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

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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) (Rivet 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 guilliermondii, Candida glabrata, and Candida kefyr 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 catemulate, Candida intermedia, Candida lamberica, 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
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 candididuria (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 processing of samples

Urine samples received in Department of Microbiology 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 Sabouraud’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. albicans isolates impart distinctive light green colonies. C. tropicalis produce blue violet smooth colonies with halo diffusing gar. C. krusei isolates 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 37°C 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-Hintong agar + 2% glucose and 0.5 g/mL methylene blue dye, (GMB medium)] was used for performing the antifungal susceptibility testing.

Preparation of inoculum: 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- 37°C. 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- 37°C 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.
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%), Itraconazole (80.20%), Ketoconazole (29.7%) and fluconazole (23.0%), 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).

**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 urethra 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 catheterization 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,

**REFERENCES**


Hi media laboratories, Technical data MV1456A, HiCrome


