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Association between drug resistance & production of biofilm in staphylococci

Sir,

Staphylococci are common cause of hospital-acquired infection and biofilm is one of its important microbial virulence factors[1,2]. Biofilm consists of multilayered cell clusters embedded in a matrix of extracellular polysaccharide, which facilitate the adherence of microorganism. The microbes forming the biofilm are difficult to treat in clinical settings. These isolates may or may not be resistant to anti-bacterial agents in laboratory setting, but due to difficulty in eradication of the biofilm formed on the surfaces of the devices/appliances and protection provided to the microorganism by protective covering of adhesive biomaterial (slime), it becomes difficult to treat infections caused by these organisms[3]. Here, we report an association between antibiotic resistance and biofilm production in clinical isolates of staphylococci.

Invasive (isolates from the blood stream), colonizing (isolates from peripheral intravenous devices) and commensal (isolates from the skin and/or nose) clinical staphylococcal isolates [*Staphylococcus aureus* and coagulase negative staphylococcus (CoNS)] collected for an earlier study[4] by our group, were selected for the present study. Isolates were grouped as biofilm producers or non biofilm producers. Biofilm production was tested by microtitre plate method[5]. Antibiotic susceptibility testing was done by disc diffusion method on Muller Hinton agar plates (Hi- Media Laboratories, Mumbai, India) according to CLSI guidelines[6]. Six antibiotics were chosen based on frequency of their use in infections; penicillin (10 U), oxacillin (1 µg) (β-lactam antibiotics), vancomycin (30 µg) (glycopeptide antibiotics), teicoplanin (30 µg) (glycopeptide antibiotics), cefazolin (30 µg) (cephalosporin) and ciprofloxacin (30 µg) (quinolones). Oxacillin resistance was taken as surrogate marker of methicillin resistance (MR). Reporting of cefazolin resistance was not simply deciphered on methicillin resistance; instead cefazolin resistance was also tested by disc diffusion test and interpreted as per CLSI guidelines[6]. Methicillin resistant *S. aureus* (MRSA) isolates which were also ciprofloxacin resistant were referred as ciprofloxacin resistant MRSA (CRM). Chi-square test was used for significance of difference in biofilm production and antimicrobial resistance pattern among invasive, colonizing and commensal staphylococcal isolates[7]. The data were analysed by SPSS software 'version 10 (SPSS Inc., USA).

A total of 79 per cent of invasive (67/84), 73 per cent of (22/30) colonizing and 28 per cent of (7/25) commensal *S. aureus* isolates were biofilm positive, while 43 per cent (7/16) of invasive, 60 per cent of (12/20) colonizing and 36 per cent (9/25) of commensal CoNS isolates were biofilm positive. The difference in biofilm production rate among all the three groups (invasive, commensal and colonizing) in both *S. aureus* and CoNS was significant ($P<0.001$)[4].

None of the *S. aureus* and CoNS isolates was resistant to glycopeptides (vancomycin & teicoplanin). The occurrence of penicillin resistant *S. aureus* varied from 66.6 to 88 per cent, followed by oxacillin resistance (44.4 to 82%), cefazolin resistance (22.2 to 63.6%) and ciprofloxacin resistance (11.1 to 54.5%) (Table I). Of the 67 biofilm producing invasive *S. aureus* isolates, 36 (53.7%) MRSA isolates were also resistant to ciprofloxacin (CRM) while only 3 of 17 (17.6%) of non biofilm producing MRSA isolates were ciprofloxacin resistant ($P<0.05$). Antibiotic resistance among colonizing *S. aureus* isolates was significantly higher in biofilm producing isolates ($P<0.05$) compared to non biofilm producing isolates. Commensal biofilm producing *S. aureus* isolates were also more frequently resistant to antibiotics than non

biofilm producing isolates but the difference was statistically insignificant (Table I).

In 61 CoNS isolates, penicillin resistance varied from 50 to 100 per cent, followed by oxacillin resistance (31.2 to 75%), cefazolin resistance (12.5 to 66.6%) and ciprofloxacin resistance (0 to 58.3%). Three of 7 biofilm producing invasive MR CNS isolates and 2 of 9 non biofilm producing MR CNS isolates were also ciprofloxacin resistant. Similarly, 7 of 12 (58.3%) biofilm producing and 3 of 8 (37.3%) non biofilm producing colonizing CNS isolates were ciprofloxacin resistant MR CNS, (P<0.05) (Table II).

Staphylococci are bacterial pathogens that usually produce biofilms during different infectious processes, which are generally difficult to treat. It has been estimated that about 65 per cent of the hospital acquired infections are associated with biofilm formation[8-10]. These infections are 10 to 1000 times more difficult to eliminate with an otherwise successful treatment[11,12]. The mechanism for enhanced antimicrobial resistance is believed to involve alteration in gene expression leading to a phenotypic difference between the planktonic and sessile forms. The sessile forms are more resistant as they produce exopolysaccharide, have different growth characteristics and take up nutrients and drugs differently from their planktonic counterparts[9,10].

de Araujo *et al*[13] reported that biofilm producing methicillin resistant *S. epidermidis* isolates from healthy individuals from the community had a higher incidence of multi-resistance than biofilm non-producers from the same population. They also noticed increased incidence of multiresistance among biofilm producers compared to non-producers, isolated from household contacts from the home care system.

It was seen that invasive CoNS were more commonly biofilm producers as compared to colonizing CoNS. CoNS colonizing intravascular devices constitute the major source of invasive isolates and consequently these are expected to have similar phenotypic profiles *in vitro*[14]. As reported earlier[4] the distribution of CoNS species in invasive and colonizing isolates is usually different due the difference in their pathogenic potential. For example, *S. epidermidis* and *S. haemolyticus* are most common invasive CoNS

Table I. Drug resistance pattern of biofilm producing and non producing *S. aureus* isolates (n=139)

Drug	Invasive (84)			Colonizing (30)			Commensal (25)		
	R/biofilm +ve isolates n=67	R/biofilm –ve isolates n=17	R/total isolates n=84	R/biofilm +ve isolates n=22	R/biofilm –ve isolates n=8	R/Total isolates n=30	R/biofilm +ve isolates n=7	R/biofilm –ve isolates N=18	R/Total isolates n=25
Pen	59 (88.0)	12 (70.5)	71 (84.5)	19 (86.3)	6 (75)	25 (83.3)	6 (85.7)	12 (66.6)	18 (72)
Oxa	55 (82.0)	7 (41.1)	62 (73.8)	16 (72.7)	5 (62.5)	21 (70)	4 (57.1)	8 (44.4)	12 (48)
Cz	42 (62.6)	5 (29.4)	47 (55.9)	14 (63.6)	2 (25)	16 (53.3)	2 (28.5)	4 (22.2)	6 (24)
Cip	36 (53.7)	3 (17.6)	39 (46.4)	12 (54.5)	2 (25)	14 (46.6)	1 (14.2)	2 (11.1)	3 (12)
CRM	36 (53.7)	3 (17.6)	39 (46.4)	12 (54.5)	2 (25)	14 (46.6)	1 (14.2)	2 (11.1)	3 (12)

Figures in parentheses are percentages
R, number of resistant isolates; Pen, penicillin; Oxa, oxacillin; Cz, cifazolin; Cip, ciprofloxacin; CRM, ciprofloxacin resistant methicillin resistant *S. aureus*

Table II. Drug resistance pattern of biofilm producing and non producing CoNS isolates (n=61)

Drug	Invasive (16)			Colonizing (20)			Commensal (25)		
	R/biofilm +ve isolates n=7	R/biofilm –ve isolates n=9	R/Total isolates n=16	R/biofilm +ve isolates n=12	R/biofilm –ve isolates n=8	R/Total isolates n= 20	R/biofilm +ve isolates n=9	R/biofilm –ve isolates n=16	R/Total isolates n=25
Pen	7 (100)	6 (66.6)	13 (81.2)	10 (83.3)	6 (75)	16 (80)	7 (77.7)	8 (50)	14 (56)
Oxa	5 (71.4)	4 (44.4)	9 (56.2)	9 (75)	5 (62.5)	14 (70)	4 (44.4)	5 (31.2)	7 (28)
Cz	3 (42.8)	2 (22.2)	5 (31.2)	8 (66.6)	3 (37.5)	11 (55)	3 (33.3)	2 (12.5)	3 (12)
Cip	3 (42.8)	2 (22.2)	5 (31.2)	7 (58.3)	3 (37.5)	9 (36)	2 (22.2)	0 (00)	1 (4)
CRM	3 (42.8)	2 (22.2)	5 (31.2)	7 (58.3)	3 (37.5)	9 (36)	2 (22.2)	0 (00)	1 (4)

Figures in parentheses are percentages
R, number of resistant isolates; Pen, penicillin; Oxa, oxacillin; Cz, cifazolin; Cip, ciprofloxacin; CRM, ciprofloxacin resistant methicillin resistant CNS

isolate while *S. saprophyticus* and *S. epidermidis* are the commonest colonizing strains. Majority of *S. saprophyticus* isolates in our laboratory were non biofilm producers while majority of *S. epidermidis* were biofilm producers[4]. It was reported that invasive and contaminant staphylococcal isolates exhibited similar susceptibilities. The same groups of invasive and contaminating isolates showed no differences in biofilm production, suggesting that resistant isolates were acquired initially as skin flora and subsequently caused invasive infections[14]. Labthavikul *et al*[15] found that MICs and MBCs were similar when CoNS were grown in the planktonic mode or as adherent monolayers. Other studies have shown that *S. aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* were significantly more resistant to both growth inhibition and killing in the adherent form than in the planktonic form. This difference could possibly be explained by different responses to antibiotics by individual species[16].

The findings of the present study show that staphylococcal isolates having biofilm propensity exhibit more resistance to antibiotics, hence are difficult to treat.

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