

EVALUATION OF ANTIFUNGAL RESISTANCE PATTERNS AND RISK FACTORS ASSOCIATED WITH CANDIDA SPECIES IN CATHETERIZED ICU PATIENTS WITH URINARY TRACT INFECTIONS

Medical Microbiology

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ABSTRACT

Introduction: Catheter associated urinary tract infection (CAUTI) is the most common hospital acquired infection which accounts for more than 80% of nosocomial urinary tract infections. Therefore, the objective of this study was to evaluate the antifungal susceptibility profile of the isolated candida species in urinary catheterized ICU patients and risk factors associated with candiduria at a tertiary care hospital at Indore Madhya Pradesh, India. **Material and Methods:** The present study was conducted in the department of Microbiology, at the Tertiary Care Hospital of Index Medical College, Indore, and Madhya Pradesh, India. Total 350 urine sample were collected during our study and were analyzed on the basis of inclusion and exclusion criteria. Before starting the study, ethical approval was obtained from the Institutional Ethics Committee. **Results:** A total of 350 urine sample were processed from catheterized ICU patients, Out of these 350 urine samples; Yeast growth (Candida species) was obtained in 71(20.28%) urine samples. Out of the total 71 isolates, NAC species showed predominance in female patients i.e. 24(53.33%) and Candida species 15(57.69%) respectively. In present study, the overall resistance pattern for anti fungal drugs of total 71 candida isolates was as follows: 64.78% resistance was observed for Fluconazole, 46.47% for voriconazole and 15.49% resistance for Ketoconazole. No resistance was observed for Amphotericin-B. **Conclusion:** In India, the scenario of antifungal drug resistance to Candida isolates is not very clear due to lack of multicentric studies over a period of many years. In our study, commonly prescribed antifungal drugs are fluconazole, voriconazole, ketoconazole and amphotericin B. Moreover, the antifungal susceptibility test may help determine the best drug therapy for each patient.

KEYWORDS

Non albicans Candida, Candiduria, Antifungal susceptibility testing, Intensive care Unit (ICU), Urinary Tract Infections (UTIs)

INTRODUCTION

Catheter associated urinary tract infection (CAUTI) is the most common hospital acquired infection which accounts for more than 80% of nosocomial urinary tract infections. The risk factors associated with CAUTI in adults mainly include intensive care unit (ICU) admission, broad spectrum antibiotics, diabetes mellitus, increased age, female sex.^{1,2} thesis

Candida species which are ubiquitous human commensals are responsible for various clinical manifestations from mucocutaneous overgrowth, urinary tract infection and bloodstream infections. The microorganisms causing CAUTI range from gram negative bacteria to gram positive cocci to candida. UTI in hospitalized patients due to Candida spp. is becoming increasingly common in ICU settings. The chances of acquiring urinary tract infections as a result of catheterization by candida species is becoming increasingly common in hospitalized settings particularly in intensive care units.^{3,4}

Various risk factors that affected the large population such as immunocompromised patients using intravenous catheters, catheterization of urinary tract/vascular system, renal failure, hepatic failure, prolonged hospital stay, prolonged use of broad-spectrum antibiotics, cytotoxic chemotherapies and transplantation are factors that contribute to the increase of these infections. Diabetes mellitus, the leading endocrine dysfunction provides a potent soil for Candidal infection.

Increased Candida species resistance to various antifungal treatments is a result of improper use of antifungal medications and a lack of adequate strategies to govern their usage, prolonged use of antifungal agents led to the emergence of multidrug resistance infection, which is a serious health issue. Hence, lack of information on antifungal susceptibility pattern of candida species that candiduria in ICU patients.⁶

Therefore, the objective of this study was to evaluate the antifungal susceptibility profile of the isolated candida species in urinary catheterized ICU patients and risk factors associated with candiduria at a tertiary care hospital at Indore Madhya Pradesh, India.

MATERIAL AND METHODS

The present study was conducted in the department of Microbiology, at the Tertiary Care Hospital of Index Medical College, Indore, and

Madhya Pradesh, India. It was a cross sectional prospective study carried out over a period of 18 months from Jan 2018 to June 2019. Before starting the study, ethical approval was obtained from the Institutional Ethics Committee.

During this study, 350 urine samples were collected from cases of symptomatic CAUTI in ICU patients on the basis of inclusion and exclusion criteria. Male and female patients of age ≥ 18 years were considered for this study. Those who had UTI after 72 hours of hospitalization & were put on Foley's catheter were included in the study. Urine samples showing concomitant pyuria on microscopic examination were included to rule out contamination. Patient's initial urine samples showed presence of candida species as a pure growth with significant colony count of more than 10^5 cfu/ml. Only those yeast isolates which showed pure growth with significant colony count were included in the study.

Patients who were catheterised before being admitted in ICU, whose Foley's catheter was removed or who were discharged before 72 hours of being catheterised were not included in this study. Urine samples where Candida species was isolated without any pyuria, colony count was $<10^5$ CFU/ml and poly microbial growth were also excluded from the study.

Urine samples were collected from Foley's catheter using aseptic technique, and a minimum of 3ml of urine was taken in a screw capped, sterile, leak proof container. The sample was transported immediately to microbiology laboratory for processing. Urine wet mount examination was done to look for the presence of pus cells, red blood cells, casts, crystals or any bacterial or fungal elements. Gram staining was done to see the presence of gram positive yeast cells. Culture was performed by semi quantitative method as per standard protocol. Species identification was done by germ tube test, corn meal agar morphology, sugar assimilation and fermentation test. Simultaneously, identification of Candida isolate was done by culture on CHROM agar.

Antifungal Susceptibility Testing

The disk diffusion test of the Kirby-Bauer technique was used to determine the antifungal drug susceptibility profiles of Candida species on Mueller Hinton agar (MHA) medium (HiMedia Lab., India) containing glucose (2%) and methylene blue dye (0.5 μg /mL) according to the guidelines of the Clinical and Laboratory Standards

Institute (CLSI) (2021). A suspension of overnight cultures of *C. albicans*, *C. krusei*, *C. tropicalis*, *C. glabrata*, and *C. dublinensis* were prepared separately in 5 mL of sterile 0.85% saline. The turbidity containing $1-5 \times 10^6$ CFU/mL of isolated *Candida* species was adjusted to McFarland 0.5 standards by using a DensiChek (bioMerieux) turbidity meter. A sterile cotton swab was moistened in the prepared suspension, and excess fluid was removed. Under aseptic conditions, the surface of Müller-Hinton agar (MHA) plate was streaked by the swab for three times and kept at room temperature for 15 minutes to dry. The antifungal discs, namely, amphotericin-B (100 Units), fluconazole (25 µg), ketoconazole (10 µg), voriconazole (10 µg), (HiMedia Lab., India), were placed over the surface of the MHA plate and incubated at 37°C for 48 h. According to CLSI (2021) recommendations, the zone of inhibition was measured in millimeters, and the results were interpreted as sensitive (S) and resistant (R) (Clinical and Laboratory Standards Institute (CLSI), 2021).

Statistical Analysis

Statistical analyses were performed with SPSS software version 24 (IBM SPSS Statistics for Windows 24.0, Armonk, NY, IBM Corp.). Categorical variables were expressed as frequencies and percentages. The descriptive statistics was used to characterise the study group.

RESULTS

In the present study a total of 350 urine samples were collected from catheterized patients (for more than 72 hours) who were admitted in the ICU of Index Medical College and Hospital, Indore Madhya Pradesh. A total of 350 urine sample were processed from catheterized ICU patients. Out of these 350 urine samples; Yeast growth (*Candida* species) was obtained in 71(20.28%) urine samples. Out of the total 71 *Candida* isolates; Female predominance i.e. 39 (61.97%). Out of the total 71 isolates, NAC species showed predominance in female patients i.e. 24(53.33%) and *Candida* species 15(57.69%) respectively.(Shown in Figure 1) The various predisposing factors along with catheterization responsible for candiduria in the study group shows that prolong use of IV catheters and antibiotic therapy are the associated risk followed by Diabetes mellitus. H/O trauma and other risk factors were the least attributes in the study group.(Shown in Table 1) In present study, the overall resistance pattern for anti fungal drugs of total 71 *Candida* isolates was as follows: 64.78% resistance was observed for Fluconazole, 46.47% for voriconazole and 15.49% resistance for Ketoconazole. No resistance was observed for Amphotericin-B. (Shown in Figure 2) In *C.albicans*; 15(57.69%) resistance was observed for fluconazole, 16(61.55%) to voriconazole, 3(11.53%) to ketoconazole. In NAC species 33(73.33%) resistance was Observed for Fluconazole, 17(37.77%) to voriconazole, 7(15.55%) to ketoconazole. Where as amphotericin-B does not show resistance to any of the *Candida* species.(Shown in Figure 3)The resistance pattern of various NAC species towards antifungal drugs was as follows: *C.tropicalis* was 9(45%) resistance to fluconazole, 7(35%) to voriconazole, 4(20%) to ketoconazole *C.krusei* was 14 (100%) resistance to fluconazole, 6 (42.85%) to voriconazole, *C.parapsilosis* was 6(54.54%) resistance to fluconazole, 4(36.36%) to voriconazole and 2(18.18%) to ketoconazole. No resistance was observed for Amphotericin-B.(Shown in Table 2)

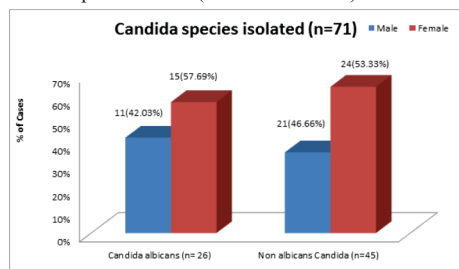


Figure 1: Distribution Of Candida Albicans And Non-albicans Candida (nac) Based On The Gender Of The Patients

Table 1: Various Risk Factors In Patients With Candiduria (n=71)

Riskfactors	Numbers	Percentage
Catheterization	68	95.71%
Antibiotics therapy	60	84.50%
Diabetes mellitus(DM)	57	80.28%
Others-H/O Previous surgery, Mechanical ventilation	21	29.57%
H/O-Trauma	06	8.57%

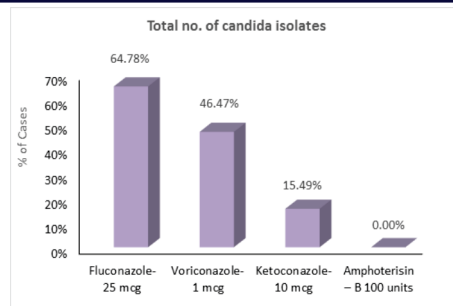


Figure 2: Over All Anti Fungal Drug Resistance Pattern Of Candida Isolates

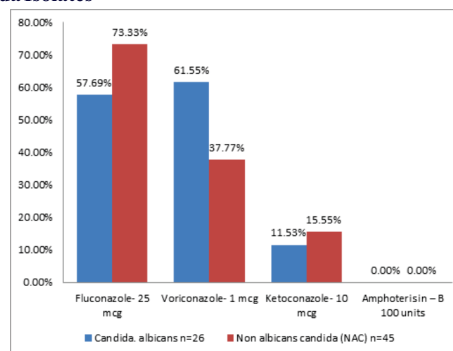


Figure 3: Over All Anti Fungal Drug Resistance In Candida Albicans (n=26) And Non Albicans Candida Species (n=45) In The Study

Table 2: Anti Fungal Drug Resistance Pattern For Various NAC Species In The Study

NAC(n=45)	ANTIFUNGALDRUGS			
	Fluconazole- (25mcg)	Voriconazole- (1mcg)	Ketoconazole - (10mcg)	Amphotericin- B 100units
<i>C.tropicalis</i> (20)	9(45%)	7(35%)	4(20%)	0
<i>C.krusei</i> (14)	14(100%)	6 (42.85%)	00	0
<i>C.parapsilosis</i> (11)	6(54.54%)	4(36.36%)	2(18.18%)	0

DISCUSSION

Over the last two decades, fungal infections have increased at an alarming rate. *Candida* species are commensals and act as opportunistic pathogen only on interruption of normal host defences. They are capable of causing a wide range of superficial and deep seated mycoses such as cutaneous, mucocutaneous, systemic candidiasis and candiduria.

The prevalence rate of candiduria caused by the species other than *C. albicans* was surprisingly high in the presence study. Changing trends in the aetiopathogenesis of urinary tract infections and considerable increase in number of non albicans *Candida* species is a matter of concern.⁷

In the last few years, various risk factors like immunocompromised status, immunosuppressive therapy, prolonged hospital stay, prolonged antibiotic therapy, prolonged catheterization have all contributed for increase in number of cases of candiduria.⁸

In this study, identified *Candida* species as the main pathogen in catheterized ICU patients representing 71(20.28%) out of 350 urine samples collected from catheterized ICU patients whose admitted in ICU for more than 72 hours. This finding is similar with earlier studies Nidhi Barot et al;(2015), this study revealed that out of 116 positive urine cultures, 28(24.1%) were positive for *Candida* species.⁹

In present study, various risk factors which includes, IV catheter, Antibiotics therapy Diabetes mellitus (DM), Others-H/O Previous surgery, Mechanical ventilation H/O-Trauma along with prolonged catheterization responsible for candiduria in the study the group that shows prolong use of catheters and antimicrobial therapy are the highest associated risk followed by Diabetes mellitus. H/O trauma and other risk factors were the least attributes in the study group.

In current study, Fluconazole-25mcg, Voriconazole-1 mcg,

Ketoconazole-10mcg and Amphotericin-B 100unit's anti fungal drugs are used to evaluate resistance and sensitivity pattern of various *Candida* isolates. The overall resistance pattern for anti fungal drugs of total 71 *Candida* isolates was as follows: 64.78% resistance was observed for Fluconazole, 46.47% for voriconazole and 15.49% resistance for Ketoconazole. No resistance was observed for Amphotericin-B. similar findings were observed by Chun Hun Ding et al; (2014).¹⁰

One other study, Sumita Rajeevan et al; (2016), in this study revealed that, all the 269 isolates were sensitive to amphotericin-B. A total of 55%, 56% and 63% of all *Candida* isolates were sensitive to fluconazole, itraconazole and ketokonazole respectively.¹¹

Our study also reveals that among NAC species the resistance pattern towards antifungal drugs was as follows: *C. tropicalis* was 9(45%) resistance to fluconazole, 7(35%) to voriconazole 4(20%) to ketoconazole *C. krusei* was 14(100%) resistance to fluconazole, 6 (42.85%) to voriconazole, *C. parapsilosis* was 6(54.54%) resistance to fluconazole, 4(36.36%) to voriconazole and 2(18.18%) to ketoconazole. No resistance was observed for Amphotericin-B.

Candiduria, if not properly diagnosed and treated has been source of morbidity and mortality. Candiduria is 5-10 times higher in catheterized ICU patients compared to general population. The increase in resistance to fluconazole is a matter of great concern as it is the most commonly used azoles for the treatment of candiduria.

CONCLUSION

In India, the scenario of antifungal drug resistance to *Candida* isolates is not very clear due to lack of multicentric studies over a period of many years. Resistance to amphotericin B among *Candida* isolates was not found in our study which is similar to other Indian studies. In our study, commonly prescribed antifungal drugs are fluconazole, voriconazole ketoconazole and amphotericin B. Resistance to azole derivative was found to be more with Non albican *Candida*. *Candida* species with fluconazole resistance have been seen more commonly in recent years. Moreover, the antifungal susceptibility test may help determine the best drug therapy for each patient. Accordingly, future investigations should focus on the occurrence of drug resistant *Candida* strains and their emergence.

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