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Urinary Isolates and Antifungal Susceptibility Pattern of *Candida species* Isolated from Patients in Tertiary Care

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Community-acquired urinary tract infections (UTIs) are fastidious growing infections caused by Candida fungal overgrowth that frequently occur in immunosuppressed hospital patients. Although many factors account for the increases in these fungal infections, the leading causes include the rise in medical device usage, immunosuppressant drugs, and interventional procedures. The emergence of drug resistant in Candidal species, which is largely attributed to use of prolonged and inappropriate empirical therapy, has further complicated patient management. The regularity of urinary tract infections (UTIs) due to *Candida* species is growing and these infections are most common clinical outcome, particularly in hospitalized patients. The microbiological invasions in any tissue of the urinary tract extend from the renal cortex to the urethral meatus. Candida UTI or candiduria is a common finding in hospitalized patients. To find out the ratio of *Candida albicans* to non- albicans *Candida* species to correlate the risk factors to the *Candida* species associated and to analyze the speciation of *Candida* isolates using the Chrom agar is a differential culture media, with sugar assimilation test. Constant surveillance of candiduria is important as *C. tropicalis* is more

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invasive, can lead to fatal candidemia. Identification of Candida up to species level is essential, as it can give an idea to the clinicians about appropriate antifungal therapy. Our study showed a predominance of non-albicans *Candida spp*. of about 86%. *C. tropicalis* (62%) was the most common isolate obtained followed by *C. albicans* (14%), *C. glabrata* (10%), *C. krusei* (9%) and *C. parapsilosis* (5%). Indwelling urinary catheter was an important associated risk factor for non-albicans candiduria.

Keywords: Candida albicans; antibacterial agents; urinary tract infection.

1. INTRODUCTION

Urinary tract infections are the most common infectious diseases in both outpatients and hospitalized patients. It is common among patients admitted to intensive care units, patients with multiple predisposing factors, including diabetes mellitus, indwelling urine catheter, long term exposure to antibiotics, and immunosuppressive therapy. Opportunistic infections by *Candida spp* are increasing globally and are commonly associated with antifungal resistance. The management and control of this disease becomes more aggressive and complicated as the severity of the infection depends largely on the treatment protocol and the patients 'own immune response. This infection generally occurs secondary to another infection or medical condition such as immunosuppression and immunodeficiencies from underlying causes such as chemotherapy, corticosteroids, surgery, malignancy, any type of manipulation, interventions, and prolonged hospital stays all increase the risk of infections due to *Candida spp.* and other fungal pathogens. There is a marked decrease in the percentage of UTI caused by *Escherichia coli*, *Proteus,* and *Pseudomonas* species and a considerable increase in the percentage of UTI caused by fungi, especially *Candida* species in critically ill patients [1].

The discovery of *Candida* species in urine samples is known as candiduria which represents a diagnostic and therapeutic challenge for physicians working in primary care or infectious diseases, as well as intensive care and surgery [2]. The presence of Candida species in the urine may represent several conditions that require careful interpretation of the report, ranging from sample contamination to urinary tract infections, including disseminated candidiasis.

Fungi are commensal organisms in plants, mammals, and insects and in humans are frequently showing too many genera of fungi through several routes. *Candida* sp., are normal commensals of humans and are commonly found on the skin, mucocutaneous membranes and in the healthy gastrointestinal tract. Usually they cause superficial mucosal infections; however if left untreated these infections may become super infections attacking tissues and yielding life threatening pathology caused by alteration of the host immune defenses. The principal causes of this increased incidence of candidal infections in the last half of 20th century are i) introduction of antibacterial agents with a broad spectrum of activity that alters the hosts normal microbial flora favoring fungal invasion and ii) increased prevalence of immunosuppressed patients due to chemotherapy and emergence of Acquired Immune Deficiency Syndrome (AIDS) [1]. Candida species are the root of a wide spectrum of clinical diseases reaching from superficial infections of the skin, nails, and mucosal surfaces to deep seated infections involving various internal organs and disseminated diseases causing significant morbidity and mortality. It commonly causes lower urinary tract infections and renal infection, in hospitalized patients. Epidemiological surveillance indicates that Candida species are now the most common pathogens causing nosocomial blood stream and urinary tract infections (UTI) [2].

Candida spp., are the most prevalent opportunistic fungal pathogens of the urinary tract and usually present as a nosocomial infection and rarely as community acquired infection. Candida spp. account for almost 9 to 40% of nosocomial UTI [3]. Nosocomial UTI"s are also associated with significant mortalities. [3-5] *C. glabrata* is less susceptible and *Candida krusei* is intrinsically resistant to fluconazole. *C. tropicalis* has the highest adherence rate to inanimate materials such as urinary and vascular catheters, and is often involved in biofilm formation, that is more resistant to antifungal agents. Resistance to azoles in *C. tropicalis* and *C. albicans* has also been increasingly reported [6-9].

Thus, it is important to know the type of Candida *spp*. causing the UTI before initiating the

treatment as non- albicans Candida species are on the rise in the hospital environment and the majority is inherently resistant to treatment with fluconazole [10-12]. The present research aims to study the Candida species diversity in urine samples from patients with urinary tract infections connected with obstructive uropathy, and investigates the correlation between the risk factors connected with the Antifungal susceptibility of the *Candida spp.* isolated.

2. MATERIALS AND METHODS

The research work was carried out in the Department of Microbiology, Central laboratory of Sree Balaji Medical College and Hospital, Chrompet, Chennai-44. The work was carried out from December 2015 to December 2016, continuously over a period of 1 year.

Study group included 100 hospitalized patients with candiduria having a colony count of more than 10^4/ml of urine.

2.1 Inclusion Criteria

- Hospitalized patients with a urine colony count of any fungal species.
  Candida species more than or equal to 10^4/ml.
- Patients older than 12 yrs of age.
- Both males and females were included.

2.2 Exclusion Criteria

- Candiduria with colony counts less than 10 4/ml of urine.
- Patients less than 12 yrs of age.
- Outpatients.

Patients satisfying the inclusion criteria were included in the study and after getting informed consent, they were assigned serial numbers. They were interviewed by structured questionnaire and their hospital records were used to understand their history, risk factors, duration of Candiduria, and treatment details. The antibiotic susceptibility was analyzed by standard disc method using amphotericin (100 units), Rifampicin (5µg) and cephalosporin (5µg).

3. SPECIMEN COLLECTION

Urine Specimens were collected in a wide mouthed, sterile, leak-proof screw capped container. The collected samples were stored in a refrigerator at 4°C or transported in a container with 1.8% boric acid and were mid-stream urine specimens, and catheter collections, that were immediately brought to the laboratory without any delay, as urine is an optimal culture medium for other microorganisms to grow. Hi-Chrom agar helps in reducing the time for diagnosis, thereby reducing the duration of the hospital stay and the cost of treatment. Further the increasing rates of resistance particularly among the non-albicans *Candida spp*. The present study emphasized the need for speciation and antifungal susceptibility testing as a routine practice in all microbiology laboratories due to the alarming increase of resistant fungal infections.

4. RESULTS

This cross-sectional study was carried out during the period of December 2015 to December 2016 in the Department of Microbiology, Central laboratory of Sree Balaji Medical College and Hospital, Chrompet, Chennai -44. Speciation of isolates from 100 hospitalized patients with candiduria Fig. 1A was done and their antifungal susceptibility testing was conducted using the disk diffusion method.

Table 1. Genderwise distribution of study population

Gender	Number	Percent
Male	54	54
Female	46	46
Total	100	100

Males contributed 54% and females contributed 46% of the total population Figs. 1 and 2. Majority of the study population belonged to the above 60 years age group (28%), followed by (21%) patients in the 20-29 & 40-49 years age groups. Females predominated in the age group of 20 -29 yrs (28.2%), whereas males predominated over 60 yr (31.4%) age group since their weak immune system changed with life style habits (smoke etc.,).

Most of the patients had asymptomatic candiduria (46%), followed by symptomatic patients (25%) and unconscious patients who were unable to tell the symptoms (29%). Symptomatic and unconscious patients were common in the age group of > 60yrs and asymptomatic patients were common in the age group of 40-49yrs and 50-59 yrs Fig. 3.

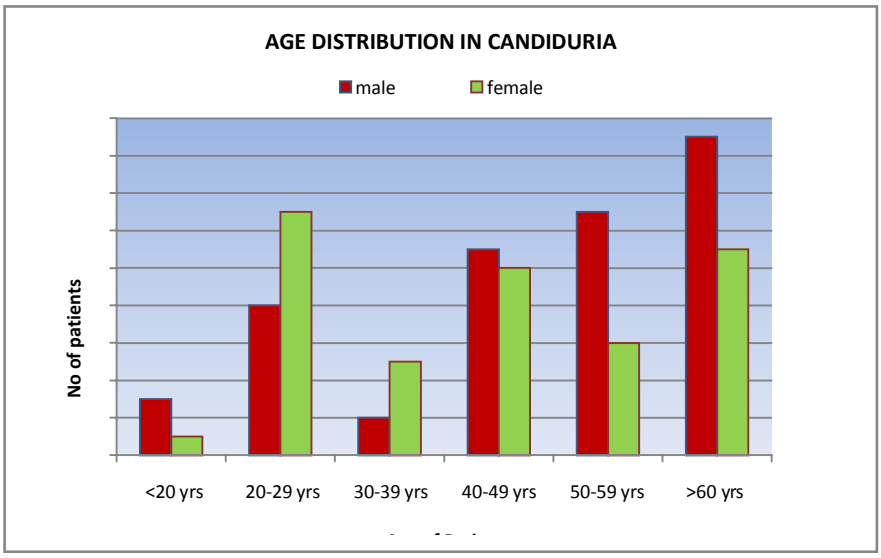

Fig. 1. Age distribution in candiduria

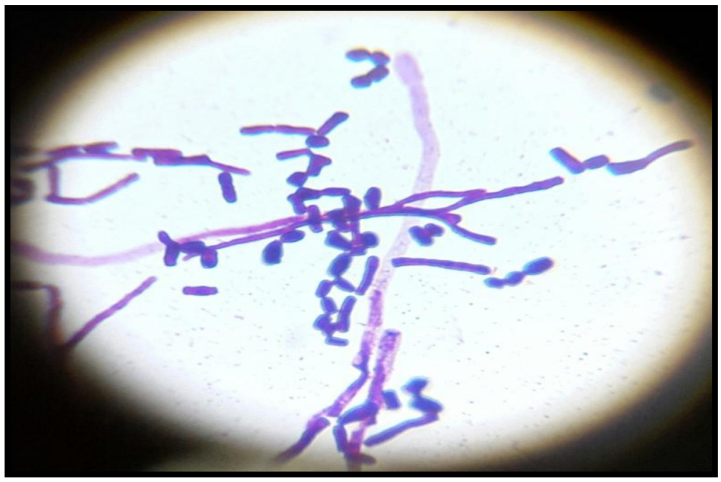

Fig. 1a. Gram's stain of centrifuged urine showing pseudohyphae of *candida spp*

73% of urine samples were catheterized urine samples followed by 27% of midstream urine samples Figs. 3A and 4. 74.07% of samples from males and 71.1% of samples from females were catheterized urine samples Figs. 5- 6) The antibiotic susceptibility was analyzed by their growth zone against the antibiotic discs. The isolates showed higher susceptibility to cephalosporin (5.9cm), amphotericin (5.4cm) and rifamycin (4cm) respectively Figs. 6 -7.

5. DISCUSSION

All identified *Candida* species were capable of producing UTIs. *C. albicans* was the most common isolated species. However, in many centers worldwide, a significant rise in frequency of Urinary Tract Infections (UTI) due to Candida species has occurred over the last decade with an upsurge of non albicans Candida species. The species level identification has clinical importance and differs in the expression of virulence factors and the antifungal susceptibility pattern. The correct identification of Candida species is of great importance, as it presents prognostic and therapeutic significance, allowing an early and appropriate antifungal therapy [13].

The current study was commenced to observe the Candida isolated from the urine samples of hospitalized patients and to find their antifungal susceptibility pattern. The work also focused on

the changes observed in species distribution and the surge of non-albicans *Candida* spp. in our hospital [14-15]. In the present study males contributed to 54% Table 1. This was similar to the results of study by Arlene O. Cantillep et al [16-17]. Although there is an increased risk in the female gender, the other associated risk factors like diabetes and chronic kidney disease were common in males in our study [18]. The present study showed that the patients were predominantly male in the age group of >60 yrs (Graph: 1) contributing to 28% of total patients, followed by 21% in 40 -49 yrs. This correlated with a study conducted by S. Krcmery et al, where the mean age was 62.4 yrs [19].

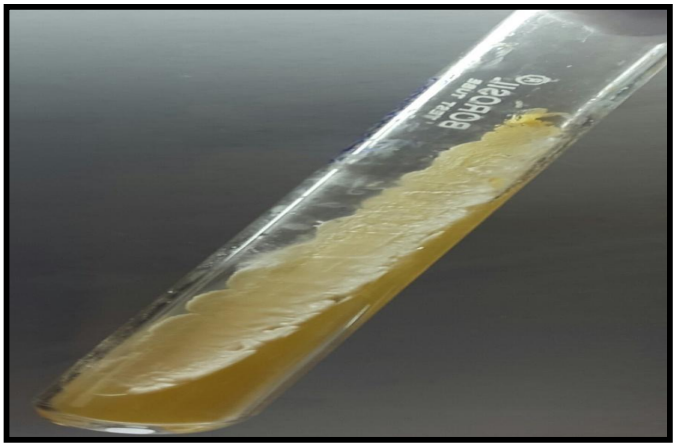

Fig. 2. Colonies of *candida* on sabouraud dextrose aga(sda) slant

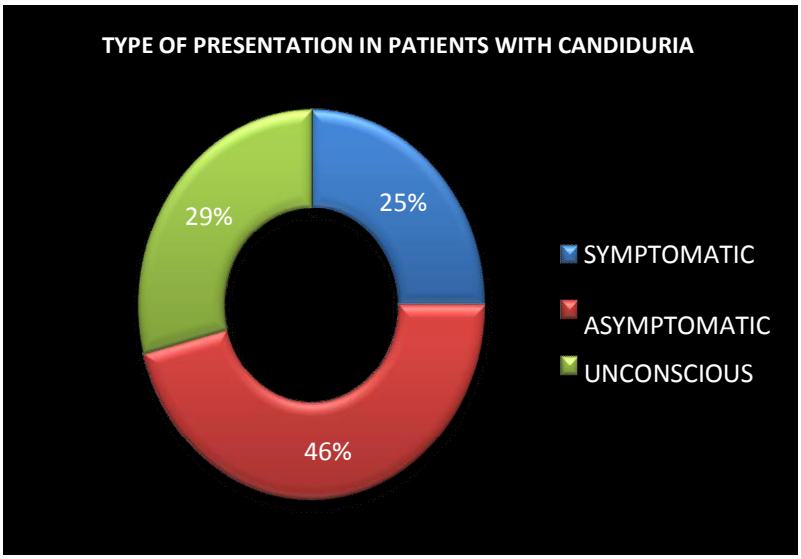

Fig. 3. Type of presentation in patients with candiduria

Table 2. Types of urine samples

Type of Sample	Total, n=100 no(%)	Male, n=54 no (%)	Female, n=44 (no%)
Catheterized urine	73 (73%)	40 (74.07%)	33 (71.1%)
Midstream urine	27 (27%)	14 (25.9%)	13 (28.26%)
Suprapubic aspiration	0	-	-
Total	**100 (100%)**	**54 (100%)**	**46 (100%)**

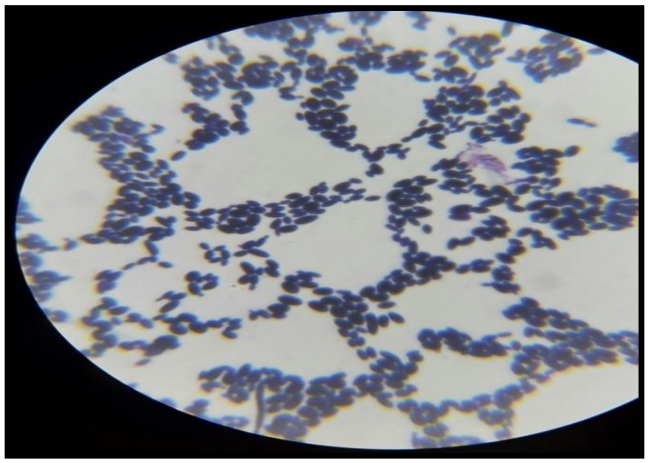

Fig. 3a. Round blastoconidia of *C. albicans*

Fig. 4. Steel blue colonies of *c. tropicalis* on chrom agar

Fig. 5. Sugar assimilation test using rapid identification kit

Diabetes mellitus (44%) was the most combined disease associated, followed by CKD (33%) and it was slightly higher than the study by Claudia Castelo Branco et al., who showed 26.7% of the patients, had diabetes probably because; Indians are more prone to diabetes. India is termed as the "Diabetes capital of the world" [20]. Immunosuppressive like steroids were also important risk factors, as they alter the natural immunity to Candida spp. These drugs were used as a therapy for transplant recipients, neurosurgery patients, and for patients with auto

immune diseases. Unconsciousness was another risk factor, as most of the patients were on prolonged indwelling catheter and antibiotic therapy [21].

The present study showed the predominance of the non- albicans Candida spp. which contributed 86% of isolates and C. albicans contributed only 14% of the isolates. This was comparable to the results obtained by Manisha Jain et al. [22-23], who reported that non-albicans Candida spp. were the predominant isolate in 71.4% of urine isolates. Biofilm formation of the *C. tropicalis* strain on the catheter surface might contribute to the colonization of patients with a urinary catheter. Biofilms of *C. tropicalis*, with an extensive, hexosamine-rich matrix, were poorly penetrated by the antifungal agents, whereas the biofilms of *C. albicans*, with a less-extensive glucose rich matrix, were more readily penetrated by the drugs [24]. The exact reason for the increase in non-albicans Candida spp. has not yet been elucidated. *C. albicans* was found in 37% of the midstream samples; however it contributed to only in 5.4% of the catheterized patients. C. albicans was more common in midstream urine samples than catheterized patients. Antifungal susceptibility testing to amphotericin B shows minimal resistance pattern and high susceptibility rate to different *Candida* species. Susceptibility rate for *C.albicans* was 85.7%, for *C. glabrata* was 100%, *C. tropicalis* was 93.4% and for *C. krusei* was 66.6%. Resistance for *C. albicans* was 14.2%, *C. glabrata* 0%, *C. tropicalis* 6.4%, and for *C. krusei* 33.33% Table 2. Our finding shows an overall susceptibility rate of Amphotericin B to be 90% which correlated with a previous study conducted by Saldanha et al and Noake et al where Candida species were more susceptible to the amphotericin B (92%) [25].

Candiduria should never be ignored as it can be the only indication of systemic or invasive candidiasis. Our study showed a predominance of non-albicans *Candida spp*. of about 86%. *C. tropicalis* (62%) was the most common isolate obtained followed by *C. albicans* (14%), *C. glabrata* (10%), *C. krusei* (9%) and *C. parapsilosis* (5%). Indwelling urinary catheter was an important associated risk factor for non-albicans candiduria. Multiple risk factors like long term antibiotic therapy, prolonged catheterization, and Diabetes mellitus were present in many patients. Hi-Chrom agar takes only 48 hrs for species identification, and is a comfortable alternative to conventional methods, that take 96 -120 hrs. Hi-Chrom agar is superior to other conventional methods available for the rapid detection of *Candida* species. Non-albicans Candida species are emerging as important pathogens with increasing rates of azole resistance and immunosuppression.

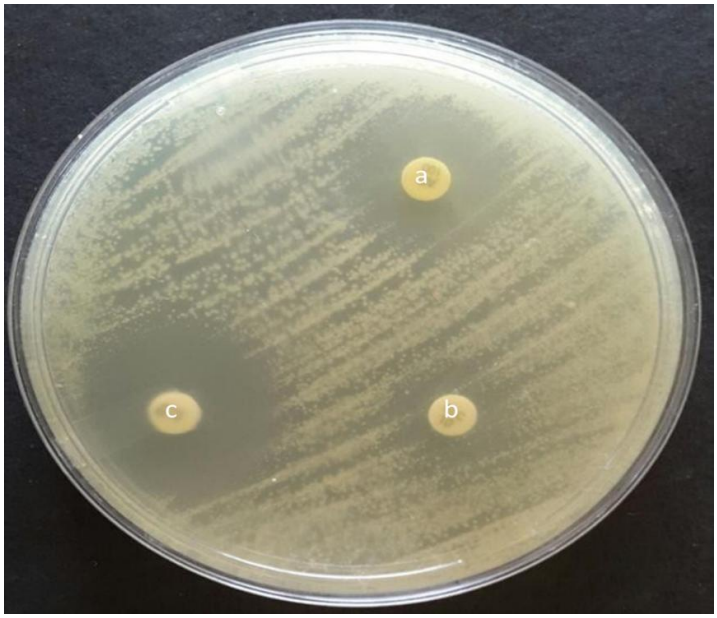

Fig. 6. Antifungal susceptibility by disk diffusion method
Legend: (a) amphotericin, (b) Rifampicin and (c) cephalosporin

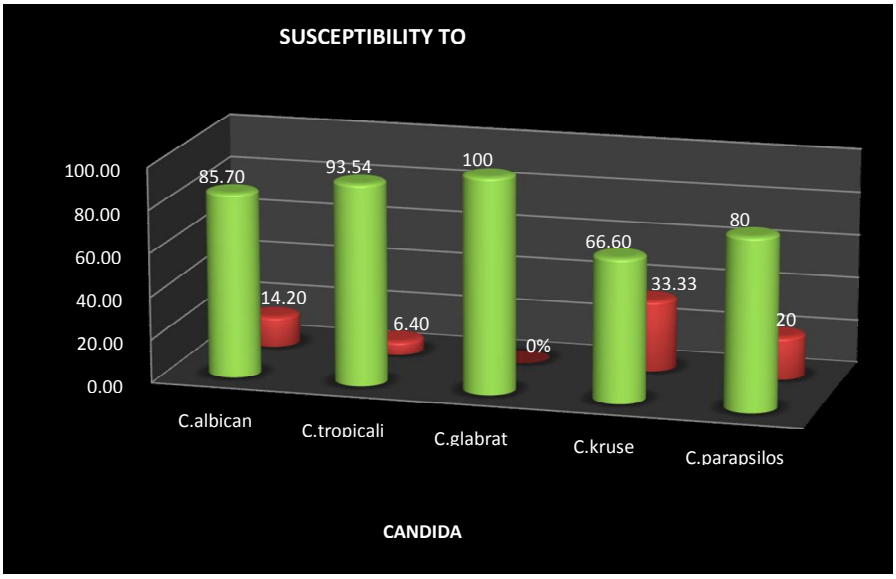

Fig. 7. Antifungal susceptibility to amphotericin b

6. CONCLUSION

Infections with *Candida spp* are usually associated with a few well-defined risk factors such as immunocompromised state, malignancy, and steroid therapy. Understanding these risk factors, and identifying the species with changing trends in antifungal resistance, and founding infection control practices to reduce morbidity and mortality in critical care areas can improve outcomes.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

The study was approved by the Institutional Ethical Committee, Sree Balaji Medical College and Hospital, Chrompet, Chennai- 44.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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