



Original Article

In Vitro Antifungal Activity of Candida Species Recovered from A Uti Patients at a Tertiary Care Hospital in Western Up, India

Dr. Jyoti Vyas¹, Dr. D. P. Singh², Dr. R.K. Verma³

¹Designation- Senior Resident of Department of microbiology U.P.U.M.S Saifai, Etawah

²Designation – Professor of Department of microbiology U.P.U.M.S Saifai, Etawah

³Designation- Professor and Head of Department of microbiology U.P.U.M.S Saifai, Etawah

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Corresponding Author:

Dr. Jyoti Vyas

Designation- Senior Resident of
Department of microbiology
U.P.U.M.S Saifai, Etawah

Email: vyasjyoti966@gmail.com

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ABSTRACT

Introduction: Candiduria is a term used to describe Candida in urine. Candida albicans is the most prevalent isolate; however, in recent years, this trend has shifted to non-albicans groups. Researchers discovered that azoles are the preferred treatment for Candida infections. However, the development of resistance to these drugs are more frequent because of excessive use.

Aim: The study aimed to know the mycological profile of Candida spp., and their antifungal susceptibility pattern in urine isolates of urinary tract infection of patients at U.P.U.M.S., Saifai, Etawah (U.P.).

Materials and methods: A total of 1100 urine samples were collected for screening out of which 200 were for process. Out of these 200 urine samples, 120 had fungal growth. Prior informed consent was taken from clinically suspected lower UTI cases.

Results: Candiduria was present in 60% of the samples. Candida albicans was the most frequently isolated species, accounting for 25% of the total study population. Voriconazole seemed more effective than Fluconazole on average. Caspofungin appeared to be the most uniformly effective drug with low MICs across all Candida species.

Conclusion: This study showed that caspofungin exhibited high sensitivity across all Candida species tested. Proper treatment of patients is necessary to prevent emergence of drug resistance and resistant fungal strains.

Keywords: Antifungal drugs, Candiduria, Urinary Tract Infection, Candida albicans, Non Candida albicans.

INTRODUCTION

Urinary tract infection occurs when any part of the urinary system — the urethra, bladder, or kidneys, becomes infected. UTIs are among the most common infections worldwide, with around 400 million cases reported each year. Although *E. coli* remains the leading culprit behind most cases, other microorganisms like *Klebsiella*, *Enterococcus*, *Pseudomonas*, various *Staphylococcus* species, and even fungi such as *Candida* can also be responsible. UTIs affect both sexes and also seen in immunocompetent and immunocompromised person. [1]

Candiduria refers when yeast levels in urine exceed 10^5 CFU/mL and are accompanied by clinical symptoms.[2] *Candida* species are some of the most commonly found fungi in urine samples. The colonization is especially common in individuals who have long-term urinary catheterization. [3]

Candida organism exists harmlessly, under certain conditions it can lead to infections. These infections can range from mild, localized issues like oral thrush or vaginal yeast infections to potentially life-threatening illnesses, such as bloodstream infections and disseminated candidiasis. [4] In cases of candiduria, the infection primarily affects the renal pelvis, kidney tubules, and ureters. In some instances, a "fungus ball" can develop, leading to blockages and causing further damage within the urinary tract and progresses to candidemia.[5] In recent years, infections caused by other *Candida* spp.

like *C. tropicalis*, *C. parapsilosis*, *C. glabrata*, and *C. krusei*, have been on the rise. These non-albicans strains are often more challenging to treat because many of them show resistance to antifungal medications. [6]

A history of frequent antibiotic use, having venous or urinary catheters in place, long stay in intensive care units, and receiving nutrition through intravenous feeding are all significant contributors.[7]

Today, a variety of methods are available for identifying yeast, ranging from traditional culture-based techniques to advanced non-culture approaches. Techniques like PCR provide rapid, highly sensitive, and accurate identification of *Candida*, and can even amplify and detect specific genes. However, while molecular tools significantly reduce diagnostic time and improve reliability.[8]

Antifungal susceptibility testing plays a crucial role in identifying the right amount of an antifungal medication needed to effectively halt the growth of a fungal organism. The resistance patterns of the isolated *Candida* spp. were assessed using the Broth Microdilution technique.

MATERIALS AND METHODS

This study was conducted at the department of microbiology, UPUMS, SAIFAI from the date of ethical clearance till march 2025 (1.5 year). A total 1100 urine sample were collected from clinically suspected cases of urinary tract infection. Prior instruction was given to patient for proper urine specimen collection.[9] Urine specimens were processed in the laboratory within 2 hours of collection refrigerated at 4°C until it was processed. [10]

The urine samples were inoculated on CLED Agar and incubated at 37°C for 24 hours. Yeasts like isolates obtained from CLED Agar were identified by germ tube test, morphological characteristics on corn meal agar Tween 80, colony color on Hi Chrom TM *Candida* differential agar (Hi Media, Mumbai, India) and further confirmation was made by Vitek-2 ® compact (BioMerieux l'Etoile, France). Antifungal susceptibility of *Candida* isolates was done by Broth Microdilution Method according to the clinical laboratory standard institute M27-A2 and M27S4 documents. Antifungal effect of Fluconazole, Voriconazole, and Caspofungin was evaluated. This study was approved by Institutional Ethics Committee (Clearance Code:28/2023-24). [12]

Inclusion criteria

Clinically suspected UTI cases from outdoor and indoor patients at UPUMS Saifai, Etawah. Pure growth of yeast isolate having colony count >10³ CFU/ml.

Exclusion criteria

Mixed growth on CLED Agar was excluded from the study. A colony count <10³ CFU/ml was also excluded.

Statistics

Data was analyzed and statistically evaluated using SPSS-PC-25 version. Quantitative data was expressed in mean ± standard deviation or median with interquartile range and depends on normality distribution difference between two comparable groups were tested by student's t-test (unpaired) or Mann Whitney "U" test while for more than two groups ANNOVA test or Kruskal Wallis H test followed by Post Hock test was used. Qualitative data were expressed in percentage. MIC range, MIC50, MIC90 were obtained for all the isolates tested. 'P' value less than 0.05 was considered statistically significant.

RESULTS

Among 120 fungal isolates the age of patients ranges from 20 to 60 year. There was a slightly higher proportion of female patients (52.5%) compared to male patients (47.5%). The largest proportion of samples originated from the Outpatient Department (OPD), accounting for 39.5% followed by General Ward constituted 37%, while the Intensive Care Unit (ICU) contributed 21%. The Emergency department representing only 2.5%. A significant portion of the patients, 30%, had diabetes, following this, 26.5% were on antimicrobial treatment. Corticosteroid use was reported in 6.5%, while hypertension was observed in only 2%. Notably, 40% of the studied population had no reported co-morbidities.

A majority of the samples (60%) showed the presence of budding yeast-like cells. Conversely, 40% of the samples did not show budding yeast-like cells. Majority of the samples (74%) showed a negative result, indicating the absence of germ tube formation. Conversely, 26% showed a positive result. Candiduria was present in 60% of the samples. Among those with candiduria, *Candida albicans* was the most frequently isolated species, accounting for 41.0% of the total study population. *Candida tropicalis* was also prevalent, found in 37.5%. (Table 1)

The data reveals a statistically significant association between *Candida* species and these co-morbidities (p < 0.001). *Candida albicans* was associated with diabetes in 40% of cases and antimicrobial use in 46% of cases. *Candida dubliniensis*, *Candida glabrata* and *Candida krusei* were highly associated (100%, 42.9%, 66.7%) with diabetes, respectively.

Table 1: Prevalence of Candiduria and their isolated *Candida* species in studied patients

Candiduria	Frequency (N=120)	Percentage (%)
<i>Candida albicans</i>	50	41.0
<i>Candida tropicalis</i>	45	37.5
<i>Candida glabrata</i>	14	11.6
<i>Candida krusei</i>	6	5.0
<i>Candida Parapsilosis</i>	3	2.5
<i>Candida dubliniensis</i>	2	1.6

Fluconazole: *C. albicans* showed Mean MIC = 3.95 µg/mL and Significant sensitivity (p<0.001). *C. glabrata*, *C. krusei* and *C. parapsilosis* show higher MICs. **Voriconazole:** Overall mean was 1.56 µg/mL. Voriconazole is more effective than Fluconazole on average. **Caspofungin:** Caspofungin appears to be the most uniformly effective drug with low MICs across all *Candida* species.

Table no 2: Comparison of Species and Fluconazole

SPECIES	Total	Sensitive Count	Sensitive %	Resistant Count	Resistant %
<i>Candida albicans</i>	50	31	62.0%	19	38.0%
<i>Candida dubliniensis</i>	1	0	0.0%	1	100.0%
<i>Candida glabrata</i>	14	8	57.1%	6	42.9%
<i>Candida krusei</i>	6	0	0.0%	6	100.0%
<i>Candida Parapsilosis</i>	3	2	66.7%	1	33.3%
<i>Candida tropicalis</i>	45	40	88.9%	5	11.1%
Total	119	81	-	38	-

Table no 3: Comparison of Species and Voriconazole

SPECIES	Total	Sensitive Count	Sensitive %	Resistant Count	Resistant %
<i>Candida albicans</i>	50	37	74.0%	13	26.0%
<i>Candida dubliniensis</i>	1	1	100.0%	0	0.0%
<i>Candida glabrata</i>	14	12	85.7%	2	14.3%
<i>Candida krusei</i>	6	4	66.7%	2	33.3%
<i>Candida parapsilosis</i>	3	2	66.7%	1	33.3%
<i>Candida tropicalis</i>	45	43	95.6%	2	4.4%
Total	119	99	-	20	-

Table no 4: Comparison of Species and CASPOFUNGIN

SPECIES	Total	Sensitive Count	Sensitive %	Resistant Count	Resistant %
<i>Candida albicans</i>	50	46	92.0%	4	8.0%
<i>Candida dubliniensis</i>	1	1	100.0%	0	0.0%
<i>Candida glabrata</i>	13	11	84.6%	2	15.4%
<i>Candida krusei</i>	6	5	83.3%	1	16.7%

Candida parapsilosis	3	2	66.7%	1	33.3%
Candida tropicalis	45	42	93.3%	3	6.7%
Total	118	107	-	11	-

DISCUSSION

UTI are a major global economic burden and one of the main causes of morbidity. Infections can range from non-life-threatening mucocutaneous conditions to invasive diseases that impact any organ. Although bacteria are the primary cause of most urinary system infections, 10% can have fungal origins, particularly *Candida* spp. But the number of *non-albicans Candida* spp. that cause different types of candidiasis has increased dramatically in recent years.

Table 3: Prevalence of *Candida* species including *Candida albicans* and *Non albicans* from Candiduria cases

Study	Year of publication	Place	Prevalence	
			<i>C. albicans</i>	<i>Non-albicans</i>
Ding CH et al[13]	2014	Malaysia	59.4%	40.6%
Jain N et al[14]	2007	New York	54%	46%
Gautam G et al[15]	2022	New Delhi	17.24%	50.86%
Eid HM et al[16]	2023	Madinah	13.7%	86.3%
Datta P et al[17]	2018	Chandigarh	58.7%	41.3%
Present study	2025	Saifai, Etawah (U.P.)	25%	75%

In our study among 200 patient 56 were diabetic and 64 were non-diabetic respectively. Female patients with candiduria are diagnosed at a higher rate than male ones. In a study conducted by [CH Ding, AA Wahab et al \[13\]](#) *Candida albicans* was 38 (59.4%) of the isolates, *Candida tropicalis* 18 (28.1%), *Candida glabrata* 6 (9.4%) and *Candida parapsilosis* 2 (3.1%). In current study, *Candida albicans* was the most frequently isolated species, accounting for 25% (50 patients). *Candida tropicalis* 22.5% (45 patients), *Candida glabrata* (7%, 14 patients), *Candida krusei* (3%, 6 patients), *Candida parapsilosis* (1.5%, 3 patients), and *Candida dubliniensis* (1%, 2 patients).

In current study, *Candida albicans* was the most frequently isolated species, accounting for 25% followed by *Candida tropicalis* 22.5%. In a study conducted by Jain Net al [14], the species distribution was as follows: *C. albicans* (54%), *C. glabrata* (36%), and *C. tropicalis* (10%). In another study conducted by Gautam G et al [15], *Candida tropicalis* (26.72%) was the most common species, followed by *Candida albicans* (17.24%), *Candida parapsilosis* (14.66%) and *Candida krusei* (9.48%).

A study conducted by Konje ET et al [18] showed that proportion of patients with candiduria due to NAC spp. were higher than those due to *C. albicans*. In a study done by Eid HM et al [16], prevalence of Candiduria was 13.7% associated with *C. albicans* while, 86.3% associated with non-*Candida albicans*. These results were similar to our study in which prevalence of Candiduria by *Candida albicans* is less than non-*Candida albicans* that is 25% and 75% respectively. In contrast to our study Mishra N et al [19] conducted a study showed candiduria cause by NAC was lower (38.1%) than that of *C. albicans* (61.9%).

In contrast to our study Zafar S et al [20] conducted a study that showed the *C. albicans* was the predominant species with 51 isolates (66.2%) followed by 26 (33.8%) of non-*Candida albicans*. Another study conducted by Eid HM et al [16] indicated that higher number of positive fungal samples were observed in female patients (47%) compared to male patients (58%). Similar to current study females (52.5%) were diagnosed more than male (47.5%) respectively.

In this study conducted by Konje ET et al [18] showed that inpatients exhibited a higher incidence of candiduria compared with outpatients, with rates of 9.4% versus 1.6%. In contrast to current study in which the largest proportion of samples originated from the Outpatient Department (OPD), accounting for 39.5%. Samples from the general Ward constituted 37%, while the Intensive Care Unit (ICU) contributed 21%.

In this study conducted by Oliveira RD et al [21] patients with diabetes mellitus were 25%. The major predisposing factors associated were previous antibiotic therapy (93%), use of corticosteroids (20%). But the current study shows that significant portion of the patients, 30%, had diabetes, making it the most prevalent co-morbidity and 26.5% were on antimicrobial treatment.

In this study conducted by Maheronnaghsh M et al [22] *Candida albicans* MIC range, MIC50, MIC90 & GM for fluconazole 64 0.06 µg/ml, 0.5 µg/ml, 64 µg/ml & 19.73345 µg/ml respectively. *Candida krusei* MIC range, MIC50, MIC90 & GM for fluconazole 64 0.25 µg/ml, 8 µg/ml, 32 µg/ml & 12.0500 µg/ml. In our study *Candida albicans* MIC range, MIC50, MIC90 for fluconazole 0.1-1.8µg/ml, 0.5µg/ml & 1.4µg/ml respectively. *Candida krusei* MIC range, MIC50, MIC90 for fluconazole 0.01-0.19µg/ml, 0.02µg/ml & 0.14µg/ml.

As far as broth microdilution is concerned in our study it was observed that *Candida albicans* MIC range, MIC50, MIC90 for fluconazole 0.1-1.8µg/ml, 0.5µg/ml & 1.4µg/ml respectively. For voriconazole 0.01-0.19 µg/ml, 0.06 µg/ml & 0.19 µg/ml respectively. For caspofungin 0.01-0.19 µg/ml, 0.04 µg/ml & 0.19 µg/ml respectively. *Candida glabrata* MIC range, MIC50, MIC90 for fluconazole 0.0001-0.0018 µg/ml, 0.0009 µg/ml & 0.0018 µg/ml respectively. For voriconazole 0.001-0.09 µg/ml, 0.01 µg/ml, 0.08 µg/ml respectively. For caspofungin 0.001-0.08 µg/ml, 0.01 µg/ml & 0.04 µg/ml respectively. In the study conducted by Badiee P et al [23] showed similar results as compare to our study.

In this study conducted by Isham NC et al [24] showed *C.dubliniensis* for voriconazole MIC range, MIC50, MIC90 0.06 µg/mL, ND µg/mL & ND µg/mL. For caspofungin MIC range, MIC50, MIC90 0.5 µg/mL, ND µg/mL & ND µg/mL. In our study *C. dubliniensis* MIC range, MIC50, MIC90 for voriconazole 0.01 µg/mL, 0.01 µg/mL & 0.01-0.01 µg/mL. For caspofungin MIC range, MIC50, MIC90 0.01-0.01, 0.01 & 0.01 respectively.

The emergence of antifungal resistance in *Candida* has risen due to the liberal use of antifungal, especially those from the azole group. In present study, Diabetes mellitus was a more prevalent co-morbidity factor. In general *Candida albicans* was the most common culprit causing Candiduria, but the rise in other *Candida* species is also of significant concern to clinicians. In this present study the prevalence of candiduria due to non *albicans Candida* was higher than *Candida albicans*. *Candida albicans* are gradually being displaced by NAC (*C. glabrata* and *C.tropicalis*) as the scenario shifts progressively.

CONCLUSION

Due to this rise in non-*albicans Candida* spp. causing UTI that are intrinsically resistant to certain antifungal agents like azoles and increasing incidence of antifungal resistance, it is essential to monitor the antifungal susceptibility profile of *Candida* spp. causing candiduria. This study showed that caspofungin exhibited high sensitivity across all *Candida* spp. tested. Proper treatment of patients is necessary to prevent emergence of drug resistance and resistant fungal strains. Depending on the patient's pathological conditions, different actions are needed to combat the infection.

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