

## Research Article

# A study of biofilm production in clinical isolates of Staphylococci at a tertiary care hospital, Bangalore

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**Received:** 01 January 2015

**Accepted:** 15 January 2015

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### ABSTRACT

**Background:** The Biofilms are densely packed communities of microorganisms consisting of layers of cell clusters embedded in a matrix of extracellular polysaccharide called polysaccharide intercellular adhesin. This layer impedes the delivery of antibiotics to the biofilm forming microbial cells leading to emergence of drug resistance. Staphylococci are commensal bacteria on the human skin and mucous membranes. So it may be easily introduced as a contaminant during the surgical intervention. So, this study was conducted to identify the Biofilm producing strains from clinical isolates of Staphylococci.

**Methods:** A total of 182 non-repetitive clinical strains of Staphylococci isolated from various clinical samples from Feb 2014 to Oct 2014 were included in the study. All the isolates were identified using standard microbiological procedures. All the samples were tested for biofilm production by modified Congo-red agar method and tube method.

**Results:** Out of 182 samples that were included in the study, a total of 90 (49.45%) samples showed biofilm formation of which 58 (75.32%) were methicillin resistant and 32 (30.47%) were methicillin sensitive. Also these strains were resistant to other antibiotics.

**Conclusion:** Our study showed biofilm production by methicillin resistant strains which were also multidrug resistant. Treatment of methicillin resistant strains of Staphylococci is one of the most challenging task for the clinicians and the microbiologists. So they should be routinely screened for biofilm formation in order to prevent emergence and spread of multidrug resistant strains.

**Keywords:** Biofilm, MRSA

### INTRODUCTION

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). PIA is involved in cell to cell adhesion and is essential for biofilm production. It also impedes delivery of antibiotics.<sup>1</sup> Research performed in many biofilm-forming organisms has revealed that the

development of a biofilm is a two-step process involving an initial attachment and a subsequent maturation phase, which are physiologically different from each other and require phase-specific factors. A final detachment (or dispersal) phase involves the detachment of single cells or cell clusters by various mechanisms and is believed to be crucial for the dissemination of the bacteria, in the case of pathogens to new infection sites in the human body.<sup>2</sup>

When cells attach to a surface, they will express a general biofilm phenotype. These biofilm cells could express increased resistance to antimicrobial agents which might be induced by nutrient limitation, certain types of stress, high cell density or a combination of these phenomena. The importance of biofilms in nosocomial infections has increased in recent times as these infections are mainly caused by drug resistant strains.<sup>3</sup> Biofilm forming bacteria cause a wide range of human infections. The resistant to antimicrobial agents among bacteria growing in biofilm are 500 to 5000 times higher than their planktonic counterparts.<sup>4</sup>

Staphylococci are recognized as the most frequent causes of biofilm-associated infections. This exceptional status is due to the fact that staphylococci are frequent commensal bacteria on the human skin and mucous surfaces. So it is probably easily introduced as a contaminant during the surgical implantation of the polymeric device. Notably, a device-related infection of Staphylococci characteristically involves biofilm formation, which generally is considered the most important factor involved in the pathogenesis.<sup>2</sup>

The Nosocomial Infections Surveillance System<sup>5</sup> recognizes *S. aureus* and CONS as the most frequently isolated nosocomial pathogens from intensive care unit patients. 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.<sup>6</sup>

In view of this, the present study was conducted to isolate biofilm forming Staphylococci from the clinical isolates, so that appropriate interventions could be done to prevent biofilm production and also to avoid emergence and spread of drug resistant strains.

## METHODS

A total of 182 non-repetitive clinical strains of Staphylococci isolated from various clinical samples from Feb 2014 - Oct 2014 were included in the study. All the isolates were identified using standard microbiological procedures. They were subjected to antibiotic susceptibility test by Kirby Bauer disc diffusion method on Mueller Hinton agar and the zones were interpreted as per CLSI guidelines.<sup>7</sup> All the samples were tested for biofilm production by modified Congo-red agar method and tube method.

### Biofilm assay

**Tube method:** Biofilm formation was determined as described by Christensen et al.<sup>8</sup> BHI with 2% sucrose was inoculated with loopful of microorganisms 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. Biofilm formation was considered positive when visible film lined the wall and bottom of the tube.<sup>8</sup>

**Modified Congo-Red Agar method (CRA):** Freeman et al.,<sup>9</sup> had described an alternative Method for detecting biofilm. Isolates were cultured on the agar containing 10g of glucose with 0.4 g of Congo-red in one liter of blood base agar-2 and incubated at 37°C for 48 h. Strains which produced black colonies considered as slime producers and strains with red colonies labeled as non-slime producers. Black colored colonies with dry crystalline consistency interpreted as positive biofilm producing strains. Red coloured colonies-interpreted as negative for biofilm production.<sup>9</sup>



**Figure 1: Tube method: a-Negative b-Positive.**



**Figure 2: Modified Congo-red agar method: a, b, c- Positive d-Negative.**

**RESULTS**

A total of 182 non repetitive clinical samples of Staphylococcus were included in the study. 63 (34.62%) of which were Staphylococcus aureus (S. aureus) and 119 (65.38%) were Coagulase Negative Staphylococci (CONS).

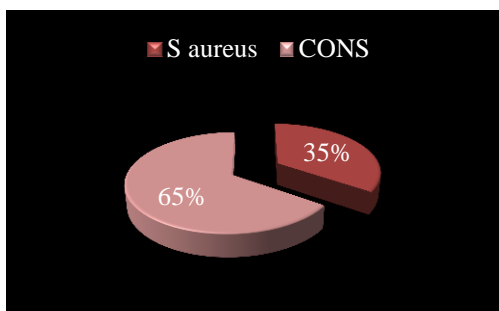
Among them, 24 (38.10%) were Methicillin resistant S. aureus (MRSA), 39 (61.90%) Methicillin sensitive S. aureus (MSSA), 53 (44.54%) Methicillin resistant Coagulase Negative Staphylococci (MR-CONS) and 66 (55.46%) were Methicillin Sensitive Coagulase Negative Staphylococci (MS-CONS).

All samples were tested for biofilm formation by modified Congo-red agar method and tube method. Samples showing biofilm formation by both methods or by tube method alone were included in the study as tube method has a sensitivity of 94.7% and specificity of 97.1% as compared to gold standard Tissue culture plate method.<sup>10</sup> All the 90 samples showed biofilm formation by tube method and 87 of them showed black colonies by modified Congo-red agar method.

In our study, a total of 90 (49.45%) samples showed biofilm formation, 58 (64.44%) were methicillin resistant and 32 (35.56%) were methicillin sensitive. Among the Methicillin resistant strains, 75.32% of produced biofilm whereas only 30.47% of methicillin sensitive strains produced biofilm.

**Table 1: Distribution of isolates.**

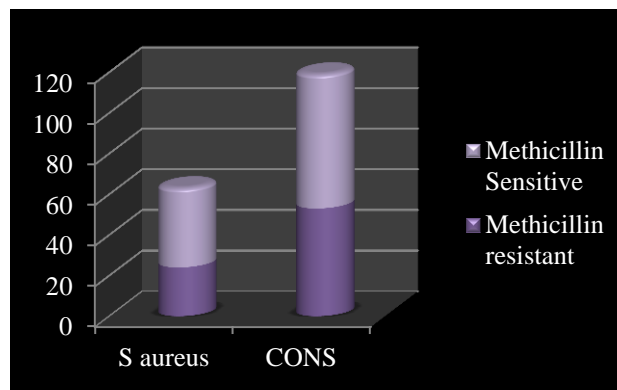
Total	S. aureus	CONS
182	63 (34.62%)	119 (65.38%)



**Figure 3: Distribution of isolates.**

**Table 2: Distribution of isolates based on sensitivity to methicillin.**

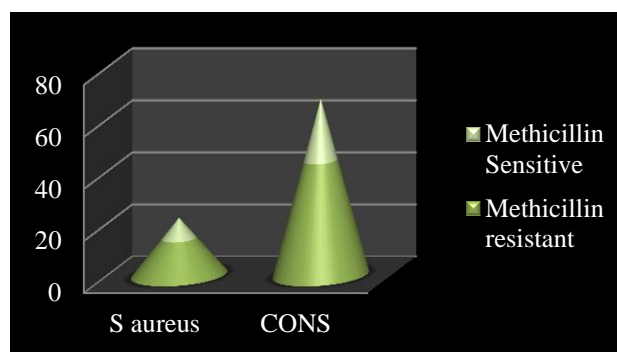
	S. aureus	CONS	Total
Methicillin resistant	24 (38.10%)	53 (44.54%)	77
Methicillin sensitive	39 (61.90%)	66 (55.46%)	105
<b>Total</b>	<b>63</b>	<b>119</b>	<b>182</b>



**Figure 4: Distribution of isolates based on sensitivity to methicillin.**

**Table 3: Distribution of biofilm producing strains.**

	S. aureus	CONS	Total
Methicillin resistant	14 (15.56%)	44 (48.88%)	58 (64.44%)
Methicillin sensitive	08 (8.89%)	24 (26.67%)	32 (35.56%)
<b>Total</b>	<b>22 (24.45%)</b>	<b>68 (75.55%)</b>	<b>90 (100%)</b>



**Figure 5: Distribution of biofilm producing strains.**

Therefore among the methicillin resistant strains, 75.32% of produced biofilm whereas only 30.47% of methicillin sensitive strains produced biofilm.

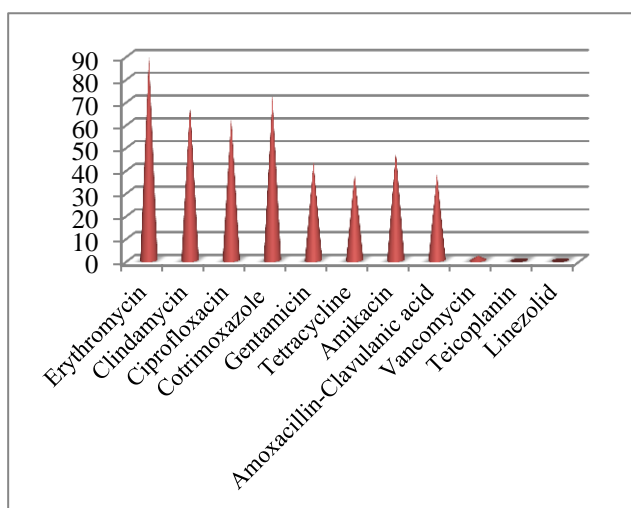
**Table 4: Strains producing biofilm in relation to sensitivity to methicillin.**

	Biofilm producing	Percentage
Methicillin resistant (77)	58	75.32%
Methicillin sensitive (105)	32	30.47%

The biofilm producing strains were also resistant to other commonly used drugs. Erythromycin (100%), clindamycin (76.66%), ciprofloxacin (70%), cotrimoxazole (80%), gentamycin (47.78%), tetracycline (42.22%), amikacin (53.33%), Amoxicillin clavulanic acid (42.22%), vancomycin (2.22%), teicoplanin (0%), linezolid (0%).

**Table 5: Resistance pattern of biofilm producing strains.**

Drug	Resistance (Percentage) n=90
Erythromycin	90 (100%)
Clindamycin	69 (76.66%)
Ciprofloxacin	63 (70%)
Co-trimoxazole	72 (80%)
Gentamicin	43 (47.78%)
Tetracycline	38 (42.22%)
Amikacin	48 (53.33%)
Amoxicillin-clavulanic acid	38 (42.22%)
Vancomycin	02 (2.22%)
Teicoplanin	0
Linezolid	0



**Figure 6: Resistance pattern of biofilm producing strains.**

**DISCUSSION**

Biofilm forming bacteria cause a wide range of human infections. Biofilm is one of the important microbial virulence factor and organisms use biofilm mechanism as a way of causing chronic infection to human.

Bacteria in biofilm are protected from antibiotics due to presence of large amount of exopolysaccharides. *S. aureus* biofilms have been associated with a variety of persistent infections which respond poorly to conventional antibiotic therapy.<sup>11</sup> 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.<sup>6</sup>

Biofilm-producing *S. aureus* is known to be more difficult to control, having greater resistance to

antibacterial agents than *S. aureus* not embedded in biofilm.

In our study, 75.32% of methicillin resistant strains produced biofilm whereas only 30.37% of methicillin sensitive strains produced biofilm. In study conducted by Dardi Charan Kaur et al., Of 231 MRSA, biofilm formation was observed in 182 (78.78%).<sup>12</sup> S. Singh reported 85.72% (36/42) of the isolates were found to be high biofilm formers.<sup>13</sup> Sasirekha B et al., also reported 61.90% of MRSA isolates to have the potential to make biofilm.<sup>14</sup> The results of these studies correlates with our study.

Our study showed a correlation between biofilm production and methicillin resistance. Our study also showed a correlation between Biofilm formation and multi drug resistance. Biofilm producing strains were resistant to more than four of the other antibiotics.

The study conducted by Maryam Rezaei et al., also demonstrated a relation between multidrug resistant MRSA and strong biofilm production.<sup>15</sup> In a study conducted by Agnes Bedie Eyoh, 35.6% of isolates were identified as biofilm formers and MDR was detected in 9 (42.9%) of biofilm forming isolates.<sup>16</sup> 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.<sup>14</sup>

**CONCLUSIONS**

Biofilm production in Staphylococci isolated from clinical samples are of clinical significance as biofilm constitutes reservoir of pathogens. Also Susceptibility to antibiotics in bacteria that are protected by biofilm is reduced because drugs are prevented from reaching the bacteria surrounded by biofilm leading to multiple drug resistance and chronic infections.

In our study, Biofilm production was detected in 49.45% (90) of isolates. Of the total isolates, biofilm production and was more in methicillin resistant strains 64.44% (58). Also among the total 77 methicillin resistant strains, 75.32% (58) produced biofilm. These strains also showed higher degree of resistance to almost all the groups of antibiotics.

To conclude, biofilm production and antibiotic resistance are inter-related. Also treatment of Methicillin resistant strains of Staphylococci is one of the most challenging task for the clinicians and the microbiologists. So they should be routinely screened for biofilm formation in order to prevent emergence and spread of multidrug resistant strains. Hence it is also advisable to screen for biofilm production since these organisms are an important cause of nosocomial infections.

*Funding: No funding sources*

*Conflict of interest: None declared*

*Ethical approval: Not required*

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DOI: 10.5455/2320-6012.ijrms20150217

**Cite this article as:** Golia S, Kamath A AS, Nirmala AR. A study of biofilm production in clinical isolates of Staphylococci at a tertiary care hospital, Bangalore. *Int J Res Med Sci* 2015;3:470-4.