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Research Article

CHARACTERIZATION AND SUSCEPTIBILITY PATTERN OF *CANDIDA* SPECIES ISOLATED FROM URINE SAMPLES IN PATTUKKOTTAI, TAMILNADU, INDIA

C. Manikandan[1]* and A. Amsath[2]

[1]*Department of Microbiology, Marudupandiyar College of Arts and Science, Thanjavur-613403, Tamilnadu, India.

[2]P.G. and Research Department of Zoology, Khadir Mohideen College, Adirampattinam-614 701, Tamilnadu, India

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ABSTRACT
Urinary tract infections are among the most common infectious diseases in humans. The incidence of candidiasis is on the rise in hospitalized patients especially due to non-*albicans Candida*. The present study was aimed to isolate, identify, and perform antifungal susceptibility testing of the yeast isolates from the urine samples. The urine samples from a total of 2400 patients in the Pattukkottai over a period of 18 months were obtained. *Candida* species were isolated from twenty two patients. The prevalence candiduria in critically ill patients in our diagnostic centre was 3.4 percent. Females were affected predominantly (81.8%). The species were identified according to morphology and color of colonies on Chromagar *Candida*. *C. albicans* (45.5%) was the highest occurring pathogens isolated, followed by *C. glabrata* (22.7%), *C. tropicalis* (18.2%), and *C. krusei* (13.6%). The antifungal sensitivity tests carried out using commercially available antifungal disc. Amphotericin B and itraconazole 21 (95.5%) was found to be the most effective antifungal agent, followed by ketoconazole, and fluconazole.

Keywords: UTIs, Urine samples, Chromagar, *Candida* species, Antifungal test.

INTRODUCTION

Candiduria is one of the most common symptoms of urinary tract infections caused by several species of *Candida*, which is a normal flora of human body. *Candida albicans* has played an important role in candiduria (Nayman *et al.*, 2011). *Candida* species are the most common cause of fungal infections leading to a range of life threatening invasive to non-life-threatening diseases (Jacqueline *et al.*, 2010). Urinary tract infections as a result of *Candida* species is becoming increasingly common in hospitalised setting particularly in intensive care units (Jain *et al.*, 2011). Epidemiological surveillance indicates that *Candida* species are now the most common pathogens causing nosocomial bloodstream and urinary tract infection (Horvath *et al.*, 2003). Yeast belonging to the genus *Candida* exists as saprophytes, colonizing mucosal surfaces and external genitalia of humans of either gender, but especially near the urethralmeatus of healthy, premenopausal women. All common *Candida* species are capable of causing urinary tract infections (UTIs), and in many centers worldwide non-albicans *Candida* species now predominate (Rivett *et al.*, 1986).

Candida species accounts for almost 9 to 40% of nosocomial urinary tract infections (Jacqueline *et al.*, 2010). About 14 *Candida* species have been implicated in human infections, with *Candida albicans* being the most prevalent among the yeast isolates. The most frequently isolated species is *Candida albicans*, but *Candida tropicalis*, *Candida glabrata*, *Candida krusei*, and *Candida parapsilosis* are also emerging as important etiologic agents of *Candida* infection (Krcmery and Barnes, 2002).

*Corresponding author e-mail: drmanikandan66@gmail.com, Mobile: + 91 9361299995

Chromagar *Candida* media can be reliably used for isolation of yeasts. Use of this medium even allows mycology laboratories to identify rapidly clinically important species. Chromagar *Candida* culture will also enable the clinician to choose appropriate antifungal drugs and there by decreasing patients mortality and morbidity (Horvath *et al.,* 2003).

Several reports showed that the frequency of urinary tract infection (UTI) due to yeasts has increased during the last decades (Laverdiere *et al.,* 2007; Saha *et al.,* 2008). Prolonged hospitalisation, long stay in ICU, urinary tract abnormality, immunocompromised patients, antibacterial therapy with broad spectrum for long time and prophylaxis by antifungal agents are presented as more important risk factors for UTI (Nayman *et al.,* 2011; Dalen *et al.,* 2005). A review of the epidemiology of candiduria including all retrospective reviews, case-controlled studies and a large prospective surveillance study on candiduria, showed that the common risk factors include urinary tract instrumentation, prior surgical procedures, recent use of antibiotics, advanced age, female sex, diabetes mellitus, immunosuppressive therapy and prolonged hospital stay (Kobayashi *et al.,* 2004 and Kauffman., 2005). The present study was aimed to isolate, identify, and perform antifungal susceptibility testing of the yeast isolates from the urine samples.

MATERIALS AND METHODS

Characterisation of *Candida* Species: Twenty two isolates of *Candida* species were used for microscopy analysis through Gram staining and culture on Chromagar media, and Sabouraud dextrose agar medium, supplemented with 50 mcg mL-1 of chloramphenicol. The cultures were incubated at 37^{o}C, for 24-48 hours, under aerobic conditions.

Chromagar media used for isolation of *Candida* species: Chromagar (Himedia) was prepared according to the manufacturer's instructions. The suspension was completely dissolved by boiling (<100°C) and mixing. The medium dose not require sterilisation by autoclave, therefore after cooling in a water bath to 45°C the agar was poured into sterile petri dishes. After allowing cooling, the plates were stored at 4°C prior to use.

Germ tube test: Small portion of an isolated colony was suspended in a test tube containing 0.5 ml of human serum then incubated at 37ºC for 2 hours then examined microscopically at 30 minutes intervals up to 2 hours for the presence of germ tube.

Sucrose assimilation: Five drops of *Candida* suspension was added to yeast nitrogen base agar after cool at 45ºC then poured into plates. Filter paper discs impregnated with saturated sucrose solution were placed on the surface of agar, and then incubate at 27-30ºC up to 48 hours. Positive growth indicated by growth of *Candida* around the assimilated sucrose.

Antifungal susceptibility test: Antifungal susceptibility testing was carried out using the disc diffusion method following the National Committee for Clinical Laboratory Standards institute (CLSI, 2004) guidelines, using fluconazole (25µg), itraconazole (50µg), ketoconazole (10µg), and amphotericin B (20µg) antifungal discs. Supplemented Mueller-Hinton agar [Mueller-Hinton agar + 2% glucose and 0.5 g/mL methylene blue dye, (GMB medium)] was used for performing the antifungal susceptibility testing.

Preparation of inoculam: Inoculum was prepared by picking five distinct colonies of approximately 1mm from 24 hours old culture grown on Saboured Dextrose Agar (SDA agar) incubated at 35- 37ºC. Colonies were suspended in 5 ml of sterile 0.85% saline.

Susceptibility test procedure: Prepared plates with Mueller Hinton Agar +2% glucose and 0.5 µg/ml methylene blue dye (GMB) medium for carrying out susceptibility of antifungal discs. The medium in the plates should be sterile and have a depth of about 4 mm. The prepared inoculum streaked in the entire agar surface of the plate with the cotton swab three times, turning the plate at 60º angle between each streaking. The inoculum allowed to drying for 5-15 minutes with lid in place. The discs were applied using aseptic technique. Deposit the discs with centers at least 24 mm apart. Inverted the plates and placed in an incubator set to 35- 37ºC within 15 minutes after the discs were applied and examined all plate after 20-24 hours of incubation. Measured the zone diameter to the nearest whole millimeter at the point at which there is prominent reduction in growth.

RESULTS

Candida species were isolated from twenty two patients**.** The species were identified according to morphology and color of colonies on Chromagar. The color of colonies on Chromagar was similar as given by the manufacturer, i.e. green colonies of *C.albicans,* steel blue colonies of *C.tropicalis* accompanied by purple pigmentation which diffuses into surrounding agar by growth, and large, fuzzy, rose colored colonies with white edges of *C.krusei*, the smooth white to light pink colonies of *C.glabrata* which later became pink (Table 1). The prevalence candiduria in critically ill patients in the study was 3.4%. Out of the total twenty two strains isolated, 18(81.8%) and 4(18.2%) *candida* strains were isolated from female and male patients respectively. *C. albicans* (45.5%) was the highest occurring pathogens isolated, followed by *C. glabrata* (22.7%), *C. tropicalis* (18.2%), and *C. krusei* (13.6%) (Table 1 and Figure 1).

Table 1. Characteristics of *Candida* species on Chromagar media and Distribution of isolated *Candida* species.

Species	Colony characteristics on Chromagar	Number of isolates	Male	Female
C. albicans	Apple green colonies; consistent	10 (45.5%)	2	8
C. glabrata	White large glossy pale pink to violet colonies	5 (22.7%)	1	4
C. tropicalis	Dull blue, to purple color that diffused into surrounding agar with pale pink edges	4 (18.2%)	0	4
C. krusei	Large, flat, spreading, pale pink colonies with matt surfaces	3 (13.6%)	1	2

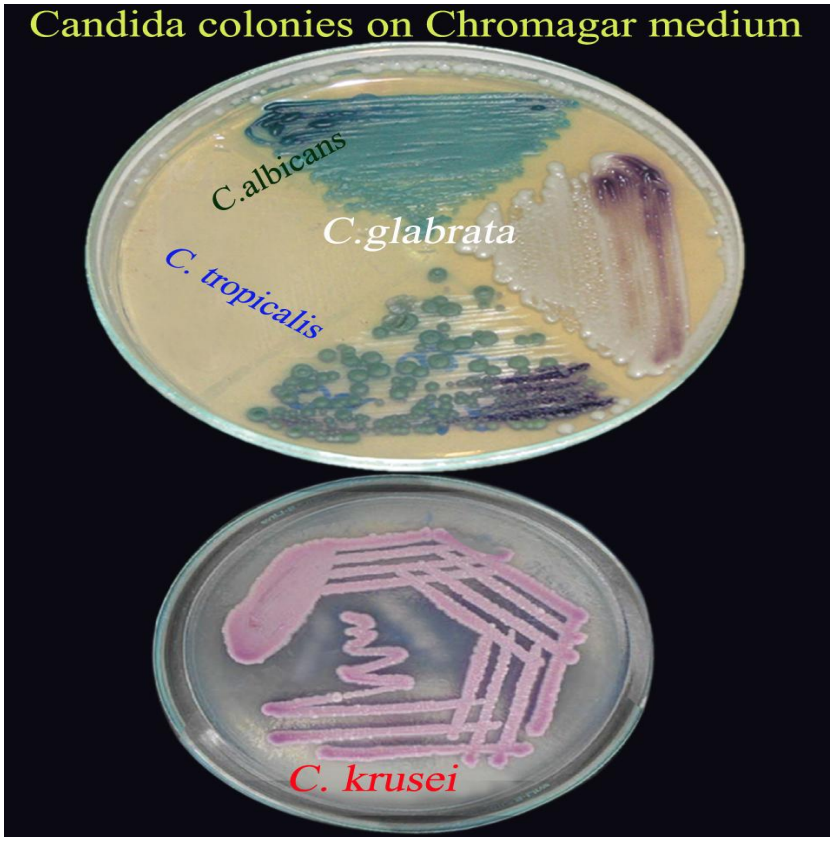

Figure 1. *Candida* species on Chromagar.

The antifungal sensitivity tests carried out using commercially available antifungal disc that 10 (100%) strains of *C. albicans* were sensitive to amphotericin B and itraconazole, while 7 (70%) strains of *C. albicans* were resistant to fluconazole and 2 (20) to Ketoconazole. Of the *C. glabrata* strains, all (100%) strains were sensitive to Itraconazole, 4 (80%) were sensitive to amphotericin B and itraconazole, whereas 3 strains (60%) were resistant to Ketoconazole. Among the *C. tropicalis strains*, four (100%) were found sensitive to amphotericin B and Itraconazole. While all four strains (100%) were found resistant fluconazole and 2 stains resistant (20%) to Ketoconazole. *C. krusei*, 3 (100%) strains were found sensitive to amphotericin B and 100 % resistant to Fluconazole and Ketoconazole, followed by 1 (33.3%) to Itraconazole antifungal agents. Amphotericin B and itraconazole 21 (95.5%) was found to be the most effective antifungal agent (Table 2; Figure 2 and 3).

Table 2: Antifungal susceptibility pattern of *Candida* species causing UTIs.

Species	Amphotericin B (20µg) Number and Percentage	Fluconazole (25µg) Number and Percentage	Itraconazole (50µg) Number and Percentage	Ketoconazole (10µg) Number and Percentage
C. albicans (n=10)	10 (100)	3 (30)	10 (100)	2 (20)
C. glabrata (n=5)	4 (80)	1 (20)	5 (100)	2 (40)
C. tropicalis (n=4)	4 (100)	0 (0.0)	4 (100)	0 (0.0)
C. krusei (n=3)	3 100)	0 (0.0)	2 (66.7)	0 (0.0)

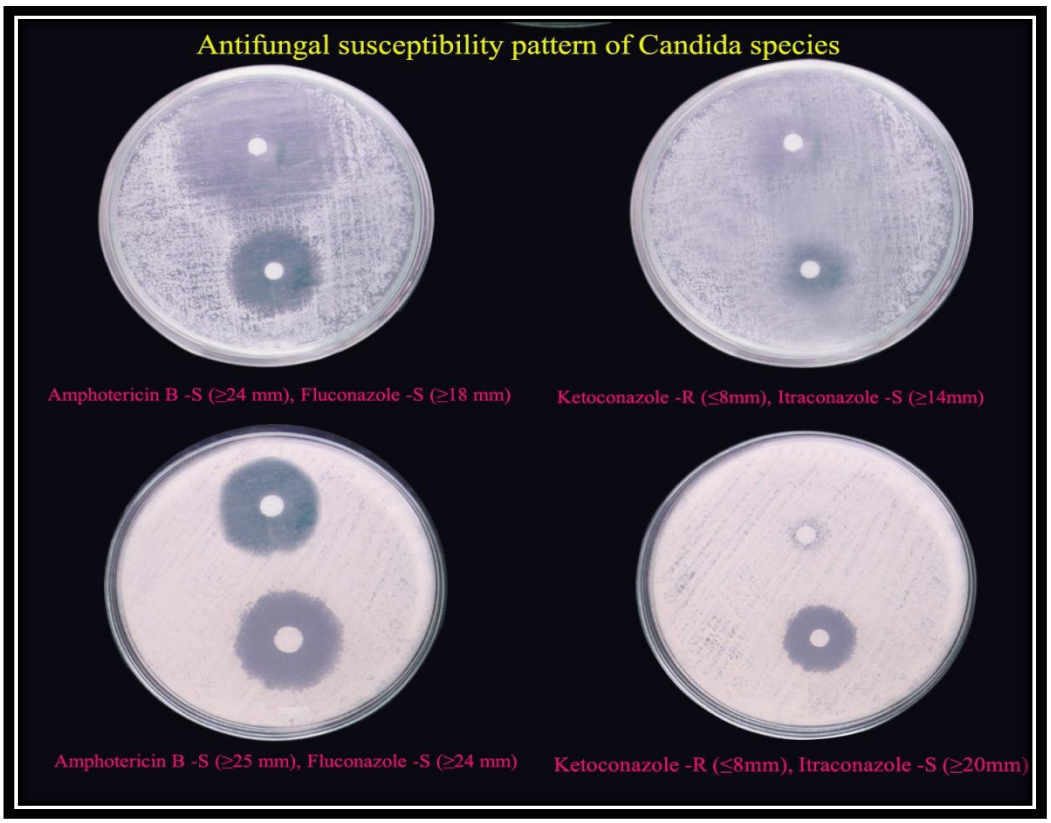

Figure 2. Antifungal susceptibility pattern of *Candida* species.

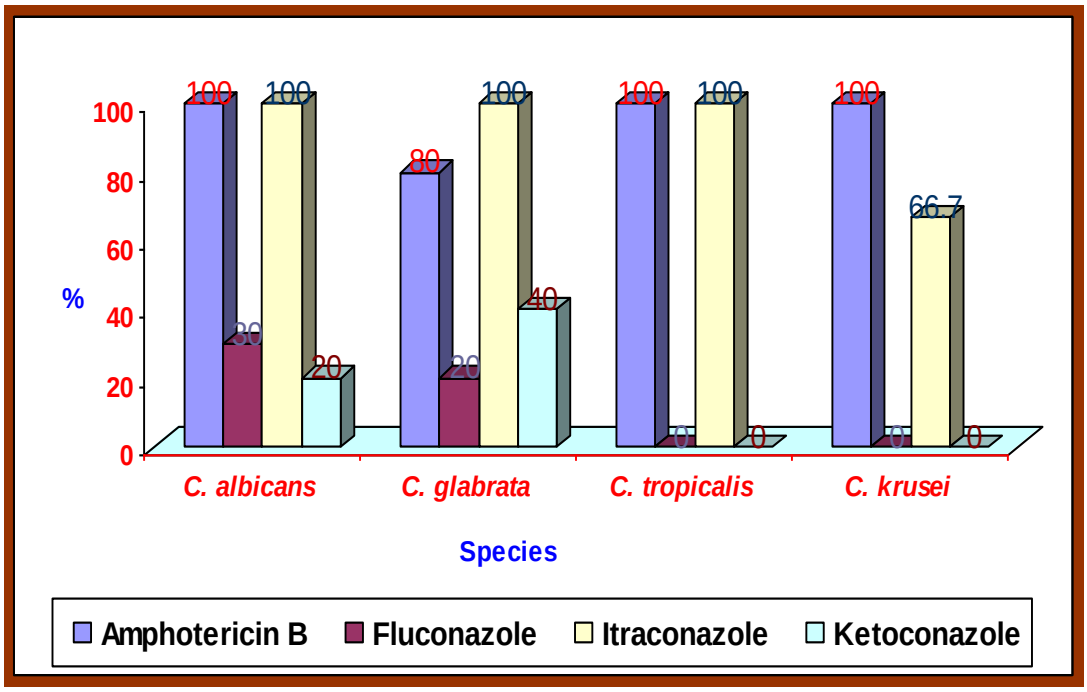

Figure 3. Antifungal sensitivity pattern of *Candida* species isolated from UTIs.

DISCUSSION

In this study, 3.4% of culture from sampled patients yielded different species of *Candida*. A total of twenty two *Candida* isolates from urine clinical specimens were included in this study, of which *C. albicans* showed the highest number of isolates (45.5%), followed by *C. glabrata* (22.7%), *C. tropicalis* (18.2%) and *C. krusei* (13.6%) respectively. According to Patel *et al.*, (2012), *Candida* species is the seventh most common nosocomial hospital wise pathogen, which caused 25% of all the urinary tract infections. Other studies have documented that hospitalised patients are relatively susceptible to candiduria (Kobayashi *et al.*, 2004; Sellami *et al.*, 2006).

The majority of candiduria in the present study were caused by *C.albicans* (45.5%), non-*albicans* species, especially *C. glabrata* (22.7%) was emerging as a nosocomial infection. Similar reports (Zarei *et al.*, 2012) from Iran showed the most common isolates were *C. albicans* (53.3%), followed by *C. glabrata* (24.4%), *C. tropicalis* (3.7%), *C. krusei* (2.2%), and *Geotrichum* spp. (0.7%). *C. albicans* had remained the major agents of candiduria until recently (Weinberger *et al.*, 2003) however; several reports show that non-albicans species, especially *C.tropicalis* and *C. glabrata* now predominate in many regions (Lagrotteria *et al.*, 2007).

In this study observed that females were affected predominantly (81.8%), contrary to the male predominance reported in the study by Paul *et al.*, (2007). Several reports show that the frequency of candiduria in women is more than men (Achkar and Fries, 2010). In this study candiduria were also more prevalent in age range 31-60 years (72.7%) followed by; 9.1% < 30 years and >61-80 years.

In the present study shows that all isolates of *C. albicans*, *C. krusei* and *C. glabrata* were resistant to fluconazole, with the exception of three isolates of *C. glabrata* that were sensitive to fluconazole. In this study indicate that both Amphotericin B and itraconazole 21 (95.5%) was found to be the most effective antifungal agent. UTIs due to *C. glabrata* have recently increased and these infections are usually resistant to fluconazole (Yang *et al.*, 2003). The susceptibility range of *Candid*a varies to antifungal drugs. *C. albicans* are usually sensitive to amphotericine B. However, several reports show that non-albicans are more resistant to antifungal, especially fluconazole (Saha *et al.*,

2008; Yanga *et al.,* 2008) believe that differences in sensitivity *Candida* species to antifungal are associated with geographical distributions.

CONCLUSION

Nosocomial urinary tract infection resulting in candiduria more common in patients with indwelling urinary catheters, systemic antibiotic use, underlying genitourinary abnormality, previous surgery and diabetes mellitus. Most cases are asymptomatic and require no treatment. Mortality with candiduria can be high in debilitated patients and those in advanced age. If treatment is indicated, the drugs of choice are Amphotericin B and itraconazole. The impact of faster identification of the species of *Candida* in patients with candidal urinary tract infection will help the clinician in selecting the appropriate antifungal agent, and thus contributing to overall reduction in the cost of treatment and the duration of hospital stay. Large-scale surveys of candiduria are needed in certain populations at risk to find the true incidence of the disease and compare the microbiology patterns, which will help in further understanding of the problem in our population.

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