Isolation, Speciation And Antifungal Drug Susceptibility Of Candida Species Isolated From Various Clinical Specimens In A Tertiary Care Hospital

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ABSTRACT:

Background-
Over the last 20 years, the rates of fungal infection have increased and Candida has emerged as a major cause of human disease. Candida species cause various clinical infections ranging from mucocutaneous infections to life threatening blood stream infections. Emergence of Candida species resistance is on the rise especially to triazoles and amphotericin B has led to use echinocandins, mostly caspofungin in the management of invasive candidiasis. The aim of the study was to identify the spectrum of Candida species and to determine the susceptibility pattern to antifungal agents.

Methods-
A total of 150 Candida isolates were isolated over a period of 2 years. Growth of each isolate was evaluated for colony appearance, Gram stain, Germ tube test, morphological appearance by Dalmau technique using cornmeal agar, sugar utilization test, Culture on CHROMagar for species identification was done for species identification. Antifungal susceptibility was performed as recommended by Clinical and Laboratory Standards Institute (CLSI) M44-A2 document.

Results-
Out of 150 Candida isolates, C. albicans was the most common species. 40% isolates obtained were from age group >60 years. Majority of Candida isolates, 52(34.67%) were from patients who had immunosuppression & chronic drug therapy as predisposing factor. Among the non-albicans Candida species C. tropicalis (27.33%) was predominant isolate followed by C. glabrata (16%). Regarding antifungal susceptibility pattern, Candida species were more resistant to fluconazole (20.67%) followed by ketoconazole (8.66%) and voriconazole (6.67%).

Conclusion-
Due to the variable clinical presentations of Candida infections, it becomes very important to identify these pathogens from all the routine culture specimens received irrespective of clinical diagnosis. Candida spp. differ in their antifungal susceptibility and virulence factors. Thus, routine identification of Candida up to species level along with antifungal susceptibility becomes very essential in diagnostic microbiology laboratory.

Keywords: Antifungal susceptibility testing, Susceptible Dose Dependent, Candida

INTRODUCTION
Fungal infections in immunocompromised individuals are a major cause of morbidity and mortality and Candida are among the most common pathogens in these patients.

Candida is an asexual, diploid, dimorphic fungus. Candida species belong to normal microbiota of an individual’s mucosal oral cavity, gastrointestinal tract and vagina[1] and are responsible for various clinical manifestations from mucocutaneous overgrowth to bloodstream infections.[2] There has been an increase in number of patients who are immunocompromised, aged, receiving prolonged antibacterial and aggressive cancer chemotherapy or undergoing invasive surgical procedures and organ transplantation; therefore, candidiasis has emerged as an alarming opportunistic disease.[3] More than 90% of invasive infections are caused by C. albicans, C. glabrata, C. parapsilosis, C. tropicalis and C. krusei.[4]

The emergence of non-albicans Candida species has however been well recognized during the past decade.[5,6] Candida species have been shown to cause a similar spectrum of disease ranging from oral thrush to invasive disease, yet differences in disease severity and susceptibility to different antifungal agents have been reported.[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.[8,9]

Thus isolation, identification, characterization and susceptibility testing of Candida species in clinical specimens have become increasingly important for management of fungal infections. The present study was designed to identify the spectrum of Candida species in clinical infections and to identify their susceptibility pattern to available antifungal agents.

MATERIAL AND METHODS
A hospital based cross sectional study was conducted for two years (November 2018 to October 2020) by Department of Microbiology, in a tertiary care hospital.

A total of 150 Candida isolates from various clinical samples were included in the study while Candida isolates from stool and sputum samples were excluded. The primary diagnosis of specimens was performed by wet mount and Gram stain. All suspected samples were inoculated on Sabouraud dextrose agar (SDA) slope supplemented with chloramphenicol and aerobically incubated at 37 °C for 24-48hrs. Any visible growth on SDA slope was processed for further identification. From isolated colony,
macroscopic examination, Gram stain, Germ tube test, morphological appearance by Dalmau technique using cornmeal agar, sugar utilization test was performed. Culture on CHROMagar was performed for species identification.

Antifungal susceptibility testing was performed by Disk diffusion method for Fluconazole (25 mcg) and Voriconazole (1 mcg) using CLSI M44A210 and for Ketoconazole (10 mcg) as per Salehei et al (2012)[11] and Infectious Diseases Society of America (2016)[12] since CLSI has no guidelines for antifungal susceptibility of this drug.

The inoculum was prepared by suspending five colonies of growth in 5 ml of sterile saline and compared the turbidity to 0.5 McFarland Standard. A cotton swab was dipped into the inoculum suspension and evenly streaked onto Mueller–Hinton agar supplemented with 2% glucose and 5 µg/ml methylene blue.[13] C. albicans ATCC 90028, C. tropicalis ATCC 750 were used as controls. Antifungal discs containing fluconazole (25 µg), ketoconazole (10 µg), and voriconazole (1µg) were placed on the inoculated media. Zone of inhibition around the disc was measured after incubating the media at 37 °C for 24 h.

Minimum inhibitory concentration (MIC) for fluconazole was performed by Etest for strains resistant to fluconazole by disk diffusion. [14-16]

RESULTS

A total of 150 Candida isolates were isolated from urine (44%), High vaginal swab (26%), Oral swab (10.66%), Pus (9.33%), Blood (5.33%) & other samples (4.66%). Gender wise distribution showed that 56.67% Candida isolates were from males and 43.33% from females. The maximum isolates (40%) obtained were from age group >60 years, followed by 20.67% from age group 21-30 years, 18.67% from 31-40 years age group. (Table 1)
Table 2: Distribution of frequency of *Candida* species in various clinical samples

<table>
<thead>
<tr>
<th>CLINICAL SPECIMEN</th>
<th>ISOLATES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C.ALBICANS</td>
</tr>
<tr>
<td>URINE (66)</td>
<td>26 (37.68%)</td>
</tr>
<tr>
<td>HIGH VAGINAL SWAB (39)</td>
<td>20 (28.98%)</td>
</tr>
<tr>
<td>ORAL SWAB (16)</td>
<td>11 (15.94%)</td>
</tr>
<tr>
<td>PUS (14)</td>
<td>7 (10.14%)</td>
</tr>
<tr>
<td>BLOOD (8)</td>
<td>3 (4.34%)</td>
</tr>
<tr>
<td>OTHER (7)</td>
<td>2 (2.89%)</td>
</tr>
<tr>
<td>TOTAL (150)</td>
<td>69</td>
</tr>
</tbody>
</table>

Overall, antifungal susceptibility profile of *Candida* species to fluconazole was found to be 70.67% susceptible (S), 8.66% susceptible dose dependent (SDD), 20.67% resistant (R). In similar way, antifungal profile (S, SDD, R) to voriconazole was (92%, 1.33%, 6.67% resp.) and to ketoconazole (80.67%, 10.67%, 8.66% resp.) as depicted in Table 3.

Table 3: Antifungal susceptibility testing of various Candida spp.

<table>
<thead>
<tr>
<th>ANTIFUNGLALS</th>
<th>C.ALBICANS</th>
<th>C.TROPICALIS</th>
<th>C.GLABRATA</th>
<th>C.PARAPSILOSIS</th>
<th>C.KRUSEI</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n= 69)</td>
<td>(n= 41)</td>
<td>(n= 24)</td>
<td>(n= 10)</td>
<td>(n= 6)</td>
<td>(n= 150)</td>
</tr>
<tr>
<td>FLUCONAZOLE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>58</td>
<td>24</td>
<td>14</td>
<td>10</td>
<td>-</td>
<td>106 (70.67%)</td>
</tr>
<tr>
<td>SDD</td>
<td>6</td>
<td>6</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>13 (8.66%)</td>
</tr>
<tr>
<td>R</td>
<td>5</td>
<td>11</td>
<td>9</td>
<td>-</td>
<td>6</td>
<td>31 (20.67%)</td>
</tr>
<tr>
<td>VORICONAZOLE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>64</td>
<td>39</td>
<td>20</td>
<td>9</td>
<td>6</td>
<td>138 (92%)</td>
</tr>
<tr>
<td>SDD</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>2 (1.33%)</td>
</tr>
<tr>
<td>R</td>
<td>5</td>
<td>2</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>10 (6.67%)</td>
</tr>
<tr>
<td>KETOCONAZOLE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Amongst the three antifungal agents, fluconazole (20.67%) showed highest level of resistance whereas highest level of susceptibility was observed in voriconazole (92%) followed by ketoconazole (80.67%) and fluconazole (70.67%).

In this study, C.tropicalis (7.33%) showed more resistant to fluconazole in comparison with C.albicans (3.33%). Voriconazole showed 6.67% resistance while ketoconazole showed 8.66% resistance.

Of the total 25 Candida isolates which were resistant to fluconazole by disk diffusion (excluding 6 C.krusei isolates which are inherently resistant to fluconazole), 4 isolates showed MIC between 16-32µg/ml (SDD) and 21 showed MIC ≥64µg/ml (resistant) depicted in Table 4.

Table 4: Minimum inhibitory concentration (MIC) of fluconazole for Candida by Etest method

<table>
<thead>
<tr>
<th>Sensitivity pattern</th>
<th>MIC range</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>≤ 8 µg/ml</td>
<td>0</td>
</tr>
<tr>
<td>SDD</td>
<td>16-32 µg/ml</td>
<td>4</td>
</tr>
<tr>
<td>R</td>
<td>≥ 64 µg/ml</td>
<td>21</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>25</td>
</tr>
</tbody>
</table>

DISCUSSION

Candida spp. are the most common cause of invasive yeast infections. At least 15 distinct species of Candida cause human diseases, although 95% of infections are caused by the five most common pathogens, C. albicans, C. glabrata, C. tropicalis, C. parapsilosis, and C. krusei.[17] Majority of the isolates in this study were obtained from urine(44%) followed by high vaginal swab (26%). Studies by Shaik N et al.,[23] and Joseph K et al.,[33] recovered maximum number of isolates from urine (60% and 46.9%, respectively) followed by respiratory samples (17.3% and 20.4%, respectively).

In this study, C.albicans (46%) was the predominant species isolated followed by C.tropicalis (27.33%) similar to other studies in literature.[18, 22-25, 28, 34, 35] Non albicans Candida (NAC) were isolated at a higher rate (54%) than C.albicans in our study which is in concordance with different studies from India suggesting that NAC are emerging microbial trend in yeast infections.[18- 21] However, some authors in their studies also observed a significant predominance of C.albicans over NAC spp.[25, 28]

When demographically distributed about 56.67% of isolates were from male patients and 40% of Candida were isolated from age group of >60 years. Predominance of Candida species in elderly group in current study might be due to presence of significant co- morbid conditions like diabetes, prolonged antibiotic therapy due to increasing infections due to increasing age. Similar findings were observed by Urvashi Chongtham et al.,[34] Bhaskaran et al.[22]

In the present study, it was observed that Immunosuppression and chronic drug therapy were the most frequently associated risk factors followed by Diabetes mellitus and pregnancy. Other significant risk factors were, preterm/low birth weight, sepsis and indwelling devices. Urvashi Chongtham et al.[34] & Shaik N et al[23] et al also observed higher rate of Candida infections in those patients.

In our study resistance of Candida against fluconazole was more (20.67%) in comparison to other antifungals used in this study. Different studies had also reported higher resistance to fluconazole.[19, 20, 24, 25, 34] While Pandita I et al.[18] & Joseph K et al[33] reported lower resistance of 1.25% & 8.3% resp. to fluconazole. Indiscriminate use of fluconazole & intrinsic resistance of few NAC species contribute to fluconazole resistance. In our study all isolates of C.krusei were resistant to fluconazole. The difference in susceptibility of C.albicans and C.nonalbicans to fluconazole was found statistically significant (p=0.000179).

In our study, voriconazole was found to be susceptible to 92% Candida isolates. Studies by different authors [34,19,33,26] reported 86%, 100%, 90.2%, 100% susceptibility to voriconazole respectively. The susceptibility pattern of Candida to voriconazole in present study was in contrast with More SR et al [27] who reported 73.43% susceptibility to voriconazole by disk diffusion method.

The antifungal ketoconazole was found to be susceptible to 80.67% isolates. Studies by Joseph K et al[33], Shaik N et al[23] reported 90.2%, 90% susceptibility to ketoconazole resp. However, Khadka et al [28] & Urvashi et al [34] reported higher resistance of 86%, 39% in their study.
REFERENCES: