ORIGINAL ARTICLE

Colonization of peripheral intravascular catheters with biofilm producing microbes: Evaluation of risk factors

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ABSTRACT

Background: Biofilms often colonize catheters and contribute to catheter-related septicemia. However, predictors of catheter colonization by biofilms remain poorly defined. The aim of this study was to evaluate clinical factors that may be associated with biofilm colonization of catheters. **Materials and Methods:** A total of 54 isolates colonizing the peripheral intravascular catheters (IVCs) were studied and their biofilm production ability was analyzed by the tube method and antimicrobial susceptibility was also done. A detailed clinical history and examination was done of each subject to know age, sex, duration of use of IVCs, site of IVCs, swelling/purulence around the IVCs, number of attempts to install the catheter, and duration of hospital stay. **Results:** 44 (81.4%) out of 54 isolates colonizing the catheters showed biofilm formation. Biofilm formations were significantly associated with duration of hospital stay of more than 7 days [odds ratio (OR) = 6.6; 95% confidence interval (CI) = 1.3-34; *P* value (*P*) = 0.02], multiple attempts to install the catheter (OR=7; CI=1.5-31.8; *P*=0.01), and multidrug resistance (OR=9.5; CI=1.8 - 51.1: *P*=0.008). *Klebsiella pneumoniae* and *Candida* spp. comprised most of the biofilm-producing isolates. The overall susceptibility to antimicrobials was low among biofilm-producing compared to nonbiofilm-producing microbes. **Conclusion:** The results of this study suggest that evaluation of predictors of biofilm production is important in order to understand, prevent or manage biofilm colonization of IVCs.

Key words: Biofilms, intravascular catheters, predictors

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INTRODUCTION

Biofilms are complex microbial communities often associated with colonization of medical devices commonly used in clinical practice, such as peripheral intravascular catheters (IVC).[1] About 82% of nosocomial septicemias are the result of colonization of IVCs predominantly by biofilm producing microbes; therefore it is necessary to know the predictors of such colonizers.[2] The colonization of IVCs by biofilm-producing bacteria is dependent upon various factors (environmental, host, microbial). The factors triggering biofilm development may vary from organism to organism. However it is clear that these factors have a profound impact on the transition of planktonic to biofilm form attributing to catheter colonization further ending up in persistent and resistant blood stream infections.[3] Therefore we studied 54 isolates colonizing IVCs of hospitalized pediatric patients and evaluated various factors to find out difference, if any, between biofilm and nonbiofilm producing ability of microbial (bacterial and fungal) isolates in a tertiary care hospital in North India.

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MATERIALS AND METHODS

A prospective study was carried out on 54 isolates colonizing IVCs of pediatric children. A detailed clinical history and examination was done of each subject to know patients' characteristics such as age, sex, duration of use of IVC, site of IVC, swelling/purulence around the IVC, number of attempts to install the catheter, and duration of hospital stay [Table 1]. The isolates leading to monomicrobial colonization on polyvinyl chloride intravascular catheters were only included in the study. The other sources of septicemia present (e.g., infusate related, catheter hub related, endogenous) were ruled out. This study was conducted after taking permission from institutional ethical committee.

Catheter colonization was defined as "Growth of organisms from a catheter segment (>15 colony forming units)" by

the semiquantitative roll plate method.[4] Semiquantitative catheter culture by the roll plate method was done on the blood agar, Mac Conkey agar, and kept at 37°C for 48 hours to obtain bacterial isolates[5] Semiquantitative catheter culture by the roll plate method was also done on Sabouraud's Dextrose Agar (SDA) plates to obtain fungal isolates, one each being kept at 25°C and 37°C, respectively.[6] The isolates obtained by semiquantitative catheter culture were identified as per standard conventional methods and tested for *in vitro* biofilm production.[5,6]

The segments of the colonized catheters were immersed in 1% glutaraldehyde and randomly 15 segments were used for scanning electron microscopy to visualize *in vivo* biofilms on the catheter surface.

In vitro biofilm forming ability of isolates obtained from catheter culture was tested by the tube method, as described by others with slight modification.[7,8] Briefly, 0.5 ml (1.5×10^8 organism/ml) of 48-hour culture saline washed suspension was inoculated into a polystyrene tube containing 4.5 ml of Luria–Bertani broth. Tubes were incubated at 37°C for 48 hours without agitation. After 48 hours, the culture broth in the tube was aspirated, and tubes were washed twice with distilled water. The walls of the tube were stained with 0.1% crystal violet after media and cells were discarded. Biofilm formation was considered positive when a visible film lined the wall and bottom of the tube. Ring formation at the liquid interface was not indicative of biofilm formation. Each isolate was tested at least three times and read independently by two different observers. Strong biofilm producer *Staphylococcus epidermidis* ATCC 35984 and nonbiofilm producer *Candida albicans* ATCC 10231 were used as a positive and negative control, respectively.

We randomly tested 15 out of 54 catheters to confirm biofilm formation on an intravascular catheter *in vivo.* The catheter segments were rinsed in a 0.1 M phosphate buffer and then placed in 1% Zetterquist's osmium for 30 minutes. The segment was subsequently dehydrated in a series of ethanol washes (70% for 10 minutes, 95% for 10 minutes, and 100% for 20 minutes), treated (two times, 5 minutes each) with hexamethyldisilizane (Polysciences Inc., Warrington, PA, USA), and finally air dried in a desiccator. The segment was coated with gold-palladium (40%/60%). After processing, the segment was observed with a scanning electron microscope (Leo 435 VP) in high-vacuum mode at 15 kV. The images were processed for display using Photoshop software (Adobe Systems Inc., Mountain View, CA, USA).

Antimicrobial susceptibility testing was also done for bacterial and fungal isolates by the disc diffusion method as per CLSI guidelines.[9,10] Multidrug resistance was defined as resistance to three or more groups of drugs.

Statistical analysis

The data were analyzed using Stat-plus software. Odds ratios and 95% confidence interval (CI) were reported for independent variables associated with the variable outcome: biofilm production [Table 1].

RESULTS

Biofilm formations were seen on 10 out of 15 randomly selected catheters *in vivo* by SEM and were fully corresponding to *in vitro* biofilm production of clinical isolates obtained from respective catheters by the tube method [Figure 1]. Out of 54 isolates studied, 44 (30 bacterial and 14 fungal) isolates were biofilm producing and 10 (9 bacterial and 1 fungal) isolates were nonbiofilm

Table 1: Characteristics of patients with peripheral intravascular catheter colonization

Age (mean)	24.4 months
Sex	
Male	28
Female	26
Duration of catheter use (mean)	
More than 48 hours	38 (76 hours)
Less than 48 hours	16 (21.4 hours)
Site	
Leg	12
Hand/forearm	42
Swelling/purulence around the catheter	
Yes	28
No	26
Attempts to install the catheter	
More than 1	36
Single	18
Hospital stay (mean)	
More than 7 days	46 (10.4 days)
Less than 7 days	8 (4.1 days)
Mutidrug resistance	
Yes	33
No	21

(*n*=54)

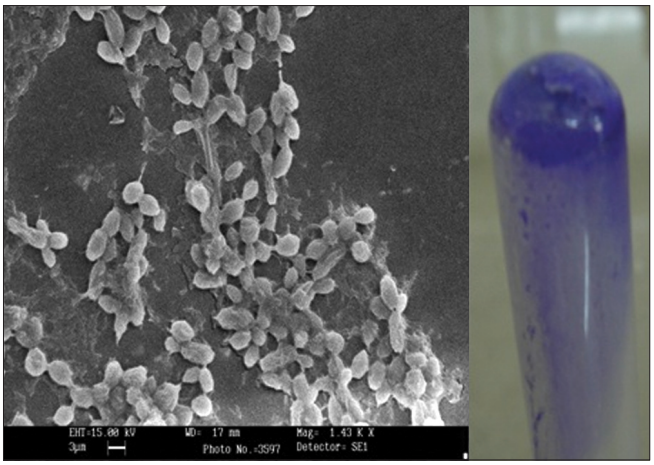

Figure 1: Biofilm formation on catheter surface as seen on scanning electron microscopy (right) and on the polystyrene tube by the tube method (left)

producers [Figure 2]. *Klebsiella pneumoniae* and *Candida* sp. comprised most of the biofilm producing isolates.

Age, sex, duration of catheter use (> 48 hours), swelling/purulence around the catheter and insertion sites (hand/forearm/leg) were not significantly associated with biofilm colonization of catheters. In the present study hospital stay of more than 7 days and multiple attempts to install IVCs were the significant factors (*P* value <0.05) and multidrug resistant microbes was a highly significant factor (*P* value<0.01) associated with biofilm production by microbes and emerged out as risk factors of colonization of IVCs by biofilm producing microbes [Table 2].

Imipenem among gram-negative bacilli, vancomycin in gram-positive cocci and voriconazole showed 100% susceptibility among *Candida* spp irrespective of biofilm producing ability of isolates. However, overall susceptibility to antimicrobials was low among biofilm producing in comparison to nonbiofilm producing microbes [Figure 2].

DISCUSSION

Catheter colonization by biofilm producing microbes is a crucial step in ensuing catheter-related sepsis. However, studies on factors facilitating biofilm production by microbes colonizing the peripheral intravascular catheters are lacking.[11] Biofilms are microbial communities that exhibit unique characteristics that must be considered when evaluating the potential of prevention or control strategies for catheter-related sepsis.[2,12]

Model systems to study biofilm formations *in vitro* are developed by various workers.[7,8] These systems usually simulate the *in vivo* or *in situ* conditions and at the same time provide reproducible, accurate results.[12,13] We have previously evaluated the tube method for biofilm formation of clinical strains *in vitro* with scanning electron microscopy for demonstration of catheter colonization with biofilms *in vivo* and in this study also we had found comparable results.[1]

An attempt was made in this study to evaluate predictors

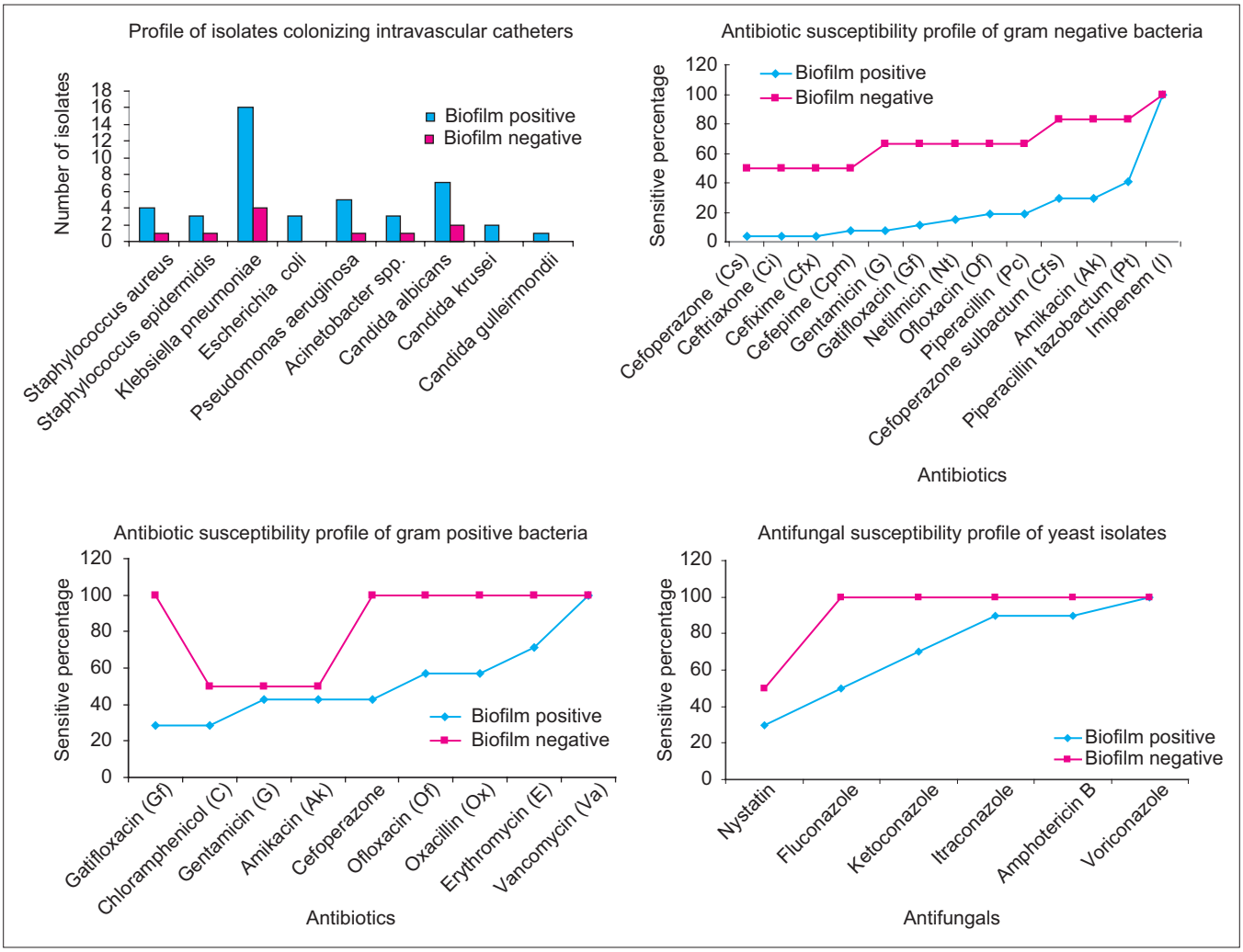

Figure 2: Microbial and susceptibility profile of biofilm producing and nonproducing isolates colonizing intravascular catheters

Table 2: Evaluation of various factors with biofilm producing ability of microbes

Parameter	Biofilm positive (*n*=44)	Biofilm negative (*n*=10)	Odds ratio	Significance
Age				
0-1 years	22	4	1.5 (0.4–6.0)	NS
1-14 years	22	6		
Sex				
Male	23	5	1.1 (0.3–4.3)	NS
Female	21	5		
Duration of catheter use				
≥ 48 hours	32	6	1.8 (0.4–7.4)	NS
≤ 48 hours	12	4		
Site				
Hand/forearm	30	8	1.8 (0.3–9.9)	NS
Leg	14	2		
Swelling/purulence around the catheter				
Yes	24	4	1.8 (0.4–7.3)	NS
No	20	6		
Attempts to install the catheter				
Multiple	33	3	7 (1.7–31.8)	0.01
Single	11	7		
Hospital stay				
More than 7 days	40	6	6.6 (1.3–34.0)	0.02
Less than 7 days	4	4		
Multidrug resistance				
Yes	31	2	9.5 (1.8–51.1)	0.008
No	13	8		

of catheter colonization by biofilm-producing microbes, which are poorly defined and inadequately discussed in the literature. Age and sex did not significantly correlate with colonization of catheters by biofilm-producing microbes in our study. However extremes of age (< 1 year) and male sex showed slight preponderance for biofilm producers. A study on nosocomial infections in a pediatric age group also showed that similar results may be because of suppression of cell-mediated immunity in infants and outnumbered male admissions compared to females in our country.[14-16] The ratio of catheter colonization in lower extremity by biofilm producing to nonproducers was 7:1 compared to 3.8:1 in upper extremity sites. However, the association between biofilm production ability and site of the catheter was not significant in our study. The higher risk for colonization by the biofilm-producers microbe in patients with lower extremity insertion sites than are upper extremity sites is because of the high density of local skin flora.[17]

A high probability of infection in the form of purulent discharge and biofilm production has been shown in a prosthetic-device-based biofilm infection model.[18] However it is noteworthy that we could not find any correlation between purulence/swelling around the catheter and biofilm production, probably because the purulence was not gross. Hospital stay of more than 7 days was an important independent predictor of catheter colonization by biofilm-producing microbes. Biofilm-associated infections are more found in patients with extended hospital stay.[2] Another avoidable but highly significant risk factor associated with biofilm colonization of IVCs was multiple attempts to install the device. In a study on colonization of intravascular catheters, multiple attempts in insertion of devices were associated with colonization by microbes.[19] Therefore peripheral catheters should be installed with full aseptic precaution and trained staff especially in children preferably in a single attempt to reduce the risk of colonization by biofilm producers.

Biofilm production has been implicated as a potential virulence factor of various bacterial (*Klebsiella pneumoniae, E.coli, A. baumanii, P.aeruginosa, Staphylococcus* species), and fungal spp (*Candida albicans* and *non albicans Candida*) ensuing catheter colonization and catheter-related sepsis.[20,21] In fact, a higher resistance to different classes of antibiotics has been associated with biofilm-producing species.[20,21] A highly significant correlation also existed between the ability of strains to form biofilms and antimicrobial resistance. Thus, it is possible that ability to form biofilm by microbes and multidrug resistance are closely linked. The underlying genetic mechanism of increased horizontal gene transfer as seen in resistant bacteria and biofilm-producing bacteria can be the basis for above observation.

To conclude, predictors of biofilm production are must to evaluate in order to prevent or mange biofilms on indwelling intravascular catheters.

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