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A Study on Biofilm Production and Antibiotic Susceptibility Profile of Clinical *Staphylococcus aureus* Isolates in a Tertiary Care Hospital

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Original Research Article

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Abstract: Staphylococcus aureus is one of the most important human pathogens and has been a leading cause of hospital and community acquired infections. Bacteria protected by biofilms are resistant to host defense mechanisms and show resistance to standard antibiotic therapy.This study was conducted to determine the ability of Staphylococcus aureus to produce biofilm and to determine the antibiotic susceptibility profile of Staphylococcus aureus isolated from various clinical samples. A total of 100 non-repetitive strains of Staphylococcus aureus isolated from various clinical samples from Jan2018 - June2018 were included in the study. All the isolates were identified using standard microbiological procedures. Antimicrobial susceptibility testing was performed by Kirby Bauer disc diffusion technique following Clinical and Laboratory Standards Institute (CLSI) guidelines. All these staphylococci were tested for biofilm production by tube method. Among 100 Staphylococcus aureus isolated from various clinical samples, 42 (42%) were biofilm positive and 58 (58%) were biofilm negative. Among 42 biofilm positive Staphylococcus aureus, 5 were from urine samples, 25 were from pus samples and 12 were from sputum samples. The resistance to penicillin,ciprofloxacin, chloramphenicol and oxacillin was significantly more($p<0.05$) in biofilm producing Staphylococcus aureus. All the staphylococcal isolates were sensitive to Vancomycin and Linezolid (100%). Our study showed that 42% of clinical staphylococcus aureus isolates were biofilm producers. Biofilm producing isolates showed higher resistance to all the antibiotics than biofilm non-producers. Thus all the clinical isolates of Staphylococcus aureus should be routinely screened for biofilm production.
Keywords: Staphylococcus aureus, Biofilm, Tube method, Brain Heart Infusion broth, Antibiotic resistance.

INTRODUCTION

Staphylococccus aureus is the most frequent cause of nosocomial and community-acquired infections and is recognized as the most frequent causes of biofilm-associated infections [1].

Staphylococcus aureus is an adaptable, pathogenic organism. In the presence of environmental challenges, it can alter its genotype and/or phenotype to adapt to its surroundings. An example of genotypic change is the acquisition of the β-lactamase gene conferring penicillin resistance. The formation of biofilm is an example of phenotypic change. Biofilms are densely packed communities of microbial cells that grow on living or inert surfaces and surround themselves with secreted polymers. This slime or biofilm consists of layers of cell clusters embedded in a matrix of extracellular polysaccharide called Polysaccharide Intercellular Adhesin (PIA) [2].

Staphylococcus aureus initially adheres to a solid substrate, after which cell–cell adhesion occurs; the bacteria then multiply to form a multilayered biofilm encased in exopolysaccharides. Biofilm formation involves the production of polysaccharide intercellular adhesin, which depends on the expression of the intercellular adhesion (*Ica*ADBC) operon that encodes three membrane proteins (*Ica*A, *Ica*D and *Ica*C) and one extracellular protein (*Ica*B) [3,1].

There are various methods described in the literature to detect biofilm production like tissue culture plate (TCP), Tube method (TM), Congo Red Agar method (CRA), modified CRA method (MCRA), bioluminescent assay, piezoelectric sensors, and fluorescent microscopic examination [4].

Bacteria protected by biofilms are resistant to host defense mechanisms and show resistance to standard antibiotic therapy [5]. Infact biofilms can resist

antibiotic concentration 10-10,000 folds higher than those required to inhibit the growth of free floating bacteria [6].

Thus this study was conducted to determine the ability of Staphylococcus aureus isolated from various clinical samples to produce biofilm and also to study their antibiotic susceptibility profile.

OBJECTIVES

- To determine the ability of Staphylococcus aureus to produce biofilm.
- To determine the antibiotic susceptibility profile of Staphylococcus aureus isolated from various clinical samples.

MATERIALS AND METHODS

This cross-sectional observational study was conducted in the Department of Microbiology, Mandya Institute of Medical Sciences, Mandya. The study was approved by the Institutional Ethical Committee. A total of 100 non-repetitive strains of Staphylococcus aureus isolated from various clinical samples from Jan2018 - June2018 were included in the study. All the isolates were identified using standard microbiological procedures [7]. Antimicrobial susceptibility testing was performed by Kirby Bauer disc diffusion technique following clinical and laboratory standards institute (CLSI) guidelines [8]. The antibiotic discs used were penicillin-G (10 units), oxacillin (1 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), erythromycin (15 µg), gentamicin (10 µg), cotrimoxazole (25 µg), vancomycin (30 µg) and linezolid (30 µg). (HiMedia Laboratories, Mumbai, Maharashtra, India). S. aureus ATCC 25923 was used as the control organism.

All these staphylococci were tested for biofilm production by tube method as described by Christensen *et al.*[9] BHI with 2% sucrose was inoculated with loopful of growth from overnight culture plates incubated for 24 hours at 37°C. Tubes were decanted and washed with phosphate buffered saline and dried. Tubes were then stained with crystal violet 0.1%. Excess stain was removed and tubes were washed with water. Tubes were then dried in inverted position and observed for biofilm formation. The results were scored visually. Visible lining of the wall and bottom of the tube by a film was considered as positive. Tubes which did not show the stained film were taken as negative. S. aureus ATCC 25923 was taken as negative control.

STATISTICAL ANALYSIS

The data was entered in Microsoft Excel and Descriptive statistics like percentage was used for analysis. Chi-square test was used for analysis of categorical data. A *P*-value of <0.05 was considered statistically significant.

RESULTS

Among 100 Staphylococcus aureus isolated from various clinical samples, 42 (42%) were biofilm positive and 58 (58%) were biofilm negative.

Among 42 biofilm positive *Staphylococcus aureus*, 5 were from urine samples, 25 were from pus samples and 12 were from sputum samples.

DISCUSSION

Biofilm producing bacteria are responsible for many recalcitrant infections and are very difficult to eradicate. They show resistance to antibiotics by various methods like restricted penetration of antibiotic into the biofilms, decreased growth rate and expression of resistance genes [10].

In our study, 42% of Staphylocoocus aureus were biofilm positive by Tube method. Taj *et al.* [11], Abirami *et al.* [12] and Chinithung *et al.* [13] have found 23.2%, 26% & 38% biofilm production by tube method in Staphylococcus aureus respectively. However, Neelusree *et al.* [14] and Ansari *et al.* [15] have reported 54.34% and 63.4% Staphylococcus aureus respectively as biofilm producers by Tube Method in their studies. Mathur *et al*. have recorded the sensitivity and specificity of tube method as 73.6% and 92.6% respectively [16]. Biofilm formation is dependent on different parameters including the characteristics of the nature of carbon source, its concentration, pH, ionic strength, and temperature, etc. [5].

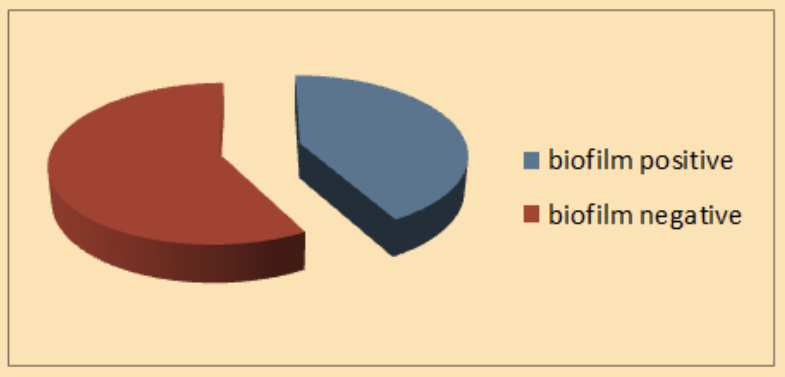

Fig-1: Percentage of biofilm positive & biofilm negative Staphylococcus aureus

Table-1: Distribution of Biofilm positive S.aureus in various clinical samples

Samples	Number	Percentage
Urine	5	11.9
Pus	25	59.5
Sputum	12	28.6

Table-2: Antibiotic susceptibility pattern of Staphylococcus aureus

Antibiotics	Biofilm positive *S.aureus*(N=42)		Biofilm negative *S.aureus*(N=58)		P value
	Resistant(%)	Sensitive(%)	Resistant (%)	Sensitive(%)	
Penicillin	40 (95.2)	2 (4.8)	46(79.3)	12(20.7)	<0.05
Erythromycin	39 (92.9)	3 (7.1)	47(81)	11(19)	>0.05
Cotrimoxazole	22 (52.4)	20 (47.6)	25(43.1)	33(56.9)	>0.05
Gentamicin	32 (76.2)	10 (23.8)	38(65.5)	20(34.5)	>0.05
Ciprofloxacin	15 (35.7)	27 (64.3)	8(13.8)	50(86.2)	<0.05
Chloramphenicol	14 (33.3)	28 (66.7)	40(68.9)	18(31.1)	<0.05
Oxacillin	35 (83.3)	7 (16.7)	25(43.1)	33(56.9)	<0.05
Vancomycin	0	42 (100)	0	58(100)	
Linezolid	0	42 (100)	0	58(100)	

Highly accurate methods like PCR for detection of *ica* gene though available to check the ability of S. aureus strains to produce biofilm, are beyond the scope of most of the microbiology laboratories in the developing countries like India.

In our study, among 42 biofilm positive Staphylococcus aureus, 5 (11.9%)were from urine samples, 25 (59.5%)were from pus samples and 12(28.6%) were from sputum samples. 65.1% biofilm positive staph aureus by Tube method from pus samples was reported by Neopane *et al*. [17]

Higher resistance to all the antibiotics used was noted in biofilm producing Staphylococcus aureus. The resistance to penicillin, ciprofloxacin, chloramphenicol and oxacillin was significantly more ($p<0.05$) in biofilm producing Staphylococcus aureus. All the staphylococcal isolates were sensitive to Vancomycin and Linezolid (100%). The higher rate of resistance in biofilm-producing Staphylococcus aureus toward erythromycin, cotrimoxazole and ciprofloxacin has been reported earlier [18, 19]. As in a study by Ansari *et al*. [19] who reported 94.7% resistance to penicillin, high rate of resistance to penicillin (95.2%) in biofilm producing staphylococcus aureus was reported in our study. Also there was no resistance to vancomycin and linezolid in their study. The study conducted by Sasirekha B et al., also reported that biofilm producing strains showed high resistance to almost all the groups of antibiotics compared to the biofilm non-producer [20].

The increased resistance of biofilm producing strains to antibiotics may be because the biofilm bacteria exhibit a slow rate of metabolism and divide infrequently resulting in decreased sensitivity to antibiotics targeted at cell wall synthesis. However, even antibiotics targeted at cellular functions such as protein and DNA synthesis which should affect cells at a quiescent state are ineffective against biofilms [21, 22]. Biofilm formations also help in the spread of antibiotic resistant traits in nosocomial pathogens by increasing mutation rates and by the exchange of genes which are responsible for antibiotic resistance [23]. Biofilm-mediated infections in the hospital environment have a significant negative impact on patient's health and place an enormous burden on the resources of the health services [24].

The limitation of this study is the small sample size and not evaluating other methods for detection of biofilms in Staphylococcus aureus.

CONCLUSION

Our study showed that 42% of clinical staphylococcus aureus isolates were biofilm producers. Biofilm producing isolates showed higher resistance to all the antibiotics than biofilm non-producers. This shows that biofilm production and antibiotic resistance are inter-related. Thus all the clinical isolates of Staphylococcus aureus should be routinely screened for biofilm production. This would not only enable improved treatment of biofilm-related infections but also slows the rate of emergence and spread of antibiotic resistant strains.

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