Comparison of biofilm formation in clinical isolates of *Candida* species in a tertiary care center, North India

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ABSTRACT

**Background and Objectives:** Biofilms are colonies of microbial cells encased in a self-produced organic polymeric matrix. The biofilm production is more important for nonalbicans *Candida* (NAC); as *C. albicans* possess many other mechanisms to establish infections. Correct identification of *Candida* species has gained importance due to persistent rise in infections caused by NAC. We sought to isolate, identify *Candida* species in clinical isolates and study biofilm formation.

**Materials and Methods:** Modified microtiter plate method was performed to study biofilm formation by isolates in Sabouraud's dextrose broth. It was then quantitatively assessed using a spectrophotometer. Biofilm formation was graded as negative, +1, +2, +3 and + 4 on the basis of percentage absorbance.

**Results:** Biofilm formation was observed in 16 of 40 (40.0%) isolates of *C. albicans* as compared to 39 of 78 (50.0%) of isolates of NAC. Strong (+4) biofilm production was seen in maximum biofilm producers in *C. tropicalis* (12 of 27) followed by *C. albicans* (8 of 16). Total biofilm producers were significantly more among high vaginal swab isolates 63.2% (12 of 19) and urine isolates 59.2% (29 of 49), when compared to blood isolates 34.2% (13 of 38) as well as other isolates 27.5% (11 of 40). **Interpretation and Conclusions:** NAC species are qualitatively and quantitatively superior biofilm producers than *C. albicans*. Biofilm production is the most important virulence factor of NAC species and compared to other lesions, it is more significantly associated with luminal infections.

**KEY WORDS:** Biofilm, *Candida albicans*, nonalbicans *Candida*, virulence factor, yeast

INTRODUCTION

*Candida* species are normal inhabitants of the skin and mucosa. The importance of epidemiological monitoring of yeasts involved in pathogenic processes is unquestionable due to the increase of these infections over the last decade; so are the changes observed in species causing candidiasis.[1] The most external layers of *Candida* cells are essential for the adherence to host surface, thereby playing a pivotal role in the pathophysiology of candidiasis.[2] Biofilms are colonies of microbial cells encased in a self-produced organic polymeric matrix and represent a common mode of microbial growth. Recently, microbial biofilms have gained prominence because of the increase in infections related to indwelling medical devices.[3] The advantages of forming a biofilm for the organism include protection from the environment, nutrient availability, metabolic cooperation, and acquisition of new genetic traits.[4]

Biofilms may help maintain the role of fungi as commensal and pathogen, by evading host immune mechanisms, resisting antifungal treatment, and withstanding the competitive pressure from other organisms. Consequently, biofilm related infections are difficult to treat.[5] The biofilm production is also associated with high level of antimicrobial resistance of the associated organisms.[6]
characteristics of candidemia, including number of positive blood cultures, presence of central venous catheter-related candidemia, total parenteral nutrition and clinical significance candidemia.\textsuperscript{[12]}

The biofilm production is more important for NAC strains and \textit{C. albicans} possess mechanisms other than biofilm production to establish infections.\textsuperscript{[13]} \textit{Candida} species are now recognized as major agents of hospital acquired infection worldwide.\textsuperscript{[14]}

This study was therefore undertaken, to study distribution of various \textit{Candida} species isolated from various clinical samples and their capacity to form biofilm.

MATERIALS AND METHODS

The present study was conducted from January 2013 to December 2013, on 118 nonrepeat samples received in clinical Microbiology laboratory of tertiary care center, in western Uttar Pradesh. \textit{Candida} species were isolated from clinical specimens received from tertiary care hospital, identified and studied for biofilm formation.

The study was approved by the research and ethics committee.

\textit{Candida} species were isolated from blood, urine, pus, high vaginal swab (HVS) and other clinical samples. The clinical isolates of \textit{Candida} were identified up to species level by standard laboratory techniques.\textsuperscript{[15]} \textit{C. albicans} ATCC 90028 strain was used as control. The modified microtiter plate method was used for biofilm formation by isolates in Sabouraud’s dextrose broth. Its formation was observed and confirmed by inverted microscope, at the end of 24 h incubation.\textsuperscript{[16]} It was then quantitatively assessed using a spectrophotometer. The percentage absorbance (%A) value for each test sample was calculated by subtracting percentage absorbance value of reagent blank from the percentage absorbance value for the sample, difference giving a measure of the amount of light blocked by biofilm when passing through the bottom of wells. Biofilm formation was graded as negative (%A: <5), +1 (%A: 5–20), +2 (%A: 20–35), +3 (%A: 35–50) and +4 (%A: More than 50).\textsuperscript{[17]}

RESULT

A total of 118 nonrepeat clinical isolates of \textit{Candida} species were included in this study and screened for biofilm production. The clinical specimens from which these species were isolated included urine 49 (41.5%), blood 38 (32.2%), HVS 19 (16.1%), pus 7 (5.9%), vault swab 3 (2.5%) and 1 (0.9%) each of bone with tissue and central line [Table 1].

The isolated \textit{Candida} species included 40 (33.9%) \textit{C. albicans} and 78 (66.1%) NAC.

ATCC 90028 strain of \textit{C. albicans} did not show biofilm formation. Biofilm formation was observed in 16 of 40 (40.0%) isolates of \textit{C. albicans} as compared to 39 of 78 (50.0%) of isolates of NAC. The Chi-square test was applied which interpreted a nonsignificant variation in the number of biofilm producers and nonproducers ($\chi^2 = 1.06$ and $P = 0.30$) [Table 2].

The NAC species were further identified up to individual species level. Various NAC species isolated were \textit{C. tropicalis} 40 (33.9%), \textit{C. parapsilosis} 22 (18.7%), \textit{C. krusei} 6 (5.1%), \textit{C. glabrata} 4 (3.4%), \textit{C. kefyr} 3 (2.5%) and \textit{C. guilliermondii} 3 (2.5%).

Biofilm formation was seen in all the isolates (3 of 3) of \textit{C. kefyr} and \textit{C. guilliermondii}. It was also more prominently observed in isolates of \textit{C. tropicalis} (67.5%), followed by \textit{C. albicans} (40%), \textit{C. glabrata} (25%) and \textit{C. parapsilosis} (22.7%). None of the isolates of \textit{C. rusei} (60%) produced any biofilm.

Among the biofilm producers, strong (+4) biofilm production was seen in maximum number in \textit{C. tropicalis} (12 of 27), followed by \textit{C. albicans} (8 of 16), \textit{C. parapsilosis} (3 of 5), \textit{C. guilliermondii} (2 of 3), \textit{C. kefyr} (1 of 3) and \textit{C. glabrata} (1 of 1). None of the \textit{Candida krusei} isolates showed biofilm production [Table 3].

Twenty-nine of 49 urine, 13 of 38 blood and 12 of 19 HVS isolates were biofilm producers. Biofilm producers among the 12 other samples consisted 1 of 7 pus, 1 of 3 vault swab, 1 of 1 central venous line and 1 of 1 bone + tissue [Table 1].

DISCUSSION AND CONCLUSIONS

The biofilm growth protects the microorganism from the host defense and antimicrobial agents. In this line, biofilm formation is a risk factor that increases the mortality rate in candidiasis in critically ill patients or immunocompromised individuals.\textsuperscript{[18]}

Recent studies have documented a shift toward NAC species from \textit{C. albicans}.\textsuperscript{[19]} Some studies have reported increasing trend of incidences of infections caused by NACs, gradually surpassing \textit{C. albicans} as cause of candidemia in some regions.\textsuperscript{[20]} Factors such as increased use of antifungal drugs and broad spectrum

| Table 1: Comparison of biofilm producers in isolates from different clinical samples |
|-------------------------|-------------------------|-------------------------|
| Sample                  | Biofilm producer | Biofilm nonproducer |
| Urine (n=49)            | 29 (59.2)          | 20 (40.8)             |
| Blood (n=38)            | 13 (34.2)          | 25 (65.8)             |
| HVS (n=19)              | 12 (63.2)          | 07 (36.8)             |
| Other (n=12)            | 04 (33.3)          | 08 (66.7)             |
| Total                   | 55                 | 63                    |

| Table 2: Distribution of isolates according to biofilm production |
|-------------------------|-------------------------|
| Isolates                | Biofilm producer | Biofilm nonproducer |
| \textit{Candida albicans} (n=40) | 16 (40)          | 24 (60)              |
| NAC (n=78)              | 39 (50)           | 39 (50)              |
| Total (n=118)           | 55 (46.7)         | 63 (53.3)            |

HVS: High vaginal swab

NAC: Nonalbicans Candida
Table 3: Distribution of different grade of biofilm in isolates of Candida species

<table>
<thead>
<tr>
<th>Candida species</th>
<th>Biofilm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grade +4 (%)</td>
</tr>
<tr>
<td>Candida albicans (n=40)</td>
<td>8 (20.00)</td>
</tr>
<tr>
<td>Candida tropicalis (n=40)</td>
<td>12 (30.00)</td>
</tr>
<tr>
<td>Candida parapsilosis (n=22)</td>
<td>3 (13.64)</td>
</tr>
<tr>
<td>Candida krusei (n=6)</td>
<td>0 (00.00)</td>
</tr>
<tr>
<td>Candida glabrata (n=4)</td>
<td>1 (25.00)</td>
</tr>
<tr>
<td>Candida kefyr (n=3)</td>
<td>1 (33.33)</td>
</tr>
<tr>
<td>Candida guilliermondii (n=3)</td>
<td>2 (66.67)</td>
</tr>
<tr>
<td>Total</td>
<td>27</td>
</tr>
</tbody>
</table>

The virulence factors vary in relation to type, site and stage of infection. We found that biofilm producers were significantly higher in numbers among HVS isolates 63.2% (12 of 19) and urine isolates 59.2% (29 of 49), compared to blood isolates 34.2% (13 of 38) as well as other isolates 27.5% (11 of 40). Biofilm as a virulence factor thus appears to contribute most in pathogenesis of urinary tract infection and other luminal infections, compared to other clinical conditions.

Due to the increasing incidence of Candida infections, there is great interest in Candida virulence factors, which are in turn important in the establishment of the strategies for control and prevention of candidiasis. 

Nonalbicans Candida species cannot be overlooked as mere contaminant or nonpathogenic commensals. Research on prevalent Candida species along with their virulence factors in a given set up would be an important tool to prove the relation between the infective species of Candida and infection. The changing patterns of the Candida isolation from various clinical samples have made identification of Candida species producing virulence factors compulsory for diagnostic Microbiology service. 

This changing trend of causative role of Candida in different studies from different parts of the world and from India and the emergence of NAC species and their association with virulence factors cannot be overlooked. Increased isolation and complete identification of Candida species in more Microbiology laboratories might be instrumental in reports of rising NAC emergence. More multilocal studies on larger sample size will definitely go a long way in revealing epidemiology, emergence and spread of NAC.

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Conflicts of interest
There are no conflicts of interest.

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