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RESEARCH ARTICLE

STUDY OF CANDIDA SPECIES COLONIZING URINARY TRACT IN CATHETERIZED PATIENTS FROM INTENSIVE CARE UNITS

***,[1]Shailesh Kumar, [1]Rakesh Kumar, [1]Harsh Priya, [1]Randhir Kumar, [1]Namrata Kumari and [2]Sudhir Kumar**

[1]Department of Microbiology, Indira Gandhi Institute of Medical Sciences, Patna, India
[2]Department of Medicine, Indira Gandhi Institute of Medical Sciences, Patna, India

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ABSTRACT

Background: The overwhelming majority of fungal infections of the urinary tract are caused by Candida species, and they usually present as complicated nosocomial infections. Colonization of the bladder most commonly is a complication of prolonged catheterization of bladder in a patient receiving antibiotics Objectives to assess the significance of candiduria in catheterized patients admitted in ICUs, to study the significance of speciation of candida isolates and determine the predominant Candida species.

Methodology: A total of 17 yeast isolates obtained from sixty catheterised urine samples collected from ICU patients after consent were included in the present study for final analysis. The candida isolates were identified upto species level by standard mycological techniques like wet mount, and culture on SDA. For speciation, rapid method – CHROM agar, cornmeal agar and germ tube test were used.

Results: The mean age of the patients was 46.9 ± 19.7 (mean ± SD). Of the 17 patients from whom the Candida spp. were isolated 9 (52.9%) were female and 8 (47.1%) were male. The mean duration of ICU stay and catheterisation were 3.8 ± 2.2 and 3.76 ± 2.3, respectively. Of the 17 patients, 7 (41.2%) had diabetes mellitus. Of the 17 yeast isolates, *Nonalbicans Candida* spp. (76.47%) was the predominant colonizer (Table 3). In our study group, *Candida tropicalis* and Candida glabrata accounted for 29.4% each, followed by C. krusei 11.7% and *C. parapsilosis* (5.88%), whereas *C. albicans* accounted for 23.57 % of the cases.

Conclusions: The Nonalbicans Candida species are more difficult to treat and chances that these strains would remain persistent are higher thus species identification should also be performed for appropriate management of ICU patients. The use of chromogenic media is an easy and reliable method for presumptive identification of commonly isolated Candida species. Speciation will help in selection of antifungal agents as *C. glabrata* and *C. krusei* are inherently resistant to azoles.

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INTRODUCTION

Fungal infections of the urinary tract encompass a broad variety of fungi, including the endemic mycosis, *Cryptococcus* species (Byrne *et al.,* 1997) and opportunistic pathogens such as *Aspergillus* species (Flechner and McAninch, 1981; Bibler and Gianis, 1987). However, the overwhelming majority of fungal infections of the urinary tract are caused by *Candida* species, and they usually present as complicated nosocomial infections. *Candida* spp. account for almost 10-15% ofnosocomial urinary tract infections (UTIs) (Kauffman, 2005; Lundstrom and Sobel, 2001; Kauffman *et al.,* 2000). Colonization of the bladder most commonly is a complication of prolonged catheterization of bladder in a patient receiving antibiotics (Hamory *et al.,* 1978). Other conditions predisposing patients to bladder colonization are diabetes mellitus, neurogenic bladder, chronic outlet obstruction from prostatic hypertrophy or pelvic irradiation for cervical carcinoma. Colonization is most often asymptomatic but it can lead to invasion of bladder wall in the presence of complete obstruction, bacterial cystitis, or damage to the bladder epithelium by cyclophosphamide or topical chemotherapy for bladder carcinoma (Kwon-Chung and John, 1992). We undertook this study to assess the significance of candiduria in catheterized patients admitted in Intensive Care Units (ICUs) and to speciate the candida isolates and determine the predominant candida species.

Corresponding author: **Shailesh Kumar,**
Department of Microbiology, Mahatma Gandhi Medical College and Research Institute, Pondicherry, India.

MATERIALS AND METHODS

This prospective and analytical study was a part of ICMR project approved by Institutional Ethical committee Ref. No. IGIMS/2015/846/Acad. done over a period of 2 months (July 2015- Sep 2015) A total of 17 yeast isolates obtained from

sixty catheterized urine samples collected from ICU patients after consent were included in the present study for final analysis. A combination of biochemical methods like fermentation of sugars, growth on mycological culture media, growth on chromogenic media and microscopic examination have been used for identification of yeasts.

Inclusion criteria

- The yeast isolates were included in the study if they were isolated as a pure growth in a significant colony count which is>10^4colony forming units/ml of urine sample (Standard loop technique).
- These isolates were obtained from catheterized patients admitted in the intensive care unit.

Exclusion Criteria

- If *Candida* spp. were isolated as a mixed growth or colony count <10^4 colony forming units/ml of urine sample
- If obtained from non-catheterized patients
- Patients on antifungal treatment. Repeat samples from same source
- Urine collected from drainage bag

Urine Sample Processing and Identification

Urine was collected in a wide mouthed sterile container after disinfecting a portion of the catheter tubing with alcohol, puncturing the tubing directly with sterile syringe and needle and aspirating the urine. The urine samples obtained were immediately processed in the microbiology laboratory by semi-quantitative method as per the standard protocols and all the yeast isolates were stocked for further microbiological characterization. Direct microscopic examination of urine sample was also done to look for the presence of pus cells, red blood cells, casts, crystals or any bacterial or fungal element. The identification of yeasts up to species level was done by standard mycological techniques like wet mount and culture on Sabouraud's dextrose agar (SDA). For speciation, rapid method – KB006Hi- Candida identification kit, CHROM agar, cornmeal agar and germ tube test were used.

Study methods: Candida isolates from catheterized urine samples were speciated by

- KB006 HiCandida Identification kit
- Germ tube test
- Colour of the colonies on CHROM agar
- Cornmeal agar for chlamydospore formation
- Gram stain
- Growth on Sabouraud's dextrose Agar(SDA)

KB006 HiCandida Identification kit

It is a standard test system that can be used for identification and differentiation of Candida species. It can also be used for validating known laboratory strains. Principle of test- Each KB006 kit is a standardised colorimetric identification system utilising 12 conventional biochemical tests. The tests are based on the principle of pH change and substrate utilization. On incubation, organisms undergo metabolic changes which are indicated by spontaneous colour change in the media from orange to yellow colour.

Germ tube test

A rapid method of identifying Candida *albicans* based on its ability to form germ tubes within 2 hours when incubated in human serum at 37 degree C (Reynolds- Braude) phenomenon.

CHROM agar

CHROMagar candida (Hi-Media)- Identification of candida sp. on CHROMagar culture produce different colour of colonies.

Cornmeal agar for chlamydospore formation- Culture on cornmeal agar at 20 degree C produce chlamydospores by *Candida albicans* in 2-4 days of incubation.

Patient's demographic details like age, sex, duration of hospital stay, duration of catheterization, clinical details and other associated risk factors like diabetes mellitus and use of broad spectrum antibiotics were maintained in a performa.

Statistical analysis

Descriptive statistics of speciation were analyzed and expressed in terms of percentage and their 95% confidence interval.

Ethical considerations

Informed consent was obtained from all the participants. Institutional Ethics Committee (IEC) approval was obtained. (Ref. No. IGIMS/2015/846/Acad)

RESULTS

The isolates were collected from July 2015 to September 2015 at a tertiary care hospital in Patna, Bihar to determine the predominant candida species in catheterised patients admitted in ICUs. A total of 17 yeast isolates from sixty catheterised urine samples collected from ICU patients were included in the present study for final analysis. The mean age of the patients was 46.9 ± 19.7 (mean ± SD). Of the 17 patients from whom the *Candida* spp. were isolated 9 (52.9%) were female and 8 (47.1%) were male. The mean duration of ICU stay and catheterisation were 3.8 ± 2.2 and 3.76 ± 2.3, respectively. The underlying illness in the study patients are summarised in Table 1.

Table 1. Underlying illness in the study patients

Underlying illness	Frequency
ARDS	1
Cerebovascular accident.	3
Cerebral encephalopathy	1
COPD	2
Fever, altered sensorium	1
Fever, headache, vomiting	1
HT , altered sensorium	1
HT with jaundice	1
Ileal perforation	1
Ischemic heart disease	1
Jaundice, fever	1
Postop	1
Seizure	1
TB, urinary incontinence	1
Total	17

Of the 17 patients, 7 (41.2%) had diabetes mellitus. The details of the antibiotics and steroid treatment of the patients prior to isolation of *Candida* spp. are summarised in Table 2.

Table 2. Frequency of antibiotics and steroid administered to the patients prior to isolation of *Candida* spp.

Drug	Frequency	Percentage
Metronidazole	6	35.3%
Ceftriaxone	7	41.2%
Moxifloxacin	3	17.6%
Levofloxacin	4	23.5%
Steroid	5	29.4%
Clarithromycin	1	5.9%
Rabeprazole	1	5.9%
Linezolid	3	17.6%
Acyclovir	2	11.8%
Vancomycin	1	5.9%
Sulbactem	3	17.6%

Table 3. Distribution of various Candida sp. from catheterised urine sample

Candida species (n=17)	Frequency	Percentage
C. *albicans*	4	23.57%)
C. *tropicalis*	5	29.4 %)
C. *glabrata*	5	29.4%)
C. *krusei*	2	11.7%
C.parapsilosis	1	5.88%

Of the 17 yeast isolates, *Nonalbicans Candida* spp. (76.47%) was the predominant colonizer (Table 3). In our study group, *Candida tropicalis and Candida glabrata* accounted for29.4% each, followed by *C. krusei* 11.7% and *C. parapsilosis* (5.88%), whereas *C. albicans* accounted for 23.57 % of the cases.

The biochemical tests done are shown in (Table 4)

Table 4. Result entry data sheet

No.	Test	C.*albicans* (n=4)	C. *tropicalis* (n=5)	C.*glabrata* (n=5)	C.*krusei* (n=2)	C.*parapsilosis* (n=1)
1	Urease	-	-	-	-	-
2	Melibiose	-	-	-	-	-
3	Lactose	-	-	-	-	-
4.	Maltose	+	+	-	-	-
5	Sucrose	-	+	-	-	-
6	Galactose	+	+	-	-	-
7	Cellobiose	-	+	-	-	-
8	Inositol	-	-	-	-	-
9	Xylose	+	+	-	-	+
10	Dulcitol	-	-	-	-	-
11	Raffinose	-	-	-	-	-
12	Trehalose	+	+	+	-	-

Table 5. Macroscopic (Colony) morphology of different Candida species on SDA

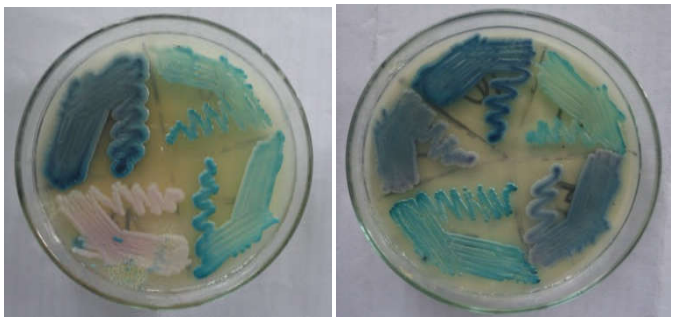
C.*albicans*	C. *tropicalis*	C.*glabrata*	C.*krusei*	C. *parapsilosis*
Entire, white, pasty, convex colonies, few with pseudohyphal fringe	White to cream, smooth, moist shiny	Glistening, smooth, cream colonies	Entire, white to dull, flat colonies, mycelial fringe around colonies	Moist, shiny white to cream coloured, smooth colonies

Table 6. Microscopic findings of different Candida species

Test	*C.albicans* (n=4)	*C. tropicalis* (n=5)	*C.glabrata* (n=5)	*C.krusei* (n=2)	*C.parapsilosis* (n=1)
Germ tube test	+	-	-	-	-
Chlamydospore Formation on corn meal agar	+	-	-	-	-

Table 7. Properties of various Candida species on CHROM agar

Candida species	Colour on CHROM agar
C. *albicans*	Light green
C. *tropicalis*	Fuzzy blue
C. *glabrata*	Light Purple to pink
C.*krusei*	Light pink fuzzy
C.parapsilosis	Creamish pink



DISCUSSION

The presence of Candida species in urine is rarely encountered in otherwise healthy people with structurally normal urinary tract. It is however a common occurence in hospitalised patients accounting for 10-15% of nosocomial UTI. In our study, *Non albicans* Candia species emerged as the predominant colonizer and was responsible for 76.47% of cases followed by *C. tropicalis* and *C. glabrata* (29.4%) each, *C.krusei* (11.7%) and *C. parapsilosis* (5.88%). *C. albicans* accounted for (23.57%). The shift towards *Non albicans* Candida is probably driven by azole use due to selection pressure (PurvaMathur, 2010). In our study, we found that candiduria was more common in females (52.9 %) as compared to the males (47.1 %). Since colonization of vulvo vestibular area with *Candida* spp. is frequent in females, they are more at risk of developing candiduria due to ascending infection (Bukhary, 2008; Lundstrom and Sobel, 2001). Diabetes is a well known risk factor for developing nosocomial UTI due to *Candida* spp. because diabetes lowers host resistance to invasion by fungi and also promotes stasis of urine in neurogenic bladder. Of the 17 patients, 7 (41.2%) had diabetes mellitus in this study which is higher than that reported by Jain *et al.* (38.6%) (Manisha Jain *et al.,* 2011). Antibiotics increase the risk of colonization of *Candida* spp. by suppressing endogenous flora and the risk of candiduria increases with prolonged antibiotic use (Fisher *et al.,* 1982). Broad spectrum antibiotics use was a universal associated risk factor in our patients. One patient was also on antituberculous drugs. Urinary catheters serve as a portal of entry and most catheters become colonized if left for longer duration (Stamm, 1991). In our study the mean duration of catherization was – 3.76 ± 2.3, days which is less than that observed by Jain etal[12]who observed it as 11.6± 6 days. Chromogenic media are frequently used in direct and rapid identification of yeasts because different Candida species produce unique colour on these media. It is a reliable method for presumptive identification of most commonly isolated Candida species.

Conclusion

Catherized patients admitted in ICU with other associated risk factors like Diabetes mellitus, old age, broad spectrum antibiotic use and steroids are at risk of developing nosocomial UTI due to Candida species. The *Nonalbicans Candida* species are more difficult to treat and chances that these strains would remain persistent are higher, thus species identification should also be performed for appropriate management of ICU patients The use of chromogenic media is an easy and reliable method for presumptive identification of commonly isolated *Candida* species. Clinical Microbiologists will be able to save time and cost for diagnosis of yeast from clinical samples Speciation will help in selection of antifungal agents as *C. glabrata* and *C. krusei* are inherently resistant to azoles.

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