Journal of Epidemiology and Public Health (2024), 09(02): 216-226
Masters Program in Public Health, Universitas Sebelas Maret

Research
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Identification and Antifungal Susceptibility Pattern of *Candida* Isolates Recovered from Urine and Blood Specimens from Patients Admitted in Wards of a Tertiary Care Hospital, North Delhi

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Received: 6 November 2023; Accepted: 30 January 2024; Available online: 16 April 2024

ABSTRACT

Background: *Candida* species are responsible for various clinical infections ranging from muco-cutaneous infection to life-threatening invasive diseases. Increased resistance to antifungal drugs during the last decade has become a serious concern. Therefore, identification of *Candida* up to species level and its antifungal susceptibility testing is very important in the management of *Candida* infections. This study aimed to identify these organisms and study their susceptibility patterns.
Subjects and Method: A retrospective study was conducted over 9 months (July 2022 to March 2023) from urine and blood samples collected from 80 IPD patients admitted to various wards of Hindu Rao Hospital. The samples were selected based on their growth on blood agar. The variables of interest are the different species of *Candida* and susceptibility to antibiotics. Identification of *Candida* species was done by Gram stain, Germ tube formation test, color on HiCrome *Candida* agar medium, chlamydospore formation on corn meal agar, and VITEK 2 Compact System. The MICs were interpreted according to the CLSI guidelines 2022.
Results: 47 and 33 of urine and blood cultures were positive for *Candida* species respectively. Most of the isolates were from the Paediatric ward (28.75%), followed by the Medicine ward (27.5%). The most common species was *C. tropicalis* (56.25%) followed by *C. albicans* (23.75%). Most species of *Candida* were sensitive to amphotericin B, fluconazole, voriconazole, caspofungin, micafungin, and flucytosine except *Candida albicans* which showed 100% resistance to amphotericin B and *Candida krusei* which showed 66% sensitivity to voriconazole and 33% to caspofungin.
Conclusion: *Candida* colonization has a considerable prevalence among patients hospitalized in our hospital. The species identification of *Candida* isolates along with their antifungal susceptibility pattern can help the clinician in better treatment of patients with candiduria and candidemia.

Keywords: Candida, bloodstream infection, minimum inhibitory concentration

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Cite this as:
Jain S, Khatri S, Kumar M, Bharara T, Yadav A (2024). Identification and antifungal susceptibility pattern of *Candida* isolates recovered from urine and blood specimens from patients admitted in wards of a tertiary care hospital, North Delhi. J Epidemiol Public Health. 09(02): 216-226. https://doi.org/10.26911/jepublichealth.2024.09.02.08.

BACKGROUND

The isolation of *Candida* spp. from urine cultures indicates colonization or urinary tract infection (candiduria), but it might be a sign of systemic candidiasis or candidemia (Kauffman, 2014; Huang et al., 2013; Colombo and Guimarães, 2007). *Candida* spp. can reach the urinary tract via the ascending route, from the urethra to the bladder, or by hematogenous spread, as *Candida* spp. is filtered by the kidneys and excreted in the urine. *Candida* as a causative agent of bloodstream infection (BSI) has become a major problem in tertiary care hospitals worldwide (Tortorano et al, 2002). Despite some improvement in fungal BSI diagnosis during recent years, diagnosis of candidemia remains difficult (Gudlaugsson, 2003). *Candida* species are the 4th most common cause of primary bloodstream infection and constitute 8th most common pathogen to cause healthcare-associated infections (Ahmed et al., 2020).

Most of the sources of candidiasis in humans are endogenous because *Candida* spp. are normal commensals of the digestive tract in many healthy people. Few conditions cause these commensals to become opportunistic, causing candidia infection in different sites of the body. Patients of advanced age, female sex, urinary tract abnormalities, drainage catheters used in hospitalized patients, diabetes, malignancies, use of broad-spectrum antibiotics, corticosteroids, and immunosuppressive agents are at high-risk conditions for developing urinary tract infections (Colombo and Guimarães, 2007; Colombo et al., 2013; Pappas et a.l, 2009). *Candida* spp. in urine, as well as blood, is frequently found in critically ill patients, in those who present with metabolic and immunosuppressive diseases, as well as in patients undergoing surgical procedures or facing long hospital stays (Fisher et al., 2011; Chenoweth et al., 2014).

From previous studies, it has been found that a patient in a critical care area who is exposed to four different antibiotics has about a 35% risk of developing candidemia, and if *Candida* is isolated from another site, such as in urine, the risk increases to 80% (Pfaller and Castanheira, 2016). Correct and timely identification of the pathogen and administration of specific antifungal therapies are crucial for patients recovery (Kauffman, 2014; Mishra et al, 2014; Yashavanth et al, 2013) as in severe systemic episodes of *Candida* infections, management of the clinical condition of the patient depends on rapid interventions. *Candida albicans* is the most common agent responsible for candiduria, however, non-*albicans Candida* (NAC) species have been increasingly reported worldwide (Yashavanth et al., 2013; Kobayashi et al., 2004).

The emergence of NAC species with reduced susceptibility or intrinsic resistance to antifungal compounds, specifically to azoles, is a major problem due to the increasing use of fluconazole (FLC) in candidiasis therapy (Sobel et al., 2011; Singla et al., 2012). In addition, studies conducted in ICU settings have reported that patients with candiduria have increased mortality rates when compared with similar patients without candiduria (Chenoweth et al., 2014). Therefore, early and accurate species identification, as well as identification of antifungal susceptibility of isolates, is very important for determining appropriate therapies for treating episodes of recurrent candiduria and/or persistent candidemia related to candiduria. Therefore, the monitoring of epidemiological data in hospitals is important for establishing efficient infection control measures. This study aimed to identify the various species of *Candida* isolated from candiduria and candidemia

episodes in a tertiary hospital in North Delhi, as well as to determine the in vitro susceptibility profiles of the species to various antifungal compounds.

SUBJECTS AND METHOD

1. Study Design

The present study was an observational retrospective cross-sectional study carried over 9 months from July 2022 to March 2023 at the Department of Microbiology, Hindu Rao Hospital and NDMC Medical College, Delhi.

2. Population and Sample

The samples were selected using some criteria. The inclusion criteria: All urine and blood culture specimens from patients admitted in various wards and intensive care units of all age groups and all genders revealed the pure growth of budding yeast cells on blood agar which was incubated at 37^0C and read at 24 hours and 48 hours intervals were included in the study. The exclusion criteria were samples from outpatient departments and patients already on anti-fungal therapy were excluded from the study. Repeat isolates of the same patient were excluded.

3. Study Variables

Variables identified in our study were the species of Candida: *C. albicans* and Non-*albicans Candida* (*C. tropicalis*, *C. pelliculosa*, *C. glabrata*, *C. krusei* and *Kodamaea ohmeri*). Susceptibility to antibiotics: fluconazole, voriconazole, caspofungin, micafungin, amphotericin-B and flucytosine.

4. Operational Definition of Variables

About 80 urine and blood samples received from in-patient departments which revealed the pure growth of budding yeast cells on blood agar (which were incubated at 37^0C and read at 24 hours and 48 hours intervals) were processed in the Microbiology Laboratory, Hindu Rao Hospital, Delhi by the semi-quantitative method as per standard protocols. Dry creamy white opaque colonies on blood agar that resemble *Candida* were confirmed by gram stain (Das et al, 2022; Mehta and Wyawahare, 2016)· *Candida* isolates were then subcultured on Sabouraoud's Dextrose Agar and Hi-Crome *Candida* agar medium for speciation.

The identification of the growth as *Candida* was done by colony morphology (cream-colored, smooth, and pasty colonies). The speciation of *Candida* was done by performing various conventional tests such as the Germ tube formation test (Das et al, 2022), chlamydospore formation on corn meal agar, and color change on HiCrome *Candida* agar medium (Mehta and Wyawahare, 2016). HiCrome Agar is a very easy and low-cost method for identification of *Candida* species. Another important advantage of HiCrome agar is its ability to detect mixed cultures (Kauffman, 2005). Colour patterns of various C*andida* species were noted on HiCrome Candida agar medium. *C. albicans* isolates impart distinctive light green colonies; *C. tropicalis* produce blue violet smooth colonies with halo diffusing into surrounding agar; *C. kruseii* isolates produce rough, fuzzy spreading big pink colonies and *C. glabrata* produce pink, glossy colonies with pale edges (Kauffman, 2005).

Species identification was further done by VITEK 2 Compact System (bioMérieux, Mumbai, India) as per the manufacturer's instruction along with antifungal susceptibility testing by VITEK 2 for fluconazole, voriconazole, flucytosine, micafungin, and amphotericin B. The MICs were interpreted according to the CLSI guidelines 2022 (CLSI, 2022).

5. Study Instruments

Blood agar, Gram stain, Sabouraoud's Dextrose Agar, HiCrome Candida agar medium, VITEK 2 Compact System.

6. Data Analysis

All categorical data were entered in a Microsoft Excel sheet and analyzed using Python (ver3.9) with Jupyter Notebook and Chi-square test and a significant P-value (<0.050) was calculated.

7. Research Ethics

Ethical approval was taken from the Institutional Ethics Committee to conduct the study.

RESULTS

Over the study period of 9 months, from July 2022 to March 2023, from 571 samples positive in blood cultures, *Candida* was isolated in 33 (5.77%) samples, and among 320 positive urine samples, 47 (14.68%) had candiduria. Out of these 80, 19 (23.75%) were identified as *C. albicans* and 61 (76.25%) were non-*albicans Candida*. Figure 1 shows the most common non-*albicans* species was *C. tropicalis* (56.25%) followed by, *C. pelliculosa* (7.5%), *C. glabrata* (6.25%), *C. krusei* (3.75%) and *Kodamaea ohmeri* (2.5%). Majority of the cases were in the age group of 1-10 years (45%) followed by >50 years of age (23.75%) and then followed by 30-50 years (20%). Figure 2 shows, 47 (58.75%) were females and 33 (41.25%) were males. Most of the patients (28.75%) were from the Pediatric Department followed by the Medicine Department (27.5%), which was not statistically significant.

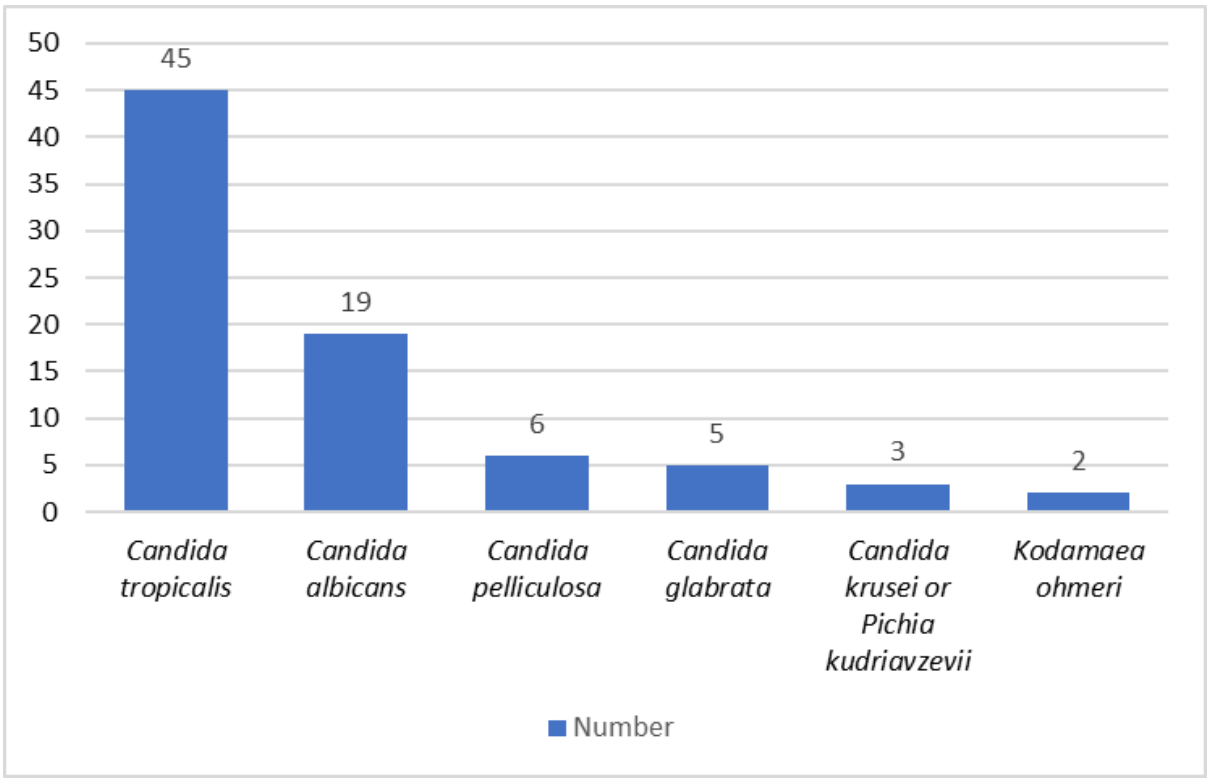

Figure 1. The isolates are identified in specimens

On correlating the clinicodemographic profile according to *Candida* spp., it was observed that most of the patients who had candiduria and candidemia were due to *Candida albicans* (23.75%) and non-*albicans Candida* spp. (76.25%) were in the age group of 1-10 years which was not found to be statistically significant (Figure 3). Figure 4 shows the difference in age between

C. tropicalis and other species was statistically significant (p=0.020).

Figure 5 shows the common predisposing factors associated with candiduria and candidemia were risk factors like diabetes mellitus and hypertension in Medicine and other wards, whereas for <15 years of patient the common risk factors were IUGR, low birth weight, and sepsis, followed by female sex (59%). According to our study, the *Candida* species isolated from urinary specimens were 56.25%, and isolates from blood were 43.75% (Table 1). This data indicates that *Candida* species isolated is dependent on the sample type, which was also statistically significant with p=0.030 (Chi-square test method).

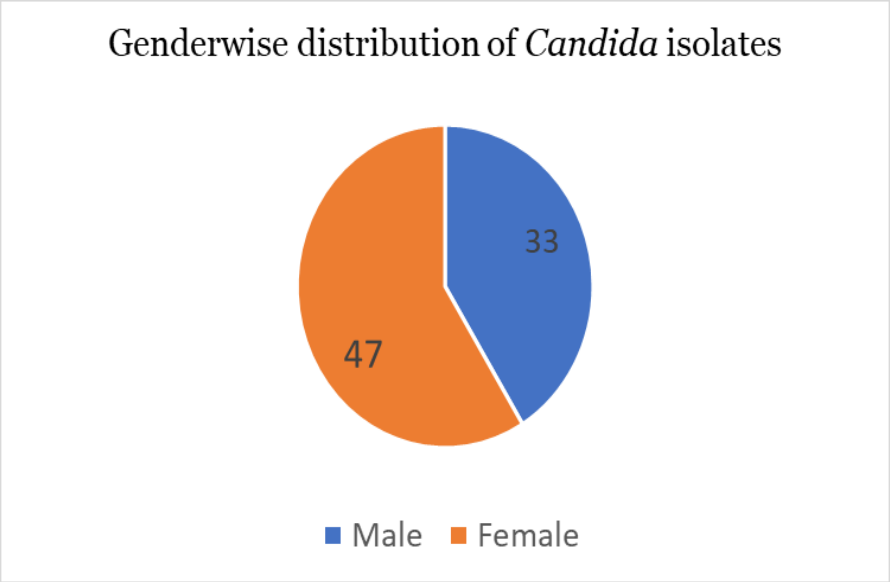

Figure 2. The genderwise distribution of Candida isolates

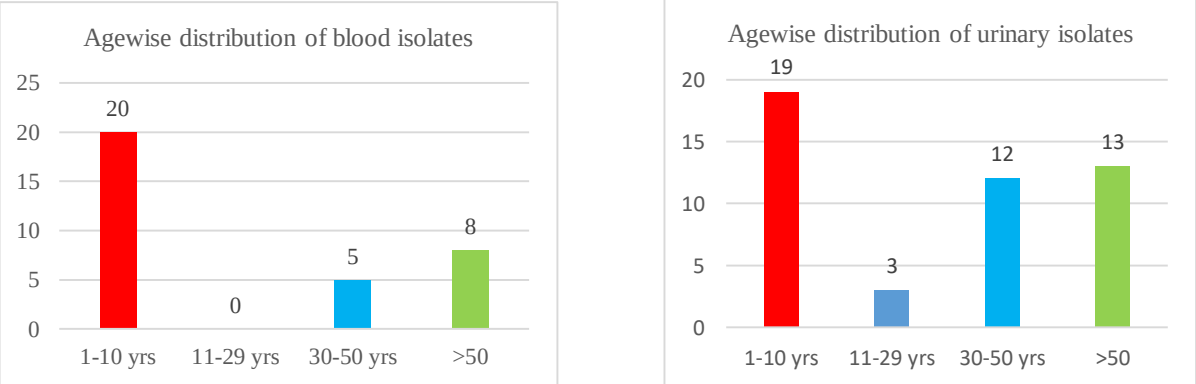

Figure 3. Agewise distribution of blood isolates and urinary isolates respectively

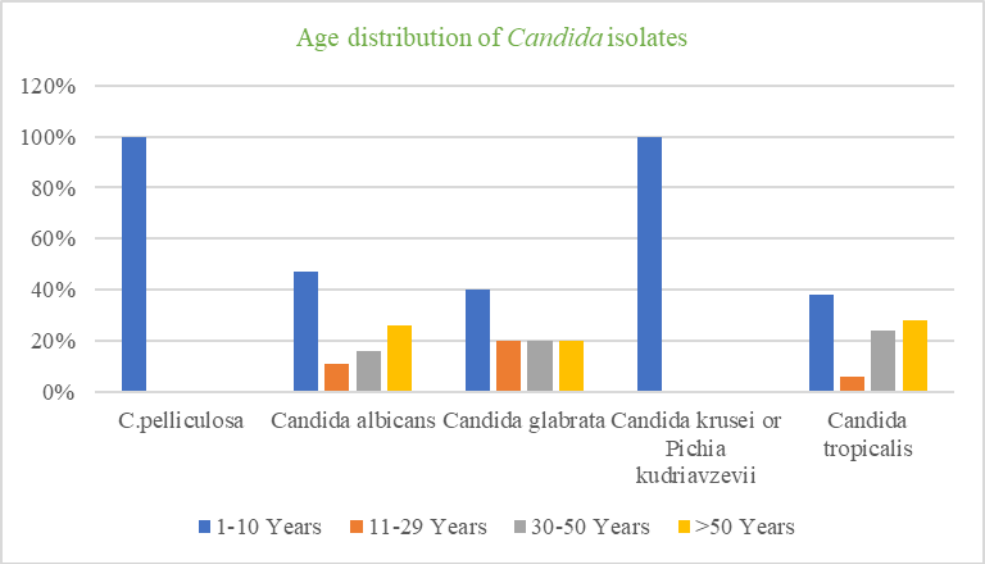

Figure 4. Age distribution of *Candida* isolates

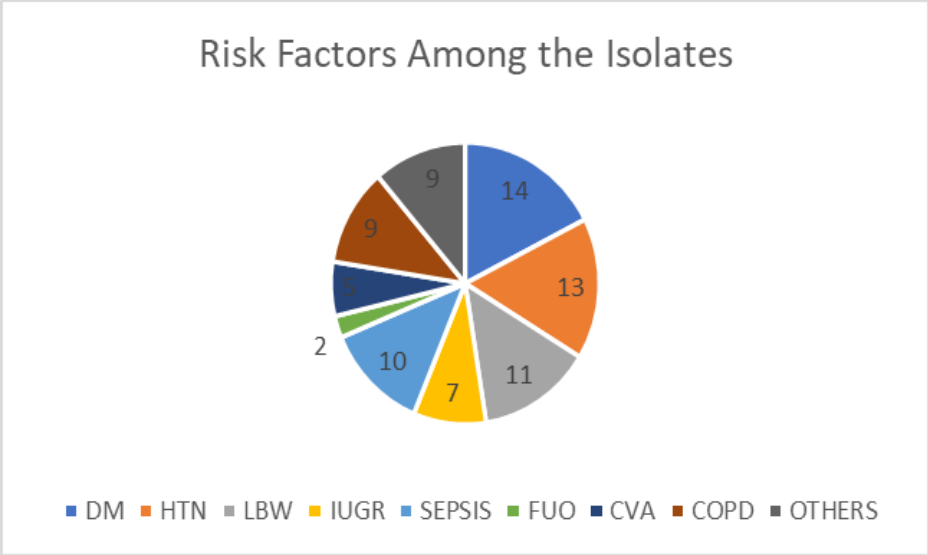

Figure 5. Risk factors among the isolates

Table 1. Distribution of different species of *Candida* from blood and urine specimens

Species	Blood Isolates	Urine Isolates
C. tropicalis	19	26
C. glabrata	1	4
C. albicans	4	15
C. pelliculosa	5	1
C. krusei	2	1
Kodamaea ohmeri	2	0

The MIC of fluconazole for most of the strains of non-*albicans Candida* was <1 and 2 while for *C. albicans* it was <0.5. The MIC of most of the strains for voriconazole was

<0.2 in both *albicans* and non-*albicans Candida* spp. For caspofungin, the MIC was <0.12 in most of the isolates in both *albicans* and non-*albicans* spp., except for *C. krusei* for which the MIC was <0.12 for 66% of isolates and rest 34%, the MIC was >1.

The MIC of micafungin was ≤ 0.25 in all isolates of *C. albicans* (100%) and *C. krusei* (100%), whereas it was ≤0.06 for all the isolates of other non-*albicans Candida* spp. The MIC of flucytosine was ≤1 for all the species of *Candida*. For amphotericin B, the MIC for most of the isolates of *C. albicans* was equal to ≤1, whereas for non-*albicans* spp., MIC was ≤ 0.5 for most strains (Table 2 and Figure 6). Interpretation of antifungal MICs for *Candida* species shows that all isolates of *C. albicans* (100%) were sensitive to all antifungal agents, except amphotericin B for which the resistance was (100%). All isolates of *C. Tropicalis* (100%) were sensitive to flucytosine, caspofungin, and micafungin; while sensitivity was 97.7% to voriconazole, amphotericin B (95.5%), and for fluconazole was 88.9%. *C. krusei* was 66% sensitive to voriconazole and 33% for caspofungin. All the isolates of *C. glabrata* (100%) were sensitive to flucytosine, caspofungin, amphotericin, and micafungin

Table 2. Sensitivity pattern of different species to various antifungals

Species	Fluconazole	Voriconazole	Caspofungin	Micafungin	Amphotericin-B	Flucytosine
C. pelliculosa	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
C. albicans	100.0%	100.0%	100.0%	100.0%	0.0%	100.0%
C. glabrata	0.0%	0.0%	100.0%	100.0%	100.0%	100.0%
C. krusei	0.0%	66.0%	33.0%	100.0%	100.0%	100.0%
C. tropicalis	88.9%	97.7%	100.0%	100.0%	95.5%	100.0%
Kodamaea ohmeri	0.0%	0.0%	100.0%	100.0%	100.0%	100.0%

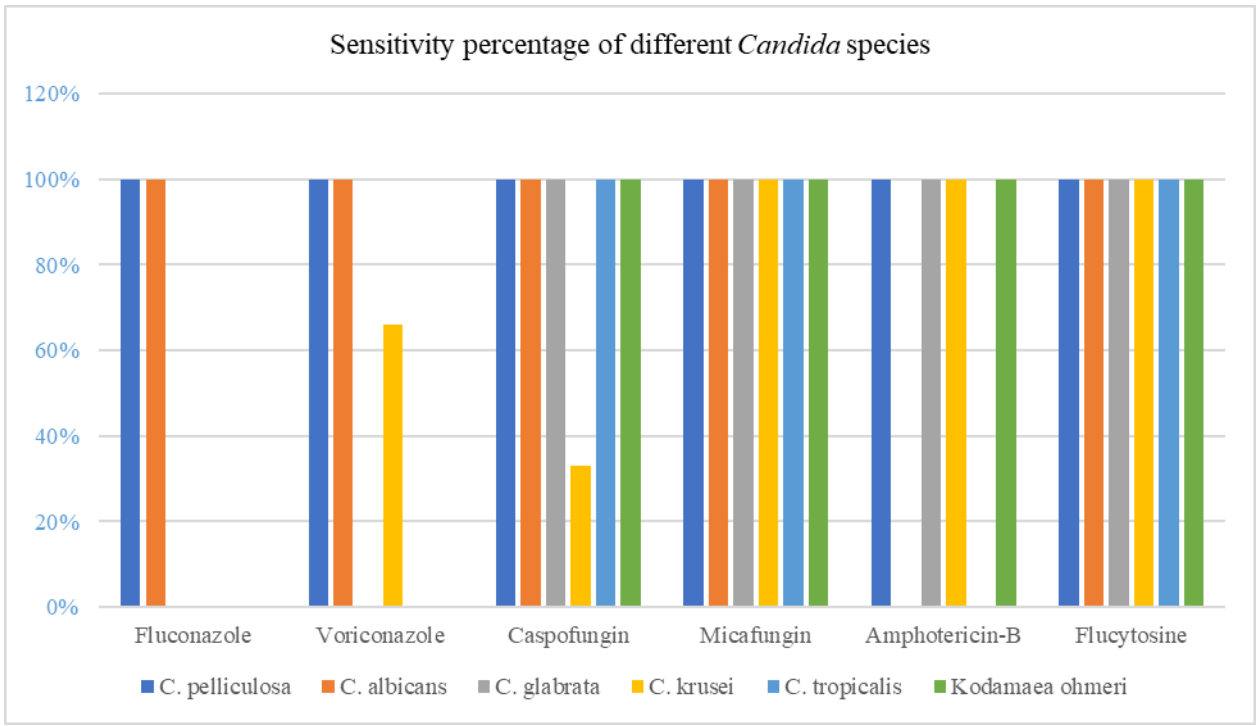

Figure 6. Sensitivity percentage of different *Candida* species

DISCUSSION

The presence of *Candida* species in urine (candiduria) is an asymptomatic condition that results from contamination during urine collection in patients with bladder colonization or upper urinary tract infection and hematogenous spread from other sites (Seifi et al., 2013). The incidence rate of candiduria and candidemia is variable and different reports have shown different frequencies. It corresponds to more predisposing factors including long stays at hospitals (especially in ICU and NICU), urinary indwelling catheters, abnormality in the urinary tract, immunosuppressive therapy in immunocompromised patients, renal transplantation, broad-spectrum antibacterial therapy, and hemodialysis (Zarei-Mahmoudabadi et al., 2012; Bukhary, 2008; Jain et al., 2011; Amar et al., 2013; Kandhari and Rama-Rao, 1969).

In the last twenty years, factors like the AIDS epidemic, an increase in the number of immunosuppressive therapy recipients, and the use of long-term antibiotic therapy have changed the epidemiology of invasive mycoses in general and of candidemia in particular. Amar et al. (2013) in their study showed that females were more predominant (60.2%) than males (39.8%) and male: female ratio was 0.66:1. In a similar study conducted by Kandhari and Rama-Rao (1969) at AIIMS, New Delhi, the incidence in female was about 61.2% while in male, it was 38.8% with a male: female ratio of 1:1.57. This study also showed similar findings with the female preponderance of 58.75% over males which accounted for 41.25% with a male: female ratio of 1:1.42.

Increased cases amongst women may be due to various factors like usage of birth control methods containing high doses of estrogen, douches or vaginal sprays, pregnancy, uncontrolled diabetes mellitus, antibiotic therapy etc. Similar results were seen in a study done by Sulaiman et al. (2014). In present study, the NAC spp. had predominance over *Candida albicans*. NAC accounted for 76.25% of the total isolates and *Candida albicans* constituted 23.75% of the isolates. Among the NAC spp. *Candida tropicalis* was the most common accounting for 56.2% followed by *C. pelliculosa* (7.5%), *C. glabrata* (6.25%), *C. krusei* (3.75%) and *Kodamaea ohmeri* (2.5%). This finding is similar to that of other studies done by Shaik et al. (2016), Pahwa et al. (2014), and Sandhu et al. (2016).

17 cases (36.1%) of candiduria were below 10 years of age in our study. Fungal UTI in children may be attributed to compromised immune systems, long-term therapy with broad-spectrum antibiotics or possible anomalies of the urinary tract. Our findings were supported by studies by Shaikh and Hoberman (2017) and Desai et al. (2016). A study by Seifi et al. (2013) showed candiduria has a prevalence rate of 5.2 % among children below 14 years of age.

According to a study conducted by Saha et al. (2008), *C. albicans* is usually sensitive to amphotericin B, whereas non-*albicans* are more resistant to antifungal drugs, especially fluconazole, which is not similar to our study showing high resistance to amphotericin B in *C. albicans* but sensitive to azoles, caspofungin, and micafungin, whereas most of the non-*albicans* spp. isolated in our study were sensitive to most of the antifungal drugs except that of *C. krusei*. Also, a study done by Aijaz and Kaur (2023) has shown a similar antifungal profile to that of Saha et al. (2008).

The resistance to fluconazole is of great concern because it is the most common azole used for the treatment of

disseminated candidiasis including candidemia. Increasing use of the azole group of antifungal drugs has led to the predominance of non-*albicans Candida* species over *C. albicans*. Resistance to voriconazole, amphotericin B, and micafungin was also seen in our study due to decreased susceptibility to fluconazole and cross-resistance to other azoles.

In conclusion, the spectrum of *Candida* urinary and blood infection has shifted dramatically from *C. albicans* to NAC spp. In serious and systemic infections, the patient's survival depends on early intervention and specific antifungal therapy. Any delay in initiating effective antifungal therapy among hospitalized patients is associated with high mortality (Kauffman, 2014). Candiduria in seriously ill patients should be carefully evaluated, as this medical condition may be the only indicator of invasive candidiasis. Its detection increases the chances of successful treatment and enhances the patient's survival.

Therefore, it is essential for an early and accurate diagnosis to be made of various species of *Candida*, since each species differs to a large extent in susceptibility to the currently used antifungal drugs. It is indispensable that antifungal susceptibility testing should be carried out routinely in the laboratory. This will assist the clinician in the timely institution of the appropriate and accurate antifungal drug to be used and will restrict the empirical use of antifungal agents as well as prevent resistance to commonly used antifungal drugs. Since the present study was confined to a very small sample size, many such kinds of studies with larger sample sizes are required to know the prevailing *Candida* spp. which may in turn help to develop guidelines on empiric therapy for invasive fungal infections.

AUTHOR CONTRIBUTION

Dr. Manoj Kumar chose the study design subjects and methods and analyzed the study critically. Dr. Shilpa Kathri- chose the sample size and processed the samples. Dr. Sanjay Jain- critically reviewed the study objectives and results. Dr. Tanisha Bharara and Dr. Abhishek Yadav- critically reviewed the study.

ACKNOWLEDGMENT

The authors would like to thank Dr. Dipti Rawat, Professor, Lady Hardinge Medical College, New Delhi, for performing VITEK.

FUNDING AND SPONSORSHIP

None.

CONFLICT OF INTEREST

None.

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