Prevalence of *Candida* Species and its Antifungal Susceptibility Isolated from Blood Culture at Tertiary Care Hospital, Ahmedabad, India

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**Abstract**

Blood infection due to *Candida* species are major cause of morbidity and mortality in hospitalised patients. The spectrum of candidemia has changed to Non albicans *Candida* species and also shows resistance to commonly used azoles drugs. To study prevalence of *Candida* species isolated from blood culture samples and to isolate and identify different *Candida* species with their antifungal susceptibility testing. Blood samples were collected and incubated in automated blood culture system. *Candida* species was isolated by culture on sabouraud dextrose agar and species identification done by standard biochemical reactions. Antifungal susceptibility testing done by disc diffusion method as per CLSI guidelines. Out of total 6455 blood samples 1399 (21.67%) samples are positive for growth. Pure growth of *Candida* species was seen in 100(7.14%) positive blood samples. Among total 100 isolates, 41(41%) was *Candida albicans* followed by *Candida glabrata* 25(25%), *Candida tropicalis* 23(23%), *Candida krusei* 5(5%), *Candida parapsilosis* 5(5%) and *Candida gullermondii* 1(1%). Antifungals drug like fluconazole shows 25% resistance, Voriconazole 23% resistance, Itraconazole 36% resistance and Miconazole 20% resistance among different *Candida* species. Antifungal susceptibility shows lower resistant (3%) to amphotericin B, where high incidence of azole resistance among Non albicans *Candida* species. 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**

Candidemia, Non albicans Candida, Antifungal susceptibility.

**Introduction**

*Candida* is the yeast like fungi which normally exist within mouth, throat, intestine, genital and urinary tract of human being (Chander et al., 2009). Candidemia is defined as at least 1 blood culture bottle positive for *Candida* species and other sign of blood stream infection. Episodes were considered separate if they occur 1 month apart or were caused by different species (Chen et al., 2003; Sandven et al., 2006). Candidemia is a life threatening fungal infection associated with mortality rate of 38% and prolongs hospital stay by as much as 30 days (Wey et al., 1998). *Candida* species infection are among the four most common causes for hospital acquired infection, catheter associated UTI and blood stream infection (Zaoutis et al., 2005). Candidemia is a manifestation of invasive candidiasis that could have originated in a variety of organs, whereas for others, candidemia originated from an infected indwelling catheter (Frikdin, 2005). The spectrum of Candidemia has changed
with the emergence of non albicans Candida (NAC) species, a strain with threat of increasing mortality and antifungal drug resistance (Horvath et al., 2003).

In all cases, Candidemia requires treatment with an antifungal drug (Pappas et al., 2009). Several studies noted the high mortality rates associated with candidemia and have shown that mortality is highest in those patients who were not treated with an antifungal drug (Fraser et al., 1992; Nguyen et al., 1995). Early and prompt diagnosis, proper treatment for microbiologist and clinician worldwide added to this is emerging drug resistance to antifungal to the Candida species.

Materials and Methods

This study was conducted in microbiology department at tertiary care hospital, Ahmedabad during July 2012 to May 2013 over 11 month’s duration. Blood sample was collected in automated blood culture bottle under total aseptic precautions. Then blood culture bottle was put in automated microbial detection system based on the colorimetric detection of CO₂ produced by microorganisms. After signalling positive for blood culture bottle, samples were inoculated on routine culture media and further tests were performed.

Primary identification done by direct smear examination blood samples by wet mount and gram stain. Sample was inoculated on Sabouraud dextrose agar (SDA) screw cap bottle and incubated at 37°C and 25°C for 48-72 hours. After growth, species identification done by Germ tube test, corn meal agar test, chrom agar inoculation, sugar assimilation test, sabouraud dextrose broth, urease test (Odds, 1998; Forbes et al., 2007; Hospenthal et al., 2006). Antifungal susceptibility testing done by disc diffusion method as described in CLSI document M-44-A(2) (Clinical Laboratory Standard institute Guidelines, 2009). Muller Hinton agar plates supplemented with 2% glucose and 0.5µg/ml methylene blue was used. Antifungal drugs like Amphotericin B (100units/disc), Fluconazole (10mcg/disc), Itraconazole (30mcg/disc), Miconazole (30mcg/disc), Voriconazole (1mcg/disc) were used for antifungal susceptibility.

Results and Discussion

During this study period total 6455 blood culture samples were processed in automated blood culture machine by colorimetric method. Out of that 1399 (21.67%) blood samples were signalling positive and processed for culture for identify organism. Out of 1399 signalling positive samples, 100 samples were identified as Candida species growth. A total of 100 samples were positive for Candida species out of total 6455 blood samples. So prevalence of Candida species was 1.54% (100/6455) in our study (Figure 1).

Out of the 100 Candida isolates, 41(41%) were Candida albicans, followed by Candida glabrata 24(25%), Candida tropicalis 23(23%), Candida krusei 5(5%), Candida parapsilosis 5(5%) and Candida gullermondii 1(1%) (Figure 2).

In this study, Candida infection was more common (73%) in 0-10 years of age group, followed by 13% in 11-40 years and 14% in 41-80 years of age group (Figure 3). Candida infection was more common in male 67% as compare to female 33% (Figure 4). Antifungal susceptibility pattern shows that Non albicans Candida species like Candida tropicalis, Candida glabrata and Candida parapsilosis tends to high resistant to azoles. Candida krusei is innately resistant to fluconazole. Amphotericin B was sensitive in 100% in Candida albicans, Candida parapsilosis and Candida krusei followed by 95.65% in Candida tropicalis and 92% in
*Candida glabrata* (Table 1). The prevalence rate of *Candida* species in blood stream infection increase in last three decades, in last few years, various factors like 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. More recently, Non albicans *Candida* species has been recovered with increasing frequency with more resistance to antifungal drugs.

In our study, the overall prevalence rate of isolation of *Candida* species from blood culture was 1.54% and 4th most common causes for blood stream infection. 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) (Table 2).

Table.1 Antifungal susceptibility pattern of different *Candida* species

<table>
<thead>
<tr>
<th><em>Candida</em> Species</th>
<th>Amphotericin B</th>
<th>Fluconazole</th>
<th>Voriconazole</th>
<th>Itraconazole</th>
<th>Miconazole</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. albicans</em> (41)</td>
<td>41 (100%)</td>
<td>36 (87.80%)</td>
<td>35 (85.36%)</td>
<td>29 (70.73%)</td>
<td>32 (78.04%)</td>
</tr>
<tr>
<td><em>C. glabrata</em> (25)</td>
<td>23 (92%)</td>
<td>16 (64%)</td>
<td>18 (72%)</td>
<td>12(52.17%)</td>
<td>21 (84%)</td>
</tr>
<tr>
<td><em>C. tropicalis</em> (23)</td>
<td>22 (95.65%)</td>
<td>18 (78.26%)</td>
<td>15 (65.21%)</td>
<td>15 (65.21%)</td>
<td>21 (91.30%)</td>
</tr>
<tr>
<td><em>C. parapsilosis</em> (5)</td>
<td>5 (100%)</td>
<td>4 (80%)</td>
<td>4(80%)</td>
<td>4 (80%)</td>
<td>3(60%)</td>
</tr>
<tr>
<td><em>C. krusei</em> (5)</td>
<td>5 (100%)</td>
<td>0(0%)</td>
<td>4(80%)</td>
<td>3(60%)</td>
<td>2(40%)</td>
</tr>
<tr>
<td><em>C. gullermondii</em> (1)</td>
<td>1(100%)</td>
<td>1(100%)</td>
<td>1(100%)</td>
<td>1(100%)</td>
<td>1(100%)</td>
</tr>
</tbody>
</table>

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* species was higher than *Candida albicans* (Table 3).
Table.2 A comparative study of prevalence of *Candida* species isolated from blood culture

<table>
<thead>
<tr>
<th>Study</th>
<th>Prevalence of <em>Candida</em> species isolated from blood culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present Study</td>
<td>1.54%</td>
</tr>
<tr>
<td>S.Giri <em>et al.</em>, (2013) Christian Medical College, Vellore, Tamil Nadu</td>
<td>0.65%</td>
</tr>
<tr>
<td>Verma <em>et al.</em>, (2003) Sanjay Gandhi Post Graduate Institute for Medical Sciences, Lucknow</td>
<td>1.61%</td>
</tr>
<tr>
<td>Oberoi <em>et al.</em>, (1988) Sir Ganga Ram Hospital, New Delhi</td>
<td>1.74%</td>
</tr>
<tr>
<td>Sahni <em>et al.</em>, (2005) Maulana Azad Medical College, New Delhi</td>
<td>6.9%</td>
</tr>
<tr>
<td>Sachin Deorukhkar <em>et al.</em>, (2012) Rural Medical College, Loni, Maharashtra</td>
<td>3.9%</td>
</tr>
<tr>
<td>Xess <em>et al.</em>, (2007) All India Institute of Medical Science (AIIMS), New Delhi</td>
<td>6.0%</td>
</tr>
</tbody>
</table>

Table.3 A comparative study of prevalence of different *Candida species* isolated from blood culture

<table>
<thead>
<tr>
<th>Species</th>
<th><em>C.albicans</em></th>
<th><em>C.glabrata</em></th>
<th><em>C.tropicalis</em></th>
<th><em>C.parapsilosis</em></th>
<th><em>C.krusei</em></th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present Study</td>
<td>41%</td>
<td>25%</td>
<td>23%</td>
<td>5%</td>
<td>5%</td>
<td>1%</td>
</tr>
<tr>
<td>Frank C. Odds <em>et al.</em>, (2007) Scotland UK</td>
<td>52%</td>
<td>22%</td>
<td>2.0%</td>
<td>11.7%</td>
<td>1.0%</td>
<td>11.3%</td>
</tr>
<tr>
<td>Peter G Pappas <em>et al.</em>, (2009) University of Alabama USA</td>
<td>46%</td>
<td>20%</td>
<td>12%</td>
<td>14%</td>
<td>2%</td>
<td>6%</td>
</tr>
<tr>
<td>David L. Horn <em>et al.</em>, (2019) Philadelphia, Pennsylvania</td>
<td>45.6%</td>
<td>26.0%</td>
<td>8.1%</td>
<td>15.7%</td>
<td>2.5%</td>
<td>2.1%</td>
</tr>
<tr>
<td>Lena Rose (2013) Asmundsdottir <em>et al.</em>, Iceland</td>
<td>56%</td>
<td>16%</td>
<td>13%</td>
<td>5%</td>
<td>4%</td>
<td>6%</td>
</tr>
<tr>
<td>Sachin Deorikhkar (2012) Maharastra</td>
<td>40.2%</td>
<td>13.9%</td>
<td>26.8%</td>
<td>2.5%</td>
<td>10%</td>
<td>6.6%</td>
</tr>
</tbody>
</table>
Table.4 A comparative study of antifungal resistance in all *Candida species* isolated from blood culture

<table>
<thead>
<tr>
<th>Study</th>
<th>Percentage resistance to antifungal drugs in all <em>Candida</em> spp. isolated from blood culture</th>
</tr>
</thead>
</table>
| **Present Study**                                   | Fluconazole (25%)  
Itraconazole (36%)  
Voriconazole (23%)  
Amphotericin B (3%)                                 |
| Kothari *et al.*, (2009) New Delhi                 | Fluconazole (36%)  
Itraconazole (24%)  
Voriconazole (56%)                                  |
| Jaswinder Kaur Oberoi *et al.*, (2012) New Delhi   | Fluconazole (21.2%)  
Itraconazole (45.7%)  
Voriconazole (11.4%)  
Amphotericin B (10.4%)                              |
| Sachin C. Deorukhkar (2012), Maharastra            | Fluconazole (19.07%)  
Amphotericin-B (4.63%)                              |
| Kumar *et al.*, (2005) Chennai                      | Fluconazole (17.2%)                                                                   |
| Gupta *et al.*, (2001) New Delhi                    | Fluconazole (37.5%)                                                                   |
| Xess *et al.*, (2007) New Delhi                     | Fluconazole (11.7%)                                                                   |
| S. Giri *et al.*, (2013) Tamil Nadu                 | Fluconazole (30.8%)                                                                   |

**Fig.1** Prevalence of various organisms isolated from blood culture

![Prevalence of various organisms](image-url)
Fig. 2 Different *Candida* species isolated from blood culture

Fig. 3 Age distribution of patients from which *Candida* species isolated

Fig. 4 Sex distribution of patients from which *Candida* species isolated
Neonates and infants have historically been populations with some of the highest rates of Candidemia (Shetty et al., 2005). In our study 72% of Candidemia were seen in neonates and infants. Candidemia is a significant cause of mortality and morbidity in neonates admitted in neonatal Intensive Care Unit. *Candida* species are the most common fungal pathogen isolated from blood culture of neonates. A numbers of risk factors like low birth weight baby, prematurity, prolonged antibiotics therapy and artificial ventilation are associated with Candidemia (Sardana et al., 2012; Rani et al., 2002). In our study all *Candida* isolates shows 25% resistance to Fluconazole, 23% resistance to Voriconazole, 20% resistance to Miconazole, 36% resistance to itraconazole and only 3% resistance to amphotericin B. In India, there is lack of multicentric studies regarding antifungal susceptibility pattern. Goel et al., (2009) and Cooper et al., (2005) reported less incidence of resistance to fluconazole. On the other hand, Kumar et al., (2005), Kothari et al., (2009), and Gupta et al., (2001) reported high incidence of resistance to fluconazole (Table 4).

Although fluconazole still remains a safe and effective choice for the treatment of candidemia, an increase trend of fluconazole resistance in *Candida* isolates from blood has been reported mainly due to the changing spectrum of *Candida* species causing candidemia from *C. albicans* to Non albicans *Candida* species especially *C. glabrata* and *C. krusei*. In our study the highest rate of resistance to fluconazole was for *C. glabrata* (36%) and for *C. tropicalis* (22%) which was consistent with other studies in which the greatest resistance to fluconazole also showed *C. glabrata* (36%).

The resistance to fluconazole is of great concern because it is the most commonazole used for treatment of disseminated candidiasis including candidemia. It is available in both intravenous and oral formulation with high bioavailability and is more cost effective than other antifungal agents. Although Amphotericin B is effective against most strains of *Candida* species, it is not the first drug of choice for the treatment of candidemia because of nephrotoxicity associated with it. Many potential mechanisms of azole resistance have been proposed. Alteration of drug efflux, reduced intracellular accumulation of fluconazole due to change CDR genes and increased expression of ATP-binding cassette transporter gene are some of the mechanism for azole resistance in *Candida* species (Loffler et al., 2003; Alberstone et al., 1996).

In conclusion, our study shows *Candida* as among most common causes for blood stream infection. A significant epidemiological shift to higher isolation of Non albicans *Candida* species was noted because of high usage of fluconazole, patient’s specific risk factors and also newer available diagnostic method. Hence it is essentials that early and accurate diagnosis is made of infecting species of *Candida* and its antifungal susceptibility testing be carried out routinely in laboratory.

**References**


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**How to cite this article:**