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STAPHYLOCOCCAL BLOODSTREAM INFECTIONS IN CHILDREN – ANTIBIOTIC RESISTANCE AND BIOFILM FORMATION

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ABSTRACT

Staphylococcus spp. is recognized as an important cause of nosocomial and community-acquired bloodstream infections in children. They are often resistant to antibiotics and the ability to form biofilm is one of the important virulence factors. Biofilms are associated with chronic and medical device-related infections. Biofilm mode of growth provides defence against immune clearance and increased tolerance to antibiotics. A total of 173 *Staphylococcus* isolates were collected over a period of 8 months from blood culture of pediatric patients and biofilm formation was detected by tissue culture plate method. Antimicrobial susceptibility was determined as per CLSI guidelines and correlation with biofilm-producing ability was seen. 42.2% *Staphylococcus aureus* and 35.5% coagulase negative staphylococci were biofilm producers. Biofilm forming isolates showed greater resistance to most antibiotics than the non-biofilm forming isolates. Biofilm formation in vivo may be responsible for device-related and persistent infections. The in vitro detection of biofilm formation can be a vital tool to guide antimicrobial therapy in such patients.

KEYWORDS: bloodstream, paediatrics, *Staphylococcus*, biofilm, resistance.

INTRODUCTION

Bloodstream infection (BSI) is defined as the presence of viable bacteria or fungi in the blood, documented by a positive blood culture result. BSIs are very common in pediatric age group and are a common cause of morbidity and mortality in neonates and children. Neonates are particularly vulnerable because of their weak immune barrier.[1] *Staphylococcus aureus* is

an important cause of bacteremia in children but is less well characterized in children than adults.[2] Although coagulase-negative staphylococci (CoNS) are commensal organisms with little pathogenicity in immunocompetent hosts, premature neonates are particularly susceptible to invasive infection. The increasing prevalence of CoNS infections is attributable to their increasing antibiotic resistance and their ability to form biofilms on foreign bodies such as intravascular catheters.[3] Attachment of *S. aureus* to host tissue and medical devices, and the establishment of mature biofilms, play an important role in the persistence of chronic infections. Biofilm infections are difficult to eradicate as the encasement of cells in polymer-based matrix prevents access of antimicrobials and immune defences to the bacteria. During infection, dispersal of cells from the biofilm can result in spread to secondary sites and contribute to dissemination of antibiotic resistance traits.[4]

Since biofilms play a significant role in device-related infections and development of drug resistance in *Staphylococcus* spp., this study was conducted to detect biofilm formation and correlate with antibiotic resistance pattern of *Staphylococcus* spp. from blood cultures of pediatric patients.

METHODS

A total of 5667 blood culture samples received in the Department of Microbiology from inpatient and outpatient pediatric patients (0 - 14 years) over a period of one year were studied. An episode of staphylococcal bacteremia was defined as a single positive blood culture specimen for *Staphylococcus* spp. in a patient with signs consistent with infection. A new episode in the same patient was recorded if the bacteremia had initially cleared but an additional blood culture performed more than 14 days after the initial positive culture was positive. The isolates were subjected to antimicrobial susceptibility testing by Kirby-Bauer disc diffusion method as per Clinical and Laboratory Standards Institute guidelines.[5] Antibiotic discs used were erythromycin (15µg), penicillin (10 units), ampicillin (10µg), cefoxitin (30µg), trimethoprim-sulfamethoxazole (1.25/23.75µg), linezolid (30µg), clindamycin (2µg), doxycycline (30µg), rifampicin (5µg), chloramphenicol (30µg), ciprofloxacin (5µg), gentamicin (10µg). *S. aureus* ATCC 25923 was used as control strain. For vancomycin susceptibility, mimimum inhibitory concentration (MIC) determination using Vancomycin Ezy MIC™ strips (HiMedia Laboratories, Mumbai) was done (Fig. 1). Statistical analyses were done using Chi-square test to determine the relationship between categorical variables. $P<0.05$ was considered statistically significant.

Fig 1: Vancomycin Ezy MIC™ strips for susceptibility to vancomycin.

Biofilm production by Tissue Culture Plate Method (TCP)[6]

Isolates from fresh agar plates were inoculated in 2 ml brain heart infusion broth with 2% sucrose and incubated overnight at 37°C in stationary condition. Individual wells of sterile, 96 well-flat bottom, polystyrene tissue culture plates were filled with 0.2 ml aliquots of 1 in 100 dilution of the cultures. Uninoculated broth was added to a blank well to check sterility and non-specific binding of the medium. After incubation for 24 hours at 37°C, the wells were washed four times with 0.2 ml phosphate buffered saline (pH 7.2) and biofilms fixed (2% sodium acetate) and stained (0.1% crystal violet). On drying, optical densities (OD) of stained adherent bacteria were determined by an ELISA reader at 570nm and graded according to the method used by Christensen et al (Table 1). Strong and moderate biofilm formation was considered as positive. *Staphylococcus epidermidis* ATCC 35984 (high slime producer) and *S. epidermidis* ATCC 12228 (non-slime producer) were used as positive and negative controls (Fig. 2).

Table 1: Grading of biofilm by TCP method[6]

Mean OD values	Adherence	Biofilm formation
< 0.120	None	None / weak
0.120 – 0.240	Moderate	Moderate
>0.240	Strong	High

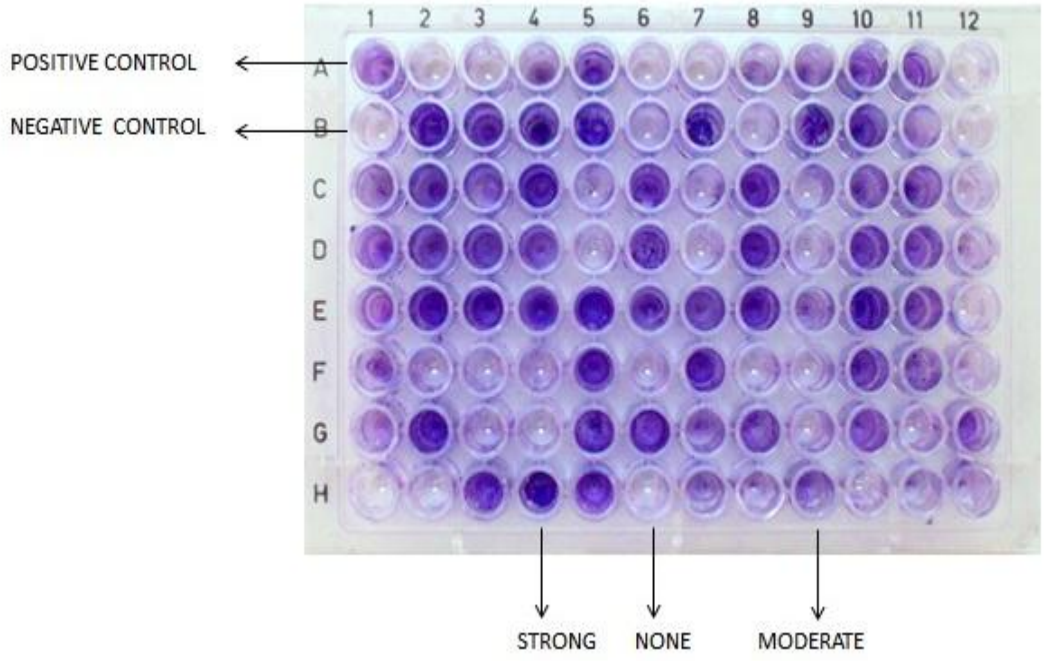

Fig 2: Tissue culture plate method for detection of biofilm formation.

RESULTS

A total of 173 *Staphylococcus* spp. isolates were identified, including 111 *S. aureus* and 62 CoNS. Both *S. aureus* and CoNS were most commonly isolated from neonates i.e. children less than 28 days old, with lower rates of isolation in older children (Chart 1). Out of 111, 46 *S. aureus* i.e. 41.4% were found to produce strong (25.2%) and moderate (16.2%) biofilm by the TCP method. Among CoNS, 35.5% isolates were biofilm producers, including 24.2% strong and 11.3% moderate biofilm producing isolates (Table 2). Among the biofilm-producing isolates, 100% of *S. aureus* were multidrug resistant, while 77.3% biofilm-producing CoNS isolates were multidrug resistant.

Biofilm producers were more resistant to various antibiotics (ampicillin, erythromycin, cefoxitin and trimethoprim-sulfamethoxazole) as compared to the non-biofilm producing isolates (Table 3). The resistance to clindamycin, chloramphenicol, ciprofloxacin and gentamicin was also higher among biofilm producing isolates ($P < 0.05$). However, doxycycline, linezolid, vancomycin and rifampicin were equally effective against both biofilm forming and non-biofilm forming isolates.

Table 2: Biofilm formation by Tissue culture plate method (TCP).

Biofilm formation	*S. aureus*	CONS
Strong	28 (25.2%)	15 (24.2%)
Moderate	18 (16.2%)	7 (11.3%)
Weak/None	65 (58.6%)	40 (64.5%)

Table 3: Antibiotic resistance pattern of biofilm forming (BF) and non-biofilm forming (NBF) *Staphylococcus* spp.

Antibiotics	Resistance in BF isolates (n=68)	Resistance in NBF isolates (n=105)	'*P*' value
Erythromycin	60 (88.2%)	41 (39.0%)	0.000
Penicillin	67 (98.5%)	95 (90.5%)	0.034
Ampicillin	62 (91.2%)	75 (71.4%)	0.002
Cefoxitin	57 (83.8%)	48 (45.7%)	0.000
Trimethoprim-sulfamethoxazole	47 (69.1%)	50 (47.6%)	0.005
Linezolid	1 (1.5%)	0	NA
Vancomycin	0	0	NA
Clindamycin	25 (36.8%)	18 (17.1%)	0.004
Doxycycline	6 (8.8%)	8 (7.6%)	0.777
Rifampicin	5 (7.4%)	4 (3.8%)	0.305
Chloramphenicol	25 (36.8%)	17 (16.2%)	0.002
Ciprofloxacin	25 (36.8%)	21 (20.0%)	0.015
Gentamicin	45 (66.2%)	49 (46.7%)	0.012

(BF- biofilm forming; NBF- non-biofilm forming).

DISCUSSION

In the present study, neonates were the most common age-group with staphylococcal bacteremia. *S. aureus* and CONS cause both early and late-onset neonatal sepsis, more frequently nosocomial than community-acquired. Shivanna et al have also reported CoNS as the leading cause of neonatal sepsis in their study.[3] 41.4% isolates of *S. aureus* in this study were biofilm producers, while 35.5% CONS produced biofilms by TCP method, which is accepted as the most accurate method of screening for biofilm production. Several authors have reported similar incidence of biofilm formation (33% - 57.8%) in *Staphylococcus* spp.[7-11] The findings indicate high biofilm forming potential of invasive and colonizing *Staphylococcus* spp. Several studies have shown that staphylococcal biofilms were more commonly produced by isolates associated with sepsis, including intravenous catheter-related bacteremia and other prosthetic device infections.[12]

In the study, biofilm producers exhibited significantly more antibiotic resistance than non-biofilm forming staphylococci. In a study by Telang et al, biofilm-producing Staphylococci

were notably resistant to erythromycin, clindamycin, tetracycline, cotrimoxazole and fluoroquinolones and also demonstrated decreased sensitivity to vancomycin, teicoplanin, and linezolid.[13] Similar results have been reported by Agarwal and Jain and Hassan et al.[14,15] The resistance to cefoxitin was also greater in the biofilm producing isolates, indicating a positive correlation between biofilm formation and methicillin resistance. Linezolid and vancomycin were found to be effective against biofilm forming staphylococci. Several in-vitro studies have demonstrated the inhibition of staphylococcal biofilms by linezolid and vancomycin.[16] However, their role in-vivo needs to be evaluated. The recalcitrance of biofilm-related infections to antibiotics leads to persistent infections and when associated with implanted devices, device removal may be the only option available.

CONCLUSION

The present study highlights a positive association between biofilm formation and antibiotic resistance in *Staphylococcus* spp. The ability of *Staphylococcus* isolates to form biofilm can be a marker for the ability to cause persistent and device-related bloodstream infections. Knowledge of the prevalence, current antibiogram and phenotypic characteristics is crucial to determining the appropriate empirical treatment for control of staphylococcal bloodstream infections.

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