

Original Research Article

DISTRIBUTION OF CANDIDA SPECIES IN URINARY TRACT INFECTIONS

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ABSTRACT

Background: Candida infections are a growing concern globally with increased drug resistance in recent year. There is also a wide geographic variation in the prevalence of type of Candida species.

Materials and Methods: The present study aims to estimate the burden of Candida infection among the urine sample received at the Microbiology Department at a Tertiary care medical college in Trichy, Tamilnadu, that have been confirmed with clinically diagnosed urinary tract infection (UTI). A total of 445 clinically diagnosed UTI urine samples were screen for candida detection. These samples were examined using direct microscopic method-gram staining and inoculated into the Sabouraud's dextrose agar for fungal detection. The colony morphology and microscopic features were identified and reported. All *Candida species* isolated were subjected to germ tube test, HiCrome differential candida agar, cornmeal agar and antifungal susceptibility testing as per the standard protocol.

Results: The prevalence of Candia infection among the 445 urine sample was 25 i.e. 5.6% (95% Confidence interval: 3.67-8.16%). The mean age of patients with fungal growth in their urine sample was 45.7 ± 5.7 years with 68% being males. The risk factors found among these patients was diabetes (42%), hypertension (22%), smoking (28%) and renal calculi (8%). Based on the *Candida species*, *C. tropicalis* (48%) was the most common followed by *C. albicans* (28%). Amphotericin and Voriconazole had 100% sensitivity among all species. Fluconazole and Itraconazole were sensitive to 78.9% and 84.2% samples. Fluconazole showed 100% resistance to *C. krusei*, while Itraconazole showed 33% resistance to *C. krusei* and *C. glabrata*. Overall, Itraconazole had the highest proportion resistance.

Conclusion: The study finding highlights that that advancing age and associated comorbidities such as diabetes are risk factors to the development of candiduria. *Non-albicans Candida species* such as *C. tropicalis* showed predominance over *C. albicans*. Antifungal drugs should be used judiciously to avoid drug resistance.

Keywords: *Candida species*, Urinary tract infection (UTI), Candiduria, Prevalence, Diabetes mellitus, Antifungal susceptibility.

INTRODUCTION

Globally, among other *Candida species*, *C. albicans* is mainly responsible for systemic candidiasis and fungal nosocomial UTIs.^[1] Among the *Candida species*, UTI in young and adult individual's three most common species include, *C. albicans*, *C.*

glabrata, and *C. tropicalis*.^[2] A common clinical finding in hospitalised patients is *Candida species* in their urine (candiduria). *Candida species* in urine may be asymptomatic (in healthy people or patients) or symptomatic. There are many clinical conditions in which patients are more prone to candiduria which includes interstitial cystitis, Epididymal-orchitis,

prostatitis, pyelonephritis, and renal candidiasis. Asymptomatic candiduria is though mostly benign and is not considered as a disease.^[3] There are many factors which includes gender (female), age, prolonged antibiotic intake, sex activities, genetic inheritance, diabetes, immunosuppression, Acquired Immunodeficiency Syndrome (AIDS), pregnancy, cancer patients, multiple clinical procedures, hypertension, hospitalisation, indwelling catheter or prosthetics, malnutrition, social behaviour, these are the predisposing factors leading to UTI candidiasis in patients.^[4] In general, inpatients are more likely to experience symptomatic candiduria, whereas outpatients and healthy adults are more likely to experience asymptomatic candiduria. Indwelling catheters are the important reservoir for these pathogens and with the help of these procedures (Catheterisation) pathogens easily reach the anatomical site and cause infections. While there are *non-albicans candida species* infections, UTIs and candidiasis caused by *C. albicans* are more prevalent.^[5] In case of candiduria, the infection finally reaches to blood stream resulting into severe disseminated bloodstream infections (Candidemia). Cases of candidiasis is increasing gradually resulting into the development of drug resistance which is major health concern worldwide. So, to control this stage monitoring at regular intervals is a very necessary and crucial point.^[6] Candiduria and UTIs by *Candida species* have different prevalence rates depending on geographical location; therefore, regional data is essential for evaluating the shift and to determine the scenario at national level. Hence, this study will be done to assess the *Candida* prevalence in UTI.

MATERIALS AND METHODS

Study Design and Setting: A hospital-based cross-sectional study was carried out among urine samples who has clinically diagnosed urinary tract infections. The study was conducted in Department of Microbiology, Trichy SRM Medical college Hospital and Research Centre, Tamil Nadu, India between May to October 2024 and Coordinating Department is General Medicine.

Sample Size: Using the formula, $n = Z^2 \times P \times (100 - P) / d^2$ where Z is the standard normal variate at 10% type I error (1.96), P is the expected proportion in the study population, and d is the absolute precision (5%). Based on a previous study [7], the prevalence of Candida infection among urinary infection patients was 23.12%, the sample size was calculated to be 445 samples.

Inclusion criteria

All age group, male & female, both in patients and outpatients' samples from clinically diagnosed Urinary tract infections (UTI)

Exclusion criteria

Those who are receiving antifungal treatment and pregnancy patients.

Methods: All the urine samples from clinically diagnosed Urinary tract infections were processed according to standard microbiology protocol. The centrifuged urine sample was cultured with a calibrated loop on Cysteine Lactose Electrolyte Deficient (CLED) agar or Mac Conkey Agar (Himedia, Mumbai, India) incubated overnight at 37°C for 24 hours, aerobically. The colony was grown as white pasty, yeasty odour colonies as *Candida spp.* And the gram stain of an isolated colony was examined by using microscopy and after confirmation of the *Candida species*, the isolates were further processed as per standard protocol. Fungal colony morphology was confirmed by using Sabouraud's Chloramphenicol Agar (Himedia, Mumbai, India), germ tube production and HiCrome Candida Differential Agar (Himedia, Mumbai, India). The Germ Tube Test (Reynolds-Braude phenomenon) was performed by inoculating a small colony of the yeast in 0.5 mL of human serum and incubating at 37°C for two to three hours. Microscopic examination was conducted to observe the formation of germ tubes, which appear as long, thin, filamentous extensions from the yeast cells without constriction at the point of origin, characteristic of *C. albicans* and *C. dubliniensis*. For species differentiation, yeast isolates were cultured on HiCrome Candida Differential Agar (HiMedia Laboratories, Mumbai) and incubated at 37°C for 48 hours. Species identification was based on distinctive colony colours and morphology: light green for *C. albicans*, metallic blue for *C. tropicalis*, purple fuzzy colonies for *C. krusei*, and cream to white for *C. glabrata*. Confirmation of species identification was performed using the corn-meal agar (Dalmau technique), where morphological characteristics such as blastoconidia, pseudohyphae, and chlamydo spores were observed after incubation at 25°C for 48-72 hours.^[8-11]

Antifungal susceptibility testing was conducted using the disk diffusion method following Clinical and Laboratory Standards Institute (CLSI) M44-A document guidelines. Mueller-Hinton agar supplemented with 2% glucose was used as the culture medium. The inoculum was prepared by suspending colonies in sterile saline to achieve a turbidity equivalent to 0.5 McFarland standard. The suspension was spread evenly on the agar surface, and antifungal disks were placed with appropriate spacing. The plates were incubated at 35°C for 24 hours for *C. albicans*, *C. tropicalis*, and *C. krusei*, and 48 hours for *C. glabrata*. Zone diameters were measured to the nearest millimetres using a calibrated scale and interpreted according to CLSI guidelines. Quality control was performed using *C. albicans* ATCC 90028 and *C. parapsilosis* ATCC 22019 reference strains. The following antifungal agents were tested: fluconazole (10 µg), voriconazole (1 µg), itraconazole (10 µg), and amphotericin B (100 units).^[12]

Ethical consideration: Institutional ethics clearance was obtained prior to the study from the institution

review board (IEC no: 504/TSRMMCH&RC/ME-1/2024-IEC No: 073) dated on 04.05.2024. Patient identifying details was anonymized.

Statistical Analysis: Data entry and analysis was done in MS Excel. Quantitative data was expressed in mean and standard deviations while qualitative data in frequency and percentage. The prevalence of candida infection was expressed in percentage with 95% confidence interval.

RESULTS

A total of 445 urine samples were received in the laboratory during the study period were diagnosed clinically as urinary tract infection. Of these 445 patients, 25 i.e. 5.6% (95% Confidence interval: 3.67-8.16%) showed fungal growth in culture. The mean age of patients with fungal growth in their urine sample was 45.7 ± 5.7 years with 68% being males. [Figure 1 and 2] The risk factors found among these patients was diabetes (42%), hypertension (22%), smoking (28%) and renal calculi (8%). [Figure 3]

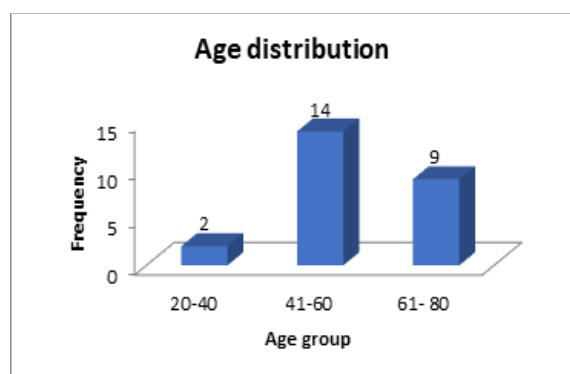


Figure 1: Age-wise Distribution of Fungus Growth in UTI Patients

Based on the *Candida species*, *C. tropicalis* (48%) was the most common followed by *C. albicans* (28%). [Table 1] The antifungal drug susceptibility is

Table 1: Candida Distribution in UTI Patients

Candida Distribution	Total (n)	Percentage%
<i>Candida albicans</i>	7	28
<i>Candida tropicalis</i>	12	48
<i>Candida krusei</i>	3	12
<i>Candida glabrata</i>	3	12

Table 2: Antifungal Susceptibility pattern of Candida species in UTI Patients

Candida species	Amphotericin		Voriconazole		Fluconazole		Itraconazole	
	S	R	S	R	S	R	S	R
<i>C. albicans</i> (n=7)	7 (100%)	0 (0%)	7 (100%)	0 (0%)	6 (85.7%)	1 (14.3%)	5 (71.4%)	2 (28.6%)
<i>C. tropicalis</i> (n=12)	12 (100%)	0 (0%)	12 (100%)	0 (0%)	10 (84.0%)	2 (16.0%)	9 (75%)	3 (25%)
<i>C. krusei</i> (n=3)	3 (100%)	0 (0%)	3 (100%)	0 (0%)	0 (0%)	3 (100%)	2 (67.0%)	1 (33.0%)
<i>C. glabrata</i> (n=3)	3 (100%)	0 (0%)	3 (100%)	0 (0%)	3 (100%)	0 (0%)	2 (67.0%)	1 (33.0%)
Total (n=25)	25 (100%)	0 (0%)	25 (100%)	0 (0%)	19 (76.0%)	6 (24.0%)	18 (72.0%)	7 (28.0%)

DISCUSSION

The present hospital based cross-sectional study was done to assess the burden of Candida infection among patients with urinary tract infection. Among the 445

urine sample confirmed with clinically diagnosed UTI, the proportion infected with *Candida species* was 5.6%. There is a gross variation in the proportion of Candida infection among UTI patients across the different studies. A similar study by Mishra et al,^[13]

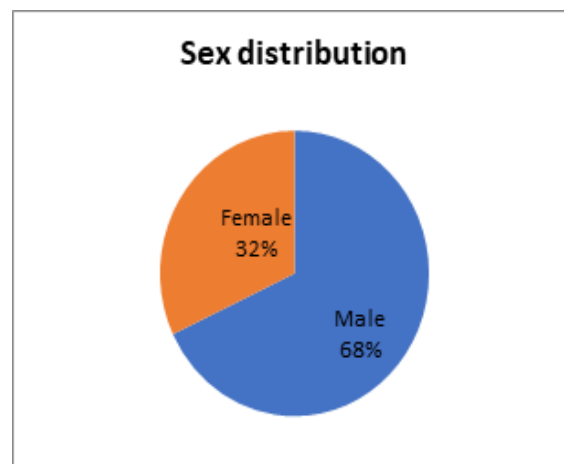


Figure 2: Sex-wise Distribution of Fungus Growth in UTI Patients.

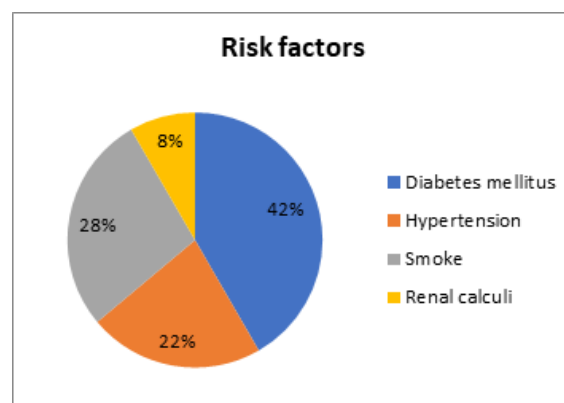


Figure 3: Risk Factors of Fungus Positive UTI patients.

urine sample confirmed with clinically diagnosed UTI, the proportion infected with *Candida species* was 5.6%. There is a gross variation in the proportion of Candida infection among UTI patients across the different studies. A similar study by Mishra et al,^[13]

done in Lucknow, reported a higher prevalence of 11.2% among the 1576 patients' urine sample. Singla et al,^[14] in their study found a very high burden of 57.5%. While another study done in Brazil,^[15] found the prevalence to be only 0.18%. This difference in proportion could be attributed to variation in the sample size, source of sample (ICU or out-patient) and geographic distribution.

In the present study most of the patients were male (68%) than female (32%). Singla et al,^[14] also found males (71.7%) to be more affected than females (28.3%). But a study from Faisalabad,^[16] and Pakistan,^[17] found females to be at a greater risk of Candida infection than male. This difference could be due the department of sample collection. Overall females are considered at greater risk than male due to pregnancy and hormonal changes. The difference between the studies could also be attributed the department from which the urine sample were received.

Diabetes (42%) was the most common risk factor associated with Candida infection in our study. Most of the other studies,^[15,16] have also documented diabetes to be a risk factor since hyperglycaemic state promotes the growth of fungal growth. But Singla et al,^[14] in their study compared the risk factors of patients with Candida infection in their urine sample versus those without and found urinary catheterization and the use of previous antibiotic to be statistically significant ($p < 0.5$) in leading to Candida infection. Apart from the above mention risk factors, genetic inheritance, sexual activities, AIDS, immunosuppressant, hypertension, surgeries, pregnancy, poor hygiene, malnutrition and social behaviour are also known to be risk factors for Candida infection.^[18]

The type of *Candida species* varies across the different regions and it is important to assess the local data. Our study demonstrated *C. tropicalis* to be most common *Candida species* (48%) followed by *C. albicans* (28%), *C. krusei* (12%) and *C. glabrata* (12%). Nadeem et al,^[17] also reported similar findings in their study with *C. tropicalis* (41.2%) being the highest followed by *C. albicans* (30.73%) and *C. glabrata* (20%). A Study by Ghasemi et al,^[16] Ortiz et al,^[19] and Gajdács et al,^[20] found *C. albicans* to be more common (74%) followed by *C. glabrata* (26%). Generally, most of the previous studies have reported *C. albicans* to be the most common species but in recent year there has been a shift to *non-Candida albicans species* becoming more common due to the widespread use of antifungals.^[21]

Amphotericin and Voriconazole were found to be 100% sensitive while Fluconazole and Itraconazole had lower sensitivity of 76% and 72% respectively. Among the species type *C. krusei* had 100% resistance to Fluconazole. Asad et al,^[22] in their study reported fluconazole to be sensitive to *C. albicans* but resistance towards other species such as *C. glabrata* was seen. Chiti et al,^[23] compared the drug susceptibility pattern among the *C. albicans* and *non-albicans candida species* like *C. glabrata*, *C. krusei*

and *C. tropicalis*. They reported a higher resistance pattern among the *non-albicans Candida species* with respect to amphotericin (20.4% versus 1.63%), voriconazole (11.1% versus 0%), and fluconazole (29.6% versus 3.27%) and Itraconazole (18.5% versus 3.27%). Fluconazole showed 62.5% resistance to *C. glabrata* while itraconazole showed 66.6% resistance to *C. krusei*. In other studies, Caspofungin was the only anti-fungal drug with nil resistance to all *Candida species* and recommended the cautious use of echinocandins to avoid their resistance in future.

CONCLUSION

The present study highlights the prevalence of *Candida species* in urinary tract infections to be 5.6% among the urine samples analysed. The diabetes mellitus was a major predisposing factor, accounting for 42% of candiduria cases, indicating the increased susceptibility to opportunistic fungal infections. The majority of patients were in the middle-aged and elderly groups, suggesting that advancing age and associated comorbidities may contribute to the development of candiduria. *Non-albicans Candida species* predominated, with *C. tropicalis* being the most frequently isolated species, followed by *C. albicans*. Amphotericin B and Voriconazole showed sensitivity to all isolates, while fluconazole resistance (100%) was observed in *C. krusei*. The findings emphasize the importance of early detection, species-level identification, and antifungal susceptibility testing in the management of candiduria, especially among patients with diabetes mellitus, to ensure appropriate therapy and prevent complications.

Limitations of the study: Our study had few limitations. It was done among a small sample size and at a single centre which limits its generalisation. We did not perform molecular identification of *Candida species* which could be more accurate than the conventional methods. Lastly, we have not taken into consideration the other potential risk factors such as prolonged antibiotic use, and duration of stay in hospital.

Recommendations: Our study findings recommend the testing of urine samples for Candia infection especially among high-risk patients such as diabetes. Species identification should be done when feasible since the burden of *non-albicans Candida species* is increasing in its prevalence. Incorporating anti-fungal susceptibility testing in routine practise will assist to guide appropriate treatment.

Authors Contribution: Prakash Babu Mohanraj (Principal Investigator) was involved in the study conception, data collection, interpretation of results, and manuscript preparation. Lalithambigai Jothi (Guide) was involved in guiding the student in sample collection and processing, interpretation and analysis of results, and manuscript writing. Viknesh Vijayasankar (Co-Guide) was involved in supervision of the research work, clinical diagnosis, and coordination of sample collection from clinically

diagnosed urinary tract infection patients. Sivakumar Karunanandham contributed to the research work through academic suggestions, and the coordinating department was General Medicine. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of Interest statement There are no conflicts of interest.

Declaration of Non-Use of AI: The authors confirm that no artificial intelligence tools were used in this study

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