Biofilm Production by *Candida*: Comparison of Bloodstream Isolates with Cervical Isolates

Sheetal U. Harakuni · S. G. Karadesai ·
Nadeemaktar Jamadar

Received: 22 July 2011 / Accepted: 1 March 2012 / Published online: 16 March 2012
© Association of Microbiologists of India 2012

Abstract The present study was undertaken to investigate biofilm formation among the clinical *Candida* isolates from blood and cervical swabs. A total of 16 *Candida* blood isolates from neonates and 21 cervical isolates from pregnant women with vulvovaginitis were included in the study. Each isolate was identified to species level by various phenotypic tests. Biofilm formation was detected by colorimetric method. *C. glabrata* and *C. albicans* were the major isolates from blood and cervical swab respectively. The biofilm formation was found in 14 (87.5 %) blood isolates and only in 4 (19.1 %) cervical isolates.

Keywords *Candida* · Biofilm · Blood stream infection · Vulvovaginitis

Biofilms (BF) are colonies of microbial cells encased in a self-produced organic polymeric matrix and represent a common mode of microbial growth. Microbes growing as biofilm are highly resistant to commonly used antimicrobial drugs. Recently, microbial BF have gained prominence because of the increase in infections related to indwelling medical devices. BF formation by *Candida* species is believed to contribute to invasiveness of the fungal species [1]. *Candida non albicans* are commonly being isolated from the blood samples [2]. BF can vary and depends on *Candida* species. Vulvovaginal candidiasis in pregnant women is a predisposing cause for candidemia in newborn [3].

The objective of this study was to investigate BF in clinical *Candida* isolates from blood of neonates and vaginal lesions of pregnant women at our tertiary care hospital.

A total of 16 *Candida* sp. were isolated from septicaemia patients admitted in neonatal intensive care units of our tertiary care hospital. Twenty-one vaginal isolates from pregnant women with vulvovaginitis were included in the study.

The samples were inoculated on Blood agar and MacConkey agar (Hi media, Mumbai) and incubated overnight at 37 °C. The cervical swab samples that grew predominantly *Candida* colonies were included in the study.

The colonies were identified to the species level as per the standard protocol [4].

Biofilm formation was detected using the microtiter plate method [5]. The assessment of biofilm formation was performed thrice in 3 wells for each strain. Biofilm positive isolates were considered those wells the A_{630} of which was higher than the mean A_{630} of negative control plus $3 \times$ SD.

The difference between the groups of blood stream and cervical isolates in their A_{630} was analyzed by student's *t* test. The difference in the number of biofilm-positive strains in both groups was assessed by a χ^2 with Yate's correction [χ^2_{yc}].

Various *Candida* species among blood isolates were identified as *C. glabrata* (56.25 %), *C. tropicalis* (37.5 %), and *C. krusei* (6.25 %). All 21 cervical isolates were *C. albicans*.

The comparison of the A_{630} of wells inoculated by the blood stream isolates against cervical isolates shows that mean A_{630} of blood isolates was higher (0.612) than cervical isolates (0.324) [student's *t* test, $P = 0.001$]. The threshold value for evaluation of biofilm-positivity was calculated as $A_{630} = 0.347$. The ratio of the biofilm-positive strains was

S. U. Harakuni (✉) · S. G. Karadesai · N. Jamadar
Department of Microbiology, J N Medical College,
Belgaum 590010, Karnataka, India
e-mail: us_c2004@yahoo.co.in

Table 1 Biofilm formation in blood stream and cervical *Candida* isolates

Strains	Biofilm positive	Biofilm negative
Blood isolates		
<i>C. glabrata</i>	7	2
<i>C. tropicalis</i>	6	–
<i>C. krusei</i>	1	–
Cervical isolates		
<i>C. albicans</i>	4	17

Difference in ability to form biofilm between the two groups was statistically significant ($\chi^2_{yc} = 14.403$; $df = 1$; $P = 0.001$)

higher in strains isolated from blood cultures (87.5 %) than in cervical isolates (19.1 %) [$\chi^2_{yc} = 14.403$; $df = 1$; $P = 0.010$; Table 1]. Biofilm formation is consistently associated with *C. tropicalis* isolates, but is variable in *Candida glabrata* and *C. albicans*.

Candida BF may contribute both to the pathogenesis of superficial and systemic candidiasis as they are notoriously resistant to antifungal drugs.

Candida species are now the fourth most common cause of nosocomial blood stream infections [6]. Although *C. albicans* remains the most common species recovered from blood in Europe and the US, emergence of non-*albicans* species has been documented [7, 8].

In earlier studies *C. tropicalis* was the predominant species in blood stream infections in India [9, 10]. In contrast to this, our study is showing predominance of *C. glabrata* species (56.25 %), followed by *C. tropicalis* and *C. krusei*. *C. glabrata* is recognized to be the emerging pathogen worldwide [11–13]. In the present study 12 neonates were preterm with low birth weight [LBW]. In LBW infants, gastrointestinal disease or multiple-site colonization is often present prior to invasive *C. glabrata* infection [14]. *C. tropicalis* is also reported to be an increasingly common cause of sepsis [9, 10, 14].

In our study none of the blood samples grew *C. albicans*. All the cervical isolates from pregnant women were *C. albicans*. This is in accordance with the other studies that state that *C. albicans* is still the commonest species being isolated from reproductive age group women [15–17]. Biofilm promotes persistence of fungal infection leading to recurrent attacks of vulvovaginitis [18]. Biofilm positive strains among the cervical isolates were only 4 (19.1 %). This shows that biofilm formation is not the main virulence factor in pathogenesis of vulvovaginitis.

Biofilm formation is highest in blood isolates when compared to cervical isolates, thus is a virulence marker in blood stream infection. Same observation is being made in other study also [19]. In conclusion, our study projects the importance of detection of biofilm formation, as a virulence factor, in blood stream isolates. The biofilm formation is not

the pathogenic marker in vulvovaginitis. As only a limited number of *Candida* isolates could be tested in this study, further clinical studies need to be performed involving a larger number of isolates to confirm the findings.

References

- Mukerjee PK, Zhou G, Munyon R, Ghannoum MA (2005) *Candida* biofilm: a well-designed protected environment. Med Mycol 43(3):191–208
- Chakrabarti A, Singh K, Das S (1999) Changing face of nosocomial Candidaemia. Indian J Med Microbiol 17:160–166
- Segal E, Soroka A, Schechter A (1984) Correlative relationship between adherence of *Candida albicans* to human vaginal epithelial cells in vitro and candidal vaginitis. Sabouraudia 22:191–200
- Larone DH (2002) Medically important fungi: a guide to identification, 4th edn. ASM Press, Washington DC, pp 109–143
- Ruzicka F, Hola V, Votava M, Tejkalova R (2007) Importance of biofilm in *Candida parapsilosis* and evaluation of its susceptibility to antifungal agents by colorimetric method. Folia Microbiol 52(3):209–214
- Pfaller MA, Diekema DJ, Jones RN, Sader HS, Fluit AC, Hollis RJ, Messer SA (2001) International surveillance of bloodstream infections due to *Candida* species: frequency of occurrence and in vitro susceptibilities to fluconazole, ravuconazole, and voriconazole of isolates collected from 1997 through 1999 in the SENTRY antimicrobial surveillance program. J Clin Microbiol 39:3254–3259
- Almirante B, Rodriguez D, Park BJ, Cuenca-Estrella M, Planes AM, Almela M, Mensa J, Sanchez F, Ayats J, Gimenez M, Saballs P, Fridkin SK, Morgan J, Rodriguez-Tudela JL, Warnock DW, Pahissa A (2005) Epidemiology and predictors of mortality in cases of *Candida* bloodstream infection: results from population-based surveillance, Barcelona, Spain, from 2002 to 2003. J Clin Microbiol 43:1829–1835
- Samonis G, Kofteridis DP, Saloustros E, Giannopoulou KP, Ntziora F, Christidou A, Maraki S, Falagas ME (2008) *Candida albicans* versus non-*albicans* bloodstream infection in patients in a tertiary hospital: an analysis of microbiological data. Scand J Infect Dis 40:414–419
- Xess I, Jain N, Hasan F, Mandal P, Banerjee U (2007) Epidemiology of candidemia in a tertiary care centre of north India: 5-year study. Infection 35:256–259
- Hasan F, Xess I, Wang X, Jain N, Bettina CF (2009) Biofilm association in clinical isolates and its association with virulence. Microbes Infect 11(8, 9):753–761
- Fairchild KD, Tomkoria S, Sharp EC, Mena FV (2002) Neonatal *Candida glabrata* sepsis: clinical and laboratory features compared with other *Candida* species. Pediatr Infect Dis J 21:39–43
- Fidel PL Jr, Vazquez JA, Sobel JD (1999) *Candida glabrata*: review of epidemiology, pathogenesis, and clinical disease with comparison to *C. albicans*. Clin Microbiol Rev 12:80–96
- Chol HK, Jeong SJ, Lee HS, Chin BS, Chol SH, Han SH et al (2009) Blood stream infections by *Candida glabrata* and *Candida krusei*: a single center experience. Korean J Intern Med 24(3):263–269
- Kaufman D, Fairchild KD (2004) Clinical microbiology of bacterial and fungalsepsis in very low birth infants. Clin Microbiol Rev 17(3):638–680
- Mohanty S, Xess I, Hasan F, Kapil A, Mittal S, Tolosa JE (2007) Prevalence and susceptibility to fluconazole of *Candida* species causing vulvovaginitis. Indian J Med Res 126:216–219

16. Ferrer J (2000) Vaginal candidosis: epidemiological and etiological factors. *Int J Gynecol Obstet* 71:S 21–S 27
17. John PV, Matthew JS, Sean GC, Jason PT, Martin EA, Eli M, Scott EG (2008) Survey of vaginal flora *Candida* species isolates from women of different age groups by use of species specific PCR detection. *J Clin Microbiol* 46(4):1501–1503
18. Jacqueline MA, Bettina CF (2010) *Candida* infections in genitourinary tract. *Clin Microbiol Rev* 23(2):253–273
19. Shin JH, Kee SJ, Shin MG, Kin SH, Shin DH, Lee SK, Suh SP, Ryang DW (2002) Biofilm production by isolates of *Candida* species recovered from nonneutropenic patients: comparison of blood stream isolates with isolates from other sources. *J Clin Microbiol* 40:414–419