RESEARCH ARTICLE

SPECIES IDENTIFICATION OF CANDIDA ISOLATES IN VARIOUS CLINICAL SAMPLES.

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Manuscript Info

Abstract

Candida species especially non albicans Candida are increasingly being isolated from clinical specimens. The aim of our study was to detect the clinical distribution of Candida species in various clinical samples in a tertiary care hospital. A total of 115 Candida species were isolated from various clinical specimens submitted to Microbiology laboratory from different units of a tertiary care centre, Aurangabad Maharashtra from January 2014 to December 2014 using conventional yeast identification method after the approval from institutional ethical committee. The percentage of isolation of non albicans Candida species was 59.1% and that of C. albicans was 40.9%. The predominant species among non albicans Candida species isolated were C. tropicalis (40%) followed by C. guilliermondii (10.43%), C. krusei (4.34%), C. glabrata (2.60%), C. kefyr and C. parapsilosis (0.87%) each. As it is possible to predict the sensitivity pattern of each Candida species with high accuracy, the accurate species identification of Candida is important.

Introduction:

Candida species are ubiquitous fungi and are the most common fungal pathogens that affect human beings. They are true opportunistic pathogens that exploit recent technological advances to gain access to the circulation and deep tissues.¹ The genus Candida comprises about 200 species, of which close to 20 have been associated with pathology in human and animal.² Candida species are the component of normal flora of human beings. They are commonly found on the skin throughout gastrointestinal tract and female genital tract particularly higher in vagina during pregnancy.³ Those that are the part of normal flora can invade tissues and cause life-threatening disease in patients whose cell mediated immunity is decreased by disease or iatrogenic intervention.⁴

Candida albicans is by far, the most common in all clinical forms of Candidiasis, representing 70-80% of all yeast isolates.⁵ Candida species especially non albicans Candida are increasingly being isolated from clinical specimens. Speciation of Candida isolates is conventionally done by germ tube test, morphology on cornmeal agar, sugar fermentation and sugar assimilation tests.⁶ Non Candida albicans like C. tropicalis, C. krusei, C. glabrata and C. parapsilosis are less susceptible to azoles, particularly fluconazole.⁷ Therefore, correct identification of Candida species is of great importance. It presents prognostic and therapeutical significance, allowing an early and
appropriate antifungal therapy. Thus, the present study was undertaken for species identification of Candida isolates in various clinical specimens using conventional method.

**Materials and Method:**

**Study population:**
All the specimens collected from IPD and OPD, patients attending various clinical services at tertiary care hospital were used in the study. These specimens were processed as per standard protocol.²

**Processing of the specimens:**
Specimens like urine, pus, vaginal discharge, blood, sputum, endotracheal tube secretion, gastric lavage and other body fluids received in the Department of Microbiology for the culture were used in the study. Brain Heart Infusion broth was used for blood culture. All the specimens were screened for growth of Candida species by standard protocol². Relevant clinical data was recorded. In the present study, total 115 isolates of Candida species were obtained.

**Identification of Candida species:**
Isolates were preliminary identified as Candida species by direct microscopy, colony morphology and Gram staining.

All the specimens for the fungal culture were inoculated on Sabouraud’s dextrose agar with chloramphenicol and incubated at 25⁰C and 37⁰C for 48 hours. Candida species were identified by standard protocol that included germ tube formation, chlamydospore production on cornmeal agar, sugar fermentation and sugar assimilation tests.

**Wet preparations:**

**Direct microscopy:**³⁸
Direct examination of specimens like urine, peritoneal fluid, ascitic fluid etc. were carried out microscopically for the presence of yeast like cells.

**10% KOH:**³⁹
The specimens like sputum and swab from lesion were examined in KOH wet mount for the presence of yeast and pseudohyphae. The yeast cells of Candida species were approximately 4-8 μm with budding and pseudohyphae.

**Gram staining:**⁹⁻¹¹
For the specimens like pus, sputum etc Gram’s stained smear was observed for the presence of oval budding yeast like cells.

**Colony morphology on Sabouraud’s dextrose agar:**³⁹
The sabouraud’s dextrose agar consisting of chloramphenicol was used for isolation of Candida species. Cream coloured pasty colonies usually appeared after 24-48 hours incubation at 35-37⁰C. The colonies had a distinctive yeast smell. Sometimes growth was observed after an overnight incubation as seen in bacteria. The lactophenol cotton blue mounts were prepared to examine detail morphological features of fungi grown on culture medium. Gram’s staining was also performed from culture isolates.

ATCC strains that were used as a control were Candida albicans ATCC 10231, Candida glabrata ATCC 15126, Candida krusei ATCC 14243 & Candida tropicalis ATCC 750

**Species identification of Candida isolates was done by following methods:**

**Germ tube test:**³⁹⁻¹¹
Candida albicans can be identified presumptively by simple germ tube test.

**Cornmeal agar morphology:**³¹⁻¹³
Cornmeal agar is used for demonstration of chlamydospores, blastospores and pseudohyphae.

**Sugar fermentation test:**³⁸
For the sugar fermentation test sugars like glucose, sucrose, maltose and lactose were used.

**Sugar assimilation test:**\(^{3,12,14}\)
Yeast Nitrogen Base Agar was used for assessing carbohydrate utilizing ability of yeasts using carbohydrate disc method. Sucrose, Lactose, Trehalose, Raffinose & cellulobiose discs were used for sugar assimilation test.

All the observations were statistically analysed.

**Result:**-
A total of 115 Candida species were isolated from various clinical specimens processed during the study period including urine 43(37.39%), sputum 34(29.56%), blood 15(13.04%), pus 10(8.69%), oral swab 4(3.47%), vaginal swab 2(1.73%), throat swab 2(1.73%), ascitic line tip 1(0.87%) and gastric lavage 1(0.87%). Candida albicans was the commonest species isolated 47(40.9%) followed by C. tropicalis 46(40%), C. guilliermondii 12 (10.43%), C. krusei 5(4.34%), C. glabrata 3(2.60%), C. kefyr 1(0.87%) and C. parapsilosis 1(0.87%). Isolation rate of non albicans Candida species were higher 68 (59.1%) as compared to Candida albicans 47 (40.9%) [Table 1]. Candida isolates were present in 60 (52.17%) males and 55 (47.82%) females with male: female ratio of 1.09:1. Table no. 2 shows age wise distribution of Candida isolates. Highest number of non albicans Candida were isolated from urine (25) followed by sputum (14) where as highest number of Candida albicans were isolated from sputum (20) followed by urine (18) [Table 3].

**Table 1:-** Distribution of Candida albicans and Non Candida albicans isolates.

<table>
<thead>
<tr>
<th>Candida isolates</th>
<th>Number of isolates</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida albicans</td>
<td>47</td>
<td>40.87%</td>
</tr>
<tr>
<td>Non albicans Candida</td>
<td>68</td>
<td>59.13%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>115</strong></td>
<td><strong>100%</strong></td>
</tr>
</tbody>
</table>

**Table 2:-** Age wise distribution of Candida isolates.

<table>
<thead>
<tr>
<th>Age</th>
<th>Total No. of Candida isolates</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10</td>
<td>24</td>
<td>20.87%</td>
</tr>
<tr>
<td>11-20</td>
<td>15</td>
<td>13.04%</td>
</tr>
<tr>
<td>21-30</td>
<td>13</td>
<td>11.30%</td>
</tr>
<tr>
<td>31-40</td>
<td>13</td>
<td>11.30%</td>
</tr>
<tr>
<td>41-50</td>
<td>8</td>
<td>6.95%</td>
</tr>
<tr>
<td>51-60</td>
<td>12</td>
<td>10.43%</td>
</tr>
<tr>
<td>&gt; 60</td>
<td>30</td>
<td>26.08%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>115</strong></td>
<td><strong>100%</strong></td>
</tr>
</tbody>
</table>

**Table 3:-** Sample wise distribution of Candida isolates.

<table>
<thead>
<tr>
<th>Type of specimen</th>
<th>No. of Candida albicans isolates</th>
<th>No. of Non albicans Candida isolates</th>
<th>Total no. of Candida isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>18</td>
<td>25</td>
<td>43 (37.39%)</td>
</tr>
<tr>
<td>Sputum</td>
<td>20</td>
<td>14</td>
<td>34 (29.56%)</td>
</tr>
<tr>
<td>Blood</td>
<td>2</td>
<td>13</td>
<td>15 (13.04%)</td>
</tr>
<tr>
<td>Pus/Discharge</td>
<td>3</td>
<td>7</td>
<td>10 (8.69%)</td>
</tr>
<tr>
<td>Oral swab</td>
<td>1</td>
<td>3</td>
<td>4 (3.47%)</td>
</tr>
<tr>
<td>Vaginal swab</td>
<td>0</td>
<td>3</td>
<td>3 (2.60%)</td>
</tr>
<tr>
<td>Throat swab</td>
<td>2</td>
<td>-</td>
<td>2 (1.73%)</td>
</tr>
<tr>
<td>Ascitic fluid</td>
<td>0</td>
<td>2</td>
<td>2 (1.73%)</td>
</tr>
<tr>
<td>Central line tip</td>
<td>1</td>
<td>-</td>
<td>1 (0.87%)</td>
</tr>
<tr>
<td>Gastric lavage</td>
<td>-</td>
<td>1</td>
<td>1 (0.87%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>47</strong></td>
<td><strong>68</strong></td>
<td><strong>115 (100%)</strong></td>
</tr>
</tbody>
</table>
Discussion:

Candidiasis is the commonest fungal disease in humans affecting skin, nails, mucosa and internal organs of the body. Candida species is endogenous and the disease represents opportunistic infections. In our study, we found highest number of Candida isolates from urine 43(37.39%). Out of 43(37.39%) isolates, 18 Candida isolates were Candida albicans and 25 were non-albicans Candida species. Among non-albicans Candida species, Candida tropicalis (21) was the most predominant species. This was in agreement with Kashid et al (2011), Patel et al (2012), Kumar et al (2013), as they found highest number of isolates from urine and among which Candida tropicalis was the most predominant species.

In our study, 34(29.56%) Candida isolates were from sputum sample. We found highest number of Candida albicans 20 from sputum sample as compared to non albicans Candida 14. This was in agreement with Patel et al (2012) who found highest number of Candida albicans from sputum sample. Candida is normal inhabitant of the mouth and can be recovered from sputum in 20% to 55% of normal subjects. The prevalence and prognosis of pulmonary candidal infection is difficult to evaluate since diagnosis were seldom confirmed. Candida isolated from sputum sample is mostly a colonizer of the respiratory tract. The role of Candida in pulmonary candidiasis and its diagnosis is still controversial.

In our study, 15 (13.04%) of the Candida isolates were from blood. Among them 10 were Candida guilliermondii, 2 were Candida albicans and Candida krusei each and one was Candida tropicalis. We observed that Candida guilliermondii was the most predominant isolate from blood. This was in agreement with study done by Patel et al (2012) Pethani et al (2013) who reported highest number of Candida guilliermondii from blood.

In the present study it was found that candidiasis can occur at all ages and in both sexes. In the present study, the number of Candida species was found to be higher in male patients 60(52.17%) as compared to female patients 55(47.82%) with male to female ratio of 1.09:1. This was in agreement with Kashid et al (2011) who reported the isolation of Candida species was higher in males 81(55.10%) as compared to females 66(44.8%) with male to female ratio of 1:0.81.

In the present study, out of 115 isolates of Candida species 89(77.39%) were from IPD and 26(22.60%) were from OPD. The continuous use of invasive monitoring and surgical technologies in the intensive care unit has increased the risk of fungal infections.

We observed that the frequent isolation of Candida species was in the age group above 60 years (26.08%) followed by age group 0-10 years (20.87%) which was similar with the study of Kashid et al (2011) who reported highest incidence in the age group above 60 years (24.48%) followed by age group 0-10 years (20.40%). Our study was also in agreement with Bineshlal et al (2011) and Yashvanth et al (2013) who also reported maximum isolation of Candida species in the age group above 60 years. Candida species remain the most important cause of opportunistic infections worldwide, affecting predominantly patients over 65 years old.
Photographs:

Fig 1: Morphology of *Candida* species on SDA.

Fig 2: Photograph showing germ tube test positive.
Chlamydospore formation on cornmeal agar (characteristics of *Candida albicans*).

Morphology of *Candida tropicalis* on Cornmeal agar.
Morphology of *Candida krusei* on cornmeal agar

Morphology of *Candida glabrata* on cornmeal agar
Sugar assimilation pattern of *Candida albicans* (Sucrose: positive, Trehalose: positive, Lactose: negative, Cellulobiose: negative and Raffinose: negative)

**Conclusion:**
An increase in the predisposing factors in recent years has resulted in an increasing incidence of *Candida* infections. Therefore, the species level identification of the *Candida* isolates can greatly influence the treatment options for the clinician because there is variation in sensitivities of various species to different antifungals and may have an impact on the patient care.
In the present study, we found higher incidence of non albinacs Candida as compared to Candida albicans. Therefore, it is necessary to identify Candida isolates up to species level for appropriate treatment.

References:-