



## A comparative study of antimicrobial susceptibility pattern of biofilm producing & non-biofilm producing staphylococcus Aureus isolated from various clinical samples at a tertiary care hospital, Jaipur

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### Abstract

**Introduction:** Biofilm is a highly resistant stage of microorganisms in which they exhibit high resistance towards antimicrobial agents and it also increases their virulence power. Staphylococcus aureus is a Gram positive cocci responsible for many serious infections including community acquired and hospital acquired infection. The severity of these infection increases with biofilm production. So, this study is focused on detection of biofilm production by Staphylococcus aureus and comparison of antimicrobial susceptibility pattern of biofilm producing and non-biofilm producing isolates.

**Material & Method:** This study was done on all clinical samples received in m Department of microbiology, National Institute of Medical Sciences and Research, Jaipur over a period of 5 months from August 2019 to December 2019. Samples were received and processed as per the standard guidelines and antimicrobial susceptibility testing was done according to the standard CLSI guidelines. Biofilm production was detected by Tube method.

**Result:** In this study 47 (48.53%) isolates were identified as Staphylococcus aureus and biofilm production was detected in 23 (48.94%) isolates by Tube method. Among the biofilm producers the sensitivity of first line drugs was very poor. Only Vancomycin, Linezolid and Teicoplanin drugs were effective against biofilm producing isolates.

**Conclusion:** Current study showed the comparison between antimicrobial susceptibility pattern of biofilm producing and non-biofilm producing Staphylococcus aureus and it will help the clinicians in treating such patients and thus it will be helpful in reducing the morbidity rate.

**Keywords:** Staphylococcus aureus, Biofilm, Tube Method, Vancomycin, Linezolid, Teicoplanin

### Introduction

Biofilms are aggregates of microbial communities in which cells are surrounded by self-produced extracellular polymer matrix <sup>[1]</sup>. These biofilms gets irreversibly attached to natural or artificial surface like teeth, epidermal cells, inside chronic wounds, intravenous lines or artificial joints. Most important thing is that bacteria showing biofilm production are highly resistant to antibiotics and also to body's defense mechanism <sup>[2]</sup>. Many important Gram Positive and Gram Negative bacteria show biofilm production. These include *Staphylococcus aureus*, *Enterococcus faecalis*, *Staphylococcus epidermidis*, *Streptococcus viridans*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Pseudomonas aeruginosa* <sup>[3]</sup>. Organisms showing biofilm production are more resistant to antimicrobial drugs as compared to non-biofilm producing organisms <sup>[4]</sup>. It has been reported that 65% of the hospital acquired infections among humans are linked with biofilm production. Studies suggest that alterations in gene expression are responsible for increased antimicrobial resistance and thus lead to a phenotype difference in between the planktonic and sessile forms <sup>[5]</sup>. *Staphylococci* are considered as the most frequent cause of biofilm associated infections in community and hospital, over the world. Biofilm production is an important virulence factor of *Staphylococcus aureus* <sup>[6]</sup>. *Staphylococcus aureus* is considered as well-known bacteria which makes biofilm on natural as well as artificial surfaces. Because of the biofilm production, it becomes difficult to

treat infections caused by such *S. aureus*. Previous studies have reported association in between antibiotic resistance and biofilm production by *S. aureus* <sup>[7]</sup>. This study was done to determine the prevalence of biofilm production in *S. aureus* and to compare the antimicrobial susceptibility pattern of biofilm producing *S. aureus* and non-biofilm producing *S. aureus*.

### Material & Methods

The present study was conducted in Department of Microbiology, National Institute of Medical Sciences & Research, Jaipur for over a period of 5 months from August 2019 to December 2019. Samples received in lab were processed by standard guidelines. Samples were inoculated on Blood agar, Mac-Conkey agar and Nutrient agar. Identification of isolates was done by using standard biochemical tests. Antimicrobial Susceptibility testing was done on Mueller Hinton agar by Kirby Bauer Disk diffusion method <sup>[8]</sup>. Biofilm detection was done by using Tube method <sup>[9]</sup>.

**Tube Method:** This is a qualitative method described by Christensen *et al.* in 1982. A loopful of test organisms inoculated in 10 ml of trypticase soy broth with 1% glucose in test tubes. Incubate the tubes at 37°C for 24 hours. After incubation, tubes were decanted and washed with phosphate buffer saline (pH 7.3) and dried. Tubes were then stained with crystal violet (0.1%). Wash excess stain with distilled

water. Tubes were dried in inverted position. The scoring for tube method was done according to the results of the control strains. Biofilm formation was considered positive when a visible thick film lined the wall and the bottom of the tube. *S. aureus* ATCC 25293 and *P. aeruginosa* ATCC 27853 were used as negative and positive controls respectively for biofilm detection.

## Results

During the study period, out of total 97 Gram Positive bacteria, 47 (48.53%) isolates were identified as *Staphylococcus aureus*. Among these, 30 isolates from male patients and 17 isolates from female patients were turned out to be *S. aureus*. Among these 47 *S. aureus*, 18 isolates were recovered from pus samples followed by 10 isolates from urine sample and 8 isolates from blood samples. Biofilm production was observed in 23 *S. aureus* isolates with a prevalence rate of 48.94%. Penicillin, Ampicillin, Cefoxitin showed high resistance while Vancomycin, Linezolid and Teicoplanin were 100% sensitive.

**Table 1:** Biofilm production

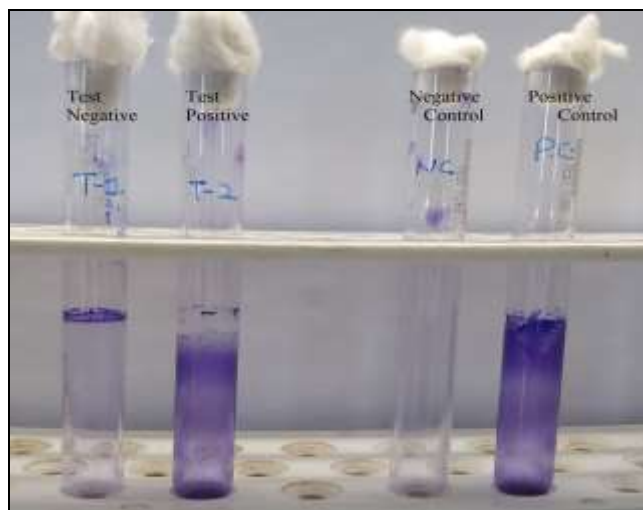
Total Number of <i>S. aureus</i>	Biofilm producer	Non-biofilm producer
47	23	24

**Table 2:** Sample distribution

Sample	Number of <i>S. aureus</i> isolates	Biofilm producer <i>S. aureus</i>	Non biofilm producer <i>S. aureus</i>
Urine	10	6	4
Sputum	4	2	2
Blood	8	3	5
Pus	18	8	10
Wound Swab	5	3	2
E. T. aspirate	2	1	1
Total	47	23 (48.93%)	24 (51.06%)

**Table 3:** Antimicrobial resistance pattern of *S. aureus* isolates.

Antimicrobial Drug	Resistance pattern of all <i>S. aureus</i> isolates (N=47)		Resistance pattern of Biofilm producing <i>S. aureus</i> isolates (N=23)		Resistance pattern of Non biofilm producing <i>S. aureus</i> isolates (N=24)	
	R	R%	R	R%	R	R%
Ampicillin	38	80.85	23	100.0	15	62.50
Penicillin	47	100.0	23	100.0	24	100.0
Cefoxitin	21	44.68	21	91.30	0	0.0
Ciprofloxacin	18	38.29	16	69.56	2	8.33
Vancomycin	0	0.0	0	0.0	0	0.0
Linezolid	0	0.0	0	0.0	0	0.0
Teicoplanin	0	0.0	0	0.0	0	0.0
Gentamycin	19	40.42	15	65.21	4	16.66



**Fig 1:** Biofilm detection by Tube method.

## Discussion

In the present study, 47 *S. aureus* isolates were obtained from 97 Gram Positive bacteria with a prevalence rate of 48.53%. This is in accordance with the result of a study conducted by Dr. Astha *et al* (2017) [10]. In her study the prevalence of isolation of *S. aureus* was 47.11%. Rania M. Abdel Halim *et al* (2018) [11] in their study showed 48% prevalence rate of *S. aureus*. Highest number of *S. aureus* were isolated from Pus sample followed by urine and blood sample in this study. Slightly variable results were seen in study of Dr. Astha *et al* (2017) [10] and Afreenish Hassan *et al.* (2011) [12].

Biofilm production is believed to be a probable cause of *Staphylococcal* infection. Biofilm production is also clinically significant as biofilm constitutes a reservoir of bacteria and also associated with increased resistance to antimicrobial drugs and chronic infections. In this study, biofilm production was detected by Tube method. Biofilm production was detected in 23(48.94%) *S. aureus* isolates out of total *S. aureus* isolates. This result was comparable with many previous studies. Rania M. Abdel Halim *et al* (2018) [11] in their study showed biofilm production in 53.2%. Dr. Astha *et al* (2017) [10] in her study reported biofilm production in 42.96% *S. aureus* isolates. Bose *et al* (2009) [13] in their study reported that 42.44% *S. aureus* isolates showed positive biofilm production.

In our study the prevalence of MRSA was 44.68%. All MRSA strains in our study were biofilm producers and showed high degree of resistance towards antimicrobial drugs. This was in accordance with the results of Puja Neopane *et al* (2018) [14].

In this study, we compared the antimicrobial resistance pattern of biofilm producing and non-biofilm producing *S. aureus* strains. The biofilm producing *S. aureus* strains showed high resistance as compared to non-biofilm

producing strains. Biofilm producing isolates showed complete resistance towards Penicillin and its derivatives. Gentamycin, Ciprofloxacin showed high resistance among biofilm producing isolates as compared to non-biofilm producing isolates. Vancomycin, Teicoplanin and Linezolid were the only effective drugs in biofilm production. They showed 100% sensitivity pattern among biofilm producing as well as non-biofilm producing *S. aureus* isolates. These results were in accordance with Hassan *et al* (2011) <sup>[12]</sup>, Garima *et al* (2015) <sup>[15]</sup> and Khan *et al* (2011) <sup>[16]</sup>.

Our results clearly state that the resistance in biofilm producing *S. aureus* isolates is more than non-biofilm producing isolates. This may be due to impaired penetration of the drugs across the biofilm, expression of resistance gene and also because the biofilm producing bacteria exhibit slow rate of metabolism and divide infrequently resulting in decreased sensitivity to drugs targeted at cell wall synthesis.

### Conclusion

Biofilm production is an important virulence factor of *S. aureus* infection and biofilm production increases the severity of the infection caused by *S. aureus*. This study clearly suggest that biofilm production is directly linked with increased antimicrobial resistance pattern. Only Vancomycin, Linezolid and Teicoplanin can be used to treat such cases according to this study. All other drugs showed high resistance. All MRSA strains showed biofilm production in our study. Furthermore studies are needed to demonstrate the relation between *mecA* gene and biofilm production. The antimicrobial susceptibility pattern will help the clinicians in prescribing appropriate drugs to treat such patients and thus it will prevent the emergence and spread of resistance.

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