Prevalence and antifungal susceptibility pattern of Candida albicans isolated from patients with suspected candidiasis – A study from South Karnataka

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Abstract

The incidence of candidiasis has increased drastically in last few decades. The distribution pattern of Candida in invasive infections varies widely in different geographical area. C. albicans is the major pathogen in most part of the world. Antifungal resistance is one of the important problems among Candida including C. albicans. Aim of this study was to determine the prevalence and antifungal susceptibility pattern of C. albicans isolated from patients. Candida isolated from various clinical specimens were identified and antifungal susceptibility test was performed by disk diffusion method. The prevalence of Candida albicans was 49%. C. albicans exhibited various degree of resistance to azoles. Resistance was highest in itraconazole followed by ketoconazole. All C. albicans isolates were sensitive to amphotericin B. Even though there is progressive shift from a predominance of Candida albicans to non-albicans Candida species in candidiasis, C. albicans remains as the most important pathogen. Since azole resistance is increasing, accurate identification of Candida species and antifungal susceptibility testing is crucial for patient management and for facilitating hospital control measures.

Key words: Candidiasis, antifungal susceptibility, disk diffusion, Candida, azole.

Introduction

Candida species are the yeast present as the opportunistic infections in immunocompromised conditions due to their great
adaptability to different host niches [1-3]. The incidence of candidiasis has drastically increased in last few decades. This is due to increased use of variety of medical implant devices such as catheters which support biofilm formation. Candida can cause a wide range of infections in man which include superficial infection to deep seated infections with a high morbidity and mortality rates [2]. In US, Candida is considered as the third most common cause of hospital acquired infection with highest mortality rate [4, 5]. The distribution pattern of Candida in invasive infections varies widely in different geographical area. Candida albicans are considered as the most common causative fungal agent in superficial and deep seated candidiasis. However, in recent years, Non Albicans Candida (NAC) species are being implicated as cause of candidiasis [2, 3]. C. tropicalis, C. parapsilosis, C. kefyr, C. krusei and C. guilliermondii are the most common non-albicans Candida species pathogenic to human [2]. There is a wide difference in pathogenicity and antifungal susceptibility among different species. Unfortunately, species identification and antifungal susceptibility testings are not done routinely in many laboratories as it is tedious and time consuming.

Even though Candida is emerging as one of the important pathogen only few drugs are available for treatment. Azole group of drugs are commonly used for treating candidiasis. But drug resistance is becoming an issue in treatment of candidiasis. Certain Non albicans Candida are intrinsically resistant to commonly used antifungal drugs such as azoles. C. albicans, which is the most common species in many part of the country exhibit secondary resistant to azole drugs [6]. Since there is a lot of variations in distribution pattern of Candida species and antifungal susceptibility pattern, close monitoring on the distribution and susceptibility of Candida species has to be done in order to optimize therapy and outcome. Identification of the optimal time point to start therapy is challenging [1] and delay in treatment is one of the reasons for treatment failure. The present study was designed to determine the prevalence and antifungal susceptibility pattern of C. albicans isolated from various clinical specimens of patients with suspected candidiasis from South Karnataka.

**Materials and Methods**

This is a prospective observational and experimental study done in diagnostic laboratory attached to a Medical College Hospital in South Karnataka. The study was conducted for a period of two years. Candida isolated from clinical specimens of patients with suspected candidiasis attending Medical college hospital was included. Polymicrobial growths from normally sterile specimen were excluded from the study.

A total of 181 isolates were obtained from various clinical specimens including blood, urine, exudate, vaginal swabs, bile, stool and oral thrush swabs of patients. Ethical clearance for the study was taken from the Institutional ethical committee.

**Isolation and identification of Candida from clinical specimen**

Specimen were inoculated on Sabouraud dextrose agar (SDA), incubated at 25°C for 48-72 hrs. Species identification were done by microscopic examination, germ tube test, Corn Meal agar test, sugar assimilation -fermentation test and by using Hi Chrome Candida differential agar, by standard procedures [8]. All the isolates and control strains were stored in glycerol broth until use.

**Antifungal susceptibility test for C. albicans**

The antifungal susceptibility test was performed for C. albicans with fluconazole,itraconazole, ketoconazole, voriconazole, miconazole and amphotericin B by standard disk diffusion method, as described by the National Committee for Clinical Laboratory Standards (CLSI) M44-A2, CLSI, US [9].

**Data management and statistical analysis:**

All the statistical analyses were done using the statistics software SPSS (Statistics Package for Social Sciences, Chicago, USA), version 15 for Windows.
Table 1: Distribution of various species of *Candida* in different clinical specimen

<table>
<thead>
<tr>
<th>Sample</th>
<th>Pus</th>
<th>Oral</th>
<th>Vaginal discharge</th>
<th>Blood</th>
<th>Urine</th>
<th>Nail</th>
<th>Ear discharge</th>
<th>Body fluids</th>
<th>Skin scraping</th>
<th>Stool</th>
<th>Tissue</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. albicans</em></td>
<td>10</td>
<td>28</td>
<td>29</td>
<td>2</td>
<td>9</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>-</td>
<td>89</td>
<td>49</td>
</tr>
<tr>
<td><em>C. tropicalis</em></td>
<td>7</td>
<td>4</td>
<td>8</td>
<td>8</td>
<td>17</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>48</td>
<td>27</td>
</tr>
<tr>
<td><em>C. guilliermondii</em></td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td><em>C. krusei</em></td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td><em>C. lambica</em></td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td><em>C. dubliniensis</em></td>
<td>3</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td><em>C. glabrata</em></td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td><em>C. lusitaniae</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td><em>C. parapsilosis</em></td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>5</td>
<td>-</td>
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<td>1</td>
<td>-</td>
<td>8</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td><em>C. kefyr</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Candida species</td>
<td>1</td>
<td>3</td>
<td>-</td>
<td>1</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>9</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>27</td>
<td>38</td>
<td>40</td>
<td>18</td>
<td>35</td>
<td>7</td>
<td>5</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>181</td>
<td>-</td>
</tr>
<tr>
<td>%</td>
<td>15</td>
<td>21</td>
<td>22</td>
<td>10</td>
<td>19</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0.5</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Sensitivity pattern of *C. albicans* in various clinical specimen

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Fluconazole (10 mcg)</th>
<th>Itrakonazole (10 mcg)</th>
<th>Ketokonazole (10 mcg)</th>
<th>Voriconazole (1 mcg)</th>
<th>Miconazole (50 mcg)</th>
<th>Amphotericin B (20 mcg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUSCEPTIBILITY</td>
<td>S</td>
<td>SDD</td>
<td>R</td>
<td>S</td>
<td>SDD</td>
<td>R</td>
</tr>
<tr>
<td>Oral (27)</td>
<td>20</td>
<td>3</td>
<td>4</td>
<td>20</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Vaginal (29)</td>
<td>27</td>
<td>-</td>
<td>2</td>
<td>26</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Pus(9)</td>
<td>8</td>
<td>1</td>
<td>-</td>
<td>8</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Bile &amp; other fluids (2)</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ear discharge (3)</td>
<td>2</td>
<td>-</td>
<td>1</td>
<td>2</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Stool (1)</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Skin scraping(1)</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Urine (9)</td>
<td>9</td>
<td>-</td>
<td>-</td>
<td>8</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Nail clipping (3)</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Total (84)</td>
<td>72</td>
<td>5</td>
<td>7</td>
<td>69</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>%</td>
<td>86</td>
<td>6</td>
<td>8</td>
<td>82</td>
<td>6</td>
<td>12</td>
</tr>
</tbody>
</table>

S- Susceptible, SDD- Susceptible Dose Dependent, R- Resistant

Results

*C. albicans* was the most frequently isolated species (49%). Among NAC, *C. tropicalis* was predominant species (27%). Other species include *C. parapsilosis, C. guilliermondii, C. dubliniensis, C. krusei, C. glabrata, C. lambica, C. kefyr* and *C. lusitaniae*. Five strains were not able to identify by conventional biochemical reactions. When considering *Candida* species among various clinical specimen, *C. albicans* was the most prevalent species except in case
of blood and urine where \textit{C. tropicalis} was the predominant (Table 1).

Figure 1: Gram’s stain

Figure 2: \textit{Candida} species on Hichrom agar

Figure 3: Sugar fermentation test

Figure 4: Sugar assimilation test

Figure 5: Chlamydosporie formation

Figure 6: Antifungal susceptibility test

Antifungal susceptibility pattern of \textit{C. albicans} revealed that 100% sensitivity to amphotericin B. But, they exhibited various degree of resistance to azoles. Resistance was
highest with itraconazole followed by ketoconazole with sensitivity 82% and 83% respectively. Among azoles, resistance was least with miconazole were 86% of *C. albicans* were sensitive (Table 2). Maximum resistance was seen among *C. albicans* isolated from oral thrush.

**Discussion**

This study was focused on 181 Candida isolates which were collected over two years period time. In this study, we observed that *C. albicans* is the most prevalent species even though there is significantly higher rate of *C. tropicalis*. Similar results were found by Shivanand Dharvad et al from South Karnataka who reported *C. albicans* as the commonest species (47%), followed by *C. tropicalis* (30%) [10]. A study by Amar C et al. from North Karnataka also reported similar prevalence rate where *C. albicans* constitute 65% followed by *C. tropicalis* (24.3%) [11]. Similar conclusions were reported from different parts of India by authors Changdeo et al, Vijaya et al and Basu et al. [12-14]. In addition, there were other studies performed in other parts of the world showing almost similar pattern as reported by Guinea J et al., Zhang J et al, Yang YL et al and Pedram Haddadi et al [15-18]. However, studies done by Pahwa et al, Jain et al and Chakrabarti et al reported *C. tropicalis* as the most prevalent species [1, 19]. In the present study, *C. tropicalis* was the predominant strain in urine as it was reported in a study on candiduria from Mangalore [20].

Monitoring antifungal resistance among Candida is useful as it helps in tracking and detection of resistance. Highest sensitivity was seen with Amphotericin B which is similar to other studies reported from India by Chaitanya et al. [2], Shivanand et al. and Vijaya et al. [13] Azole resistance was reported between 0-15% in this study with highest resistance for Ketoconazole (15%). But Shivanand et al reported little higher resistance rate for azoles (0-22%) [10]. It may have been because of less number of *C. albicans* they have performed antifungal susceptibility test. There is a wide difference in sensitivity to Fluconazole among *C. albicans* which varied from 0-22% in South Karnataka [2, 10] compared to the present study which is only 8%. However, studies from other parts of the country have shown resistance up to 28% [18].
Conclusion
The increase in the predisposing conditions in recent years has resulted in an increase in incidence of Candida infections. Therefore, the species level identification of the Candida isolates and antifungal susceptibility will help the clinician in patient care. Periodic surveillance of antifungal susceptibility testing should be carried out.

Acknowledgement
This work was supported by grant from Yenepoya University (YU/Seed Grant/2013-021).

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For Citation: