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## Original Research Article

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

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## ABSTRACT

**Aim and Objective:** The purpose of this study is to isolate, identify and specification of various the Candida species from various clinical samples in a tertiary care hospital, and to characterize various the isolated Candida species.

**Materials and Methods:** A study was conducted on people of different age groups from January 2019 to December 2019. Candida species isolated from different patients by using Potassium Hydroxide mount and processed by BacTAlert 3D (Biomérieux) automated blood culture system. Further culture identification of Candida species were done on Sabouraud dextrose agar (SDA). Speciation of Candida was done using Germ tube test, CHROM agar Candida Medium, Cornmeal agar, Sugar Fermentation test and Sugar Assimilation test.

**Results:** In our study *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 common followed by 9(16.7%) of *C.glabrat* and 5(9.3%) of *C.krusei*. 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%).

**Conclusion:** 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.

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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.<sup>1</sup>

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.<sup>2</sup>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.<sup>3</sup>

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.<sup>4</sup> It include various species such as *C.glabrata*, *C.tropicalis*, *C.parapsilosis*. Among these, *C.tropicalis* is one of the most common non albicans Candida species

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isolated from various clinical types of candidiasis.<sup>5</sup>

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.<sup>6</sup>

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.<sup>7</sup>

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.<sup>8</sup>

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.<sup>3</sup>

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 devices.<sup>9</sup>

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

The concentration of KOH was increased depending upon nature of clinical materials

The wet mount of KOH was prepared by the following two methods:

- 1) Slide KOH
- 2) Tube KOH

#### 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.<sup>3</sup>

### 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.<sup>4</sup>

### 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.<sup>10</sup>

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, Chlamyospore formation on Corn meal agar, growth on CHROM agar and the biological test were sugar fermentation, sugar assimilation for final confirmation of species.<sup>4</sup>

### 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.<sup>10</sup>
- 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. parapsilopsis* 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.<sup>10</sup>
- 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.<sup>11</sup>
- 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.<sup>11</sup>

### 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.glabrat* 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

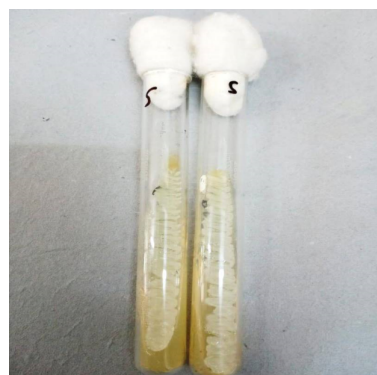
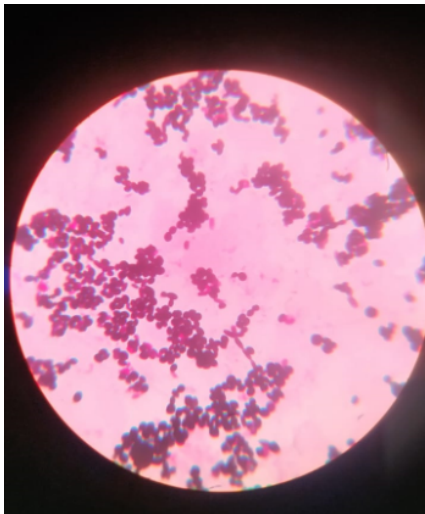


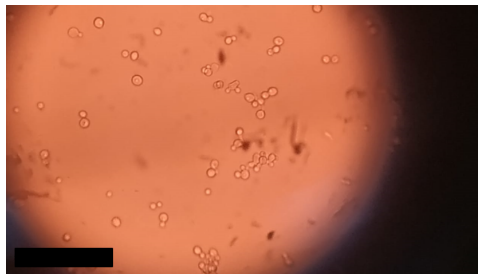
Fig. 2: Growth of Candida species in SDA



**Fig. 3:** Gram stained smear showing budding yeast cells under oil immersion lens (100X)



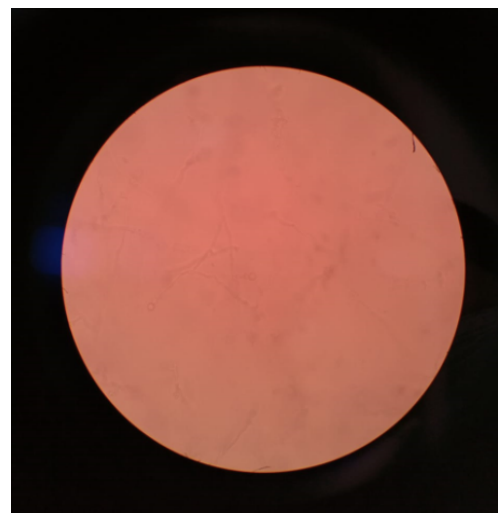
**Fig. 6:** Corn meal agar in petri dish showing Dalmau culture technique.



**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. 7:** Microscopic appearance of chlamydoconidia 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

<b>Candida species</b>	<b>Color change</b>
<i>C. albicans</i>	Light green
<i>C. glabrata</i>	Pink to Purple
<i>C. krusei</i>	Pink
<i>C. tropicalis</i>	Blue with pink hole

**Table 2:** Comparison of species identification by Conventional method & HI Chrome agar (n=54)

Candida species	No. of Candida species identified by	No. of Candida species identified using CHROM agar
	Conventional method	
<i>Candida albicans</i>	22	21
<i>Candida tropicalis</i>	19	19
<i>Candida glabrata</i>	9	9
<i>Candida krusei</i>	4	5
Total	54	54

#### 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.<sup>12</sup>

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<sup>13</sup> in which 54.6% were in males and 45.3% in females. Unlike this study from Amar C.S et al<sup>14</sup> 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<sup>15</sup> 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<sup>16</sup> 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,<sup>6</sup> 50% by Manmeet Gill et al<sup>17</sup> and 57.7% by Raminder Sandhu et al.<sup>18</sup> This study is not similar with Sankarankutty Jaya et al<sup>19</sup> 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<sup>20</sup> 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<sup>6</sup> 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<sup>21</sup> that 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<sup>22</sup> in 2014 and Mr Joginder et al<sup>23</sup> 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<sup>24</sup> in which the isolation rate of *Candida* from neonatal ICU was 18.86% and by Raminder Sandhu et al<sup>18</sup> 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

The authors declare no potential conflict of interest with respect to research, authorship, and/or publication of this article.

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