

Original Article

Epidemiology and antifungal susceptibility of *Candida* species in a tertiary care hospital, Kolkata, India

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Abstract

Background and Purpose: The incidence of fungal infection as well as candidemia has increased significantly, contributing to morbidity and mortality in the developed countries. The alarming increase in infections with multidrug resistant bacteria is due to overuse of a broad spectrum antimicrobials, which leads to over growth of *Candida* spp.; thus, enhancing its opportunity to cause the disease. A shift has been observed in the relative frequency of each *Candida* spp. Antifungal agents available for the treatment of systemic and invasive candidiasis are restricted to polyenes, allylamines, azoles, and the recent echinocandin class of molecules. In the past few decades, the incidence of resistance to antifungal treatment of *Candida* spp. has increased rapidly, which is of serious concern for healthcare professionals. Studies on prevalence of infections and antifungal susceptibility testing can help with deciding on clinical strategies to manage this problem. Herein, we aimed to identify the epidemiology of *Candida* spp. among blood culture isolates and to investigate the susceptibility pattern of these species to antifungal agents.

Materials and Methods: *Candida* spp. were isolated from blood cultures from 70 patients in a tertiary care hospital, Kolkata, India. The growth of *Candida* spp. on sabouraud dextrose agar was confirmed by Gram staining, where gram-positive budding fungal cells were observed. The species identification as well as antifungal susceptibility testing were performed with VITEK 2 compact automated system using VITEK-2 cards for identification of yeast and yeast-like organisms (ID-YST card). Antifungal susceptibility testing was carried out with VITEK 2 fungal susceptibility card (AST-YS07) kit.

Results: Out of 70 samples, *Candida albicans* were isolated from 34 (48.57%) samples. The remaining 36 (51.43%) were non-*albicans Candida* (NAC). Out of 34 *C. albicans*, antifungal susceptibility was detected in 28 isolates, all of which were sensitive to fluconazole (FLC). Resistance to amphotericin B (AMP), flucytosine (5FC), voriconazole (VRC), and itraconazole (ITC) was observed in 44.12%, 52.94%, 8.82%, and 17.65% of the cases, respectively. For other *Candida* spp. (other than *C. albicans*), antifungal susceptibility was evaluated for 36 isolates, among which resistance to AMP, FLC, 5FC, VRC, and ITC was found in 30.56%, 61.11%, 33.33%, 19.44%, and 38.89% cases, respectively.

Conclusion: Species-level identification of *Candida* and their antifungal sensitivity testing should to be performed to achieve better clinical result and to select an appropriate and effective antifungal therapy. High resistance to antifungal agents is an alarming sign to the healthcare professionals.

Keywords: *Candida albicans*, Fluconazole, Non-*albicans Candida*, Voriconazole, Itraconazole

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Introduction

Candida spp. are the normal flora of human skin and mucosa, but have been reported more frequently as pathogen due to risk factors such as excessive consumption of a broad spectrum of antibiotics, underlying malignant diseases, HIV infection, organ transplantation, prolonged hospital stay, and exposure to invasive procedures [1, 2].

Candida spp. can cause a wide range of infections including blood stream infections (BSIs) and

disseminated candidiasis. In spite of advances in the diagnosis and treatment of candidiasis, among the pathogens involved in BSI, *Candida* ranks fourth in the United States and seventh in Europe [3-6]. Only few studies from India have reported candidemia rates (6-18%) [7-9] and increase in isolation of non-*albicans Candida* (NAC) from BSIs [10, 11]. In a recent study, the incidence rate of candidemia has been reported to be 6.9 per 1000 in intensive care unit (ICU) patients, and 7.5% of ICU patients receiving

antifungal therapy [12, 13]. Candidemia increases mortality rate by 20-49% [14, 15] and nosocomial candidiasis are associated with crude mortality rate of over 60%, while the attributable mortality rate may be as high as 49% [16, 17], but still there are many questions regarding management of candidiasis that remain unanswered.

The genus *Candida* is comprised of a heterogeneous group of organisms, and more than 17 different *Candida* spp. are known as etiological agents of human infection. However, more than 90% of invasive infections are caused by *Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis*, and *Candida krusei* [18].

A shift has been observed in the relative frequency of each *Candida* spp. isolated from blood. There are published data from various centers regarding the incidence and relative frequency of *Candida* spp. [19]. NAC are also implicated in the recent years [20-22].

Antifungal agents available for the treatment of systemic and invasive candidiasis are restricted to polyenes, allylamines, azoles, and the recently developed echinocandin class of molecules [23, 24]. Fluconazole (FLC) is an antifungal agent most commonly used for prophylaxis as it can be administered orally and is comparatively cheaper than other antifungal agents. Nonetheless, selection of appropriate empiric therapy is complicated considering the increasing prevalence of NAC species [25]. Adverse side effects, toxicity, and emergence of drug resistance are the limitations for use of polyenes, allylamines, and azoles. Emergence of drug resistance in *C. albicans* is reported all over the world [23, 24].

Incidence of antifungal resistance to *Candida* spp. has been on a growing trend over the past decade [26, 27]. Studies on the prevalence rate of infections and antifungal susceptibility testing can help with deciding on clinical strategies [28]. The potential clinical importance of species-level identification has been recognized as *Candida* spp. differs in expression of putative virulence factors and antifungal susceptibility [29, 30]. Rapid identification of *Candida* spp. can also help with early management of antifungal therapy. In the current study, we aimed to identify the epidemiology of *Candida* spp. among blood culture isolates and to investigate the susceptibility pattern of these species to antifungal agents.

Materials and Methods

This retrospective, observational study was performed in the microbiology laboratory of a

tertiary care hospital in Kolkata, India, during January 2011-March 2015. Ethics Committee permission was obtained prior to the study. From a total of 1735 positive blood cultures, only 70 *Candida* spp. were isolated from blood cultures.

Specimen collection

Specimens were collected under aseptic precaution, and blood culture was carried out by BacTAlert3D (Biomérieux, France) automated blood culture system.

Isolation and identification of *Candida* and antifungal susceptibility testing

Clinical isolates of *Candida* spp. obtained from blood culture using automated blood culture system were sub-cultured onto Sabouraud dextrose agar (HiMedia, India) and blood agar plates (HiMedia, India) after getting gram-positive budding yeasts on Gram stain of blood culture broth. Suspected colonies of *Candida* were confirmed through Gram stain and Germ tube test, and then were identified with VITEK 2 Compact (Biomérieux, France) using VITEK 2 cards for identification of yeast and yeast-like organisms (ID-YST cards) kits. Antifungal susceptibility testing was performed with AST YS07 Kits on VITEK 2 Compact. Standard operative procedures as described by the manufacturer were followed. Antifungal susceptibility testing was performed by NCCLS M44-A2 Disc diffusion method [31].

Results

All the 70 *Candida* spp. found to be microscopy and culture positive on both blood agar and SDA for *Candida* spp. were considered. Out of 70 samples, *C. albicans* were isolated from 34 (48.57%) and the remaining 36 (51.42%) samples were positive for NAC species. Among NAC, *Candida tropicalis* 17 (24.28%) followed by *Candida haemulonii* 6 (8.57%) had the highest frequency; the remaining NAC isolates are illustrated in Table 1 and Figure 1.

Table 1. Species distribution of *Candida*

Species	No. (%)
<i>Candida albicans</i>	34 (48.57%)
<i>Candida tropicalis</i>	17 (24.28%)
<i>Candida haemulonii</i>	6 (8.57%)
<i>Candida glabrata</i>	4 (5.71%)
<i>Candida pelliculosa</i>	2 (2.86%)
<i>Candida sake</i>	3 (4.29%)
<i>Candida rugosa</i>	2 (2.86%)
<i>Candida famata</i>	2 (2.86%)

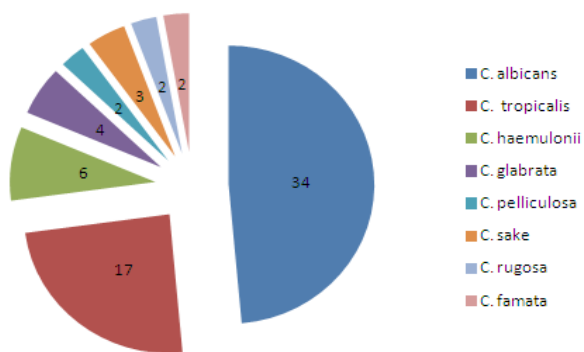


Figure 1. Species distribution of *Candida* isolates obtained from blood isolates

Table 2 demonstrates age and gender-wise distribution of patients. Out total 34 patients having isolates of *C. albicans*, 7 (20.59%) were female and 27 (79.41%) were male. Whereas, among the patients having NAC isolates, 19 (52.77%) were female and 17 (47.22%) were male.

Figure 2 shows *Candida albicans*; most of the patients were within the age group of 51-60 years, whereas for NAC, most of the patients were aged 31-60 years; it also shows that candidiasis is more prevalent in females than males.

Table 3 exhibits susceptibility pattern of

Table 2. Age and gender-wise distribution of *Candida albicans* and non-albicans *Candida* species

Age	<i>Candida albicans</i>		Non-albicans <i>Candida</i>		Total
	Male	Female	Male	Female	
0-10	1	1	4	2	8
11-20	2	2	2	0	6
21-30	6	0	2	1	9
31-40	0	1	0	7	8
41-50	1	0	2	5	8
51-60	7	2	5	2	16
61-70	7	1	0	2	10
71-80	3	0	2	0	5
Total	27	7	17	19	70

Table 3. Susceptibility of *Candida* species to antifungal drugs

Name of the organisms	Antifungal drug														
	Amphotericine B			Fluconazole			Flucytosine			Voriconazole			Itraconazole		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
<i>C. albicans</i> (n=28) (%)	13 (46.4)	0 (0.0)	15 (53.6)	28 (100.0)	0 (0.0)	0 (0.0)	10 (35.7%)	0 (0.0)	18 (64.3)	25 (89.3)	0 (0.0)	3 (10.7)	22 (78.6)	0 (0.0)	6 (21.4)
<i>C. tropicalis</i> (n=17) (%)	12 (70.59)	0 (0.0)	5 (29.41)	7 (41.18)	1 (5.88)	9 (52.94)	8 (47.06)	0 (0.0)	9 (52.94)	10 (58.82)	2 (11.76)	5 (29.41)	9 (52.94)	2 (11.76)	6 (35.29)
<i>C. haemulonii</i> (n=6) (%)	2 (33.3)	0 (0.0)	4 (66.67)	2 (33.3)	0 (0.0)	4 (66.67)	6 (100.0)	0 (0.0)	0 (0.0)	4 (66.7)	0 (0.0)	2 (33.3)	2 (33.3)	0 (0.0)	4 (66.67)
<i>C. glabrata</i> (n=4) (%)	4 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	4 (100.0)	4 (100.0)	0 (0.0)	0 (0.0)	4 (100.0)	0 (0.0)	0 (0.0)	4 (100.0)	0 (0.0)	0 (0.0)
<i>C. pelliculosa</i> (N=2) (%)	2 (100.0)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)
<i>C. sake</i> (n=3) (%)	3 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (100.0)	0 (0.0)	0 (0.0)	3 (100.0)	3 (100.0)	0 (0.0)	0 (0.0)	1 (33.33)	2 (66.66)	0 (0.0)
<i>C. rugosa</i> (n=2) (%)	2 (100.0)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100.0)
<i>C. famata</i> (n=2) (%)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	2 (100.0)	2 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	2 (100.0)
Total (n=64) (%)	38 (59.38)	0 (0.0)	26 (40.63)	41 (64.06)	1 (1.56)	22 (34.38)	34 (53.13)	0 (0.0)	30 (46.88)	50 (78.13)	2 (3.1)	12 (18.75)	40 (62.5)	4 (6.25)	20 (31.25)

S=Sensitive, R=Resistance, I=Intermediate

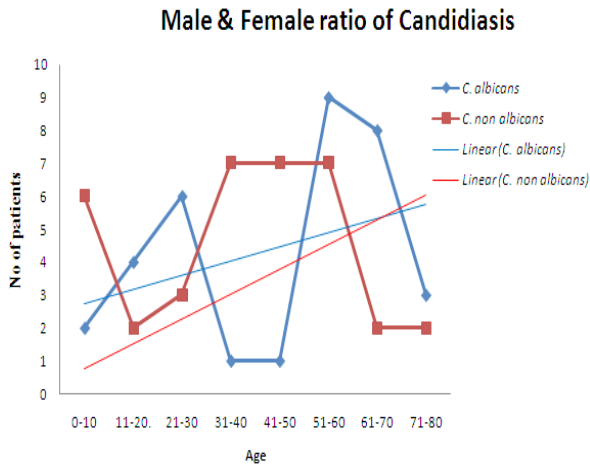


Figure 2. Male to female ratio of Candidiasis

Candida spp. to amphotericin B (AMP), FLC, flucytosine (5FC), voriconazole (VRC), and itraconazole (ITC). Out of all *Candida* spp, resistance to AMP, FLC, 5FC, VRC, and ITC was identified in 40.63%, 34.38% 46.88%, 18.75%, and 31.25% of the cases, respectively. *C. albicans* was found to be sensitive to FLC 100.0%, and resistance to AMP, 5FC, VRC, and ITC was detected in 53.6%, 64.3%, 10.7%, and 21.4% of the cases, respectively.

For NAC, resistance to AMP, FLC, 5FC, VRC, and ITC was found in 30.56%, 61.11%, 33.33%, 19.44%, and 38.89% of the cases, respectively. *C. albicans* showed maximum number of sensitive cases to FLC, whereas susceptibility pattern of few NAC species to caspofungin shows 100% sensitivity pattern, which may help healthcare professionals to treat *Candida* infection caused by NAC in the near future.

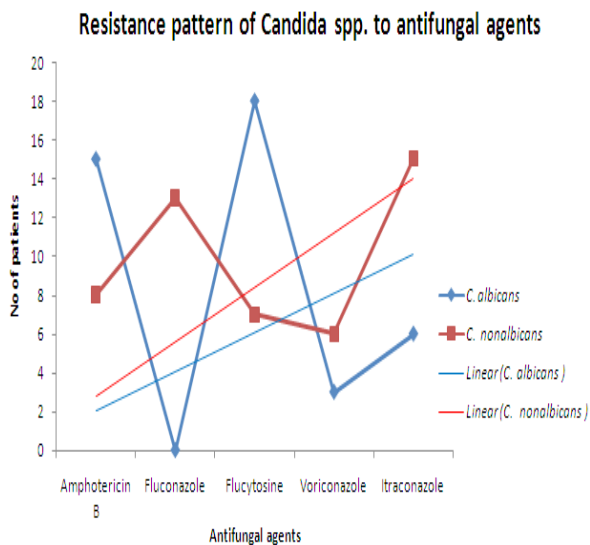


Figure 3. Resistance pattern of *Candida* species to different antifungal agents

Figure 3 exhibits the resistance pattern of *Candida* spp. to different antifungal agents. According to this figure, NACs are more resistant to antifungal agents compared to *Candida albicans*.

Discussion

Infection represents a frequent complication among the patients admitted to tertiary care hospitals. *Candida* spp. infections have increased in the last few decades, particularly those caused by the NAC, indicating the importance of laboratory diagnosis for the correct identification of species involved and initiation of timely and adequate treatment [32].

In our study, the *Candida* spp. were isolated from 4.03% total positive blood culture for growth. Great variation in the prevalence and incidence of candidemia has been reported from India. Kumar et al. [33] from South India reported incidence of 5.7% for candidemia among children with hematological malignancy; Verma et al. [34] reported incidence rate of 1.61%, whereas Xess et al. [8] found a prevalence rate of 6% for candidemia. A study by Sahin et al. [35] performed in Moulana Azad Medical College, New Delhi, India, found incidence rate of 6.9% for *Candida* spp.

The variables considered in this study include age, gender, and the hospital units (Figure 4), in which patients were infected. Furnaleto et al. [36] observed that infection caused by *Candida* spp. were more frequent in the elderly, those aged less than one year old, and ICU patients [37, 38]. The age and gender distribution of our study correlates the observation of other researchers in India [34, 39]. In our study, *C. albicans* has traditionally been the

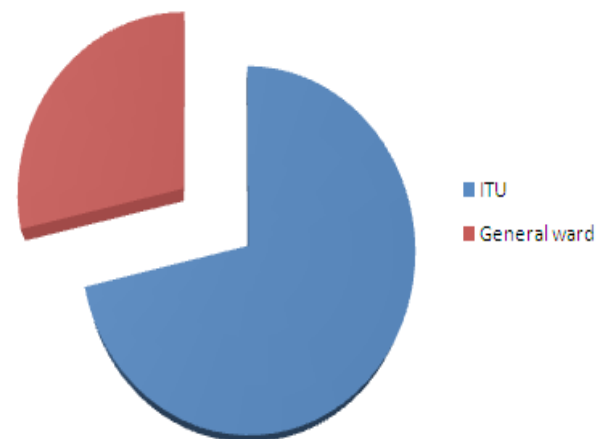


Figure 4. The Distribution of *Candida* spp. in different wards

leading cause of candidemia worldwide, but this study shows that NAC infection is on a growing trend [40-42]. Although significant geographic variation is observed among cases of candidemia in different parts of the world, which appears to follow a specific pattern. In our study, BSIs caused by NAC were more common than those caused by *C. albicans*, more specifically in Asia, South Europe, South America, and the Indian subcontinent [43, 44].

In the present study, the incidence rate of BSIs caused by *C. albicans* was 48.57%, which is similar to the reports from United States, Europe, and Brazil [45-47]. Herein, we found *C. tropicalis* following *C. albicans* was the most predominant species, which is in line with other Indian studies showing *C. tropicalis* (35-45%) as the predominant isolates [8, 9, 11, 44, 48, 49]. We did not identify *C. parapsilosis*, which is the causative agent in the most cases of BSI and candidemia [8, 10, 41, 50-52], and *C. krusei*, which has low prevalence in all settings and geographical regions [43].

C. glabrata has emerged as an important opportunistic pathogen worldwide. It is the second most common yeast isolated as part of normal flora and its role as a pathogen has only been recognized in the past few decades. Trick et al. [41] reported a remarkable increase in the incidence rate of isolation of *C. glabrata* from BSI patients, and there is concern over the increase in azole resistance among *C. glabrata* strains.

With the increased incidence of candidemia and the growing number of antifungal agents, laboratory aids with the accurate selection of antifungal therapy. The standardized broth microdilution method is expensive, laborious, and cumbersome for use in clinical microbiology laboratories. Recently, disc diffusion method has been approved by the Clinical and Laboratory Standards Institute (CLSI) for antifungal susceptibility testing of yeasts. We have confirmed the susceptibility pattern by VITEK 2 automated system since VITEK 2 system is a reliable technique for antifungal susceptibility testing of yeast species; this technique can be clinically useful for determining susceptibility of *Candida* spp. and other yeast species. It is also a reliable technique for in-vitro identification of azole and AMP resistance. It has the advantage of being more rapid and easier than the alternative procedures developed by either the CLSI or the EUCAST [53] and the disk diffusion method [31].

Even though CHROM agar helps with

identification at a lower cost compared to VITEK 2, which is useful in low resource countries, its main drawback is the long duration that it takes for complete identification. As early and rapid instillation of treatment can save the life of patients with fungal infections, rapid diagnosis is of utmost importance. Accordingly, VITEK 2 can be applied for rapid identification [54]

In our study, FLC resistance rate of *Candida* spp. was 34.8%, which is in line with other Indian studies [9, 33]. Resistance to FLC is of great concern as it is the most common azole used for the treatment of disseminated candidiasis including candidemia. FLC is available in both intravenous and oral formulations with high bioavailability and is more cost-effective than other antifungal agents. Although AMP is effective against most strains of *Candida* spp., it is not the first-line treatment for candidemia due to the nephron toxicity associated with it. ITC is used for the treatment of mucosal candidiasis [55]; studies regarding its role in the treatment of candidemia are sparse. In a study by Kothari et al. [9], 24% of *Candida* isolates were resistant to ITC, which is consistent with our result.

The decrease in susceptibility of *Candida* isolates to FLC (75%) is a matter of concern although VRC, AMP, and 5FC continue to show good efficacy. Western data have revealed that *Candida* species are reliably susceptible to polyenes, azoles, and echinocandins, but Indian studies demonstrate very high resistance to FLC for all *Candida* isolates although AMP susceptibility is high [56], which is in agreement with our findings.

In this present study, caspofungin shows 100% sensitivity pattern to few NAC species, which may be useful for healthcare professional to treat the *Candida* infection caused by NAC. Widespread use of FLC in various clinical conditions is the major cause of NAC dominance over *C. albicans* [27].

With various types of antifungal agents available in the market, performing antifungal susceptibility testing and reporting their therapeutic outcome seems to be necessary. Evaluation of the recent antifungal agents is required, as well.

Our study is limited to a single institution's experience, other shortcomings of this study include retrospective design and scarcity of data on the costs associated with *Candida* infection and virulence among *Candida* species in different age groups. Additionally, the small number of isolates of *Candida* spp. has limited our ability to evaluate the relative significance of these specific

non-albicans species.

Further studies with richer clinical data and larger samples are required to evaluate the costs associated with albicans and NAC species. In summary, the prevalence of *Candida* BSI has shifted dramatically from *C. albicans* to NAC spp. Therefore, early and accurate diagnosis of *Candida* infection is essential since each species varies significantly in susceptibility to the currently used antifungal drugs. Conducting antifungal susceptibility testing in the laboratories can aid clinicians with timely administration of the appropriate and accurate antifungal agents, it may also restrict the empirical use of the current antifungal agents.

Conclusion

The changing epidemiology of candidemia highlights the need for close monitoring of *Candida* species distribution and susceptibility to optimize treatment and outcome. We should also develop guidelines for empiric therapy based on the disease epidemiology in India.

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Author's contribution

P. B. designed the study, and drafted and prepared the manuscript.

Conflicts of interest

None declared.

Financial disclosure

There was no financial interest related to the materials of the manuscript.

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