Species distribution, virulence factors and antifungal susceptibility profile of Candida isolates from various clinical samples

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Abstract

Introduction: Historically, most of infections due to Candida species are attributed to Candida albicans. C. albicans is considered as the most pervasive and pathogenic species of genus Candida. However, recent studies on Candida have documented a shift in the epidemiology of candidiasis towards non albicans Candida (NAC) species. NAC spp. produce clinical manifestations similar to C. albicans however, they differ in respect to epidemiology, virulence factors and most importantly antifungal susceptibility pattern.

Methods: Species distribution, virulence factors and antifungal susceptibility pattern of Candida isolates from various clinical specimens was studied.

Observation and results: NAC spp. were the predominant isolates. Maximum isolates were from urine samples followed by vaginal swabs. NAC spp. produced virulence factors similar to C. albicans. Antifungal resistance was more in NAC spp. compared to C. albicans.

Conclusion: Emergence of non albicans Candida species along antifungal resistance highlights the importance of species identification with antifungal susceptibility testing. NAC spp. like C. tropicalis produce virulence factors once only attributed to C. albicans.

Key words: Antifungal resistance, Candida, C. albicans, non albicans Candida species, virulence factors

Introduction

In recent years, mycotic diseases are becoming more and more important because of various reasons like increased in immunocompromised patients, use of broad spectrum antibiotics and immunosuppressive drugs and presence of indwelling medical devices. The severity of mycotic infection ranges from mild to fatal and is dependent on various factors like virulence of infecting strain and host predisposing factors. Candidiasis is one of the most prevalent mycotic infections worldwide. The spectrum of clinical manifestations of Candida infection ranges from mucocutaneous overgrowth to fulminant systemic infections like candidemia and meningitis. Historically, most of infections due to Candida species are attributed to Candida albicans. C. albicans is considered as the most pervasive and pathogenic species of genus Candida. However, recent studies on Candida have documented a shift in the epidemiology of candidiasis towards non albicans Candida (NAC) species. NAC spp. produce clinical manifestations similar to C. albicans however, they differ in respect to epidemiology, virulence factors and most importantly antifungal susceptibility pattern.

Candida is a commensal flora of human. It colonizes the gastrointestinal and genitourinary tract. The transition of Candida from harmless commensal to potent pathogen is mediated by number of virulence factors like production of extracellular enzymes, biofilm formation and haemolysin production.

The present study was carried out in tertiary care academic hospital with an aim to study species distribution, virulence factors and antifungal susceptibility pattern of Candida isolates from various clinical specimens.
Materials and methods

A total of 307 Candida spp. isolated from various clinical specimens were included in the study. Candida isolates were identified up to species level by standard mycological techniques like germ tube test, carbohydrate assimilation and colony color on Hichrom Candida agar.

The Candida isolates were screened for production of virulence factors like production of extracellular enzymes (phospholipase and proteinase), biofilm formation and haemolysin production.

(1) Extracellular enzymes

(1.1) Phospholipase production: The isolates were screened for in vitro phospholipase activity by using egg yolk agar. The procedure described by Samaranayake et al (1984) was used. Standard inoculum (5 µl) of each isolate was aseptically inoculated onto egg yolk agar. Inoculum contained 10⁸ cells/ml of isolate to be tested. Presence of zone of precipitate around the colony was considered as indicator of phospholipase production. Phospholipase activity (Pz) was expressed as the ratio of the colony to the diameter of the colony plus the precipitation zone. As suggested by Deorukhkar et al (2014), A Pz value of 1 denoted no phospholipase production by isolate whereas Pz<1 denoted no phospholipase activity. C. albicans ATCC 10231 and C. kefyr ATCC 25412 were used as positive and negative controls, respectively.

(1.2) Proteinase production: Proteinase activity in Candida isolates was screened by the method described by Aoki and colleagues (1990). Bovine serum albumin (BSA) agar was used for screening proteinase activity. Proteinase activity (Prz) was measured in the terms of the ratio of the colony to the diameter of unstained zone. As described by Deorukhkar et al (2014), A Prz value of 1 denoted no proteinase production by isolate whereas Prz<1 denoted no proteinase activity. C. albicans ATCC 10231 and C. kefyr ATCC 25412 were used as positive and negative controls, respectively.

(2) Biofilm formation

Biofilm formation in Candida isolates was detected by using polystyrene tube method as suggested by Yigit et al (2011). Presence of visible adherent film on the wall and the bottom of the tube indicated biofilm formation by the isolate. C. albicans ATCC 90028 and C. albicans ATCC 10231 were used as positive and negative controls, respectively.

(3) Haemolysin production.

Haemolysin production in Candida isolates was screened on sheep blood Sabouraud dextrose agar plate as suggested by Luo et al (2001). The presence of a distinct translucent halo around the colony indicated haemolysin activity. As suggested by Deorukhkar et al (2014), Haemolytic activity was determined by calculating the ratio of the diameter of the colony to the translucent haemolytic zone. C. albicans ATCC 90028 and C. parapsilosis ATCC 22019 were used as positive and negative control, respectively. Additionally, one strain each of Streptococcus pyogenes and Streptococcus sanguis were used as controls for β and α haemolysis respectively.

Antifungal susceptibility profile of Candida isolates was studied by Clinical and Laboratory Standard Institute (CLSI) Reference Method for Antifungal Disc Diffusion Susceptibility Testing of Yeasts. Antifungal discs were procured Himedia Laboratories Pvt. Ltd Mumbai. The results of antifungal susceptibility tested were interpreted as per the approved CLSI guidelines. C. albicans ATCC 90028 and C. parapsilosis ATCC 22019 were as control strains.
Results

In the present study, out of 307 Candida isolates, a total of 214 (69.7%) isolates belonged to NAC spp. whereas 93 (30.3%) were C. albicans. As shown in figure 1. Maximum isolates were from urine samples (33.2%) followed by vaginal swabs (31.9%).

![Figure 1: Clinical specimen wise distribution of Candida isolates.](image)

The species wise distribution of NAC isolates is shown in figure 2. C. tropicalis followed by C. krusei and C. glabrata were predominant isolates.

![Figure 2: Species wise distribution of non albicans Candida isolates.](image)

Table 1, shows species and clinical specimen wise distribution of Candida isolates. Majority of C. dubliniensis isolates were isolated from oral swabs. These isolated were from HIV infected patients having oropharyngeal candidiasis. A total of 2 C. dubliniensis isolates were from non-HIV infected individuals with vulvovaginal candidiasis (VVC). In this study, all cases of candidemia were due to NAC spp.
Table 1: Clinical specimens and species wise distribution of Candida isolates.

<table>
<thead>
<tr>
<th>No. of isolates</th>
<th>C. albicans</th>
<th>C. tropicalis</th>
<th>C. krusei</th>
<th>C. glabrata</th>
<th>C. kefyr</th>
<th>C. parapsilosis</th>
<th>C. dubliniensis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>38</td>
<td>41</td>
<td>15</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>102</td>
</tr>
<tr>
<td>Vaginal swab</td>
<td>31</td>
<td>46</td>
<td>7</td>
<td>6</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>98</td>
</tr>
<tr>
<td>Oral swab</td>
<td>21</td>
<td>11</td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>18</td>
<td>64</td>
</tr>
<tr>
<td>Blood</td>
<td>0</td>
<td>5</td>
<td>2</td>
<td>4</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>Pus</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>2</td>
<td>8</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>Total</td>
<td>93</td>
<td>114</td>
<td>34</td>
<td>23</td>
<td>12</td>
<td>11</td>
<td>20</td>
<td>307</td>
</tr>
</tbody>
</table>

As shown in table 2, a total of 209 (68.1%) Candida isolates showed phospholipase activity. Proteinase and haemolysin production was seen in 71.3% and 73.3% of Candida isolates respectively. Biofilm formation was seen in a total of 222 (72.3%) isolates. Virulence factor production was high among C. tropicalis isolates followed by C. albicans.

Table 2: Virulence factors production in Candida species.

<table>
<thead>
<tr>
<th>Candida spp.</th>
<th>Phospholipase production (%)</th>
<th>Proteinase production (%)</th>
<th>Biofilm formation (%)</th>
<th>Haemolysin production (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>76 (36.7)</td>
<td>74 (33.7)</td>
<td>89 (40.1)</td>
<td>91 (40.5)</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>102 (48.8)</td>
<td>112 (51.1)</td>
<td>109 (49.1)</td>
<td>111 (49.3)</td>
</tr>
<tr>
<td>C. krusei</td>
<td>18 (8.6)</td>
<td>19 (8.7)</td>
<td>16 (7.2)</td>
<td>11 (4.9)</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>06 (2.9)</td>
<td>01 (0.4)</td>
<td>02 (0.9)</td>
<td>02 (0.9)</td>
</tr>
<tr>
<td>C. kefyr</td>
<td>03 (1.4)</td>
<td>01 (0.4)</td>
<td>02 (0.9)</td>
<td>01 (0.4)</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>03 (1.4)</td>
<td>01 (0.4)</td>
<td>02 (0.9)</td>
<td>01 (0.4)</td>
</tr>
<tr>
<td>C. dubliniensis</td>
<td>01 (0.8)</td>
<td>11 (5.1)</td>
<td>02 (0.9)</td>
<td>08 (3.6)</td>
</tr>
<tr>
<td>Total</td>
<td>209</td>
<td>219</td>
<td>222</td>
<td>225</td>
</tr>
</tbody>
</table>

The antifungal susceptibility profile of Candida isolates is shown in table 3. Azole resistant was more common in NAC spp. as compared to C. albicans. All C. krusei isolates demonstrated resistance to fluconazole. Amphotericin B resistance was low.
Table 3: Antifungal susceptibility profile of *Candida* isolates.

<table>
<thead>
<tr>
<th><em>Candida</em> spp. (N)</th>
<th>Fluconazole Resistant (%)</th>
<th>Itraconazole Resistant (%)</th>
<th>Ketoconazole Resistant (%)</th>
<th>Amphotericin B Resistant (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans (93)</td>
<td>06 (6.4)</td>
<td>04 (4.3)</td>
<td>12 (12.9)</td>
<td>01 (1.1)</td>
</tr>
<tr>
<td>C. tropicalis (114)</td>
<td>15 (13.2)</td>
<td>06 (5.3)</td>
<td>18 (15.8)</td>
<td>01 (0.9)</td>
</tr>
<tr>
<td>C. krusei (34)</td>
<td>34 (100)</td>
<td>21 (61.8)</td>
<td>30 (88.2)</td>
<td>03 (8.8)</td>
</tr>
<tr>
<td>C. glabrata (23)</td>
<td>16 (69.6)</td>
<td>10 (3.5)</td>
<td>14 (60.9)</td>
<td>04 (17.4)</td>
</tr>
<tr>
<td>C. kefyr (12)</td>
<td>01 (8.3)</td>
<td>-</td>
<td>02 (16.7)</td>
<td>-</td>
</tr>
<tr>
<td>C. parapsilosis (11)</td>
<td>01 (9.1)</td>
<td>01 (9.1)</td>
<td>02 (18.2)</td>
<td>-</td>
</tr>
<tr>
<td>C. dubliniensis (20)</td>
<td>05 (25)</td>
<td>-</td>
<td>04 (20)</td>
<td>-</td>
</tr>
<tr>
<td>Total (307)</td>
<td>78 (25.4)</td>
<td>42 (13.7)</td>
<td>82 (26.7)</td>
<td>09 (2.9)</td>
</tr>
</tbody>
</table>

Discussion

Fungus belonging to the genus *Candida* is unique among various mycotic pathogens as it exists in both commensal and pathogenic state and has broad spectrum of clinical manifestations. In accordance to recent national and international studies, predominance of NAC spp. was noted in the present study. NAC spp. is a heterogeneous group of *Candida* with about 19 medically important species. The emergence of NAC spp. as an important cause of infections can be attributed to various reasons, but improvement in identification techniques involving use of chromogenic media, commercially available kits and molecular techniques appear to be more important.

In the present study, *C. tropicalis* followed by *C. krusei* and *C. glabrata* were major isolates from NAC group. This observation is in accordance to other researchers. *C. tropicalis* alone or in association with other species of *Candida* is increasingly reported. *C. glabrata* is the only known haploid *Candida* spp. and is associated with high mortality rates.

Similar to recent study conducted by Deorukhkar and Roushani (2017), majority of *Candida* spp. were isolated from urine samples. Candiduria is now recognized as an important subgroup of nosocomial urinary tract infections. A total of 98 (31.9%) *Candida* spp. were isolated from vaginal swabs. Vulvovaginal candidiasis (VVC) is a common manifestation of mucocutaneous candidiasis. In recent years, *Candida* spp. have emerged as important cause of blood stream infections (BSI). *Candida* spp. is 3rd most common etiological agent of nosocomial BSI in the US. In the present study, all isolates from BSI belonged to NAC spp. Recent national and international studies have reported similar findings.

Various virulence factors like adhesion to host tissues and surfaces of indwelling medical devices, biofilm production, thigmotropism, phenotypic switching and secretion of hydrolytic enzymes attributes to pathogenicity of *Candida* spp.

Extracellular hydrolytic enzymes like phospholipase and proteinase facilitate the *Candida* invasion by damaging host cellular contents. Phospholipase cleaves phospholipids of host cell membrane. In the present study, phospholipase activity was noted in 209 (68.1%) *Candida* isolates. Among *Candida* spp., high phospholipase activity was noted in *C. tropicalis* isolates whereas, *C. dubliniensis* demonstrated low phospholipase production. Similar findings were noted by Deorukhkar et al (2014). Proteinase aids *Candida* colonization and invasion by deteriorating host’s epithelial and mucosal barrier proteins. In the current study, a total of 219 (71.3%)
Candida spp. demonstrated in-vitro proteinase production. Silva et al (2012) reported high proteinase activity in C. tropicalis isolates in medium containing bovine serum albumin as a sole nitrogen source. In recent years, medical device-associated infections due to Candida spp. have increased dramatically. As per Seneviratne et al (2008) Candida spp. can form biofilm on most, if not all currently used medical devices. In the present study, biofilm formation was high in C. tropicalis isolates. This finding is similar to that of Deorukhkar et al (2014). Haemolysin is one of the putative virulence factors contributing to pathogenicity of Candida spp. It degrades hemoglobin and aids recovery of the elemental iron from host’s red blood cells. In this study, haemolysin production was seen in 73.3% of Candida isolates.

Emergence of NAC spp., has highlighted the importance of antifungal susceptibility testing. In the present study, all isolates of C. krusei were resistant to fluconazole. Many national and international studies have reported similar findings. C. krusei is considered to be innately resistant to fluconazole. Fluconazole resistance was seen in 69.6% of C. glabrata isolates. Similar finding was reported by Sanglard et al (1999). Amphotericin B resistance was very low in our study.

Conclusion
Emergence of non albicans Candida species along antifungal resistance highlights the importance of species identification with antifungal susceptibility testing. NAC spp. like C. tropicalis produce virulence factors once only attributed to C. albicans.

References

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