ABSTRACT
Objective: The incidence of Candida has been on the rise worldwide. Urinary tract infections [UTIs] as a result of Candida species are becoming common in hospitalised patients. Clinicians face dilemma in differentiating colonization from true candiduria. The species identification of Candida is important, as non albicans Candida species are increasing in number and more resistant to antifungal drugs. The aim of the study was to find out the frequency of Candida among uropathogens, their speciation and to determine the susceptibilities to antifungal drugs of Candida species isolated from candiduria.

Material and Methods: A total of 2900 urine samples were analysed in a tertiary care hospital. Candida species isolated from urine samples were subjected to speciation using CHROM agar and standard yeast identification protocol. Antifungal susceptibility testing for fluconazole, voriconazole, flucytosine, amphotericin B was carried out using VITEK-2 compact system of Biomerieux.

Result: A total of 66[2.27%] Candida species were isolated from 2900 urine samples. Among them non albicans Candida species were predominant [69.7%] compared to Candida albicans [30.3%]. The Candida isolated were more susceptible to amphotericin B [91%] and flucytosine[82%] compared to voriconazole[72.72%] and fluconazole [66.66%].

Conclusion: The present study reiterates the prevalence of Candida species among UTIs and their antifungal susceptibility pattern. Prevalence of non albicans Candida was more than Candida albicans. Non albicans Candida species are more resistant to antifungal drugs compared to C.albicans. Therefore, the species identification of Candida isolates along with their antifungal susceptibility pattern can help the clinicians in better treating the patients with candiduria.

Keywords: Candiduria, Non albicans Candida, Antifungal susceptibility testing

INTRODUCTION
The frequency of urinary tract infections (UTIs) due to Candida species is increasing and these infections are now being the most common clinical finding, particularly in hospitalised patients [1]. Since 1980s there has been increase in the prevalence of Candida species causing urinary tract infections. It is common in the patients admitted in intensive care units, individuals with multiple predisposing factors, including diabetes mellitus, indwelling urine catheter, long term exposure to antibiotics and immunosuppressive therapy. The emergence of drug resistant Candida species, which is largely attributed to use of prolonged and inappropriate empirical therapy, has further complicated the patient management [2]. Candida species account for almost 10-15% nosocomial UTIs[3,4]. Candiduria not properly diagnosed and treated has been source of morbidity and mortality [5]. All Candida species are capable of causing UTIs, in many centers worldwide non albicans Candida species have replaced Candida albicans as the predominant pathogen. Non albicans Candida species appear better adopted to the urinary tract environment and are more resistant to antifungal drugs compared to C. albicans. In this context present study was carried out to know the prevalence of Candida species causing UTIs and their antifungal susceptibility pattern in a tertiary care hospital.

MATERIAL AND METHODS
A total 2900 urine samples were collected from patients attending to outpatient department and admitted in the hospital at A.J. Institute of Medical Science, Mangalore from November 2010 to October 2012. Permission from the institutional ethical committee was taken.

Inclusion criteria
Male and female patients of all age groups were considered for our study.

Both outpatients and inpatients who presented with signs and symptoms of urinary tract infections were included. Pure growth of yeast isolates with significant colony count was included in the study.

Exclusion criteria
The urine samples, where Candida species was isolated in the absence of pyuria, Candida with colony count <1000 CFU/ml and mixed growth (polymicrobial growth) were excluded from analysis.

Method: The urine samples were collected in a sterile leak proof container with screw capped lids and transported immediately to microbiology laboratory. Urine wet mount examination was done to look for the presence of pus cells, red blood cells, casts, crystals or any bacterial or fungal elements. The urine samples were inoculated on Cysteine Lactose Electrolyte Deficient (CLED) and Mac Conkey agar by calibrated loop technique delivering 0.001ml of urine as per standard protocol for urine culture. The culture plates were incubated aerobically at 37°C for 24 to 48 hours. Candida species isolated on culture plates with colony count >10000 CFU/ml were considered significant [6,7]. The Candida isolates (66) were further speciated by Gram stain, culture on sabouraud’s dextrose agar, germ tube test, chlamydospore formation on conmeal agar. Apart from identifying the isolates by the conventional methods, they were also inoculated on to CHROM agar (HiMedia -HiCrome Candida differential Agar) for identification of Candida species [7,8]. After inoculation on to CHROM agar, the plates were incubated for 24-48 hours at 30°C and the results were read according to the standard instruction from the manufacturers [9]. The colonies were identified based on the color of the colonies (chromogenic reaction) produced by the Candida species on the CHROM Agar. Light green colonies -
Candida albicans, blue colonies with pink halo- Candida tropicalis, cream to white colonies- Candida glabrata, purple fussy colonies- Candida krusei [10].

Further confirmation of identification and antifungal susceptibility testing was done for 33 Candida isolates using VITEK-2 compact system of biomerieux. The antifungals which were tested included fluconazole, voriconazole, flucystosine and amphotericin B [11].

STATISTICAL ANALYSIS

The data were obtained and entered using SPSS software version 16.0 for statistical analysis. The descriptive statistics was used to characterise the study group. Fischer’s exact test was used for comparing the difference between the two groups. p value of 0.05 was considered as statistically significant.

RESULTS

A total of 66(2.27%) Candida species were isolated from 2900 urine samples. Among them non albicans Candida species 46(69.7%), were predominant compared to C. albicans 20(30.3%). Non albicans Candida species included C.tropicalis (30), C. krusei (10) and C. glabrata (6), [Table/Fig-1]. The rate of isolates of Candida species were more in males, 41 (62.12%) than in females 25 (37.87%). The highest isolation rates of Candida among uropathogens were found in age group above 60 years [Table/Fig-2]. Antifungal susceptibility testing was done for 33 Candida isolates. The Candida isolates were more susceptible to amphotericin B (91%) and flucytosine (82%) compared to that of voriconazole (72.72%) and fluconazole (66.66%), [Table/Fig-3]. Resistance to azoles were more in non albicans Candida group when compared to C. albicans [Table/Fig-4].

DISCUSSION

The prevalence of candiduria caused by the species other than C. albicans was surprisingly high in the given study. Changing trends in the aetiopathogenesis of urinary tract infections and considerable increase in number of non albicans Candida species is a matter of concern [12]. In the last few years various factors like immunocompromised status, immunosuppressive therapy, prolonged hospital stay, prolonged antibiotic therapy, catherisation have all contributed for increase in number of cases of candiduria [13, 14, 15]. Catheterisation process increases chances of UTIs by allowing migration of the organisms into the bladder from external perurethral surface. The indiscriminate, inadequate use of antifungal drugs, especially azole group have all contributed for increase in emergence of resistance strains of Candida [16].

In the present study, isolation rate of Candida species from urine samples were 2.27%, which is slightly higher than the observation of Ragini et al (1.37%) [17]. Studies have shown that there is considerable increase in number of non albicans Candida species among candiduria. Our study showed that isolation rate of non albicans Candida was 69.7%, which is higher than C.albicans 30.3%, this finding is in concordance with the studies done by Iman et al [18]. Identification of Candida species is important as non albicans Candida are more resistant to azoles compared to that of C.albicans. C.krusei is intrinsically resistant to fluconazole. Antifungal susceptibility pattern showed that Candida isolates were more susceptible to amphotericin B and flucytosine to that of azoles. The increase in resistance to fluconazole is a matter of great concern as it is the most commonly used azoles for the treatment of candiduria [19]. In our study C. albicans showed more susceptibility to azoles compared to that of non albicans Candida, which is similar to the results of studies done by Shivanand et al., [20].

### Table/Fig-1: Distribution of Candida species

<table>
<thead>
<tr>
<th>Candida species</th>
<th>Total number = 66</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>20</td>
<td>30.3</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>30</td>
<td>45.45</td>
</tr>
<tr>
<td>C. krusei</td>
<td>10</td>
<td>15.15</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>06</td>
<td>9.09</td>
</tr>
</tbody>
</table>

### Table/Fig-2: Age and Gender wise distribution of Candida isolates

<table>
<thead>
<tr>
<th>Age group</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-15</td>
<td>03</td>
<td>01</td>
<td>04</td>
</tr>
<tr>
<td>16-30</td>
<td>03</td>
<td>05</td>
<td>08</td>
</tr>
<tr>
<td>31-45</td>
<td>07</td>
<td>08</td>
<td>15</td>
</tr>
<tr>
<td>46-60</td>
<td>15</td>
<td>03</td>
<td>18</td>
</tr>
<tr>
<td>&gt;60</td>
<td>13</td>
<td>08</td>
<td>21</td>
</tr>
<tr>
<td>Number of isolates</td>
<td>41 (62.12%)</td>
<td>25 (37.87%)</td>
<td>66</td>
</tr>
</tbody>
</table>

### Table/Fig-3: Antifungal susceptibility testing pattern of Candida species - 33 isolates

<table>
<thead>
<tr>
<th>Species</th>
<th>Total No. 33</th>
<th>Fluconazole</th>
<th>Voriconazole</th>
<th>Fluucytosine</th>
<th>Amphotericin B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S (%)</td>
<td>I</td>
<td>R</td>
<td>S (%)</td>
<td>I</td>
</tr>
<tr>
<td>C. albicans</td>
<td>10</td>
<td>8(80)</td>
<td>1</td>
<td>1</td>
<td>8(80)</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>15</td>
<td>10(66.6)</td>
<td>2</td>
<td>3</td>
<td>11(73.33)</td>
</tr>
<tr>
<td>C. krusei</td>
<td>5</td>
<td>2(40)</td>
<td>-</td>
<td>3</td>
<td>3(60)</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>3</td>
<td>2(66.6)</td>
<td>-</td>
<td>1</td>
<td>2(66.6)</td>
</tr>
<tr>
<td>Overall % sensitivity to each antifungal drugs</td>
<td>66.66%</td>
<td>72.72%</td>
<td>82%</td>
<td>91%</td>
<td></td>
</tr>
</tbody>
</table>

### Table/Fig-4: Percentage wise sensitivity to Azoles

<table>
<thead>
<tr>
<th>Candida species</th>
<th>Fluconazole</th>
<th>Voriconazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans (10)</td>
<td>80%</td>
<td></td>
</tr>
<tr>
<td>Non-albicans Candida (23)</td>
<td>60.86%</td>
<td>69.56%</td>
</tr>
</tbody>
</table>
CONCLUSION
This study furnishes much needed information on various species of Candida causing urinary tract infection and their antifungal susceptibility pattern in this region. Hence species level identification of Candida and their antifungal susceptibility pattern will help in accurate treatment of candiduria.

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REFERENCES

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