Distribution of Candida albicans and the Non-Albicans Candida Species in Different Clinical Specimens from South India

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Abstract: Distribution of Candida species in different clinical specimens was studied. As many as 100 isolates were recovered from 602 various clinical specimens. Among the 100 isolates of Candida recovered from various clinical specimens 66 were non-albicans Candida species and 34 were C. albicans. Out of the non-albicans isolates C. glabrata (30%) was the most dominant, followed by C. tropicalis (20%), and C. kefyr (16%). Out of 500 urine specimens, 66 (13.2%) yielded candida isolates. Among the 66 Candida species isolated from urine, 43 were non-albicans Candida species and 23 were C. albicans. Among the 43 non-albicans Candida species isolated from urine, 16 were C. glabrata, 14 were C. kefyr and 13 were C. tropicalis. Out of 20 intravascular catheter tips, 8 (40%) were colonised with Candida species. Among the 8 isolates recovered from catheter tips, 5 were C. albicans, 2 were C. glabrata and one was C. tropicalis. In conclusion, the present study showed the distribution of Candida species in different clinical specimens and the predominance of non-albicans Candida species.

Key words: Distribution of Candida • Clinical Specimens • Non-Albicans Candida Species • C. glabrata

INTRODUCTION

Candidiasis is the most common cause of fungal infections, leading to a range of life-threatening invasive to non-life-threatening mucocutaneous diseases. Number of candidiasis cases have been increased in the past few decades [1]. Excessive use of broad-spectrum antibiotics and the emergence of AIDS are important among the various contributing factors [2]. Until recently, C. albicans was considered as the predominant species in most countries, causing most of the cases of candidiasis. However, during last few decades, many countries around the world reported a change in the epidemiology of candida infections, characterized by a progressive shift from a predominance of C. albicans to non-albicans Candida species such as C. tropicalis, C. glabrata and C. krusei [3-7]. The clinical manifestations produced by various pathogenic Candida species are not distinguishable. Many of the non-albicans Candida species such as C. glabrata and C. krusei exhibit resistance to traditional triazole antifungals like fluconazole and may also demonstrate cross-resistance to newer triazoles [8]. Hence, identification of Candida isolates to the species level has become mandatory to aid the selection of appropriate antifungal agents in treatment.

In last few decades, there are many reports of candidiasis from India. However, in many of these reports, Candida isolates were not identified based on a detailed study of their biochemical characteristics. Because of considerable regional variability, local epidemiological knowledge is critical in the effective management of
infections caused by Candida species. To our knowledge, there are not many reports of candidiasis from South India. The objective of the present study was to investigate the prevalence of different types of candida infections and to determine isolation pattern, species distribution of various isolates.

**MATERIALS AND METHODS**

**Sample Collection:** As many as 602 clinical specimens were collected from Department of Microbiology, Sri Venkateswara Institute of Medical Sciences, Tirupati during October 2011 to March 2012 for a period of six months. The clinical specimens investigated include urine-500, pus-30, intravascular catheter tips-20, respiratory tract secretions (sputum-10, bronchoalveolar lavage-10, Blood-10, stool-10, skin scrapings-06 and CSF-06). The 602 patients were grouped into 5 age groups, less than 20 years, 21-40 years, 41-60 years, 61-80 years and 81-100 years.

**Culture of Specimens:** All clinical specimens were inoculated on MacConkey and Blood Agar. Biphasic blood culture bottles containing Brain Heart Infusion broth and agar (HiSafe™ Blood Culturing System, Hi Media, Mumbai) were used for blood samples. All the inoculated plates were incubated at 37°C. Out of 500 urine specimens, 66 (13.2%) yielded candida isolates. Among the 66 Candida species isolated from urine, 43 were non-albicans Candida species and 23 were *C. albicans* (Table 2). Out of 20 intravascular catheter tips, 8 (40%) were colonised with Candida species. Among the 8 isolates recovered from catheter tips, 5 were *C. albicans*, 2 were *C. glabrata* and one was *C. tropicalis* (Table 2). Two out of ten blood samples yielded Candida species.

**Identification of Isolates:** The colonies were initially studied for their morphological characters such as: colony appearance, colony colour, colony shape and colony texture. Colonies suggestive of Candida species were further identified and speciated by Gram’s staining, germ tube test, chlamydospores formation on cornmeal agar, sugar fermentation test, sugar assimilation test and colour production on CHROM agar (HiMedia, Mumbai) [10, 11]. Gram’s staining, sugar fermentation and sugar assimilation tests were performed using standard protocol.

For testing germ tube production, one or two colonies of candida isolates were suspended in individual tubes containing 1 ml of pooled human serum. The test tubes were incubated at 37°C for 2 hrs. One drop of each yeast-serum suspension was placed on a glass slide, covered with a cover slip and examined under the microscope for the presence of germ tubes.

For testing chlamydospores production, each isolate was picked up with a straight wire, streaked on corn meal agar plate and covered with a cover slip to produce a relative anaerobic condition. Then the plates were incubated at 25°C for 3 days. The plates were examined under microscope at every 24 hrs interval for the presence of chlamydospores.

**RESULTS**

Total number of Candida isolates that were identified from 602 various clinical specimens was 100. Among these 48 isolates were from males and 52 isolates were from females (Table 1). Among females, most number of Candida species were isolated from the age group 41-60 (Table 1). Where as in case of males, most number of Candida species were isolated from age groups 41-60 years and 61-80 years, each age group contributed 17 (32.6%) isolates (Table 1).

Out of 500 urine specimens, 66 (13.2%) yielded candida isolates. Among the 66 Candida species isolated from urine, 43 were non-albicans Candida species and 23 were *C. albicans* (Table 2). Among the 43 non-albicans Candida species isolated from urine, 16 were *C. glabrata*, 14 were *C. kefyr* and 13 were *C. tropicalis* (Table 2). Out of 20 intravascular catheter tips, 8 (40%) were colonised with Candida species. Among the 8 isolates recovered from catheter tips, 5 were *C. albicans*, 2 were *C. glabrata* and one was *C. tropicalis* (Table 2). Two out of ten blood samples yielded Candida species.

Among the 100 isolates of Candida species isolated from various clinical specimens 66 were non-albicans Candida species and 34 were *C. albicans* (Table 2). Out of the non-albicans isolates *C. glabrata* (30%) was the most common, followed by *C. tropicalis* (20%) and *C. kefyr* (16%) (Table 2).

**DISCUSSION**

Candidiasis is the most common cause of fungal infections, leading to a range of life-threatening invasive to non-life-threatening mucocutaneous diseases. Until recently, *C. albicans* was considered as the predominant species causing candidiasis in most of the
Table 1: Isolation of Candida species from different age group male and female patients

<table>
<thead>
<tr>
<th>Age in years</th>
<th>Females</th>
<th>Males</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;20 years</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>21-40</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>41-60</td>
<td>16</td>
<td>17</td>
</tr>
<tr>
<td>61-80</td>
<td>11</td>
<td>17</td>
</tr>
<tr>
<td>81-100</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>48</td>
<td>52</td>
</tr>
</tbody>
</table>

Table 2: Species distribution of different Candida isolates among various clinical specimens

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Urine</th>
<th>Catheter tips</th>
<th>Sputum</th>
<th>Pus</th>
<th>CSF</th>
<th>Skin scrapings</th>
<th>Blood</th>
<th>BAL</th>
<th>Stool</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>23</td>
<td>5</td>
<td>5</td>
<td>1</td>
<td>Nil</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>Nil</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>13</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>Nil</td>
<td>1</td>
<td>1</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>16</td>
<td>2</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>Nil</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>C. kefyr</td>
<td>14</td>
<td>Nil</td>
<td>Nil</td>
<td>1</td>
<td>1</td>
<td>Nil</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

countries. Over the past decade, there has been a significant increase in the number of reports of systemic and mucosal yeast infection with Candida species other than C. albicans [3-7]. The potential clinical importance of species level identification has been recognized as Candida species differ in the expression of putative virulence factors and antifungal susceptibility. Thus, it has become imperative to identify all yeast isolates up to the species level in routine microbiology laboratories. Molecular methods such as real-time PCR [12], multiplex-tandem PCR [13] and Ribosomal Intergenic Spacer Fingerprinting methods [14] are being increasingly employed for the identification of Candida isolates to the species level. However such methods are very expensive and not suitable for routine use in clinical laboratories. Hence, an attempt to identify clinical isolates of Candida up to species level based on biochemical methods such as Gram’s staining, germ tube test, chlamydospores formation on cornmeal agar, sugar fermentation test, sugar assimilation test and colour production on CHROM agar (HiMedia, Mumbai), was undertaken.

A total of 100 isolates from 602 various clinical specimens were studied. The predominant species identified in the present study was C. albicans and it was isolated from all kinds of clinical specimens taken into consideration. In the present study 66% of isolates belong to non-albicans Candida species and 34% were C. albicans (Table 2). Non-albicans Candida species are generally considered as normal flora of cutaneous and mucocutaneous surfaces and only rarely are incriminated as agents of infection. However, in last few decades many researchers have reported the increased isolation and significance of non-albicans Candida species [5, 15]. Out of the non-albicans isolates C. glabrata (30%) was the most common, followed by C. tropicalis (20%) and C. kefyr (16%) (Table 2). Several researchers have also reported that C. glabrata and C. tropicalis as the most common pathogens among non-albicans Candida species [16-19].

Candida species in measurable quantities in the urine (candiduria) are found in <1% of clean voided specimens in healthy persons [20], but account for 5% of all urine culture results in the general hospital setting and 10% of urine isolates in tertiary care facilities [21]. In the present study, 13.2% of urine samples yielded Candida isolates. Bouza and colleagues reported that the incidence of candiduria in non-catheterized subjects was 6.6% [22]. Among the 66 Candida species isolated from urine, 43 were non-albicans Candida species and 23 were C. albicans (Table 2). Non-albicans Candida species appear better adapted to the urinary tract environment with many studies reporting that >50% of urinary Candida isolates belong to non-albicans species [23, 24]. Among the 43 non-albicans Candida species isolated from urine, 16 were C. glabrata, 14 were C. kefyr and 13 were C. tropicalis (Table 2). Kauffman [24] reported C. glabrata as the dominant species among urinary isolates of Candida. Our result is in agreement with this report. C. glabrata appears to be adapt well to selected urine properties such as substrate availability, osmolality and pH [25]. Paul and colleagues have identified C. tropicalis as the most prevalent fungal isolate from urine specimens [26]. This study reports that C. tropicalis as the third most dominant fungal isolate from urine.

In the present study, 40% of intravascular catheter tips were colonised with Candida species. Previous reports suggest that Candida colonization of intravascular devices is associated with candidemia [27, 28]. Leenders and colleagues [29] suggested pre-emptive antifungal treatment based on catheter tip cultures. Previous reports suggest that 8% of cases of nosocomial blood stream infections were caused by
Candida species and nearly half of the isolates were non-albicans Candida species [30, 31]. In this study, 20% (2/10) of blood samples yielded Candida species. Out of 2 isolates, one was *C. albicans* and the other one was *C. tropicalis* (Table 2).

In conclusion, the present study showed the distribution of Candida species in different clinical samples and the predominance of non-Candida albicans such as *C. glabrata*, *C. tropicalis* and *C. kefyr*.

**REFERENCES**


