prevalence of *Candida* species and their Susceptibility to Triazoles in Clinical Isolates from a Tertiary Care Hospital

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Abstract

In the recent past, the incidence of Candidiasis has witnessed a concerning upsurge, resulting in a significant healthcare challenge. These infections are further exacerbated by factors like the widespread use of broad-spectrum antimicrobials, chemotherapy-induced neutropenia, and the presence of medical devices. The present study is designed to address the critical need for identifying the *Candida* species responsible for clinical infections and assessing their susceptibility to key antifungal drugs Fluconazole, Voriconazole, and Itraconazole. Two hundred clinical samples from Rohilkhand Medical College & Hospital, Bareilly were analyzed. Using Vitek-2 Compact (Biomerieux, France), the *Candida* spp. and the antifungal drug sensitivities were identified for Fluconazole and Voriconazole. E-test was done to identify Itraconazole sensitivity. This study found that *C. albicans* accounted for 21.5% while Non-albicans *Candida* (NAC) constituted 78.5%. Prolonged medication was the most common factor making susceptible for Candidiasis (43.5%), followed by indwelling biomedical devices (23%), Diabetes mellitus (16%), surgical causes (5.5%), trauma (5%), pregnancy (5%), and HIV (2%). Antifungal susceptibility testing showed that 68.5%, 72%, and 69.5% of *Candida* spp. isolates were sensitive to Fluconazole, Voriconazole, and Itraconazole, respectively. In conclusion, non-albicans *Candida* infections are increasing due to predisposing conditions, and some of these species are inherently resistant to the routinely used antifungal drugs. The study emphasizes the importance of identifying *Candida* spp. and their susceptibility to antifungals. This can limit the indiscriminate use of antifungal drugs, aid in selecting appropriate treatments, and reduce treatment costs, hospital stays, and patient morbidity and mortality.

Keywords: Antifungal Susceptibility, Azoles, *Candida*, Non-albicans *Candida*

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INTRODUCTION

The prevalence of *Candida* infections has reached alarming levels in recent past, leading to significant mortality and morbidity. *Candida* species, including *Candida albicans* and Non-albicans *Candida* spp. such as *C. tropicalis, C. parapsilosis, C. glabrata, C. krusei, C. guilliermondii, C. dubliniensis*, and *C. auris* are major contributors to human fungal infections. Candidiasis, once rare, has become increasingly common among bedridden and critically ill patients, despite improvements in medical care. These fungal infections pose a substantial risk to both healthy individuals and those with compromised immune systems.[1] *Candida* spp. are also a leading cause of nosocomial infections. *Candida* overcomes immune defenses, invades tissues, and causes severe infections, often facilitated by medical devices. Factors such as broad-spectrum antibiotics, chemotherapy-induced neutropenia, reduced phagocytic activity, and parenteral nutrition contribute to the growth and invasion of *Candida*. Among *Candida* spp., *Candida albicans* and Non-albicans *Candida* spp. such as *C. tropicalis, C. glabrata, C. parapsilosis*, and *C. krusei* account for as high as 90% of all the infections. However, *Candida auris* a notoriously multiple drug resistant species, pose a great challenge, particularly in critically ill patients. Another interesting aspect is that the diversity of *Candida* spp. differ from multicenter studies to single center studies.

Candidiasis, caused by various *Candida* spp., necessitates a diverse range of antifungal drugs for effective treatment. Polyenes, azoles, echinocandins, nucleoside analogues, and allylamines are commonly used drug classes. *Candida spp.* exhibit variations in virulence and drug sensitivity patterns. Notably, *C. krusei* displays intrinsic resistance to fluconazole due to low fluconazole-ERG11 affinity, rendering it ineffective for treatment. Studies have shown high resistance rates among *C. krusei* and *C. glabrata* to fluconazole and azoles, respectively. Timely identification of *Candida* spp. is crucial prior to initiating treatment, as empirical therapy can prolong the disease and lead to increased morbidity and mortality. Determining the sensitivity pattern of *Candida* spp. to antifungal drugs is vital for selecting appropriate and targeted therapy. Although significant progress has been made in the management of Candidiasis, the increased prevalence of drug-resistant *Candida* strains poses a significant hurdle, impacting treatment outcomes. Despite the introduction of novel antifungal agents, the survival rates of critically ill patients with *Candida* infections remain largely unchanged, which can be partly attributed to the emergence of drug resistance.

As the *Candida* spp. differ in the antifungal sensitivity pattern, it is important to identify the species of *Candida.*

The study aims to find the distribution of *Candida* spp. in clinical samples and find the susceptibility pattern to triazole antifungal drugs namely Fluconazole, Voriconazole and Itraconazole.

MATERIALS AND METHODS

A cross sectional study was done from the year 2019 to 2022 at Rohilkhand Medical College & Hospital, A Tertiary Care Hospital in Bareilly. A total of 1437 culture positive samples collected from patients in Intensive Care Units (ICU), In-Patients Wards (IP) and Out Patients Departments (OPD). 200 isolates of *Candida* spp. obtained from these samples. The samples included respiratory samples (sputum, Endotracheal (ET) aspirate, bronchoalveolar lavage (BAL), throat swabs), blood, urine, vaginal swabs, CSF, pus and catheter tips.

Sample collection

Enough sample was collected from active lesions under strict aseptic conditions using sterile instruments following the universal safety precautions. All the samples were transported immediately to the laboratory without delay.

Sample processing

The samples (Urine, Respiratory samples, Vaginal swabs, CSF, Pus, Catheter tips) were subjected to direct Gram's Staining and were inoculated into Blood Agar and Mac Conkey Agar and incubated at 37°C for 24-48 hours. If there is no growth after 48 hours of incubation, the sample is considered sterile for aerobic organisms. If there is growth, the colonies were picked up

and a Gram's stain is done. In the Gram's stain preparation, the appearance of Gram positive, oval, budding yeast like fungi with pseudohyphae are presumptively identified as *Candida* spp. Additionally, the samples were inoculated in Saboraud's Dextrose Agar and incubated at 25°C for 48 hours to one week. Blood samples were incubated in BacTAlert3D (Biomerieux, France) automated blood culture system. A positive blood culture is subcultured in Blood Agar and Mac Conkey Agar and processed like explained above for other samples.

Candida species identification

HiCrome™ Candida Differential Media (HiMedia, Mumbai) was used to differentiate *Candida* species based on the color of the colonies after 48 hours of aerobic incubation at 37°C. As the color is purely subjective, when there is any ambiguity, sugar fermentation, sugar assimilation and microscopic morphology on Corn Meal agar media and Vitek-2™ yeast identification cards were used to correctly identify the species.

Antifungal sensitivity testing

A few colonies from the pure culture of *Candida* were transferred to 3ml of sterile saline and vortexed until the turbidity of the suspension is in the range of 1.80-2.20 McFarland units. The suspension was inoculated into YS09™ (Biomerieux, France) Yeast Antifungal Susceptibility cards and processed in Vitek-2™(Biomerieux, France) system. Sensitivity for the drugs Fluconazole and Voriconazole were obtained in this method. For the sensitivity of Itraconazole, E-test method was used. CLSI guidelines were followed for testing and interpretation of the antifungal sensitivity results.

RESULTS

A total of 200 *Candida* spp. were isolated from different clinical samples. Of these, 116 (58%) were from Respiratory samples, 34 (17%) from urine, 23 (11.5%) from Blood, 22 (11%) from vaginal swab, 5 (2.5%) from pus samples. The distribution of different *Candida* spp. from various samples is summarized in Table 1.

Table 1. Distribution of *Candida* spp. in various samples

	Respiratory Samples	Urine	Blood	Vaginal Swab	Pus	Total
C. tropicalis	69	16	9	9	1	104
C. albicans	25	8	4	5	1	43
C. dubliniensis	6	3	7	5	2	23
C. krusei	7	2	2	1	0	12
C. gulliermondii	6	4	1	1	0	12
C. glabrata	3	1	0	1	1	6
Total	116	34	23	22	5	200

Table 2. Prevalence of *Candida albicans* and Non-*albicans Candida* among various predisposition factors

Predisposing factor	*C. albicans*	Non-*albicans Candida*	Total
Prolonged medication	17	70	87
Biomedical Devices (IV Catheters, Central line tips, Ventilator, ET tube)	8	38	46
Diabetes Mellitus	6	26	32
Trauma	4	6	10
Surgical Causes	3	8	11
Pregnancy	5	5	10
HIV	0	4	4
Total	43	157	200

Table 3. Triazole antifungal drug susceptibility pattern to *Candida* spp.

	Candida albicans	Non-*albicans Candida*	Total
Fluconazole			
Resistant	12 (27.9%)	51 (32.5%)	63
Sensitive	31 (72.1%)	106 (67.5%)	137
Total	43	157	200
Voriconazole			
Resistant	10 (23.2%)	46 (29.3%)	56
Sensitive	33 (76.7%)	111 (70.7%)	144
Total	43	157	200
Itraconazole			
Resistant	13 (30.2%)	48 (30.6%)	61
Sensitive	30 (69.8%)	109 (69.4%)	139
Total	43	157	200

Of all the *Candida* spp. isolated, *C. albicans* were 43 (21.5%) and Non-albicans *Candida* (NAC) were 157 (78.5%). Species wise, *C.tropicalis* 104 (52%) was the most commonly isolated species, followed by *C. albicans* 43 (21.5%), *C. dubliniensis* 23 (11.5%), *C. krusei* 12 (6%), *C. gulliermondii* 12 (6%) and *C. glabrata* 6 (3%).

84% of the total infections were seen in 21-70 age group. It is also evident that males are more prone to Candidiasis than females with a frequency of 130 (65%) and 70 (35%), respectively. The predisposing factors leading to Candidiasis are summarised in Table 2. The ratio of incidence in Male versus Female is 1.86:1 (M:F).

Prolonged medication 87 (43.5%) was the most common predisposing factor for Candidiasis, followed by biomedical devices 46 (23%), Diabetes mellitus 32 (16%), Surgical causes 11 (5.5%), Trauma 10 (5%), Pregnancy 10 (5%) and HIV 4 (2%).

The drug sensitivity of Candida spp. to Fluconazole (FLU), Voriconazole (VOR) and Itraconazole (ITR) were 68.5%, 72% and 69.5%, respectively. Detailed susceptibility pattern is summarized in Table 3.

DISCUSSION

Fungal infections to the hospitalized and the immunocompromised is an emerging problem to be tackled. During the last three decades, infections due to *Candida* spp., especially by the non-albicans species, increased to a great extent.[2] The reason for this increased incidence can be attributed to both fungal virulence and host susceptibility factors. Extended use of antibacterials which eventually alter the normal flora, use of corticosteroids, surgeries, malnutrition and hormonal imbalance contribute to predisposition to Candidiasis in the immunocompetent individuals.[3] Prolonged use of routinely used antifungal drugs alter the prevalence of *Candida* spp.[4,5]

In concordance with the previous studies, it is found that the Non-albicans *Candida* spp. prevalence has surpassed that of the of *C. albicans.*[6-10] C. tropicalis was the most isolated species in all samples, followed by *C. albicans*. This finding of *C. tropicalis* dominance is in concordance with other studies in India.[6,11-15] Furthermore, the Non-albicans *Candida* spp. were found to be the causative agent for invasive or deep rooted Candidiasis. Whereas, *C. albicans* caused superficial and mucosal infections mostly in skin, urinary tract and other mucosal areas.

Prolonged Medication, for more than a week was found to be the predominant risk factor in this study. This is in concordance with Chakrabarthi A *et al.* study which found that patients on antibiotics for more than a week and/or patients receiving multiple antibiotics, are prone to Candidiasis.[14]

In this study, many *Candida* spp. isolation were from respiratory samples. This deviates from the previous studies where majority of the *Candida* isolates were from Urine sample. As the study was done during the COVID-19 pandemic, there was a natural predilection towards respiratory complications and infections. This respiratory predilection was also confirmed by another study conducted in between 2018-

2022 by Froidefond *et al.*[16] It is also found that 92% of all COVID-19 patients who were given broad spectrum antibiotics as part of COVID-19 management were infected by *Candida* spp.[17]

In the present study, 68.5%, 72% and 69.5% of all the *Candida* spp. isolates were susceptible to Fluconazole, Voriconazole and Itraconazole, respectively. A comparison of studies by Jayalakshmi *et al.*, Wang *et al.*, Sukumaran *et al.*, and Kashid *et al.*[18-21] also denotes that 30% resistance to Fluconazole is not an outlier but is becoming common.

If seen as *C. albicans* versus Non-albicans *Candida* spp., 27.9%, 23.2% and 30.2% of *C. albicans* were resistant to Fluconazole, Voriconazole and Itraconazole, respectively, and 32.5%, 29.3% and 30.6% of Non-albicans *Candida* were resistant to Fluconazole, Voriconazole and Itraconazole, respectively.

20 isolates of *C.tropicalis,* 10 *C. albicans* isolates, 5 isolates of *C. dubliniensis*, 4 isolates of *C. krusei* and one each of *C. glabrata* and *C. gulliermondi* were resistant to all the 3 azole drugs tested.

CONCLUSION

This study showed that Non-albicans *Candida* spp. prevalence is higher compared to that of *C. albicans*. As the number of predisposing factors increased, the incidence of Non-albicans *Candida* infections has also increased.

As the Non-albicans *Candida* spp. are inherently resistant to routine antifungal drugs like Fluconazole, prompt identification of different *Candida* spp. and simultaneous testing of their susceptibility to antifungal medications will have a dual impact: it will limit the indiscriminate application of antifungal drugs and significantly shape the available treatment choices for medical professionals, ultimately benefiting the patients.

Prompt speciation and susceptibility pattern of *Candida* spp. will aid the clinicians in selecting the appropriate antifungal drug, and thus contributing to overall reduction in the cost of treatment & the duration of hospital stay and potentially reduces the patient's morbidity and mortality.

A periodic surveillance to susceptibility to antifungal drugs is essential as it would throw light on the rational use of antibiotics and thus preventing the emergence of drug resistance.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This study was approved by the institutional Ethics Committee, Rohilkhand Medical College, India, with Reference number IEC: BIU/REG/PhD/722"

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