

Biofilm as Virulence Marker in Candida Isolated from Blood

¹M. Vinitha and ²Mamata Ballal

¹Department of Microbiology, R.V. Dental College, CA 37,
24th Main Road, Jpnagar, First Phase, Bangalore, 560078, India

²Department of Microbiology, KMCIC, Manipal, India

Abstract: Biofilm production has been implicated as a potential virulence factor of some candida species responsible for catheter related candidaemia in ICU patients with indwelling devices. Early detection of slime production by the candida species may be useful for clinical decisions. We therefore have aimed at demonstrating the formation of biofilm by candida species isolated from blood samples collected from ICU patients and patients with indwelling devices (dialysis). 34 species of Candida were isolated from the blood samples and the isolates include *C. krusei* (38.23%), *C. albicans* (20.58%), *C. parapsilosis* (11.76%), *C. guilliermondii* (5.88%), *C. glabrata* (11.76%), *C. tropicalis* (5.88%) and *C. pseudotropicalis* (5.88%). The organisms were grown on sabouraud's liquid medium containing 8% glucose. Biofilm production was determined. The biofilm formation by *C. albicans* was less frequent (42.85%), than that by non *C. albicans* (63.33%). The data suggest that the capacity of candida species to produce biofilm invitro may be a reflection of the pathogenic potential of the isolates to cause central venous catheter related candidaemia in ICU patients and patients on dialysis.

Key words: Biofilm • candidaemia • slime production

INTRODUCTION

The number of nosocomial blood stream infections due to Candida species has increased over the past few decades. Candida is the fourth most common cause of blood stream infection in hospital patients [1]. Candida strains possess a number of virulence factors which enable the organism to cause hematogenously disseminated infections in susceptible hosts. One amongst them is slime production. Biofilms are the structured microbial communities that are attached and encased in a matrix of exopolymeric material [2] and are important for the development of clinical infection. A typical laboratory fungal model of biofilm formation involves two operational steps: (a) adhesion and (b) biofilm growth and maturation [3].

In cases of weakening of surface host defenses, fungal elements may penetrate into the intimacy of tissues, blood and spread over in the host. The considerable increase of deep seated candidosis is mostly observed in hospitals and particularly in ICU's, oncology, organ transplant and other departments, where

most patients are subject to heavy therapeutic protocols and suffer from immunodeficiency. 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 [4].

Candida produces large quantities of viscid slimy material in glucose containing solutions. The ability to form extensive biofilms on the catheters and other prosthetic devices, also contribute to the prevalence of the organism as an etiologic agent of intravascular nosocomial infections [5]. Central venous catheters are considered as the most common risk factor for the development of candidaemia in patients [6]. Hence, slime is considered as one of the virulent factors produced by candida strains which will enable the organism to cause haematogenously disseminated infection in susceptible hosts with their persistence and colonization of the host tissues [7, 8]. The intravascular devices become colonized by the organisms that forms a biofilm of cells; the detachment of which can result in septicaemia [9]. The production of biofilm is also associated with high level of antimicrobial resistance of the associated organisms [10].

MATERIALS AND METHODS

A total of 120 blood samples were collected from the ICU and dialysis units in the hospitals and nursing homes, in and around Bangalore. The samples were collected from patients who have had no antifungal drug exposure during hospitalization. A total of 34 candida species isolates were recovered from the blood samples. The isolates were tested for biofilm production. The 34 isolates include, *C. albicans*, *C. tropicalis*, *C. krusei*, *C. glabrata*, *C. parapsilosis*, *C. pseudotropicalis* and *C. guilliermondii*. The identification of the species was conducted by assessing the germ tube formation, chlamyospore formation, sugar fermentation and assimilation patterns. The comparison study was done by using the standard strains provided by P.G.I.M.E.R. Chandigarh.

Slime production: slime production was determined by using a method proposed by Brachini *et al.*, [11]. A loop full of organisms from the SDA plate was inoculated into a tube containing 10ml Sabouraud’s liquid medium supplemented with glucose (final concentration of 8%). The tubes were incubated at 37°C for 24 h after which the broth was aspirated out and the walls of the tubes were stained with safranin. Slime production was scored as negative, weak positive (1+), moderate positive (2+) or strong positive (3+).

RESULTS

34 candida species were isolated from 120 blood samples collected. Amongst the 34 isolates, 13 were *C. krusei*, 7 were *C. albicans*, 4 were *C. glabrata*, 4 were *C. parapsilosis*, 2 were *C. pseudotropicalis*, 2 were *C. tropicalis* and 2 were *C. guilliermondii*. Slime production of 34 strains isolated from blood were

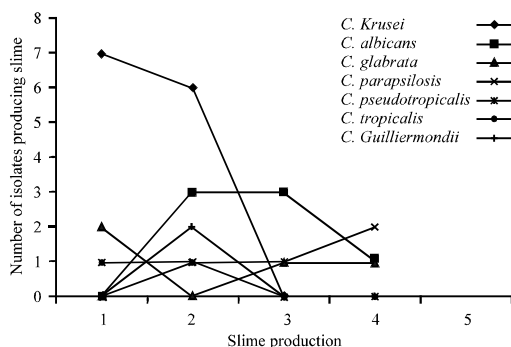


Fig. 1: Slime production by Candida species isolated from blood

Table 1: Slime production by Candida species isolated from blood

Candida species	Number of isolates	Slime production			
		3+	2+	1+	0
<i>C. krusei</i>	13 (38.23)	7	6	0	0
<i>C. albicans</i>	7 (20.58)	0	3	3	1
<i>C. glabrata</i>	4 (11.76)	2	0	1	1
<i>C. parapsilosis</i>	4 (11.76)	0	1	1	2
<i>C. pseudotropicalis</i>	2 (5.88)	1	1	0	0
<i>C. tropicalis</i>	2 (5.88)	1	1	0	0
<i>C. guilliermondii</i>	2 (5.88)	0	2	0	0

0 -Negative, 1+ -Weak positive, 2+ -Moderate positive, 3+ -Strong positive

determined in this study. All the isolates and standard strains showed moderate to high slime production. Amongst the 34 strains, slime production was found in 30 strains(88.23%). Strong slime production was seen in *C. krusei* and weak slime production was seen in *C. parapsilosis*. Strong slime production (3+) was found in 11 strains and weak slime production (1+) was found in 5 strains. The results are shown in the Table 1 and Fig. 1.

DISCUSSION

Over the past few years the yeasts of the genus candida continue to be amongst the important etiologic agents of nosocomial infection. Most catheter related septicemia are caused by micro organisms that invade the intracutaneous wound during catheter insertion or there after. The proportion of such infection due to non candida albican species is persistently rising. The isolation of non candida albicans has been frequently encountered in the past few decades [12]. A biofilm is a community of microorganisms and their extracellular polymers that are attached to a surface [13]. The ability to form biofilms is associated with the ability to cause infections and as such should be considered as an important virulence determinant during candidiasis. Fungal biofilms may try to maintain their niche as a commensal and pathogen of humans namely, by evading host immune mechanisms, resisting antifungal treatment and withstanding the competitive pressure from other organisms. Consequently, biofilm related infections are difficult to treat [14]. Indwelling intravascular catheters represent a risk factor that is associated with nosocomial candida infection. The devices become colonized by the candida that forms biofilm, the detachment of which can result in candidemia.

Candida albicans isolates recovered from blood demonstrated lower percentage of biofilm positivity than

other candida species isolates in this study. Biofilm positivity occurred most frequently in isolates of *C. krusei* followed by *C. tropicalis*, *C. pseudotropicalis*, *C. guilliermondii*, *C. glabrata*, *C. albicans* followed by *C. parapsilosis*.

This result suggests that the slime production is more important for non candida albicans strains and Candida albicans possess mechanisms other than biofilm production to establish blood stream infections.

ACKNOWLEDGEMENT

The authors thank Dr. K.S. Nagesh, Dr. Kamath and Dr. Deshpande for providing assistance to carry out the study. Dr. Karthik for his help in procuring samples. Dr. Shivprakash M.R. assistant professor, P.G.I. M.E.R Chandigarh for providing standard strains.

REFERENCES

1. Arunaloke Chakrabarti, Kavitha Singh and Sanchita Das, 1999. Changing face of nosocomial candidaemia. Ind. J. Med. Microbiol., 17: 160-166.
2. D' Antonio, D., F. Romani, E. Pontieri and G. Carruba, 2002. Catheter related candidaemia caused by candida lipolytica in a patient receiving allogenic bone marrow transplantation. J. Clin. Microbiol., 40: 1381-1386.
3. Baillie, G.S. and L.J. Douglas, 1999. Role of dimorphism in the development of candida albicans biofilms. J. Med. Microbiol., 48: 671-679.
4. Jean- Marcel Senet, 1998. Candida adherence phenomenon from commensalisms to pathogenicity. Intl. Microbiol., 1: 117-122.
5. Matsumoto, F.E., R.F. Gandra, L.S. Ruiz, M.E. Auler, S.A.V. Marques, F.C. Pires, W. Gambale and C.R. Paula, 2001. Yeast isolated from blood and catheter in children from a public hospital of Sao Paulo. Brazil, Mycopathologia 154: 63-69.
6. Shin *et al.*, 2002. Biofilm production by isolates of candida species recovered from nonneutropenic patients; comparison of blood stream isolates from other sources. J. Clin. Microbiol., 40: 1244-1248.
7. Gordon Ramage, P. Stephen Saville, P. Derek, Thomas and L. Jose, 2005. Lopez- Ribot, Candida biofilms; an update. Eukaryotic Cell., 4: 633-638.
8. Gordon Ramage, Kacy Vande Walle, Briass L. Wiekes and Jose L, Lopez- Ribot, 2001. Biofilm formation by candida dubiliensis. J. Clin. Microbiol., 39: 3234-3240.
9. Khun, D.M., J. Chandra, P.K. Mukherjee and M.A. Ghannoun, 2002. A comparison of biofilm formed by *C. albicans* and *C. Parapsilosis* on bioprosthetic surfaces. Infect. Immuno., 70: 878-888.
10. Semina Ozkan, Fatima Kaynak, Ayse kalkanci, Ufak Abbasoglu and Semra Kustimur, 2005. Slime production and proteinase activity of candida species isolated from blood samples and comparison of these activities with minimum inhibitory concentration values of antifungal agents. Mem. Inst. Oswaldo Cruz, Rio de Janeiro, 100: 319-324.
11. Branchini, M.L, M.A. Pfaller, J. Rhine-Chalkberg, T. Frempong and H.D. Isenberg, 1994. Genotype variation and slime production among blood and catheter isolates of *C. Parapsilosis*. J. Clin. Microbiol., 32: 452-456.
12. Segal, E. and D. Elad, 1998. Candida species and Blastoschizomyces capitatus. In: Agillo, L. and R.J. Hay (Eds). Microbiology and Microbial Infection. New York, Arnold, pp: 423-460.
13. Pfaller, MA., 1996. Nosocomial candidiasis: Emerging species, reservoirs and modes of transmission. Clin. Infec. Dis., 22: S89-S94.
14. Baillie, G.S. and L.J. Douglas, 1999. Candida biofilm and their susceptibility to antifungal agents. Methods Enzymol, 310: 644-656.