Detection of biofilm production in *Candida* species isolates recovered from bloodstream patients

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**Abstract**

**Introduction:** *Candida* Bloodstream Infections (CBSIs) are the fourth most common infections among hospitalized patients. *Candida* biofilm results from an initial attachment of cells to glycoprotein-coated host cells and tissue or biomaterial surfaces.

**Material and Method:** *Candida* spp. was identified by conventional method and biofilm formation was detected by tube method.

**Result:** *C. tropicalis* was the most common species followed by *C. haemulonii, C. albicans, C. parapsilosis, C. glabrata, C. pelliculosa, C. guilliermondii* and *C. kruisi*. 125 of the isolates were tested for biofilm formation of which 67(53.6%) were found to be capable of forming biofilms. Of these 55(82%) biofilm forming isolates were those recovered from patients with Catheter Related Candidemia (CRC) while 12 (17.9%) biofilm forming isolates were without CRC. Most common biofilm producing species were *C. pelliculosa* followed by *C. tropicalis, C. haemulonii, C. parapsilosis, C. glabrata, C. albicans, C. kruisi* and *C. guilliermondii*.

**Conclusion:** Our study found more biofilm production in Central Venous Catheter (CVC) Candidemia and most of the CVC related Candidemia were found in diabetic and neutropenic patients and hence care should be taken in those patients who are at risk of developing biofilm production before applying the external appliances.

**Keywords:** Catheter Related Candidemia (CRC), *Candida* Bloodstream Infections (CBSIs), Non-Albicans Candida (NAC), Central Venous Catheter (CVC).

1. **Introduction**  
*Candida* bloodstream infections (CBSIs) are the fourth most common infections among hospitalized patients [1], accounting for 8% to 15% of hospital-acquired BSIs [2]. They are considered high-morbidity infections [3, 4], with significant hospital costs [5, 6], largely due to increased hospital length of stay and costs for antifungal therapy [2]. Fungal biofilm are increasingly common as a result of the widespread use of antibiotics, medical devices and the increase in the number of immunocompromised patients [7-9]. *Candida* biofilm results from an initial attachment of cells to glycoprotein-coated host cells and tissue or biomaterial surfaces. The second phase (proliferation and biofilm formation) is characterized by the generation of a three-dimensional structure [10-12], which is highly dependent on the conditions under which the biofilm is formed (e.g., type of implanted device and its location) [13-15]. This infection is highly serious because biofilms are thought to be recalcitrant to antifungal (e.g., fluconazole) therapy [16] and only two classes of agents (i.e. amphotericin B and echinocandins) appear to have *in vitro* efficacy against *Candida* biofilms [17,18].

2. **Material & Method**

A total of 141 *Candida* species isolated from fungemia suspected patients were included in the study. All positive blood cultures were Gram-stained for preliminary identification of the microorganism and subculture on SDA agar and incubated at 37°C for 24 hrs. Identification of the species was done by Germ tube test, Hi-Chrom agar and confirmed by morphology on Corn Meal Agar (CMA) and Sugar fermentation tests as per standard methods.

Of the total of 141 *Candida* strains biofilm production was performed with 125 strains as 16 strains were contaminated during storage. Biofilm production was determined by tube methods proposed by Branchini et al. [19]. Inoculum of *Candida* isolates was prepared in Sabouraud’s Dextrose Broth supplemented with 8% glucose.
and turbidity adjusted to McFarland’s 0.5. Ten millilitre broth in tubes were incubated at 37°C for 24 hrs, after which the broth was aspirated out and stained with 1% safranin. The tubes were then kept still for 7 min. Safranin was then removed, and the tubes were examined for biofilm production. In tubes, biofilm production was observed visually by two separate observers and correlated. The adherent biofilm layer was scored visually as negative, weak positive, or strong positive as described by Shin et al. In our study, all positive results, including weak or strong, were considered as positive. We used C. albicans ATCC 90028 strain as control.

3. Result

A total of 141 Candida strains C. tropicalis 32 (22.7%), was the most common, followed by C. haemulonii 30 (21.2%), C. albicans 26 (18.4%), C. parapsilosis 25 (17.7%), C. glabrata 13 (9.2%), C. pelliculosa 7 (5%), C. guillermondii and C. krusei 4 (3%) [20], (Table-1).

The main underlying condition in patients forming biofilm production was catheterization, diabetes and neutropenia and prolonged antibiotic (Table 2).

125 of the isolates were tested for biofilm formation of which 67 (53.6%) were found to be capable of forming biofilms. Of these 55 (82%) biofilm forming isolates were those recovered from patients with Catheter Related Candidemia (CRC) while 12 (17.9%) biofilm forming isolates were without CRC. Most common biofilm producing species were C.pelliculosa (71.4%) followed by C.tropicalis (66%), C.haemulonii (56%), C. parapsilosis (54.5%), C. glabrata (53.8%), C. albicans (37.5%) C. krusei (33.3%) and C. guillermondii (25%) (Table 3).

Table 1: Distribution of Candida species

<table>
<thead>
<tr>
<th>Species</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.tropicalis</td>
<td>32</td>
<td>22.7%</td>
</tr>
<tr>
<td>C.haemulonii</td>
<td>30</td>
<td>21.2%</td>
</tr>
<tr>
<td>C. albicans</td>
<td>26</td>
<td>18.4%</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>25</td>
<td>17.7%</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>13</td>
<td>9.2%</td>
</tr>
<tr>
<td>C. pelliculosa</td>
<td>7</td>
<td>5%</td>
</tr>
<tr>
<td>C. guillermondii</td>
<td>4</td>
<td>3%</td>
</tr>
<tr>
<td>C. krusei</td>
<td>4</td>
<td>3%</td>
</tr>
</tbody>
</table>

Table 2: Characteristics of patients with biofilm forming isolates

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Biofilm production</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>67</td>
<td>53.6%</td>
</tr>
<tr>
<td>Catheterization</td>
<td>55</td>
<td>82%</td>
</tr>
<tr>
<td>Diabetics</td>
<td>32</td>
<td>47.7%</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>31</td>
<td>46%</td>
</tr>
<tr>
<td>Prolonged antibiotic</td>
<td>26</td>
<td>38.8%</td>
</tr>
</tbody>
</table>

Table 3: Distribution of Candida spp. forming biofilm production among the Catheter-Related Candidemia (CRC) and non CRC isolates

<table>
<thead>
<tr>
<th>Candida species</th>
<th>Total</th>
<th>Biofilm production</th>
<th>Percentage</th>
<th>CRC</th>
<th>Non-CRC</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.pelliculosa</td>
<td>7</td>
<td>5</td>
<td>71.4%</td>
<td>3(60%)</td>
<td>2(40%)</td>
</tr>
<tr>
<td>C.tropicalis</td>
<td>27</td>
<td>18</td>
<td>66.6%</td>
<td>12(66.6%)</td>
<td>6(33.3%)</td>
</tr>
<tr>
<td>C.haemulonii</td>
<td>25</td>
<td>14</td>
<td>56%</td>
<td>12(85.7%)</td>
<td>2(14.2%)</td>
</tr>
<tr>
<td>C.parapsilosis</td>
<td>22</td>
<td>12</td>
<td>54.5%</td>
<td>12(100%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>C.glabrata</td>
<td>13</td>
<td>7</td>
<td>53.8%</td>
<td>6(85.7%)</td>
<td>1(14.3%)</td>
</tr>
<tr>
<td>C.albicans</td>
<td>24</td>
<td>9</td>
<td>37.5%</td>
<td>9(100%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>C.krusei</td>
<td>5</td>
<td>1</td>
<td>33.3%</td>
<td>1(100%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>C.guillermondii</td>
<td>4</td>
<td>1</td>
<td>25%</td>
<td>0(0%)</td>
<td>1(100%)</td>
</tr>
</tbody>
</table>

4. Discussion

A wide range of biomaterials used in clinical practice are shown to support colonization and biofilm formation by Candida species [21], making device-related Candida infections relatively refractory to medical therapy [22]. It has been reported that certain Candida species in the presence of glucose-containing fluids or lipid emulsion might produce “slime” (now commonly referred to as biofilm), potentially explaining the increased proportion of CBSIs among patients receiving parenteral nutrition [23-25].

In the present study C. tropicalis, was the most common species followed by C. haemulonii, C. albicans, C. parapsilosis, C. glabrata, C. pelliculosa, C. guillermondii and C. krusei. While, a study by Shyamala et al [26] reported C. albicans as the most common species followed by C. tropicalis, C. glabrata, C. krusei, C. dubliniensis, C. guillermondii, C. kefyr and C. parapsilosis.

In our study, we found risk factors of Catheterization, Diabetes, Neutropenia and prolonged antibiotics that were specifically associated with biofilm-forming CBSI. Diabetes mellitus has previously been reported to be a general risk factor for Candida infections [22]. Yet, glucose is thought to serve as the carbohydrate energy source required by Candida for biofilm formation [25], perhaps necessary to produce the polysaccharide matrix [27], in which organized communities of yeast, hyphae, and pseudohyphae are enclosed [28]. In a study by Bhatt et al[29] they reported administration of broad spectrum antibiotics, indwelling catheter and patients on mechanically ventilator were the major risk factor.

C. pelliculosa was found to be the most common Candida species which formed maximum number of biofilm production followed by C. tropicalis, C. haemulonii, C. parapsilosis, C. glabrata, C. albicans, C. krusei and
C. guilliermondii. However, Shin et al [23] reported C. tropicalis was most common isolates followed by C. parapsilosis, C. glabrata, and C. albicans. While, another study by Bhatt et al [29] reported C. parapsilosis and C. tropicalis were strong biofilm producers whereas C. albicans and C. krusei were identified as weak producers.

5. Conclusion

Advanced patient management has seen that increased use of prosthetic biomaterials and changing epidemiology of Candidemia are responsible for biofilm production. Our study found more biofilm production in CVC Candidemia and most of the CVC related Candidemia were found in diabetic and neutropenic patients and hence care should be taken in those patients who are at risk of developing biofilm production before applying the external appliances.

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Conflict of Interest

The authors declare there is no conflict of interest.

Reference


