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RESEARCH ARTICLE

CHARACTERIZATION AND ANTIFUNGAL SUSCEPTIBILITY PATTERN OF *CANDIDA* SPECIES ISOLATED FROM URINE SAMPLES IN A TERTIARY CARE CENTRE

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ABSTRACT

Introduction: Urinary tract infections are among the most common infectious diseases in humans. The incidence of candidiasis is on the rise in hospitalized patients. The incidence of *Candida* has been on the rise worldwide. Urinary tract infections as a result of *Candida* species are becoming common in hospitalized patients. The species identification of *Candida* is important, as non-albicans *Candida* species are increasing in number and more resistant to antifungal drugs. The present study was aimed to speciate, characterize and perform antifungal susceptibility testing of the *Candida spp.* isolates from the urine samples. **Material and methods**: The study was conducted in the Department of Microbiology, Patna Medical College, Patna) from october-2017 to December 2018 over 1 year 3 months period. A total of 2251 urine samples were analysed and isolated Candida species were subjected to speciation and antifungal susceptibility was performed according to standard procedures. **Results:** A total of 2251 urine samples were screened and 100 *Candida spp.* were isolated The incidence of Candiduria was 4.44 %. Female predominance (71%) was noted in the present study. In cases of females, the maximum number of patients was in the age group of > 60 years. The most common predisposing factors responsible for candiduria was Foley's catheter (87%) followed by diabetes (71%), IV catheter (53%), and frequent use of antibiotics (53%). *C. albicans* was the most common species isolated (58%). It was observed that all species showed maximum susceptibility to Caspofungin (100%), Amphotericin (97%), Voriconazole (92.94%), Posaconazole (93.72%) followed by itraconazole (80.20%). **Conclusion:** From this study, it is concluded that, candida albicans was the most common type of candida isolated from Candduria. NAC species have emerged as common cause of UTI. UTI is more common in female in >60 years age group, catherterized patients, diabetes and systemic antibiotic use. For treatment of candiduria, antifungal drugs like Voriconazole, Posaconazole and itraconazole can be given judiously while Caspofungin and Amphotericin B keep as reserve drug.

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INTRODUCTION

Candida spp. are the most common cause of fungal infections, leading to a range of life-threatening invasive to non-life-threatening mucocutaneous diseases (Jacqueline *et al.,* 2010). *Candida* species is a part of human microflora and it becomes pathogenic when certain conditions are present and cause an opportunistic infections. The genus *Candida* exists as saprophytes, colonizing mucosal surfaces and external genitalia of humans of either gender, but especially near the urethral meatus of healthy, premenopausal women (Al-Oebady, 2015). All common *Candida* species are capable of causing urinary tract infections (UTIs) (Rivett *et al.,* 1986). *Candida* species accounts for almost 9 to 40% of nosocomial urinary tract infections (Jacqueline *et al.,* 2010). The major etiological agent is *Candida albicans*, whereas different *Candida* species can cause a variety of infections including *Candida tropicalis, Candida dubliniensis, Candida parapsilosis, Candida krusei, Candida guillermondii, Candida glabrata,* and *Candida kefyer* which represent many clinical forms of candidiasis. Some of these species are encountered as secondary infections to another species, for example; *Candida parapsilosis* is secondary infection only when *C. albicans* as a cause of *Candida* endocarditic. Still other species of *Candida* have been occasionally isolated from clinical isolates such as *Candida catenulate, Candida intermedia, Candida lambica,* and *Candida zeylanoides.* These species are therefore not considered as agents of opportunistic infections (Emilio *et al.,* 2015; Krcmery *et al.,* 2002). The yeast begins to invade and colonize the body tissues by releasing powerful chemicals into the bloodstream causing such varying symptoms as lethargy, chronic diarrhea, yeast vaginites, bladder infections, muscle and joint pain, menstrual problems, constipation, and severe depression. The medical term for this overgrowth is candidiasis. Candidiasis is responsible for 90% of the cases of infectious vaginites (Ryan and Ray, 2004). Urinary tract infections as a result of *Candida* species is becoming

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increasingly common in hospitalised setting particularly in intensive care units (Jain *et al.,* 2011). Anatomic defects of urinary tract, urinary tract instrumentation, prior surgical procedures indwelling urinary drainage devices, abdominal surgery, ICU stay, broad spectrum antibiotic therapy, diabetes mellitus, increased age and female sex, immunosuppressive therapy are risk factors associated with candiduria (Nayman *et al.,* 2011; Dalen *et al.,* 2005). The aim of this study was to isolate, identify, and perform antifungal susceptibility testing of the *candida spp* isolates from the urine samples.

MATERIALS AND METHODS

This study was conducted in the Department of Microbiology, Patna Medical College, Patna from october-2017 to December 2018 during which 100 cases of candida spp were isolated.

Inclusion criteria

Male and female patients of all age groups were considered in present study. Both outpatients and inpatients who presented with signs and symptoms of urinary tract infection were included. Pure growth of yeast isolates with significant colony count was included in the study.

Exclusive criteria

The urine samples from where *candida* species were isolated in the absence of pyuria, *Candida* with colony count ≤1000 cfu/ml and mixed growth (polymicrobial growth) were excluded.

Collection and procesing of samples

Urine samples receivedin Department of Mcrobiology were inoculated by calibrated loop (0.01 ml) onto Blood agar and Mac Conkey agar medium, incubated at 370 C and read at 24 hours and 48 hours interval. Dry creamy white opaque colonies on blood agar and tiny dry lactose fermenting pink colonies on Mac Conkey agar medium that resemble *Candida* were confirmed by gram stain (Chander, 2009; Chander, 2002). *Candida* isolates were then subcultured on Sabouraoud's Dextrose Agar and CHROM agar candida medium for speciation. Various *Candida* species produce various Colour pattern of colony of were noted on CHROM agar medium. C.allbicans isolates impart distinctive light green colonies. *C.tropicalis* produce blue violet smooth colonies with halo diffusing gar. *C.krusei* ioslates produce rough,fuzzy spreading big pink colonies with pale edges. *C. glabrata* imparts small pink coloured colonies (Hi media laboratories). Germ tube test was performed for preliminary identification of *C.albicans* and *C.dublinesis*, further confirmation was done by following tests:

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 (Chander, 2009).

Carbohydrate fermentation test

An inoculum pool was prepared by emulsifying a heavily loaded loop full of the strain to be identified in 5ml of sterile saline. The test organism was inoculated by adding one drop of the inoculum suspension into each sugar fermentation tube. It was incubated for 48-72 hours at 30o C. The ability to ferment sugar was shown by the presence of acid and gas in the Durham's tube. *C. albicans* ferments glucose and maltose with gas production. *C. tropicalis* ferments glucose, sucrose and maltose with gas production and *C. krusei* and *C. glabrata* ferments glucose with gas production (Kwon-hung *et al.,* 1992).

Carbohydrate assimilation test

The organism was inoculated on a carbohydrate free medium. Carbohydrate containing filter paper disks were added and utilization was determined by the presence of growth round the disc. It consist sugar disk of 4% concentration (Milne *et al.,* 1996. *C. albicans* assimilate glucose, maltose, trehalose, sucrose, lactose and cellobiose. *C. krusei* assimilates glucose only. *C. tropicalis* assimilates glucose, sucrose, maltose, trehalose and cellobiose and *C. glabrata* assimilate glucose and trehalose only (Chander, 2009).

Antifungal susceptibility test: Antifungal susceptibility testing was carried out using the disc diffusion method following the National Committee for Clinical Laboratory Standards institute (CLSI, 2017) guidelines, using fluconazole (25µg), itraconazole (50µg), ketoconazole (10µg), and amphotericin B (20µg) and Caspofungin 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. Measurements of zone of inhibition were taken as per CLSI guideline (CLSI, 2017).

RESULTS

A total of 2251 urine samples were screened and 100 *Candida spp.were* isolated and identified on the basis of microscopic and stained smear examination, cultural characteristics and biochemical tests. The incidence of Candiduria in our study was 4.44 %. Female predominance (71%) was noted in the present study. In cases of females, the maximum number of patients was in the age group of > 60 years.

Table 1. Colony 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	58 (58.0%)	2 1	37
C. tropicalis	Dull blue, to purple color that diffused into surrounding agar with pale pink edges	23(23.0%)	4	19
C. krusei	Large, flat, spreading, pale pink colonies with matt surfaces	13 (13.0%)	4	9
C. glabrata	White large glossy pale pink to violet colonies	6(6.0%)	2	4

Table 2. Antifungal susceptibility pattern of Candida species causing UTIs

	Fluconazole	Itraconazole	Voriconazole	Posaconazole	Ketoconazole	Amphotericin B	Caspofungin
C. albicans (58)	32 (55.17%)	47 (81.03%)	49 (84.48%)	51 (87.93%)	33 (56.89%)	58 (100%)	58 (100%)
C. tropicalis (23)	9 (39.13%)	18 (78.26%)	20 (86.95%)	20 (86.95%)	11 (47.82%)	19 (82.60%)	23 (100%)
C. krusei (13)	0 (0.00%)	8 (61.53%)	13 (100%)	13 (100%)	4 (30.76%)	13 (100%)	13 (100%)
C. glabrata (6)	0 (00.00%)	6 (100%)	6 (100%)	6 (100%)	2 (33.33%)	6 (100%)	16 (100%)
Total	23.57%	80.20%	92.94%	93.72%	29.7%	95.5%	100%

The most common predisposing factors responsible for candiduria was Foley's catheter (87%) followed by diabetes (71%), IV catheter (53%), frequent use of antibiotics (53%), surgical procedures (29%). *C. albicans* was the most common species isolated (58%) followed by *C. tropicalis* (23%), *C.krusei* (13%) and *C.glabrata* (6%). Antifungal susceptibility of *Candida* isolates is presented in following table. It was observed that all species showed maximum susceptibility to Caspofungin (100%), Amphotericin (97%), Voriconazole (92.94%), Posaconazole (93.72%) followed by itraconazole (80.20%) and fluconazole (23.57%). *C. albicans*, *C.* and *C. glabrata* species showed 100% sensitivity to Amphotericin. Maximum resistance to Fluconazole was shown by *C. krusei* (100%) species. Sensitivity to itraconazole was maximally shown by *C. glabrata* (100%) least by *C. krusei* (61.53%)

DISCUSSION

In the present study the incidence of *Candida* in urine was found to be 4.44%. Manikandan *et al.* (2015), Goyal *et al.* (2016) and Yashavanth *et al.* (2013) obtained 3.4%, 2.36% and 2.27% respectively. Which are lower incidence rate than our study However, Singhal *et al.* (2015) and Kobayashi, Claudia *et al.* (2004) obtained higher incidence rate of 10.2% and 22%, this may be varies due to considerably different hospital setting (Lundstrom *et al.,* 2001). In this study observed that females were predominantly affected (71%) as in the study of Manikandan *et al.* (2015), this may occur most probably due to short urethera in females. Most common age group affected with candiduria was > 60 years which was similar to as stated by Yashavanth *et al.* (2013), Kobayashi *et al.* (2004). Urinary catherization increases chances of UTI and the most common predisposing factor in present study is the Foley's catheter (87%) Above finding is in accordance with Kobayashi, Claudia et al (84.4%) (Kobayashi *et al.*, 2004). The majority of candiduria in the present study were caused by *C.albicans* (58.0%), non-*albicans* species, especially *C.tropicalis* (23.0%), *C. krusei* (13.0%), *C. glabrata* (6.0%) was emerging as a nosocomial infection. Similar reports (Zarei *et al.* 2012*)* from Iran showed the most common isolates were *C. albicans* (53.3%). 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. Yashavanth *et al.* (2013) and Singhal *et al.* (2015). Found that Isolation of non-albicans *Candida* (68.0%) was more than *C.albicans* (32.0%). This is consistent with emergence of predominance of non-albicans *Candida* species all over world, Pfaller *et al.* (1999). Identification of *Candida* species is important as non-albicans is more resistant to azoles as compared to *C. albicans*. *C. krusei* and *C. glabrata* is intrinsically resistant to fluconazole. Antifungal susceptibility pattern showed that *Candida* isolates were more susceptible to Caspofungn (100%). Amphotericin (95.5%) s more susceptible than azoles (Voriconazole (92.94%), Posaconazole (93.72%), itraconazole (80.20%), Ketoconazole (29.7%) and fluconazole (23.57%). as in the study of Manikandan *et al.* (2015), Yashavanth *et al.* (2013). In this study we found that Voriconazole (92.94%) and Posaconazole (93.72%) were more sensitive than itraconazole (80.20%), Ketoconazole (29.7%) and fluconazole (23.57%), which are comparable to Sadeghi *et al* (2018) study in Iran. Although fluconazole has a broad treatment spectrum and low toxicity, long-term or repeated administration of this drug with low doses significantly increases the resistance of candida species, including albicans (Lopez *et al.,* 2001). AmphotericinB used to be considered as the standard treatment for invasive fungal infections and our study showed that the susceptibility of this drug is still high. However, unfortunately due to some major complications of this drug, such as nephrotoxicity, it is used with a tint of caution today (Fluckiger *et al.,* 2006).

Conclusion

From this study, it is concluded that, candida albicans was the most common type of candida isolated from Candduria. NAC species have emerged as common cause of UTI. UTI is more common in catherterized patients, diabetes and systemic antibiotic use. Females in >60 years age group are more commonly affected. For treatment of candiduria, antifungal drugs like Voriconazole, Posaconazole and itraconazole can be given judiously while Caspofungin and Amphotericin B keep as reserve drug. 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.

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Hi media laboratories, Technical data MV1456A, HiCrome Candida differential HIVeg Agar Base Method.

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