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**ORIGINAL ARTICLE****Changing trends of candidemia: A study from western Uttar Pradesh***Vidhi Kardam<sup>1</sup>, Peetam Singh<sup>2\*</sup>, Anita Pandey<sup>2</sup>*

<sup>1</sup>Department of Microbiology, Chaudhary Charan Singh University, Meerut-250005 (Uttar Pradesh), India, <sup>2</sup>Department of Microbiology, Subharti Medical College, Meerut-250005 (Uttar Pradesh), India

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**Abstract:**

**Background:** Candidemia is one of the common health care associated Blood Stream Infections (BSI). Frequency of isolation of various *Candida* species causing candidemia and their antifungal drug resistance trends in a health care setting can help in timely institution of appropriate empirical antifungal treatment. **Aim and Objectives:** To observe the species distribution of various *Candida* species isolated from suspected cases of BSI and their antifungal susceptibility pattern in a tertiary care hospital from western Uttar Pradesh. This study was conducted to speciate *Candida* isolates isolated from blood culture samples and their antifungal susceptibility testing. **Material and Methods:** This study was conducted in the Department of Microbiology, Subharti Medical College and associated Chhatrapati Shivaji Subharti Hospital, Meerut for a period of one year. Blood culture was done by BacT/Alert automated blood culture system from BioMerieux, France. *Candida* isolates recovered from blood culture were further processed for speciation using conventional morphological and biochemical characteristics and antifungal susceptibility testing by Kirby Bauer disk diffusion method. **Results:** Out of total 70 *Candida* isolates isolated during the study period *C. tropicalis* was the predominant isolate accounting for 34.28% followed by *C. parapsilosis*. The occurrence of candidemia was higher in Intensive Care Units (ICUs) patients (70.83%). Antifungal drug voriconazole was found to be sensitive against all the *Candida* isolates. **Conclusion:** Mycological shift towards the isolation of NAC species indicates a changing trend and changing antifungal drug resistance profile accordingly have to be considered before initiation of empirical antifungal treatment.

**Keywords:** Candidemia, *Candida*, Blood Stream Infections, Antifungal susceptibility test

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**Introduction**

Candidemia is a Blood Stream Infection (BSI) caused by *Candida*. *Candida* infection can spread from bloodstream to other parts of the body such as eyes, kidney, liver, or brain known as invasive candidiasis [1]. Candidemia is one of the most common health care associated BSIs [2]. Immunocompromised states including elderly, pregnancy, diabetes mellitus, and individuals with chronic renal failure, alcoholics and patients admitted in the Intensive Care Unit (ICU) are at risk of developing BSIs [3].

*Candida* is normally considered as a common commensal organism of gastrointestinal tract and skin but the disruption of these anatomical barriers can lead to invasive infections [4]. *Candida* includes over 200 species but only a small proportion of them is medically important. The medically significant *Candida* species include *Candida albicans*, *Candida tropicalis*, *Candida parapsilosis*, *Candida glabrata*, *Candida krusei*, *Candida kefyr*, *Candida guilliermondii*, and *Candida dubliniensis* [5].

As candidemia is a serious life-threatening condition that can lead to significant morbidity and mortality especially due to delayed institution of specific antifungal therapy, timely institution of antifungal treatment is very crucial. Delayed diagnosis and non-availability of antifungal susceptibility testing facility in routine laboratories further complicates the scenario leading to delayed initiation of treatment [6]. As fungal culture and antifungal susceptibility testing are time consuming and not routinely performed by most of the laboratories, it is of utmost importance to know the prevalent species causing invasive fungal infections including BSI and their antifungal susceptibility pattern for early institution of empirical as well as specific treatment [7].

*Candida albicans* has been considered as the most common cause of candidemia [2]. There are many studies showing the predominance of Non-albicans *Candida* (NAC) species over *Candida albicans* [8-9]. The epidemiology of species causing candidemia is dynamic and evolving.

Recent studies have reported an increase in the proportion of BSI cases caused by NAC [8, 10, 11]. This change in epidemiology has been related to increased use of antifungal agents for prophylactic and empirical treatments with many species found to be less susceptible to azoles and costly alternatives (e.g., echinocandins) being used for NAC [8, 11]. Therefore, early and accurate identification of *Candida* is important for the reduction of morbidity and mortality, especially among infants [11]. This study was planned to observe the species distribution and highlighting the changing trends in the distribution of various *Candida* species isolated from suspected cases of BSI and their antifungal susceptibility pattern in a tertiary care hospital from western Uttar Pradesh.

### Material and Methods

This cross-sectional hospital based observational study was conducted in the Department of Microbiology, Subharti Medical College and associated Chhatrapati Shivaji Subharti Hospital, Meerut for a period of one year from June 2021 to May 2022. This study was approved by Institutional Ethics Committee, Subharti Medical College, Swami Vivekanand Subharti University, Meerut. The paired blood samples were collected from patients in BacT/Alert automated blood culture bottles designated for that particular age group such as green capped bottles for adults and yellow capped bottles for paediatric patients by trained phlebotomists following standard precautions following inclusion and exclusion criteria. The volume of blood for blood culture collected was 10 mL for adults and 2 mL to 5 mL but not more than 1% of total blood volume for paediatric patients [12].

**Inclusion criteria:** Patients of all age groups and genders, patients suspected of having blood stream infections such as undetermined fever (body temperature more than or equal to 38°C) or hypothermia (body temperature less than or equal to 36°C), shock, chills or rigors.

**Exclusion criteria:** Patients with the history of recent antimicrobial therapy, samples collected after initiation of antimicrobial therapy, blood cultures showing growth of microorganisms other than *Candida* species

### Sample size calculation

We used following formula for calculating sample size for cross-sectional studies:

$$\text{Sample size} = Z^2 p(1-p)/d^2$$

Where,

Z is standard normal variate (usually 1.96 at 5% error), p is expected proportion in the population and d is the precision (5%).

Considering the value of p from north India as 1.31% [13] appropriate sample size for our study was calculated and found to be 20.

$$\text{Sample size} = (1.96)^2 \times 0.0131(1-0.0131) / (0.05)^2 \\ = 19.87 \approx 20$$

### Sample processing

The blood culture bottles were incubated in BacT/ALERT 3D automated blood culture system from bioMerieux, France. The blood culture bottles were incubated for a maximum period of 5 days to declare no growth if not flagged positive by the system. A total of 8428 blood samples received during the study period of one year from the suspected cases of BSI were processed. The blood culture bottles flagged positive by the system were further processed by subculturing on to Blood Agar (BA), Chocolate Agar (CA), MacConkey Agar (MA) and Sabouraud's Dextrose Agar (SDA). Any growth on subculture was further processed for presumptive identification using conventional microbiological procedures including Gram stain to rule out commensals and contaminants.

Presumptive *Candida* isolates were further processed for species identification based on germ tube test, pigment production on chromogenic agar *Candida* (CHROMagar), carbohydrate assimilation, carbohydrate fermentation and chlamydo-spore formation on cornmeal agar [14]. Antifungal Susceptibility Testing (AFST) was done by Kirby Bauer disk diffusion method on Mueller Hinton Agar (MHA) supplemented with 2% dextrose and methylene blue using commercially available

antifungal discs from HiMedia, India as per CLSI guidelines for antifungal susceptibility 2017. Antifungal susceptibility of candida isolates was tested against ketoconazole, fluconazole, clotrimazole and voriconazole [15].

### Statistical analysis

The data generated in this study was statistically analysed by using statistical package for the social sciences (SPSS) software version 26 (IBM Corp., Armonk, NY, USA). Fisher's exact test was used to calculate p-value and interpretation was done considering p-value of <0.05 as statistically significant. Categorical variables were compared using proportions and percentage.

### Results

Out of total 8428 blood samples received for blood culture during one year of study period, 2796 were flagged positive by BacT/Alert system. The blood culture positivity rate was found to be 33.2%. Out of total 2796 blood culture samples flagged positive, bacterial isolates were 2726 (97.49%) while 70 (2.50%) were *Candida* isolates. Among these total 70 *Candida* isolates, *C. albicans* and Non-albicans *Candida* species were found to be 9% and 91% respectively. *C. tropicalis* 26 (37%) was the predominant *Candida* species isolated followed by *C. parapsilosis* 20 (29%) (Figure 1).

Maximum number of isolates were isolated from neonates and adults, comprising of 22 (31%) followed by elders. Age wise distribution of all *Candida* isolates is shown in figure 2 and age groups were categorised as defined by National Institute of Health.

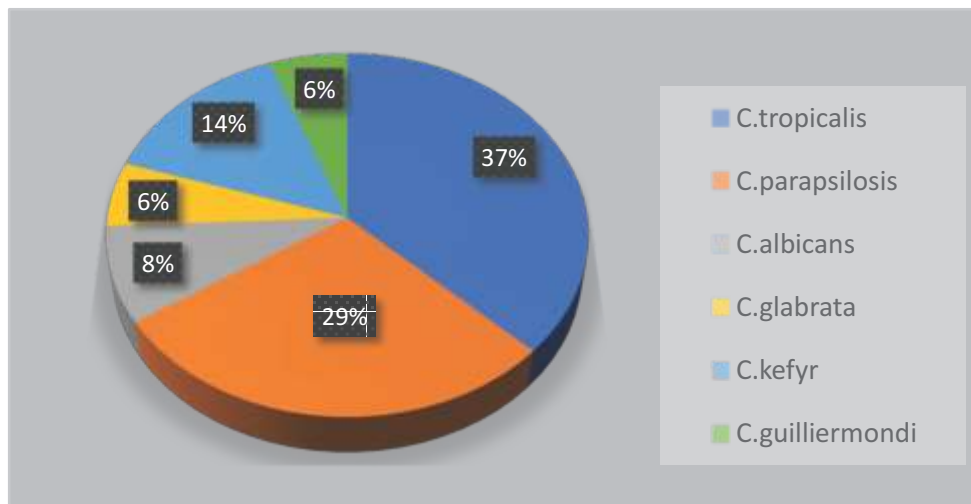


Figure 1: Distribution of various Candida species

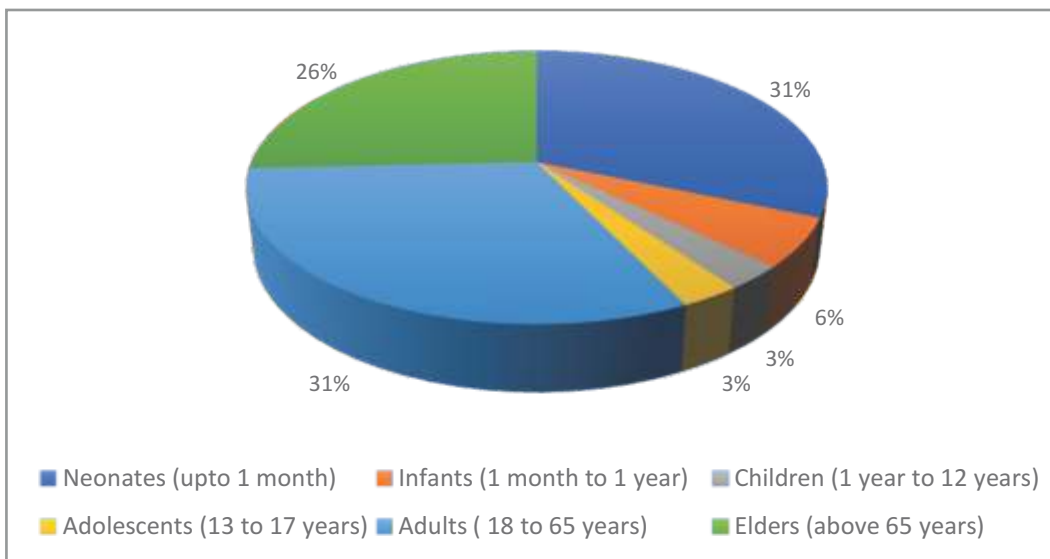


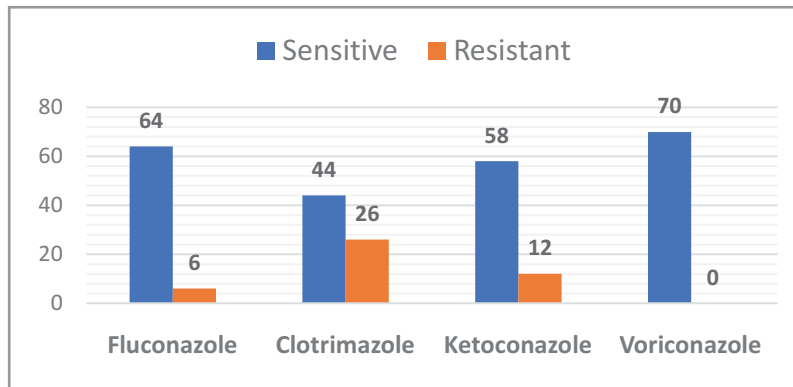
Figure 2: Age wise distribution of isolates

*Candida* isolates were predominantly isolated from male patients (71.42%) as compared to female patients (28.57%). In this study samples received from IPD and OPD were 68.57% and 31.42% respectively. Maximum number of isolates was from ICU (70.83%) as compared to other wards (29.16%). The comparison of distribution profile of *Candida albicans* and NAC according to age groups, gender and location are shown in table 1.

On antifungal susceptibility testing against four antifungal agents ketoconazole, fluconazole, clotrimazole and voriconazole tested, all the isolates were found to be 100% susceptible against voriconazole. Highest level of resistance of 30.14% was observed against clotrimazole followed by resistance against ketoconazole (17.14%) and fluconazole (8.6%) (Figure 3).

**Table 1: Comparison of distribution profile of *Candida albicans* and NAC**

| Parameter                    | <i>Candida albicans</i> | NAC | <i>p</i> |
|------------------------------|-------------------------|-----|----------|
| <b>Age</b>                   |                         |     |          |
| Neonates (up to 1 month)     | 0                       | 22  | 0.17     |
| Infants (1 month to 1 year)  | 0                       | 4   |          |
| Children (1 year to 12 year) | 0                       | 2   |          |
| Adolescents (13 to 17 years) | 0                       | 2   |          |
| Adults (18 to 65 years)      | 2                       | 20  |          |
| Elders (above 65 years)      | 4                       | 14  |          |
| <b>Gender</b>                |                         |     |          |
| Male                         | 4                       | 46  | 0.56     |
| Female                       | 2                       | 18  |          |
| <b>Location</b>              |                         |     |          |
| ICU                          | 2                       | 32  | 0.07     |
| Other wards                  | 0                       | 14  |          |
| OPD                          | 4                       | 18  |          |



**Figure 3: Summary of antifungal Susceptibility pattern (n=70)**

Highest resistance among various *Candida* species was observed in the case of *C. guilliermondi*, which was found susceptible only against voriconazole, while it was 100% resistant against

fluconazole, clotrimazole and ketoconazole. *C. kefyr* and *C. glabrata* were 100% sensitive against all the four antifungals (Table 2).

Table 2: Species specific antifungal susceptibility of all *Candida* isolates

| Candida species               | Fluconazole<br>N (%) | Clotrimazole<br>N (%) | Ketoconazole<br>N (%) | Voriconazole<br>N (%) |
|-------------------------------|----------------------|-----------------------|-----------------------|-----------------------|
| <i>C. albicans</i> (n=6)      | 6 (100%)             | 4 (67%)               | 4 (67%)               | 6 (100%)              |
| <i>C. tropicalis</i> (n=26)   | 24 (92%)             | 16 (67%)              | 22(85%)               | 26 (100%)             |
| <i>C. parapsilosis</i> (n=20) | 18 (90%)             | 14 (70%)              | 16 (80%)              | 20 (100%)             |
| <i>C. kefyr</i> (n=10)        | 10 (100%)            | 10 (100%)             | 10 (100%)             | 10 (100%)             |
| <i>C. glabrata</i> (n=4)      | 4 (100%)             | 4 (100%)              | 4 (100%)              | 4 (100%)              |
| <i>C. guilliermondi</i> (n=4) | 0                    | 0                     | 0                     | 4 (100%)              |

## Discussion

Blood stream infections are important cause of serious morbidity and mortality and are among the most common healthcare associated BSIs. This condition can be life threatening and more commonly seen in immune compromised and critically ill patients admitted to intensive care units of the hospitals. In the present study, a total of 8428 samples of blood culture received in microbiology section of central laboratory, over a period of one year were analysed.

In this study, the blood culture positivity rate was found to be 33.17%. Similar positivity rate was also observed in a study conducted by Sharma *et al.* (33.9%) [16]. However, a study by Khanal *et al.* [17] reported a higher positivity rate of 44%. Lower positivity rate was reported by Alam *et al.* (20.09%) [18] and Roy *et al.* (16.4%) [19]. Variation in blood culture positivity rate may be due to many factors which involve disruption of microflora of patients, hospital-based settings and immune compromised status of patients. This study revealed that the percentage of *Candida* species was 2.50% from the positive blood

samples. In a study conducted by Sudan *et al.* [20] showed the higher percentage of *Candida* isolates (4.48%) among the culture positive samples. The frequency of *Candida* species varies among hospitals depending upon diagnostic approach, studied population, exposure to the risk factors and availability of diagnostic procedure. It was found that NAC species accounted for 91.4% of the cases of candidemia whereas *C. albicans* responsible for 8.57% of cases. These findings reported mycological shift towards the NAC species. In 2016, a study conducted by Sudan *et al.* [20] reported 65.38% cases of candidemia caused by NAC species while 34.61% by *C. albicans*. In another study conducted by Ahmad *et al.* in 2020 from Aligarh [8], the proportion of *Candida* species causing candidemia was reported as 85.91% of NAC species as compared to 14.09% of *Candida albicans*. The predominance of NAC species among Candidemia have been observed by various other studies such as studies by Chakraborti *et al.* in 1996 [21] and Mokaddas *et al.* in 2007 [22]. After data analysis of previous studies

we observed continuously increasing proportion of NAC species over *Candida albicans* from 52.6% in 1992 to 85.91% in 2020. We observed further increased predominance of NAC species up to 91.4% in present study. These findings are consistent with the findings of studies conducted by Rajmane *et al.* and reported around 50% and 77% of NAC species respectively during 2010-11 and 2011-12 in their study [23-24]. Another study done by Gautam *et al.* from north India recently during 2021-22 also reported the predominance of NAC comprising of 82.76% in comparison to 17.24% of *Candida albicans* [9]. Speciation of the recovered isolates revealed *C. tropicalis* to be the predominant isolate accounting for 37.14% followed by *C. parapsilosis* (28.57%), *C. kefyr* (14.28%), *C. albicans* (8.57%), *C. guilliermondii* (5.71%) and *C. glabrata* (5.71%). Similarly, a study conducted by Jose *et al.* in 2017 from Bangalore [25] reported that *C. tropicalis* was the

predominant species (53.3%) followed by *C. parapsilosis* (20%), *C. krusei* (16.7%) and *C. albicans* (10%). Our findings were consistent with the study carried out by Gandham *et al.* in 2016 [26] from Maharashtra. They reported *C. tropicalis* (34.48%) predominantly followed by *C. parapsilosis* (27.585%), *C. albicans* (10.34%) and *C. krusei* (10.34%). These findings of % distribution of various *Candida* species are consistent with the findings from other studies including the findings of studies conducted by Rajmane *et al.* during 2010-11 and 2011-12 [23-24] showing the predominance of *C. tropicalis*. The distribution of NAC versus *Candida albicans* as reported from various studies is shown in table 3 in chronological order which further reveals the predominance of NAC over *Candida albicans* and predominance of *C. tropicalis* among NAC species.

**Table 3: Distribution of NAC versus *Candida albicans* reported from various studies**

| Study conducted by         | Year | <i>Candida albicans</i> (%) | NAC (%) | Most common NAC species (%)    |
|----------------------------|------|-----------------------------|---------|--------------------------------|
| Gandham <i>et al.</i> [26] | 2016 | 10.34%                      | 89.66%  | <i>C. tropicalis</i> (34.4%)   |
| Tejan <i>et al.</i> [11]   | 2017 | 20.8%                       | 79.2%   | <i>C. tropicalis</i> (42.1%)   |
| Kumar <i>et al.</i> [10]   | 2019 | 21.3%                       | 78.7%   | <i>C. parapsilosis</i> (29.8%) |
| Ahmed <i>et al.</i> [8]    | 2020 | 14.08%                      | 85.92%  | <i>C. tropicalis</i> (35.2%)   |
| Sharma <i>et al.</i> [16]  | 2020 | 10.8%                       | 89.2%   | <i>C. auris</i> (32.4%)        |
| Gautam <i>et al.</i> [9]   | 2021 | 17.4%                       | 82.76%  | <i>C. tropicalis</i> (26.7%)   |
| This study                 | 2022 | 8.57%                       | 91.43%  | <i>C. tropicalis</i> (37.14%)  |

In this study, the highest frequency of candidemia was observed in adults and neonates comprising of 31% each. Other studies conducted by Shettigar *et al.* [27] and Rani *et al.* [28] reported 9.59% and 34.27% frequency of candidemia in neonates respectively. The higher frequency of candidemia in neonates may be due to immune status as in this age group the immunity is not completely developed. The clinically significant *Candida* isolates were predominantly isolated from male patients (71.42%) as compared to female patients (28.57%). We observed maximum candidemia cases of 68.6% from IPD patients as compared to 31.4% cases from OPD. Among IPD patients the highest frequency of candidemia was observed from ICU patients (70.83%). It has been observed from various studies that frequency of candidemia is significantly higher in the ICUs than in wards including the study conducted by Gandham *et al.* in 2016 [26]. The frequency of isolation of candida from BSI patients depends on many factors including hospital related factors and various patient related factors such as hospital infection control practices followed, immune status and genetic factors related to the patients, which may vary from patient to patient and hospital to hospital. Patients admitted to ICU are more prone to acquire invasive infections including BSI due to various comorbidities associated with the patient or various invasive devices predisposing the patients for these infections [8-9].

Antifungal susceptibility testing against four azole drugs ketoconazole, fluconazole, clotrimazole and voriconazole revealed that all *Candida* species were completely sensitive to voriconazole. As evidenced from the results, two isolates of *C. glabrata* and five of *C. kefyr* were completely sensitive to these azole drugs. Similar observations

in the trends of Antifungal susceptibility pattern were also reported in a study conducted by Gandham *et al.* in 2016 [26]. In a study by Giri *et al.* [29] revealed 30.8% *Candida* isolates resistant against fluconazole whereas in another study by Parvez *et al.* [30] revealed 65% and 24% resistance against fluconazole and clotrimazole respectively. The efficacies of these drugs also depend on the integrity of the hosts' immune system, site and severity of infection and their pharmacokinetics. Therapeutic failures may result from emergence of resistance against antifungals.

### Conclusion

Although *C. albicans* was considered as the most common cause of candidemia, there has been a changing trend towards more frequent isolation of NAC species in recent years. Mycological shift towards the isolation of NAC species indicates a changing trend and changing antifungal drug resistance profile accordingly have to be considered before initiation of empirical antifungal treatment. Strict implementation of antibiotic stewardship programme along with policymaking and drafting of antifungal stewardship is the need of the hour.

### Limitations of the study:

There were certain limitations of the study including:

1. This study was limited to a single healthcare facility limited to a small geographical area hence the findings cannot be generalised to entire population.
2. Molecular characterization of species level identification and detection of antifungal resistance genes was not attempted due to limited resources.



**Future recommendations of the study:**

*C. albicans* is still considered as predominant species from candidemia patients as compared to NAC. As there is a shifting trend in the predominance of NAC over *C. albicans* as evident from various studies from different geographical

areas, more studies covering large geographical area have to be conducted. It is also recommended that a particular healthcare facility should consider its own microbial flora related to BSI before institution of antimicrobial chemotherapy.

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**\*Author for Correspondence:**

Dr. Peetam Singh, Department of Microbiology, Subharti Medical College, NH-58, Delhi Haridwar Bypass Road, Meerut-250005, Uttar Pradesh, India  
Email: kgmclco@gmail.com Cell: 7906228710

**How to cite this article:**

Kardam V, Singh P, Pandey A. Changing trends of candidemia: A study from western Uttar Pradesh. *J Krishna Inst Med Sci Univ* 2023; 12(2):102-111

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Submitted: 07-Jan-2023 Accepted: 16-Mar-2023 Published: 01-Apr-2023

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