

Original Research Article

Cell Surface Hydrophobicity and Biofilm Formation among Clinical Isolates of Candida

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ABSTRACT

The different Candida species differ in their capacities to form biofilms which help them for development of clinical infection and subsequently chronic infection. The biofilm formation also confers them ability to resist to antifungal therapy and to withstand host immune defences. Biofilm formation is a step by step and finely regulated process in which the adhesion phase is a crucial event and cell surface hydrophobicity (CSH) facilitates adhesion to substrate. The present study was undertaken to test the relationship between CSH and biofilm formation among the clinical isolates of Candida species. A total of 127 Candida strains isolated from various clinical specimens representing four different species C. albicans (77), C. tropicalis (23), C. parapsilosis (18) and C. krusei (09) were included in the study. The biofilm formation & CSH among included Candida strains were determined by a method proposed by Brachini et al & Rodrigues et al respectively. Non albicans Candida were more biofilm producers (62.00%) as compared to C. albicans (48.05%) with C. krusei (77.78%) as a highest biofilm producer. Biofilm producing strains of C. albicans, C. tropicalis, C. parapsilosis and C. krusei showed significantly higher percentage of hydrophobicity than the non biofilm producing strains. The findings of the present study showed that the positive correlation exists between CSH and biofilm formation in case of C. albicans, C. parapsilosis, C. tropicalis and C. krusei. However, as such correlation in Candida species is less studied and it carries important clinical repercussions, requires new and more rigorous studies.

Key words: Candida, Biofilm, Cell surface hydrophobicity.

INTRODUCTION

The different Candida species differ in their capacities to form biofilms which helps them for development of clinical infection and subsequently chronic infection. ⁽¹⁻³⁾ The formation of Candida biofilms carries important clinical repercussions because of their increased resistance to antifungal therapy and the ability of cells within biofilms to withstand

host immune defences. Also, the biofilm formation on medical devices can negatively impact the host by causing the failure of the device and by serving as a reservoir or source for future continuing infections. ^(4,5) The net effect is that Candida biofilms adversely impact the health of these patients with increasing frequency and severity and with soaring economic sequelae. ⁽⁶⁻⁹⁾ Biofilm formation is a step-by-step and finely

regulated process in which the adhesion phase is a crucial event.⁽¹⁰⁾ Before the biofilm formation, the initial step in the interaction with the host is the attachment or adherence of the Candida to the host tissue. The cell surface hydrophobicity (CSH) of *Candida* provides the hydrophobic interactions needed to turn this initial attachment between the Candida and the surface into a strong bond for successful colonization and invasion of host tissue by *Candida*.⁽¹¹⁻¹³⁾ Studies showing the positive correlation between CSH and biofilm formation are limited. Therefore, the present study was undertaken to test the relationship between CSH and biofilm formation among the clinical isolates of *Candida* species.

MATERIALS & METHODS

The present prospective study was conducted at microbiology department of Mahatma Gandhi Institute of Medical Sciences, Sevagram, Maharashtra, India over a period of 1 year from January 2011 to December 2011. A total of 127 *Candida* strains isolated from urine, high vaginal swab, blood, sputum, brochoalveolar lavage, drain, stool representing four different species *C. albicans* (77), *C. tropicalis* (23), *C. parapsilosis* (18), *C. krusei* (09) were included in the study. The isolates were identified by standard diagnostic procedures (germ tube production, chlamydospore formation and sugar assimilation).⁽¹⁴⁾ Biofilm formation was determined by using a method proposed by Brachini et al.⁽¹⁵⁾ A loopful of *Candida* colonies from the surface of Sabouraud's dextrose agar plate was inoculated into tubes containing 10 ml of Sabouraud's broth supplemented with glucose (final conc. 8%) and the tubes were incubated at 37°C for 24 hours. The broth was aspirated out and the walls of the tubes were stained with 1% saffranin. The stain on the walls was observed and compared with controls. Cell surface hydrophobicity (CSH)

measured as described by Rodrigues et al.⁽¹³⁾ Briefly, the yeast cells were harvested and washed twice in 10 mmol /L phosphate buffer (Ph 7.0). A yeast suspension was prepared in the same buffer, to hold an optical density (A0) of 0.4-0.6. 150 ul of hexadecane was added to 3ml of this yeast suspension in acid washed spectrophotometer glass tubes. The tubes were incubated at 30°C for 10 min and then vortexed twice for 30 sec. The phase separation was allowed for 10 min. and then measured the optical density of the lower aqueous phase (A1). The % of cells in hexadecane layer (adhered cells) was used to estimate the hydrophobicity, using the formula: Percent cell adhesion = {1-(A1/A0)} X 100. The data were analyzed using the statistical program SPSS version 16.0 (Statistical Package for the Social Sciences) and MINITAB 16. Statistical calculations were based on a confidence level >95% (P < 0.05 was considered statistically significant).

RESULTS

Out of 127 clinical isolates of *Candida*, 54% were biofilm producers and 46% were non biofilm producers. Non *albicans* *Candida* were more biofilm producers (62.00%) as compared to *C. albicans* (48.05%) as illustrated in Table 1. However these correlation was found to be statistically insignificant (P-Value = 0.118).

Table1: Biofilm formation among *C. albicans* and non *albicans* *Candida*.

<i>Candida</i> species	Positive (BP) N (%)	Negative (NBP) N (%)
<i>Candida albicans</i> (77)	37 (48.05)	40 (51.95)
Non <i>albicans</i> <i>Candida</i> (50)	31 (62.00)	19 (38.00)
Total (127)	68 (53.54)	59 (46.46)

The highest biofilm production seen in *C. krusei* (77.78%) among all the identified *Candida* species was not found statistically significant (Table 2).

Table 2: Biofilm formation among Candida species

Candida species	BP (%)	NBP (%)	P-value
C. albicans (77)	37 (48.05)	40 (51.95)	0.272
C. tropicalis (23)	14 (60.87)	09 (39.13)	0.564
C. parapsilosis (18)	10 (55.56)	08 (44.44)	0.857
C. krusei (09)	07 (77.78)	02 (22.22)	0.256

Biofilm producing strains of C. albicans, C. tropicalis, C. parapsilosis and C. krusei showed significantly higher percentage of hydrophobicity than in non biofilm producing strains (Table 3).

Table 3: Cell surface hydrophobicity (%) in (BP) and (NBP) Candida species

Candida sp.	Mean CSH (%) BP(37)	Mean CSH (%) NBP(40)	Mean difference (BP- NBP)	t-test	P-value
C. albicans	39.51	35.50	04.01	2.912	0.005
C. tropicalis	64.37	27.25	37.12	23.834	< 0.0001
C. parapsilosis	73.36	39.89	33.47	20.034	< 0.0001
C. krusei	80.17	40.10	40.07	9.211	< 0.0001

DISCUSSION

Biofilm formation is a step by step and finely regulated process in which the adhesion phase is a crucial event. CSH facilitates adhesion to substrate. ⁽¹⁶⁾ Hydrophobic interactions believed to contribute to adherence to a wide variety of surfaces by maintaining the fidelity of the adhesion receptor bonds. ⁽¹⁷⁾ CSH could be involved in the specific ligand receptor linkages, facilitating approach and adhesion between yeasts and host cells. Studies demonstrated that CSH is involved in adherence of most isolates of C. albicans to human epithelial cells. ^(16,17) In the study of Borghi E et al, ⁽¹⁸⁾ the cell surface hydrophobicity in biofilm producing strains of C. tropicalis, C. parapsilosis and C. glabrata was significantly higher than non biofilm producing strains. Regarding C. albicans, no significant differences were observed in cell wall hydrophobicity between biofilm producers and non biofilm producing strains. Study further explained the reason of relatively low hydrophobicity of C. albicans due to the armamentarium of adhesions that can make up for it, promoting adhesion process and biofilm formation in such extremely evolved species. In the present study, we noted similar trends of significantly higher cell surface hydrophobicity in biofilm producing strains of C. tropicalis, C. parapsilosis and C. krusei than non biofilm producing strains.

However, in contrary to the study by Borghi E et al, ⁽¹⁸⁾ the significant difference between the cell surface hydrophobicity of biofilm producing and non biofilm producing strains of the C. albicans noted in our study was in synchronization with the positive correlation between biofilm and CSH observed in a study by Xiaogang Li et al ⁽¹⁹⁾ in 115 strains of C. albicans. The results in this study were consistent and extended previous observations of positive correlation between adhesion to plastic surfaces and CSH and Candida species. Based on the findings of the present and previous studies, it could be said that CSH is an important pathogenic factor involved in adherence and may be in initial steps of biofilm development. ⁽¹⁶⁾ However, relative contributions of hydrophobicity to the different steps of biofilm formation are not known and further; the development of biofilm may be influenced by the other factor like the filamentation of the fungus. ^(16,19)

CONCLUSION

The findings of the present study showed that the positive correlation exist between CSH and biofilm formation in case of C. albicans, C. parapsilosis, C. tropicalis and C. krusei. However, as such correlation in Candida species is less studied and it carries important clinical repercussions, requires new and more rigorous studies.

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