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## Original Research Article

## Epidemiology of urinary candidiasis and antifungal susceptibility pattern of various *Candida species* at a rural tertiary health care centre of Puducherry, South India- An observational study

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## ABSTRACT

**Introduction:** Urinary tract infection (UTI) is a common health problem across the globe. Although majority of them are bacterial in origin, there has been an increasing trend in the incidence of UTI due to the yeast like fungi, *Candida*. UTI due to *Candida species* are in large proportion nosocomially acquired and of growing concern is the development of resistance to the commonly used azole group of drugs for their treatment. Since the resistance is more commonly reported among non- albicans *Candida species*, routine species identification and antifungal susceptibility testing is crucial for successful clinical outcomes.

**Aims and Objectives:** This study was conducted to analyse the distribution and risk factors associated with Urinary candidiasis and also to determine the resistance patterns of different *Candida species* to various antifungal agents using phenotypic methods.

**Materials and Methods:** A hospital based observational study was conducted from September 2016 to December 2017 on patients presenting with symptomatic UTI. *Candida* isolates were speciated using phenotypic methods like germ tube test and growth character on chromagar *candida*. Antifungal susceptibility to fluconazole, voriconazole, ketoconazole and amphotericin B were determined using disc diffusion method.

**Statistical Analysis:** All data were analyzed using EpiData Analysis software version 2.2.2.186.

**Results:** A total of 101 *Candida species* were isolated. The incidence of Urinary candidiasis was more among females (66.4%) than in males (33.6%). People above 50 years (38.6%) were commonly affected followed by people in the age group of 21-30 years (22.7%). The incidence among hospitalized patients was 86.7% and urinary catheterization (43.5%) was the most commonly associated risk factor. The most common isolates were *Candida tropicalis* (31.6%) followed by *Candida albicans* (21.7%). The overall resistance patterns among various *Candida species* were 50.5%, 32.7%, 19.9% and 2% for fluconazole, ketoconazole, voriconazole and amphotericin B respectively.

**Conclusion:** Hospitalization and urinary catheterization are the important risk factors for developing urinary candidiasis. The antifungal susceptibility varies among different *Candida species* and hence, identification of *Candida* to species level along with antifungal susceptibility testing should be practiced as a routine in all clinical mycology laboratories.

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### 1. Introduction

*Candida* is yeast like fungi consisting of more than 150 species which can cause a range of infections from

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superficial infections to deep invasive infections. The incidence of urinary tract infections caused by *Candida species* has increased substantially in the recent times.<sup>1</sup> Although community acquired urinary candidiasis is rare, nosocomial UTIs are more common.<sup>2</sup> *Candida species* can account for 9-40% of all nosocomial urinary tract infections.<sup>3</sup> Various factors like female gender, pregnancy, diabetes mellitus, urinary catheterization, prolonged use of broad spectrum antibiotics, corticosteroid therapy and immunocompromised states have been associated with the risk of urinary candidiasis.<sup>4</sup>

Among the pathogenic *Candida species*, *C.albicans* is the most commonly isolated species from urinary tract infections. However in the recent past many *Candida* non-albicans species such as *C.tropicalis*, *C.glabrata*, *C.parapsilosis* and *C.krusei* are frequently more isolated<sup>5</sup>

Azole group of drugs such as fluconazole are commonly used in the treatment of urinary candidiasis. Unfortunately many *Candida* non-albicans species are increasingly becoming resistant to fluconazole.<sup>6</sup> Few *Candida species* like *C. krusei* are also known to be intrinsically resistant to fluconazole.<sup>7</sup> In view of this, routine species identification and antifungal susceptibility testing of *Candida species* is crucial for successful clinical outcomes.

This study was undertaken to analyse the distribution and risk factors associated with urinary candidiasis and also to determine the resistance patterns of different *Candida species* to various antifungal agents using phenotypic methods.

## 2. Materials and Methods

This is an observational study conducted over a period of 15 months from September 2016 to December 2017 in the department of microbiology of a rural tertiary health care centre in Puducherry. All urine samples from patients with UTI attending the hospital were included in the study. All non-repeat urine samples yielding pure growth of *Candida species* with more than 10<sup>5</sup> colony forming units (CFU) were included in the study. Samples which did not yield a pure growth of yeast, samples with colony count equal to or less than 10<sup>4</sup> CFU/ml and samples with growth other than *Candida* were excluded from the study. The institute ethics committee approval (No: SMVMCH-EC/DDO/AL/225/2016) was obtained prior to commencement of the study.

### 2.1. *Candida* isolation and identification

The urine samples submitted were inoculated on to Cysteine Lactose Electrolyte Deficient agar (CLED) and incubated at 37°C for 24-48 hours. White creamy colonies on CLED confirmed to be yeasts by Gram stain were subcultured on to Sabouraud's dextrose agar (SDA) plates and were incubated at 37°C for 48 hours. Presumptive identification

of the *Candida species* was done by urease test and germ tube test. Final identification of the *Candida species* was derived based on the growth character on Chrom agar *Candida*. The isolates were inoculated on HiCrome *Candida* Differential Agar (Himedia labs, Mumbai) and the species were identified by characteristic growth and colour after 24 to 48 hours of incubation at 37°C. Light green smooth colonies- *C.albicans*, metallic blue colonies- *C.tropicalis*, cream to white smooth colonies-*C.glabrata*, purple fuzzy colonies- *C.krusei*, and white creamy colonies- *C.papirapsilosis*.

### 2.2. Antifungal susceptibility testing

Disk diffusion method was employed for antifungal susceptibility testing following the Clinical Laboratory Standards Institute (CLSI) guidelines (M44 A-2 document).<sup>8</sup> The media used for testing was the Muller Hinton agar with 2% glucose and 0.5 mcg/ml methylene blue. The media and the antifungal disks were procured from HiMedia Laboratories, Mumbai. The colonies from 24 hour old cultures of *Candida species* on SDA were suspended in 5ml of 0.85% saline and the turbidity was matched to 0.5 McFarland standards. A lawn culture of the suspension was made on the test medium using sterile cotton swab. Antifungal disks with 25 mcg fluconazole, 1 mcg voriconazole, 10 mcg ketoconazole and 100 U amphotericin B were placed on the lawn cultures, and the plates were incubated for 24 hours at 37°C. After 24 hours the zones of inhibition around each disk were measured. The interpretation of zone diameters for fluconazole and voriconazole were done using the CLSI M44-S3 document guidelines.<sup>9</sup> The interpretation of zone diameters for ketoconazole and amphotericin B were done using manufacturer instructions as there are no established interpretive criteria. For fluconazole, more than or equal to 19 mm was interpreted as Susceptible (S), 15 to 18mm as Susceptible-Dose Dependent (SDD), and less than or equal to 14 mm as Resistant (R). For voriconazole, more than or equal to 17 mm was interpreted as S, 14 to 16 mm as SDD and less than or equal to 13 mm as R. For ketoconazole, more than or equal to 32 mm was interpreted as S and less than or equal to 20 mm as R. For amphotericin B, more than or equal to 15 mm was interpreted as S and less than or equal to 10 mm as R. *C.albicans* (ATCC 90028) was used as the quality control strain for the antifungal susceptibility testing.

### 2.3. Statistical analysis

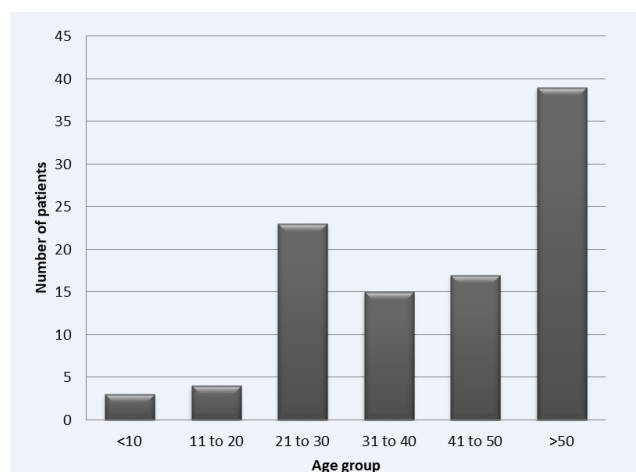
All data were entered in Microsoft Excel and was analysed using EpiData Analysis software version 2.2.2.186. The association between various risk factors and urinary candidiasis was assessed using the chi square test and a P value less than 0.05 is considered significant.

### 3. Results

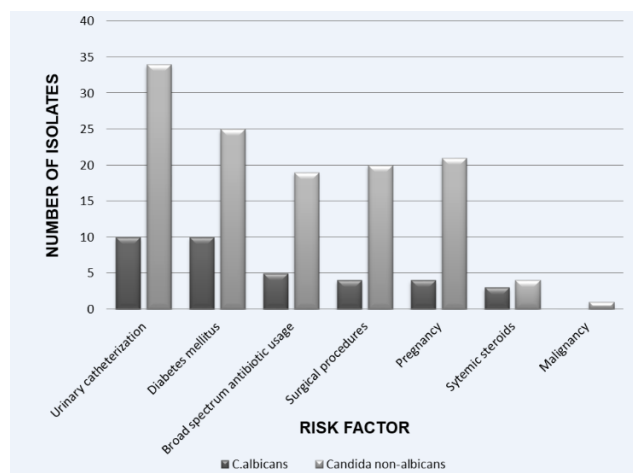
A total of 6930 urine samples were received during the study period and 101 *Candida* isolates were obtained from these samples. *C.tropicalis* was the most common species isolated followed by *C.albicans* (Table 1). Females had more incidence of Candiduria (66.3%) compared to males (33.6%). *C.albicans* was isolated in 7 males (20.6%) and 15 females (22.4%) whereas *Candida non-albicans* was isolated in 27 males (79.4%) and 52 females (77.6%). Candiduria was predominantly seen above 50 years of age (38.6%). (Figure 1) Urinary candidiasis was seen in 87(86.1%) hospitalized patients whereas it was present only in 14(13.8%) patients treated on outpatient basis. Urinary catheterization was the predominant risk factor associated with urinary candidiasis in 44(43.5%) patients. (Figure 2) Among the *Candida* species 50 (49.5%) isolates were found to be susceptible to fluconazole, 81(80.1%) isolates were found to be susceptible to voriconazole, 68(67.3%) isolates were found to be susceptible to ketoconazole, and 99(98%) isolates were found to be susceptible to amphotericin B. Among azoles, voriconazole showed highest susceptibility both to *C.albicans* and *Candida non-albicans*. (Figure 3) Among different *Candida species* isolated, *C.parapsilosis* and *C.dublinensis* showed the least susceptibility to the azole group of drugs. Table 2

**Table 1:** Various species of *Candida* isolated from urine samples

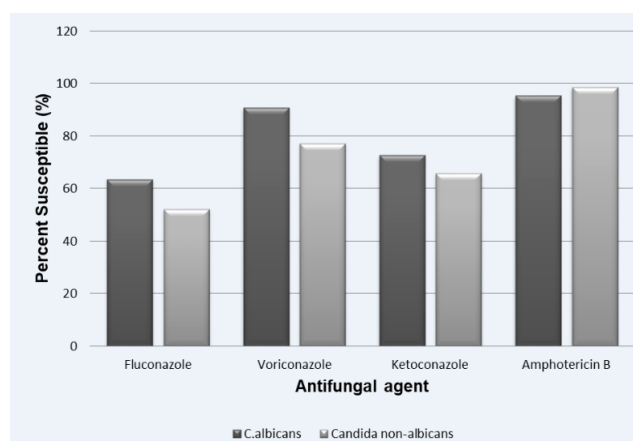
<i>Candida species</i>	Number isolated (n=101)	Percentage (%)
<i>C.tropicalis</i>	32	31.6 %
<i>C.albicans</i>	22	21.7%
<i>C.glabrata</i>	18	17.8%
<i>C.parapsilosis</i>	12	11.8%
<i>C.krusei</i>	10	9.9%
<i>C.dublinensis</i>	07	6.9%



**Fig. 1:** Distribution of urinary candidiasis in different age groups



**Fig. 2:** Distribution of risk factors for *C.albicans* and *Candida non-albicans*



**Fig. 3:** Susceptibility patterns of *C.albicans* and *Candida non-albicans* to different anti-fungal agents

### 4. Discussion

Urinary tract infections by *Candida species* are becoming increasingly common.<sup>10</sup> In normal adults, yeasts are found in less than 1 % of clean voided urine samples. In this study 101 urine samples (1.45%) yielded growth of *Candida species* from a total of 6,930 samples. Similar *Candida* isolation rates (1.37%) were shown in previous studies conducted in South India.<sup>11</sup>

The prevalence of urinary candidiasis was higher among females (66.3%) when compared to males (33.6%) in this study. This is similar to the observations of N Jain et al, and N Safdar et al, where 77.4% and 77% females respectively had candiduria in their studies.<sup>12,13</sup> However, a few studies have reported a higher prevalence of candiduria in males compared to females.<sup>14,15</sup> Urinary candidiasis was found to be more prevalent among patients over 50 years of age (38.6%) in our study. A similar

**Table 2:** Antifungal susceptibilities of different *Candida species*

<i>Candida species</i> (n=101)	Fluconazole			Voriconazole			Ketoconazole		Amphotericin B	
	S (%)	SDD (%)	R (%)	S (%)	SDD (%)	R (%)	S (%)	R (%)	S (%)	R (%)
<i>C.tropicalis</i> (n=32)	65.6	-	34.4	81.2	-	18.8	84.3	15.7	100	0.0
<i>C.albicans</i> (n=22)	63.6	-	36.4	90.9	-	9.1	72.7	27.3	95.4	4.6
<i>C.glabrata</i> (n=18)	66.6	-	33.4	83.3	-	16.7	55.5	44.5	100	0.0
<i>C.parapsilosis</i> (n=12)	16.6	-	83.4	58.3	-	41.7	41.6	58.4	91.6	8.4
<i>C.krusei</i> (n=10)	ND	ND	ND	90.0	-	10	80.0	20.0	100	0.0
<i>C.dublinensis</i> (n=7)	14.2	-	85.8	57.1	-	42.9	28.5	71.5	100	0.0

S= Susceptible, SDD= Susceptible Dose Dependant, R=Resistant, ND=Not Determined

observation has been recorded in previous studies.<sup>16,17</sup> Urinary candidiasis was observed in 87 (86.1%) patients who were hospitalized prior to the onset of the disease in this study. This was also observed in many previous studies where hospitalized patients are at a higher risk of contracting urinary candidiasis.<sup>18,19</sup>

The most common risk factors for urinary candidiasis observed in this study are urinary catheterization (43.5%), diabetes mellitus (33.6%), surgical procedures (22.7%), use of broad spectrum antibiotics (22.7%), pregnancy (21.7%), use of systemic corticosteroids (6.9%) and malignancy (0.9%). Similar observations where urinary catheterisation is the predominant risk factor for urinary candidiasis is also shown in previous studies by Jain N et al, and Navin Paul et al, where the risks were were 61.8% and 66.6% respectively.<sup>12,20</sup>

In our study, out of the 101 *Candida species* isolated, *Candida tropicalis* predominated (31.6%) followed by *Candida albicans* (21.7%), *Candida glabrata* (17.8%), *Candida parapsilosis* (11.8%), *Candida krusei* (9.9%) and *Candida dubliniensis* (6.9%). Many recent studies on candiduria have also shown a considerable increase in the incidence of non albicans *Candida species* as compared to *Candida albicans*.<sup>21</sup>

Among azole group of drugs, both *C.albicans* and *Candida non-albicans* showed highest susceptibility to voriconazole (90.9% and 77.2% respectively) whereas fluconazole showed the least susceptibility (63.6% and 47.8% respectively) in the antifungal susceptibility testing. For amphotericin B, one isolate each of *C.albicans* and *C.parapsilosis* was found to be resistant. Among the *Candida non albicans*, *C. parapsilosis* and *C. dublinensis* showed the least susceptibility to the azoles fluconazole, voriconazole and ketoconazole (16.6%, 58.3%, 41.6% and 14.2%, 57.1%, 28.5% respectively). Recent studies on urinary candidiasis reveal that many *Candida species* exhibit higher rates of resistance to fluconazole whereas they still remain susceptible to voriconazole and amphotericin B.<sup>22</sup>

## 5. Conclusion

Hospitalization and urinary catheterization are the important risk factors for developing urinary candidiasis. The

antifungal susceptibility varies among different *Candida species* and hence, identification of *Candida* to species level along with antifungal susceptibility testing should be practiced as a routine in all clinical mycology laboratories.

## 6. Conflicts of Interest

All contributing authors declare no conflicts of interest.

## 7. Source of Funding

None.

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