Speciation of candida species isolated in clinical samples in a tertiary health care centre in Northern India

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1. Introduction

Candida is yeast like fungus. It is a ubiquitous human commensal. It becomes pathogenic and causes infections when the host’s resistance to infection is lowered either locally or systemically.¹

The genus Candida comprises of about 150 yeast species, which is composed of a heterogeneous group of organisms & consists of more than 17 different Candida species that are responsible for different human infections.² The commonest pathogenic species of this genus is Candida albicans, it has namely A and B, on basis of their differences between mannan components of cell wall.³

Candida is the most opportunistic fungal pathogen, which results in various manifestations ranging from mucocutaneous lesions to life threatening invasive diseases. Although Candida albicans is the most common cause of candidiasis accounting for about 60-80% of infections, a shift towards non albicans Candida species is evident in recent years.⁴ It include various species such as C.glabrata, C.tropicalis, C.parapsillosis. Among these, C.tropicalis is one of the most common non albicans Candida species
isolated from various clinical types of candidiasis.\textsuperscript{5}

The morbidity and mortality associated with candidal infections are significant and have emerged as important public health problems. Increasing use of broad-spectrum antibiotics, intravascular catheters, cytotoxic chemotherapies, invasive surgical procedures and long duration of hospital stay are few of the pre-disposing risk factors.\textsuperscript{6}

The emergence of non albicans Candida species may represent selection of less susceptible species like C. glabrata and C.krusei. C. glabrata is less susceptible and C. krusei is intrinsically resistant to Fluconazole. C. tropicalis has the highest adherence rate to inanimate materials such as urinary and vascular catheters, and is often involved in biofilm formation, that is more resistant to antifungal agents. Resistance to azoles in C. tropicalis and C. albicans has also been increasingly reported.\textsuperscript{7}

The azole drugs have been commonly used to treat many forms of Candida infections for a long time. Resistance to the azole is seen more in non albicans Candida species as compared to C. albicans. Although many new antifungal drugs have been licensed in recent years, their resistance is becoming a major concern during treatment of such patients.\textsuperscript{8}

Among the most studied Candida species, Candida albicans has several known virulence factors contributing to its pathogenicity that include adherence to epithelial and endothelial cells, proteinase production, pseudohyphae formation, phenotypic switching, phospholipase production and antigenic modulation as a result of pseudo hyphae formation. After yeast cells of Candida encounter a particular host tissue, colonization takes place at the local sites or it invades deeper into the host tissue.\textsuperscript{3}

Candidiasis is an opportunistic fungal disease found in humans who affect the skin, nails, mucosa and various internal organs. There are several types of candidiasis including mucosal candidiasis, cutaneous candidiasis and systemic candidiasis and the incidence of all these infections has been increased due to many factors such as more people living with HIV, the excessive use of antibiotics, organ transplantation and the use of invasive devises.\textsuperscript{9}

The purpose of this study is to isolate and identify the Candida species from various clinical samples in a tertiary care hospital, to identify the spectrum of the Candida infections in clinical samples and to characterize the isolated Candida species.

2. Materials and Methods

A study was conducted in the department of Microbiology in our institute. Various samples obtained from people of different age groups with suspected candidiasis from IPD & OPD from January 2019 to December 2019 were processed in mycology section.

Candida species isolated from different patients were included in the study. The samples were collected using aseptic conditions and the patient’s information such as name, age, sex, occupation, duration of hospitalization, ward, underlying medical conditions, associated risk factors such as presence of urinary catheter, mechanical ventilation, central line insertion, duration of antibiotic therapy, antifungal prophylaxis, exposure to invasive procedures, and use of corticosteroids were obtained from clinical records and analyzed.

Various tests performed for the identification of Candida species are given below:-

2.1. Direct examination

Direct microscopic examination was done by Potassium Hydroxide mount (KOH) to see the presence of fungal elements. It was prepared from Potassium hydroxide (10gm), Glycerol (10ml) and Distilled Water (80ml).

2.2. Procedure

(a) Preparation of KOH mount
(b) Examination of KOH mount

2.2.1. Slide KOH Procedure

1. Placed a small amount of sample on a clean glass slide.
2. Poured a drop of 10%KOH on the specimen and placed a coverslip over it.
3. Passed the slide gently over flame.
4. Left the slide for few minutes.
5. Examined first under low power lens (10x) of light microscope then under high power lens (40x).

2.2.2. Tube KOH procedure

1. This procedure was used for nail clippings and skin biopsies which dissolves with difficulty.
2. The homogenized tissue material was dissolved in 10% KOH in test tube and examined after overnight incubation at 37°C.\textsuperscript{3}

2.3. Blood culture

Blood samples (5 -10ml) were collected in blood culture bottles using aseptic and antiseptic precautions and incubated in BacTalert 3D (Biomerieux) automated blood culture system. Samples were processed using standard mycological procedure as shown in Figure 1.\textsuperscript{4}

2.4. Culture Identification of candida

For the isolation of Candida species the samples were inoculated on Sabouraud dextrose agar (SDA) with
antibiotics and incubated aerobically one at 22°C in Biological Oxygen Demand (BOD) and other at 37°C in incubator for 48hrs. The growth was identified as yeast like organisms by smooth, cream colored, white and pasty colonies on SDA after 3 to 4 days as shown in Figure 2. The further confirmation was done by Gram Staining to observe the presence of yeast cells and pseudohyphae as shown in Figure 3, Germ tube test, Chlamydospore formation on Corn meal agar, growth on CHROM agar and the biological test were sugar fermentation, sugar assimilation for final confirmation of species.

2.5. Speciation of Candida

Speciation of Candida was done by following methods-

1. **Germ tube test**: This was used for the preliminary identification of *Candida albicans*. It is also known as Reynold Braude phenomenon as shown in Figure 4.

2. **CHROM agar Candida Medium**: CHROM agar is new chromogenic differential isolation medium that facilitates presumptive differentiation of some of clinically important yeast like organisms and can be used for simultaneous isolation and presumptive identification of various Candida species like *Candida albicans*, *C. krusei*, *C. tropicalis*, *C. glabrata*, *C. parapsilosis* and *C. dubliniensis*.

   The CHROM agar Candida shows different colors of colonies after incubation of 48 to 72 hours at 30°C as shown in table 1 and Figure 5.

3. **Cornmeal agar**: It is a nutritionally deficient medium being low in nutrients and hence suppresses vegetative growth and stimulates sporulation in fungi. It is also used for preservation of fungi as stock cultures as shown in Figures 6 and 7.

4. **Sugar Fermentation test**: This test was done to observe the ability of Candida species to ferment a number of carbohydrates and thus produces acid, gas and hence pink color in presence of Andrade’s indicator.

5. **Sugar Assimilation test**: Yeasts and yeast like fungi utilize specific carbohydrates substrate alone. Organisms were inoculated into a carbohydrate free medium and carbohydrate containing filter paper discs were placed. Utilization was determined by presence of growth around the discs. Characteristic carbohydrate utilization profiles were used to identify species of yeast.

3. Results

In the present study, a total of 54 Candida species were isolated from various clinical samples which included 31 from blood (57.4%), 17 from sputum (31.5%), 4 from urine (7.4%), 1 from catheter tip (1.9%) and 1 from skin (1.9%).

*Candida albicans* was the most common species isolated among non albicans Candida i.e. 21 (38.9%); 19(35.2%) of *C. tropicalis* was the most commonest followed by 9(16.7%) of *C. glabrata* and 5(9.3%) of *C. krusei*.

Candida isolates were present in 35 males (64.8%) and 19 females (35.2%). The rate of isolation of the Candida species was more in male patients than female patients.

The youngest patient was less than one year old and the oldest one was 60 years old. Maximum number of patients i.e. 29 (53.7%) belonged to the age group of <1 year and minimum number of patients i.e. 4 (7.4%) belonged to the age group of >60 years.

Maximum number of Candida isolates were obtained from NICU i.e. 27(50.0%) followed by 11 from Med (20.3%), 7 from E/W (13.0%), 2 from BICU (3.7%), 2 from Skin (3.7%), 1 from PICU (1.9%), and 1 from R/R (1.9%).

The results of the Conventional method & HI Chrome Candida differential agar for various species are as shown in the Table 2.

![Fig. 1: Blood culture bottle](image1)

![Fig. 2: Growth of Candida species in SDA](image2)
Fig. 3: Gram stained smear showing budding yeast cells under oil immersion lens (100X)

Fig. 4: Wet mount preparation of serum showing germ tube formation by Candida spp.

Fig. 5: Chrom agar medium showing growth of different colored colonies of Candida spp.

Fig. 6: Corn meal agar inpetridish showing Dalmau culture technique.

Fig. 7: Microscopic appearance of chlamydomspores produced by Candida spp. on cornmeal agar

Table 1: CHROM agar Candida shows different colours of colonies after incubation of 48 to 72 hours at 30°C

<table>
<thead>
<tr>
<th>Candida species</th>
<th>Color change</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>Light green</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>Pink to Purple</td>
</tr>
<tr>
<td>C. krusei</td>
<td>Pink</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>Blue with pink hole</td>
</tr>
</tbody>
</table>
4. Discussion

Candida species are one of the major fungal pathogens of humans causing a diverse range of diseases. Most notable among invasive candidiasis is candidemia. Nosocomial bloodstream infections due to Candida species are associated with a mortality rate of 5% to 71%. According to the findings of the Centers for Disease Control and Prevention, Candida infections are held responsible for 11% of all nosocomial infections. *Candida albicans* remains the leading cause of Candida bloodstream infections; however, the prevalence of non albicans Candida infections has increased worldwide, particularly *C. tropicalis*, *C. glabrata*, *C. parapsilosis* and *C. krusei* as seen by Bac N.D et al.\(^\text{12}\)

In this study, most of the Candida isolates were found to be higher in male patients (55.10%) as compared to female patients (44.8%). This correlates well with the study of RA Kashid et al in 2011 who reported that the isolation of Candida species were higher in males (55.10%) as compared to females (44.8%) with male to female ratio of 1:0.81 and Renuka Devi et al\(^\text{13}\) in which 54.6% were in males and 45.3% in females. Unlike this study from Amar C.S et al\(^\text{14}\) in 2013, isolated Candida species were more from female (60.2%) than male (39.8%) patients in ratio of 0.6:1 (M>F).

In this study, Candida was isolated most commonly in the age group of 0-1year (53.7%), followed by 41-60years (31.5%), 21-40years (7.5%) and >60years (7.4%) which is similar to the study conducted by L. Sumitra Devi et al\(^\text{15}\) in which Candida was mostly isolated in the age group of 0-<1year old (60.9%), followed by 21-40 years (12.5%), >60 years (10.9%),1-20 years (7.8%) and 41-60 years (7.8%). This study does not correlate with the study done by Soumya Kaup et al\(^\text{16}\) in which majority of Candida isolates from patients aged between 61-70 years followed by 41-50 & 51-60 years.

In this study, 54 Candida species were isolated from various clinical sample which include blood (57.4%) followed by sputum (31.5%), urine (7.4%), Catheter tip (1.9%), Skin (1.9%). Maximum number of Candida species were isolated from blood sample. This study correlates with the observation of other authors who showed that majority of Candida were isolated from blood 33.6% by Tavleen Jaggi et al,\(^\text{6}\) 50% by Manmeet Gill et al\(^\text{17}\) and 57.7% by Raminder Sandhu et al.\(^\text{18}\) This study is not similar with Sankarankutty Jaya et al\(^\text{19}\) who proved that majority of Candida isolates were from urine (48%) followed by respiratory sample (17%).

Data from surveillance and control of pathogens of epidemiological importance (SCOPE) surveillance system confirms that Candida species have become the fourth leading cause of blood stream infections. A recent study done by MN Chowta et al\(^\text{20}\) shows that candidemia is associated with increased cost and attributable mortality of 38%. Although *Candida albicans*...
is the most frequently encountered organism, a number of reports have documented non albicans Candida species such as *C. tropicalis*, *C. glabrata*, *C. parapsilosis* and *C. krusei* and other filamentous fungi as emerging pathogens in recent years. Intravascular catheters, broad-spectrum antibiotics therapy, mucosal colonization, neutropenia, previous surgical procedures (particularly complicated abdominal surgery), total parenteral nutrition and concomitant bacteremia have been identified as significant risk factors for invasive candidal infection in various epidemiologic studies.

In this study, out of 54 Candida isolates obtained from various clinical samples, majority were *Candida albicans* (38.9%). Among non albicans, *Candida, C. tropicalis* (35.2%) followed by *C. glabrata* (16.7%), *C. krusei* (9.3%) which is similar with another study conducted by Tavleen Jaggi et al in which *Candida albicans* was the commonest species isolated causing (44%) of the infection followed by *Candida tropicalis*, *Candida parapsilosis* causing (26.4%) and (12.8%) of the infection respectively and also by other authors such as B.S.G Sailaja et al which showed higher incidence of *Candida albicans* (64%) over non *albicans Candida*(36%) but it is discordant with the study carried out by Mokaddas et al in 2007 showed that non albicans Candida incidence (60.5%) were higher than that of *C.albicans* (39.5%) and also by Rachana Mehta et al in 2016 showed that the isolation rate of non albicans Candida (59.1%) were higher as comparatively *C.albicans* (40.9%).

In this study, all the 54 isolates of Candida species namely C. albicans, C. tropicalis, C. glabrata, and C. krusei were identified by conventional method. Candida isolates were inoculated on HI Chrome Candida differential agar. We observed that performance of HI Chrome Candida differential agar for identification of the above four species were exactly parallel to that of conventional method. Similar findings were observed in various studies like Amar C.Sajjan et al in 2014 and Mr Joginder et al in 2020.

In this study, maximum number of Candida isolates were obtained from various departments including NICU (50.0%) followed by Medicine (20.3%), E/W (13.0%), BICU (3.7%), Skin (3.7%), PICU (1.9%), R/R (1.9%). Similar studies were conducted by other authors such as Asifa Nazir et al in which the isolation rate of Candida from neonatal ICU was 18.86% and by Raminder Sandhu et al in 2015 who also observed that the majority of Candida isolates were obtained from NICU(24%) followed by respiratory medicine ward(11%), MICU(7%).

5. Conclusions

This study emphasizes the requirement of precise isolation and identification of Candida species from various clinical samples. Prevalence of candidiasis was found to be higher in patients associated with predisposing factors like indwelling vascular catheters, prolonged antibiotic therapy and diabetes mellitus. Our study showed that *Candida albicans* is the most common isolates species. Among non *albicans Candida, C.tropicalis* was found to be the most common isolate followed by *C.glabrata, C.krusei*. Children less than 1 year are most affected with maximum number of Candida species were obtained from NICU department.

HiChrom Candida is proven to be more useful as differential agar, as it provides for the rapid isolation and identification of medically important candida species in a resource-limited setting and it is cost effective.

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7. Conflicts of Interest

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