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Microbiological Trends of Candidemia and Antifungal Susceptibility Pattern: Seven Year's Experience at a Multi Super - Specialty Center in Northern India

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Abstract: ***Introduction: The threat of Invasive fungal infections has been increasing in the contemporary health care scenarios, with candidal infections being the most common. Candidal bloodstream infections (BSIs) account for one-fourth of the nosocomial BSIs. Candida albicans once the most common has now been replaced by nonalbicans Candida (NAC), accounting for majority of cases of candidal infections. The knowledge and understanding of specific risk factors, epidemiology, prophylactic measures, and outcomes with relation to speciation are changing rapidly. Emergence of antifungal resistance is a cause of concern. With this background, a retrospective study was undertaken. Aims and Objectives: To isolate Candida spp. from blood cultures of patients with clinically diagnosed or suspected septicemia; to determine incidence of candidemia; to identify and speciate Candida isolates and carry out the antifungal susceptibility test. Materials and Methods: This retrospective study was conducted at the Department of Microbiology, CMC and Hospital, Ludhiana from January 2014 to December 2020. Blood cultures received during this period by BACTEC automated culture system; Becton Dickinson were included in the study. The positive blood culture bottles were cultured on Sabouraud dextrose agar. Recovered Candida isolates were speciated and antifungal susceptibility testing was performed as per Clinical and Laboratory Standards Institute guideline (CLSI). Results: A total of 396 out of 52, 066 blood cultures were culture positive for Candida species. Therefore the overall prevalence rate of isolation of Candida species was 0.76% in our study. The incidence of blood stream infection caused by Non albicans Candida species (72.98%) was higher than Candida albicans (27.02%). Among NAC spp. Candida tropicalis (28.28%) was the most common, followed by Candida parapsilosis (18.94%), Candida glabrata (11.87%), Candida krusei (9.09%), and Candida gullermondii (4.30%) and Candida dubliniensis (0.5%). Candida spp. demonstrated higher resistance to azole group of antifungal agents, as compared to amphotericin B. Resistance was significantly higher among NAC spp., Itraconazole, Clotrimazole, Fluconazole, Ketoconazole and Amphotericin B- 46.37%, 42.21% 39.10%, 34.60%, and 4.15%, 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: Blood stream infections, candidemia, Candida albicans, nonalbicans Candida (NAC), Fluconazole, Antifungal resistance

1. Introduction

The last few decades have witnessed a significant rise in the incidence of infections due to mycotic pathogens. Fungal infections have emerged as one of the most important cause of morbidity and mortality in immunocompromised and terminally ill immunocompetent individuals. [1]Among various pathogenic fungi, *Candida* spp. is the most pervasive pathogen capable of causing a broad spectrum of clinical manifestations ranging from mucocutaneous overgrowth to disseminated infections.[2] *Candida* spp. are normal commensals of human skin and mucosa, but have been reported more frequently as pathogens in blood stream infections (BSI) due to a wide range of risk factors such as excessive consumption of a broad spectrum of antibiotics, underlying malignant diseases, HIV infection, organ transplantation, prolonged hospital stay, total parenteral nutrition and exposure to invasive procedures such as central venous catheterization and urinary catheterization. [3, 4] In spite of advances in the diagnosis and treatment of candidiasis, *Candida* ranks fourth in the United States among the leading causes of BSI. European studies on candidiasis have reported Candida as 6th to 10th cause of nosocomial BSI.[5-8] As only few single centric and multi-centric studies are available from India the scenario of candidemia remains largely unclear.

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.* [9]Recent literature on invasive candidiasis clearly documents a shift towards non albicans *Candida* (NAC) species. The emergence of NACspp. has raised concern because NAC spp. frequently demonstrates intrinsic or acquired or both resistances to commonly used antifungal drugs.[10] However the search through available literature has revealed paucity of data regarding differences between the *C. albicans* and NAC spp. BSI.[11]

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.[12, 13] 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.[14] Adverse side effects, toxicity, and emergence of drug resistance are the

limitations for use of polyenes, allylamines, and azoles. Emergence of antifungal resistance to *Candida* spp. has been on a growing trend over the past decade.[15, 16] Studies on the prevalence rate of infections and antifungal susceptibility testing can help with deciding on clinical strategies.[17] The potential clinical importance of species-level identification has been recognized as *Candida* spp. differs in expression of putative virulence factors and antifungal susceptibility.[18, 19] Rapid identification of *Candida* spp. can also help with early management of antifungal therapy.

Aims & Objectives

The current study was conducted in a tertiary care teaching hospital in North India where we aimed to investigate the epidemiology of candidemia, further analyse the species distribution and investigate the susceptibility pattern of these species to common antifungal agents.

2. Materials and Methods

This retrospective study was conducted at the Department of Microbiology, Christian Medical College and Hospital, Ludhiana from January 2014 to December 2020. Blood cultures received during this period by Becton Dickinson BACTEC 9120 and 9050 automated culture system; were included in the study. Blood sample was collected in automated blood culture bottle under proper aseptic conditions. Then blood culture bottle was put in automated microbial detection system based on the colorimetric detection of CO2 produced by microorganisms. After signaling positive for blood culture bottle, samples were inoculated on routine culture media and further tests were performed. The isolates of Candida were identified to the species level. Primary identification was done by direct smear examination of blood samples by gram stain. Sample was inoculated on Sabouraud dextrose agar (SDA) and incubated at 37°C and 25°C for 48-72 hours. Typical Candida colonies, characterized by smooth, creamy, and pasty appearance on SDA were speciated using standard tests such as germ tube test, sugar assimilation test, sugar fermentation test, microscopic morphology on corn meal agar and color production on CHRO Magar media (Himedia Laboratories Pvt. Ltd. Mumbai, India). The Candida isolates were also subjected to antifungal susceptibility testing using the disk diffusion method on Müller–Hinton agar with 2% glucose and 0.5 lg/mL methylene blue, according to the standard Clinical and Laboratory Standards Institute (CLSI) guidelines. Antifungal drugs like Amphotericin B (100units/disc), Fluconazole (10mcg/disc), Itraconazole (30mcg/disc), Ketoconazole (10mcg/disc) and Clotrimazole (10mcg/disc) were used for antifungal susceptibility. For interpretation of sensitivity, zone sizes recommended by the CLSI guidelines corresponding to year were referred to.[20, 21]American Type Culture Collection (ATCC) strains *C. albicans* 90028, *Candida parapsilosis* 22019 and *Candida krusei* 6258 were used as controls.

Statistical Analysis-Descriptive statistics was used to summarize demographic and other clinical features of patients. Qualitative and quantitative data values were expressed as frequency along with percentage.

3. Results

During this study period total of 52, 066 blood culture samples were processed via automated blood culture machine BD BACTEC system. A total of 396 samples were positive for *Candida* species. Therefore, the overall prevalence rate of isolation of *Candida* species was 0.76% in our study. The prevalence rate of *Candida* species in blood stream infections has increased over the last four decades, various factors like; the AIDS epidemic, increases in the number of immunosuppressive therapy recipients and use of long term antibiotics therapy have altered the epidemiology of invasive mycoses, particularly in candidemia.

The numbers of different *Candida* species isolated from blood stream infection has been increasing during the last few years in different parts of world. More than 17 species of *Candida* species have been implicated in human infections till date and list of reported species continue to grow. In our study, the incidence of blood stream infection caused by Non albicans *Candida (*NAC) species was higher than *Candida albicans*. Among the 396*Candida* isolates on SDA (Figure1), 107 (27.02%) isolates were positive for germ tube test (Figure 2) and were identified as either *C. Albicans* or *C. dubliniensis*. To further identify *Candida albicans,* growth at 45°C and Chlamydospore formation on Corn Meal Agar (CMA) was carried out (Figure 3). Isolates which showed growth at 45°C and chlamydospore formation were identified as *Candidaalbicans.* whereas those which were negative for all the above tests were identified as NAC spp.289 (72.98%).

Based on these sugar fermentation and assimilation assays the NAC *spp.* Were identified: glucose was fermented by all *Candida* species, while sucrose was not fermented by *C.albicans, C. glabrata, C.krusei* and *C. parapsilosis*, trehalose was fermented only by *C. glabrata*. Lactose was not fermented by any of the isolated species. In sugar assimilation assays, again glucose was assimilated by all Candida species, sucrose and maltose by all except *C. krusei & C.glabrata* and lactose assimilated by only *C. albicans (*Table 1).

Candida tropicalis 112 (28.28%) was the most common, followed by *Candida parapsilosis* 75 (18.94%), *Candida glabrata* 47 (11.87%), *Candida krusei*36 (9.09%), *Candida gullermondii*17 (4.30%) and *Candida dubliniensis*2 (0.50%). (Figure 4)

Figure 1: Test Tube with SDA Agar Showing Smooth Cream Pasty Colonies

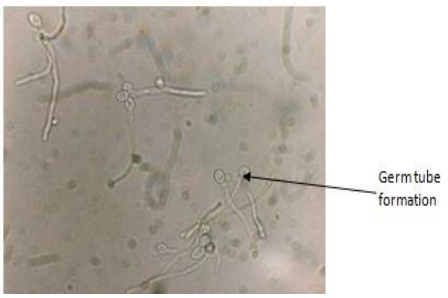

Figure 2: Germ Tube Formation in Human Serum Seen at 40x Magnification

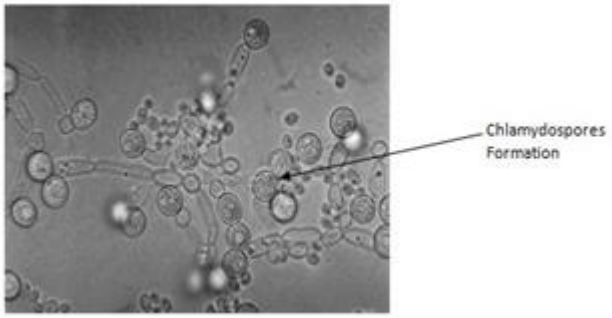

Figure 3: *C.albicans* Showing Branched Pseudo Hyphae and Chlamydospores Formation at 40 X Magnification

On growth on CHROM Agar Candida medium, dark green coloured colonies were seen for all isolates of *Candida albicans*, metallic blue coloured for all *C. tropicalis*, smooth cream colonies for Candida glabrata, purple fizzy colonies for *C. krusei*, light pink colored colonies for *C. parapsilosis* isolates, Pinkish purplecolonies for *C. gullermondii* and bluish green*C. dubliniensi (*Table 2, Figure 5).

On comparison between results obtained by sugar assimilation, fermentation and CHROM Agar methods, it was observed that both the methods showed similarresults for identification of *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. glabrata*, *C. krusei*, *C. gullermondii* and *C. dubliniensi*.

Table 1: Identification of Candida Species by Sugar Assimilation and Fermentation Reaction (Total No. of Isolates Tested =396)

Carbohydrate Assimilation Test					Carbohydrate Fermentation Test				*Candida* Spp. Identified (No.)
GLU	SUC	LAC	TRE	RAFF	GLU	SUC	MAL	LAC	
+	+	+	+	-	AG	-	AG	-	*C. albicans (107)*
+	+	-	+	-	AG	AG	AG	-	*C.tropicalis (112)*
+	+	-	+	-	AG	-	-	-	*C.parapsilosis (75)*
+	-	-	+	-	AG	-	-	-	*C. glabrata (47)*
+	-	-	-	-	AG	-	-	-	*C.krusei (36)*
+	+	-	+	+	AG	AG	-	-	*C. gullermondii (17)*
+	+	-	+	-	AG	-	AG	-	*C. dubliniensi (2)*

Table 2: Type of Coloured Colonies Produced by Various Candida Species on CHROM Agar (TotalNo. of Isolates Tested: 396)

Colour of the Colonies Produced on CHROM Agar	*Candida* Spp. Identified* (No.)
Dark green	*C. albicans* (107)
Metallic blue	*C. tropicalis* (112)
Light pink	*C. Parapsilosis* (75)
White to Cream	*C. glabrata* (47)
Purple fuzzy	*C. krusei* (36)
Pinkish purple	*C. gullermondii (17)*
Bluish green	*C. dubliniensi (2)*

*** As per manufacturer's instructions**

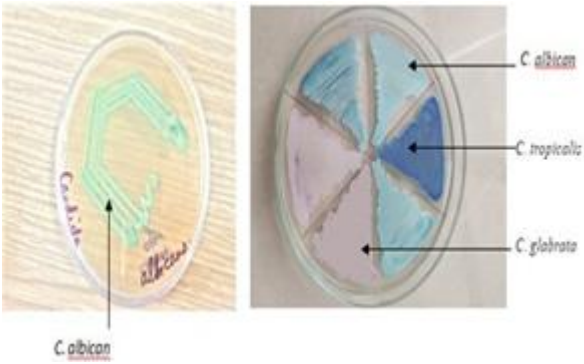

Figure 5: Growth of Candida Species on CHROM Agar

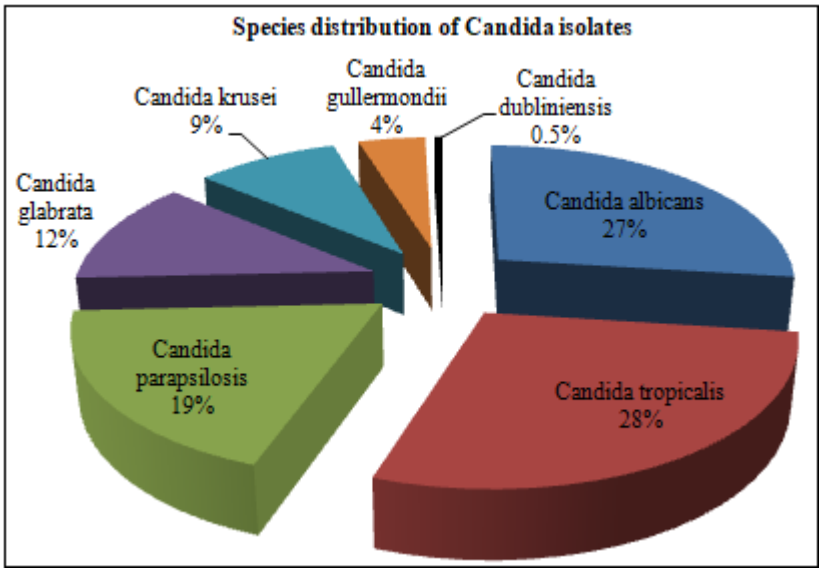

Figure 4: Species distribution of Candida isolates obtained from blood isolates

Majority of *Candida* spp. were isolated from adults patients (74%) followed by patients of age group <1 year (14%) whereas 12% strains were isolated age group 1-15 years. We noted a predominance of male patients (69%). The male to female ratio was 2:1.

Antifungal susceptibility profile of *Candida* spp. is shown in Table 2. As compared to Amphotericin B, *Candidaalbicans* demonstrated higher resistance to azole group of antifungal agents. Among azoles, *C. albicans*, demonstrated good sensitivity against Ketoconazole (80.37%) and Fluconazole (79.44%). Azole resistance was significantly higher among NAC spp. like *Candida tropicalis*, *Candida glabrata* and *Candida parapsilosis*. Because *Candidakrusei* is intrinsically resistant to azoles, it was not analyzed for sensitivity to the azole group of drugs. Amphotericin B was sensitive in 100% isolates of *Candida albicans*, *Candida parapsilosis* and *Candida krusei*, followed by *Candida tropicalis* (96.43%), *Candida glabrata* (93.62%) and *Candida gullermondii* (82.35%). The *Candida dubliniensis* isolates were resistant to Amphotericin B. For NAC *spp*., resistance to Amphotericin B, Fluconazole, Ketoconazole, Itraconazole and Clotrimazole was found in 95.85%, 60.90%, 65.40%, 53.63%, and 57.79% of the cases, respectively.

Table 3: Comparison of antifungal susceptibility between NAC and *C. albicans*

Candida Species	Fluconazole	Ketconazole	Itraconazole	Clotrimazole	Amphotericin B
C.albicans (107)	85 (79.44%)	86 (80.37%)	77 (71.96%)	73 (68.22%)	107 (100%)
NAC (289)	176 (60.90%)	189 (65.40%)	155 (53.63%)	167 (57.79%)	277 (95.85%)
Total (396)	261 (65.91%)	275 (69.44%)	232 (58.59%)	240 (60.61%)	384 (96.97%)

Table 4: Antifungal susceptibility patterns of Candida isolates

***Candida* Species**	Fluconazole	Ketconazole	Itraconazole	Clotrimazole	Amphotericin B
C. albicans (107)	85 (79.44%)	86 (80.37%)	77 (71.96%)	73 (68.22%)	107 (100%)
C. tropicalis (112)	78 (69.64%)	89 (79.46%)	79 (70.54%)	80 (71.43%)	108 (96.43%)
C. parapsilosis (75)	58 (77.33%)	65 (86.67%)	46 (61.33%)	48 (64%)	75 (100%)
C. glabrata (47)	29 (61.70%)	23 (48.94%)	20 (42.55%)	28 (59.57%)	44 (93.62%)
C. krusei (36)	0	0	0	0	36 (100%)
C. gullermondii (17)	9 (52.94%)	12 (70.59%)	10 (58.82%)	9 (52.94%)	14 (82.35%)
C. dubliniensi (2)	2 (100%)	0	0	2 (100%)	0

4. Discussion

Many reports in recent years have highlighted increase in the incidence of mycoses in general and candidiasis in particular. Among various clinical types of candidiasis, candidemia is usually associated with high mortality rates. [22]

Intravascular catheterization, prolonged antibiotic exposures, long-term bedridden patients, and various immunocompromised conditions increase the chances of opportunistic pathogens and nosocomial agents to predominantly cause candidemia. It also significantly increases health-care costs and duration of hospital stay. Candidemias are reported as the fourth common cause of BSIs in the Intensive Care Units (ICUs) and account for 10% of all BSIs. [23, 24] In another study, this statistic was 8–15%. [25]In the present study, candidemia accounted for 0.76%. Several studies done in India shows prevalence rate of Candidemia varies from 0.65% to 6.9% (Giri *et al*., 2013; Verma *et al*., 2003; Sanhi *et al*., 2005; Deorukhkhar *et al*., 2012; Behera *et al*., 2020). [26- 30]

Majority of *Candida* spp. were isolated from adults male patients followed by patients of age group <1 year (14%) whereas 12% strains were isolated age group 1-15 years.

These findings are similar to a number of reports from all around the Indian subcontinent. [31, 32]

In accordance to various studies from different parts of world, the present report also documents the predominance of NAC spp. (73%) over *C.albicans (*27%). Several factors are implicated for emergence of NAC spp. these include empirical prophylactic and therapeutic use of azoles, use of chromogenic media and commercially available user-friendly kits for rapid identification of yeasts and yeast like fungi. [33] Epidemiological studies from India have reported NAC spp. in as many as 67-90% cases of *Candida* BSI. [30, 34, 35] Previously, *C.albicans* accounted for 70–80% of candida disease. However, in the last 10 years, infections due to Nonalbicans species account for a majority of invasive candidal infections. Available literature on species distribution of Candida has pointed out the significant variation with respect to frequency of isolation of NAC spp. from BSI. The highest proportion of *C. parapsilosis* is reported from some hospitals of North America and Europe by contrast the incidence of infection due to *C. glabrata* was reported to high in studies from US and North and Central Europe. The species distribution in Asia varies greatly by the geographic region and type of health-care setup. [36]More than 90% of invasive infections are attributed to five species: *C. tropicalis*, *C. albicans*, *C. glabrata*, *C. parapsilosis*, and *C. krusei*. [34] Majority of studies worldwide quote, *C. tropicalis* as the now most common NAC. [16, 30, 37, 38] In the present study also, *C. tropicalis* was the most common isolate (29.5%), followed by *Candida albicans (*26.5%), *Candida parapsilosis (*20.2%), *Candida glabrata (*12.3%), *Candida krusei (*8.2%), *Candida gullermondii (*3%) and *Candida dubliniensis (*0.3%). This finding is in consistent to that of other researchers from India. [32, 38, 39]

Studies on the NAC spp. show a marked difference in behavior between different *Candida spp*. This includes factors which affect the ability to cause disease and development of mechanisms of drug resistance. Therefore, to apply the findings in *C.albicans* to the NAC species would be a fallacy. [40]

Several classes of antifungal drugs (azoles, echinocandins and polyenes) are available for treatment of candidemia. The choice of antifungal drug depends on various factors; the local epidemiology and the patient's co-morbidities. The emergence of NAC spp. has initiated the need of antifungal susceptibility testing of Candida isolates. Antifungal resistance once rarely documented has increased rapidly in recent years. The problem is compounded by aggressive immunosuppression (acquired or induced), an ageing population, and the emergence of virulent and intrinsically resistant organisms. [41] In this study, NAC spp. demonstrated significantly high resistance to azoles compared to *C. albicans*. Similar findings were found in a study from the Himalayan region of northern India and also from Maharashtra.[33, 39] Resistance to azole group of antifungal agents can be due to quantitative or qualitative modifications of target enzymes, low access of the drug to the target, or a combination of these mechanisms. [42] In contrast to *C. albicans*, antifungal susceptibility varies significantly in NAC spp. Some NAC *spp*. are inherently or secondarily resistant to antifungal agents. [16] Fluconazole resistance was observed in 33% of Candida isolates. Various studies in India have reported fluconazole resistance between 4.9% and 37.5% among all Candida isolates. [23, 34] Resistance to fluconazole is of concern because it is one of the most widely used first line antifungal agents for treatment and prophylaxis of all forms of candidiasis, especially in low birth weight infants.[33] We noted, Fluconazole resistance was almost twice as high among NAC (39.1%) in comparison to *C. albicans* (20.6%). Also, among *C.tropicalis* isolates fluconazole resistance was 30.36%. *C. tropicalis* was initially regarded as a fluconazole susceptible species; however, the scenario has changed over the period of last few years.[42] The increasing rate of fluconazole resistance in *C.tropicalis* is important because it is one of the most commonly isolated NAC spp. As the reason for rapid emergence of fluconazole resistance in *C. tropicalis* is unclear, the need of further studies is underscored.

Our study showed, amphotericin B resistance in only 3.03% Candida isolates. The maximum resistance was demonstrated by *C. dublinensis* (100%), *C. gullermondii (*17.65%) and *C. glabrata* (6.38%) isolates. Montagna et al. reported high amphotericin B resistance in *C. glabrata* isolates compared to other NAC spp.[43] On the other hand Amphoricin B resistance by *C. gullermondii* is rare.[44]*C. albicans* was 100% susceptible to amphotericin B, mimicking the sensitivity pattern noticed in other studies around the world.[45, 46, 47] There have been a few reports of emerging resistance Amphotericin B in various formulations has been used for the treatment of disseminated candidiasis and candidemia.[48, 49, 50] Although amphotericin B hasa rapid cidal action against most strains of Candida species (especially *C. albicans*), it is not the first choice for treatment of cases of candidemia because of the nephrotoxicity associated with it.[51]Secondary resistance to amphotericin Balso appears to be an uncommon development. There have been reports of some cases of disseminated infections due to *C. glabrata*, *C. krusei*, and *C. albicans* isolates that developed amphotericin B resistance during treatment. [13] The mechanism of amphotericin B resistance appears to bean alteration or a decrease in the amount of ergosterol in the cell membrane.[52] Since each species varies significantly in susceptibility to the currently used antifungal drugs. Therefore, early and accurate diagnosis of *Candida* infection is essential. 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. Our study differs from the previous studies in the fact that all the previous studies were restricted to small sample size. In this study, we have examined the sensitivity pattern in large number of various clinical isolates. Reduced susceptibility as well as frank resistance to drugs such as azoles, as observed in our study, is an issue of crucial importance in treatment of immune compromised patients with serious infections. Hence, antifungal susceptibility testing is a promising tool for predicting the efficacy of a given agent.

5. Conclusion

In the present study, we identified the magnitude of candidemia from the blood culture sample received from patients in a tertiary care hospital and characterized the *Candida* isolates. Emergence of NAC spp. and their resistance to antifungals is a matter of concern. The changing epidemiology of candidemia emphasizes the need for monitoring of distribution of *Candida* species and antifungal susceptibility testing to develop guidelines on empiric therapy for invasive candidiasis, based on the epidemiology of infection which will help us to recognize the emerging fungal pathogen and drug resistance.

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