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Original Research Article

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. krusei.* 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.krusei* 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^{o}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 37^0C 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. guilliermondii* 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. guilliermondii* (25%) (Table 3).

Table 1: Distribution of *Candida* species

Species	Number	Percentage
C.tropicalis	32	22.7%
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	4	3%
C. krusei	4	3%

Table 2: Characteristics of patients with biofilm forming isolates

Characteristics	Biofilm production	Percentage
Number of patients	67	53.6%
Catheterization	55	82%
Diabetics	32	47.7%
Neutropenia	31	46%
Prolonged antibiotic	26	38.8%

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

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

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. guilliermondii 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.

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