SPECIATION AND ANTIFUNGAL SUSCEPTIBILITY PATTERN OF CANDIDA ISOLATES IN A TERTIARY CARE HOSPITAL

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Abstract:
There has been a dramatic increase in infections caused by candida worldwide. They are most commonly treated by fluconazole, which has led to emergence of fluconazole resistant candida. Also C krusei show innate resistance and C. glabrata & C. tropicalis show reduced susceptibility to fluconazole. Hence we undertook this study to speciate and determine antifungal susceptibility pattern of Candida isolates.
Candida species isolated from various clinical samples were identified by standard yeast identification protocol and Antifungal susceptibility test was performed by disc diffusion method. Identification and susceptibility pattern was also confirmed by Vitek Compact 2 system.
The rate of candidal infection in our study was 1.9%. A very high rate of infections due to Candida non albicans species (87.5%), was observed, whereas infections due to Candida albicans was 12.5%. C. tropicalis (50%) was the major Candida non albicans species. All our isolates were sensitive to voriconazole, amphotericin B & caspofungin.
Interpretation & Conclusion:
Infections caused by Candida non albicans and yeasts other than candida is emerging. Hence accurate identification and susceptibility pattern is necessary for management of candidal infections.

Keywords:
Antifungal susceptibility pattern, Candida albicans, Candida non albicans, Speciation, Stephanosascus ciferrii, Trichosporon asahii.

INTRODUCTION:
Candida species are significant opportunistic pathogens which cause a wide variety of infections in humans ranging from trivial intertriginous infection to fatal candidemia. The commonest species that are implicated in infections are C.albicans, C.tropicalis, C.parapsilosis, C.krusei and C.glabrata.1 C.albicans is the most predominant species followed by C.tropicalis, which is the commonest cause of nosocomial infection particularly in immunocompromised patients. But, recently candida non albicans are emerging as human pathogens causing variety of infections. 2,3,4

Candidiasis is an opportunistic infection occurring in presence of predisposing factors such as extensive and prolonged use of broad-spectrum antimicrobials, corticosteroids, immunosuppressive agents and cytotoxic drugs, diabetes mellitus, immunosuppression, chronic renal failure, hemodialysis, renal transplantation and indwelling urinary catheter.4

Immune status has a definite impact on candida carriage. In immunocompetent persons, candidiasis is usually a much localized infection of the skin or mucosal membranes, including the oral cavity (thrush), the pharynx, the oesophagus, the urinary bladder or the genitalia. Candidal infections in immunocompromised patients are often severe, rapidly progressive and difficult to treat. Infants and children are highly susceptible to candidiasis. Early detection and effective antifungal therapy prevents progression to fatal candidemia and save the precious life.

Candida species were initially susceptible to ‘Azoles’, but now several species have developed resistance to the azoles. Widespread use of fluconazole for the prophylaxis and treatment of candidiasis has led to a reduction in the number of cases of infections caused by Candida albicans but has also resulted in the emergence of candidal infections caused by fluconazole-resistant Candida non albicans species.5 Resistance to fluconazole, itraconazole, voriconazole, flucytosine was as frequent as potential resistance to amphotericin B. Few strains of Candida albicans, C. glabrata & C.
tropicalis resistant to caspofungin has also been reported. Hence antifungal susceptibility of clinical isolates of candida has gained significance in the management of candidal infections. Moreover, early detection and speciation of the candida will play a crucial role for administering appropriate antifungal therapy at the earliest. Hence we undertook this study to speciate candida isolates causing various clinical infections and to determine their antifungal susceptibility pattern, which helps in early initiation of appropriate antifungal drugs and also to know the susceptibility pattern of isolates prevalent in this part of our country.

MATERIALS AND METHODS:
A Prospective study was conducted for 6 months from May 2013 to December 2013 in the department of Microbiology, JSS Medical College Hospital. All clinical samples which yielded the growth of Candida were included in the study and samples which yielded organisms other than Candida were excluded from the study.

All clinical samples i.e., blood (660 samples), urine (850 samples), pus (540 samples), endotracheal tube suction (35 samples) & throat swab (15 samples) were examined by KOH preparation (Figure 1) cultured onto Sabouraud’s Dextrose Agar and incubated aerobically at 37°C. The cultures were examined everyday for the growth of cream coloured pasty colonies suggestive of Candida species (Figure 2).

Speciation of Candida:
Candida species were speciated by performing the Germ Tube Test, Corn meal agar inoculation for Chlamydosporo formation, sugar assimilation (Figure 3) and sugar fermentation test.9

Antifungal susceptibility testing of Candida: 10
All candida strains isolated were tested for antifungal susceptibility by disc diffusion method (M 44-A, CLSI, USA). The inoculum was prepared by suspending five colonies in 5ml of sterile saline and matching the turbidity to Mac Farland 0.5. A cotton swab was dipped into the inoculum suspension and evenly streaked onto Mueller Hinton agar with 2% glucose and 0.5μg/ml of Methylene Blue dye. Discs containing Fluconazole 25 μg, Voriconazole 1 μg, amphotericin B 10 μg, itraconazole 8 μg and ketoconazole 15 μg were placed on the inoculated plates. Zones of inhibition around the disk were measured after incubating the plates for 24 hours at 35-37°C. The results were interpreted according to CLSI criteria M44A. Candida albicans ATCC 90028 was used as control. The sensitivity pattern against fluconazole, voriconazole, flucytosine, amphotericin B & Caspofungin and identification of the isolates was also confirmed by Vitek Compact 2 (Biomerieux).

RESULTS:
A total of 2100 various clinical samples were collected during the study period from May 2013 to December 2013 from various wards. 1008 (48%) were collected from male patients and 1092 (52%) were collected from female patients. Of the total 2100 clinical samples, 850 were urine samples, 660 were blood culture samples, 540 were pus samples, 35 were endotracheal tube suction samples and 15 were throat swab samples. Among 2100 various clinical samples, 40 samples (1.9%) yielded the growth of Candida isolates. Table I shows the isolation of Candida species from various clinical samples.

Among 40 candida isolates, 10 (25%) were isolated from children and 30 (75%) strains were isolated from adult patients. The most common risk factors associated with candidiasis in our study were broad spectrum antibiotic therapy (92.5%), followed by catheterization (42.5%) and diabetes mellitus (25%). Figure 4 shows the risk factors associated with candidiasis.

Candida isolates were speciated based on germ tube production, corn meal agar morphology, sugar assimilation and sugar fermentation tests. The different species of candida isolated from various clinical samples in our study is given in Table II.

All candida isolates in our study were sensitive to all drugs except two strains of Candida tropicalis which were resistant to fluconazole, C.krusei resistant to fluconazole, itraconazole & ketoconazole and Stephanoascus ciferrii which showed resistance to Amphotericin B and intermediate sensitive to Fluconazole.

DISCUSSION:
Candida is one of the most common emerging pathogen in immunocompromised patients and in patients with underlying risk factors. The rate of candidal infection in our study was 1.9%.
A very high percentage (8.57%) of candida infection was observed among endotracheal tube suction samples, which indicates that patients on ventilators are at high risk of developing candidal infections especially ventilator associated pneumonia.

In our study, candidal infections were more common among adults (75%) than children (25%). Urinary tract infections & ventilator associated pneumonia due to candida was more common among adults while neonatal septicemia was the more common candidal infection among children. This may be due to underlying risk factors like diabetes mellitus, catheterization and usage of broad spectrum antibiotics, which is more commonly observed among adults. All the four candida isolates from blood in our study were from neonates. Identification of a single colony of Candida in the blood is clinically significant. In spite of its poor sensitivity, blood culture continues to be the standard diagnostic test. Accurate, rapid, and sensitive diagnosis of invasive Candida disease is needed for the neonatal population, in whom the burden of disease is high and outcomes are poor.

About 92.5% of patients in our study were on broad spectrum antibiotics, which have emerged as the major risk factor associated with candidal infections, which correlates with the study by Gupta et al and Kashid et al. Use of broad spectrum antibiotics has not only resulted in emergence of multidrug resistance strains, but are also responsible for susceptibility to opportunistic infections like candidal infections. Irrational use of broad spectrum antibiotics has to be curtailed.

Recent studies have reported an increase in the rate of Candida non albicans species. In our study also, we noticed a very high rate of infections due to Candida non albicans species (87.5%), whereas infections due to Candida albicans constituted only about 12.5%. Patel et al have also reported high rate of non candida albicans infection (62.6%) and only 37.4 % of infections caused by Candida albicans. Our study also correlates with the study by Chakrabarti et al, who has reported in increase in non albicans Candida species from 52.6 % in 1992 to 89.5% in 1995. The emergence of Candida non albicans species could be because of frequent use of antifungal agents like fluconazole for prophylaxis & therapy, which results in selection of less susceptible species.

The study by Chenz et al has reported a high rate of 57.6% of invasive infections in critically ill patients due to Candida albicans. Few studies like Mendiratta et al have reported high rate (45.9%) of colonization of preterm babies with Candida albicans and Narain have reported Candida albicans (53.3%) as the major species causing neonatal systemic candidiasis followed by Candida tropicalis (23.3%) & Candida krusei (23.3%).

Candida tropicalis species has emerged as the major Candida non albicans species (50%) in our study, followed by Candida glabrata (17.5%). Our study correlates with the study by Patel et al (40.9%), and Kashid et al (46.25%) who have also reported Candida tropicalis as the most common Candida non albicans species. The predominant isolates in the study by chetana et al on faecal samples of non HIV patients with antibiotic associated diarrhea was also Candida tropicalis, followed by Candida albicans & Candida krusei. Gupta et al have reported a high rate of Candida glabrata (42.1%) candidemia in neonatal ICU. In our study, neonatal candidemia was caused by C. tropicalis in three patients and by C. albicans in one case.

Eventhought many studies have reported an increase in resistance to antifungal drugs, in our study, only two strains of candida tropicalis (10%) were resistant to fluconazole & C.krusei was resistant to fluconazole, itraconazole & ketoconazole and all other isolates were found to be susceptible to azoles, amphotericin B, flucytosine & Caspofungin. Patel et al have reported that Azole group showed 25.5% sensitive among C. albicans and 18.7% sensitive among C. tropicalis to fluconazole while in Amphotericin B, sensitivity varied from 75.6% to 100% to all isolated spp. of candida. The antifungal susceptibility pattern in the study by Kashid et al showed 30.6% resistance to fluconazole, 28.5% resistance to clotrimazole, 11.56% resistance to nystatin and all isolates (100%)were sensitive to amphotericin B.

Frequent use of fluconazole selects for the emergence of candida krusei as a commonly isolated opportunistic pathogen. Furthermore, this organism is intrinsically resistant to fluconazole both invivo and invitro. In our study also, one strain of candida krusei, which was isolated from endotracheal suction tube showed resistance to fluconazole, itraconazole & ketoconazole, but was sensitive to voriconazole, flucytosine, amphotericin B & caspofungin.

All our isolates were sensitive to caspofungin, eventhough caspofungin resistance in c.albicans, c.tropicalis and c.glabrata has been reported in other studies.
Stephanosascus ciferrii isolated from throat swab of a child with odynophagia showed resistance to Amphotericin B and intermediate sensitivity to Fluconazole. Stephanosascus ciferrii is a teleomorph of Candida ciferrii. Stephanosascus ciferrii has also been reported from an aural discharge of a patient with intractable otitis media and also from toe nail. We also isolated other non candida yeast like Trichosporon asahii from urine sample of a patient with indwelling urinary catheter. Trichosporon asahii causing nosocomial urinary tract infection has also been reported by Sun et al and Kumar et al. Non candida yeasts are also now emerging as pathogens causing human infections.

CONCLUSION:

Candida tropicalis is the most common emerging Candida non albicans species causing various clinical infections especially in patients on broad spectrum antibiotics. Not all yeast like cells seen in the microscopy are Candida, they can be yeasts other than candida. Yeasts other than candida should also be identified to species level as in the direct microscopy both are seen as yeasts and now they are emerging as human pathogens. Hence speciation and Susceptibility testing of the candida isolates plays an important role in the management of candidal infections.

FIGURE LEGENDS:

Figure 1: Urine KOH preparation showing yeast cells with pseudohyphae

Figure 2: candida tropicalis growth on Sabouraud’s Dextrose agar

Figure 3: Sugar assimilation test

### Table I: Candida species from various clinical samples

<table>
<thead>
<tr>
<th>Sl No.</th>
<th>Clinical samples</th>
<th>No. of samples</th>
<th>Candida species</th>
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</thead>
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<tr>
<td></td>
<td></td>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>1</td>
<td>Urine</td>
<td>850</td>
<td>32</td>
</tr>
<tr>
<td>2</td>
<td>Blood</td>
<td>660</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>Pus</td>
<td>540</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Endotracheal suction tube</td>
<td>35</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>Throat swab</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>2100</td>
<td>40</td>
</tr>
</tbody>
</table>
Table II: Distribution of Candida species among various clinical samples

<table>
<thead>
<tr>
<th>Sl no</th>
<th>Clinical samples/ Candida species</th>
<th>Urine</th>
<th>Blood</th>
<th>Pus</th>
<th>ET suction tube</th>
<th>Throat swab</th>
<th>Total No. of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Candida tropicalis</td>
<td>17</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>20(50%)</td>
</tr>
<tr>
<td>2</td>
<td>Candida glabrata</td>
<td>6</td>
<td>-</td>
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<td>1</td>
<td>-</td>
<td>7(17.5%)</td>
</tr>
<tr>
<td>3</td>
<td>Candida albicans</td>
<td>3</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>5(12.5%)</td>
</tr>
<tr>
<td>4</td>
<td>Candida parapsilosis</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3(7.5%)</td>
</tr>
<tr>
<td>5</td>
<td>Candida krusei</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>1(2.5%)</td>
</tr>
<tr>
<td>6</td>
<td>Candida famata</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1(2.5%)</td>
</tr>
<tr>
<td>7</td>
<td>Candida colliculosa</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1(2.5%)</td>
</tr>
<tr>
<td>8</td>
<td>Trichosporon asahii</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1(2.5%)</td>
</tr>
<tr>
<td>9</td>
<td>Stephanoascus ciferrii</td>
<td>-</td>
<td>-</td>
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<td>1</td>
<td>-</td>
<td>1(2.5%)</td>
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<tr>
<td>Total</td>
<td></td>
<td>32</td>
<td>4</td>
<td>-</td>
<td>3</td>
<td>1</td>
<td>40</td>
</tr>
</tbody>
</table>

Figure 1: candida tropicalis growth on Sabouraud’s Dextrose agar

Figure 2: Urine wetmount showing yeast cells with pseudohyphae
REFERENCES:


