

## Tube adherence test as a screening tool for detection of biofilm formation among *Staphylococcus*

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### Abstract

**Background:** Due to large number of infections caused by biofilm producing organism, a reliable and reproducible method for its detection is necessary. Specific methods like Polymerase Chain Reaction (PCR) are available for detection of biofilm, but these are beyond the scope majority of laboratories in developing nations like India.

**Objective:** To test reliability of Tube Adherence test as a screening tool for detection of biofilm formation among *Staphylococcus*.

**Materials and Methods:** Various clinical samples were collected with universal precautions from patients with indwelling medical device for more than 48 hours. Screening for biofilm detection was done using Tube adherence method.

**Results:** Tube method was 97.5% sensitive and 100% specific for detection of biofilm formation.

**Conclusion:** Tube method can be used as a general screening method for detection of biofilm formation by *Staphylococci*.

**Keywords:** Biofilm, Predictive Value, Sensitivity, Specificity, *Staphylococci*, Tube Method.

### Introduction

Biofilm formation is an important factor in most of the nosocomial infections. In modern clinical medicine role of *Staphylococci* on indwelling medical devices has had considerable impact.<sup>(1)</sup> Chronic infections of indwelling medical devices are most often associated with *Staphylococci* and identified as the most common cause of biofilm-associated infections.<sup>(2)</sup> This exceptional status among biofilm-associated organism is due to the fact that *Staphylococci* are normal commensal on skin and mucous membrane. Thus, *Staphylococci* are among the most likely organism to infect any medical device when being inserted during surgery. The differentiation of biofilm phenotype by *Staphylococci* might help to elucidate the impact of *Staphylococci* in the diagnosis of infections related to indwelling medical devices and these findings may be useful in the prevention of medical device related infections. In infection produced by biofilm producing *Staphylococci*, the differentiation with respect to biofilm phenotype might help to modify the antibiotic therapy and to prevent infection related to indwelling medical devices. A reliable, suitable and reproducible method is necessary for screening of biofilm producers in any hospital setting.<sup>(3)</sup> In our study an attempt was made to test Tube adherence test as a screening tool for detection of biofilm formation among *Staphylococcus*.

### Material and Methods

Out of 100 non-repetitive, *Staphylococcus* strains isolated from various clinical samples from different departments of Vijayanagar Institute of Medical Sciences (VIMS), Bellary, India. The study was conducted for a period of one year from Jan 2013 to Dec 2013. Various clinical samples such as pus, urine,

sputum, blood, body fluids, corneal scrapping, and indwelling urinary catheter were collected and processed. Consent was taken from all patients after explaining them briefly about the study.

### Inclusion Criteria:

1. All isolates of *Staphylococcus spp.* from patients hospitalized for more than 48 hrs.
2. All *Staphylococcus spp.* isolated from out-patients, with in-dwelling medical devices.

### Exclusion Criteria:

1. All out-patients without in-dwelling medical devices.
2. Patients hospitalized for less than 48 hrs.

**Tube Adherence Test<sup>(4)</sup> (TM):** (Fig. 1) The broth containing Brain Heart Infusion (BHI) was inoculated by tested strains in test tubes were incubated aerobically at 35°C for 48 hrs. Then the test tubes were stained with 0.1% safranin after discarding the supernatants, washed with distilled water three times and dried. A positive result was defined as the presence of a layer of the stained material which adhered to the inner wall of the tubes. The exclusive observation of a stained ring at the liquid-air interface was considered as negative. Experiments were done in triplicate for three times and read as absent, weak, moderate and strong.

**Tissue culture Plate Method<sup>(1)</sup> (TCP):** Fresh culture samples were inoculated in BHI broth with 2% sucrose and incubated at 37°C for 24 hrs in a stationary condition and diluted 1 in 100 with fresh medium. Individual wells of sterile, polystyrene, 96 wells-flat bottom tissue culture plates wells were filled with 0.2 ml of the diluted cultures and only broth served as control to check sterility and non-specific binding of media. The tissue culture plates

were incubated for 24 hours at room temperature. After incubation content of each well was gently removed by tapping the plate. The wells were washed four times with 0.2 ml of phosphate buffer saline (PBS pH7.2) to remove free-floating 'planktonic' bacteria.

Biofilm formed by adherent 'sessile organism in plate were fixed with sodium acetate (2%) and stained with safranin (0.1%). Excess stain was rinsed off by through washing with deionized water and plates were kept for drying. Adherent *staphylococcal* cells usually formed biofilm on all side wells and were uniformly stained with safranin.

Optical densities (OD) of stained adherent bacteria were determined with an ELISA reader at wavelength of 570 nm. These OD values were considered as an index of bacteria adhering to surface and forming biofilm. Experiment was performed in triplicate and repeated three times, the data was then averaged. To compensate for background absorbance, OD readings from sterile medium, fixative and dye were averaged and subtracted from all test values. The mean OD value obtained from media control well was deducted from all the test OD values.

Classification of bacterial adherence was based on OD values. When the mean OD values were <0.120 it was classified non/weak biofilm formation, OD<sub>570</sub>0.120–0.240 was classified moderate biofilm formation,

OD<sub>570</sub>> 0.240 was classified strong biofilm formation. Experiments were performed in triplicate and were repeated 3 times, the data were then averaged.

### Statistical Analysis

Statistical analysis was done by considering the percentages and simple ratios.

### Results

Out of 100 samples, 66 (66%) samples were from male patients and 34 (34%) were from female patients. The male to female ratio in the present study was 6.6:3.4. Age ranged from 3 months to 91 years, with a median age of 35 years, and majority of the patients were in the age group of 21-30 years, consisting 22 % of the samples. Of the 100 clinical strains of *Staphylococci* : 63 (63%), 23 (23%), 5 (5%), 4 (4%) and 4 (4%) were isolated from the following specimens: urine, pus, blood, sputum, and miscellaneous. Miscellaneous consisted of corneal scrapping, throat swab and vaginal secretion.

Of the 100 *Staphylococcus* samples collected, 57(57%) were *Staphylococcus aureus*, 21 of which were Methicilin Resistant *Staphylococcus aureus* (MRSA). 43 (43%) were Coagulase Negative *Staphylococcus* (CONS).

**Table 1: Biofilm detection by Tube Method**

Observation	Inference	<i>S.aureus</i>		CONS	
		Number	Percentage	Number	Percentage
3+	Strong	07	12.28 %	16	37.21 %
2+	Moderate	29	50.88 %	22	51.16 %
0/1	Negative	21	36.84 %	05	11.63 %
Total		57	100 %	43	100 %

A total of the 57 isolates tested for biofilm by tube method, 07 (12.28%) were strongly positive. maximum number of isolates 29 (50.88%) were showing moderately positive and 21 (36.84%) did not show any biofilm formation. Out of the 43 CONS isolates tested for biofilm by tube method, 16 (37.21%) were strongly positive. However maximum number of isolates 22 (51.16%) were moderately positive and 05 (11.63%) did not show any biofilm formation.

**Table 2: Comparison of results from TM and TCP tests on *S.aureus* using TCP as the gold standard**

<i>S.aureus</i>	Tissue Culture Plate Method			
	Positive	Negative	Total	%
Tube Method Positive	36	00	36	63.16
Negative	01	20	21	36.84
Total	37	20	57	100

Sensitivity – 97.30%

Specificity – 100%

Positive Predictive Value (PPV) – 100%

Negative Predictive Value (NPV) – 95.24%

Kappa – 0.96

When the results of Tube method for biofilm formation was compared with TCP, it was found that the sepcificity and sensitivity was 100% and 97.30%.

**Table 3: Comparison of results from TM and TCP tests on CONS, using TCP as gold standard**

CONS	Tissue Culture Plate			
	Positive	Negative	Total	%
Positive	35	03	38	88.37
Negative	00	05	05	11.63
Total	35	08	43	100

Sensitivity – 100%

Specificity – 62.5%

Positive Predictive Value (PPV) – 92.1%

Negative Predictive Value (NPV) – 100%

Kappa – 0.74

When the results of Tube method for biofilm formation was compared with TCP, it was found that the sepcificity and sensitivity was 62.5% and 100%.

### Discussion

Understanding of biofilm provides a clearer picture of the limiation of conventional therapies for treating biofilm associated infections.<sup>(5)</sup> The susceptibility of biofilms to antimicrobial agents cannot be determined by means of standard microdilution testing. Susceptibility must be determined against biofilm associated organisms preferable under circumstances that stimualte biofilm formation. In all isolates of MRSA and in nosocomial *Staphylococcal* infections patients screening for biofilm production should be done routinely by TCP method as this is an affordable method with no subjective errors and requires less experience.<sup>(6)</sup> Studies by these authors laid the importance of biofilm detection in routine practice and since then search for a reliable and reproducible method is going on. Totally there are 16 different methods and 17 different variations of these methods have been proposed for the detection of biofilm, but these methods are subject to severe analytical limitations.<sup>(1)</sup> TM and Congo Red Agar (CRA) are to be used as a general screening method for detection of biofilm producing bacteria in laboratories as they are more qualitative and reliable method to detect biofilm.<sup>(7)</sup> In the study done by oliveria et al 100 CONS isolates were, in which 82% tested positive by tube test, same as PCR is the gold standard. In the same study 81% isolates tested positive by TCP. Knobloch JK et al evaluated TCP assay and tube test using two basic media trypticase soy broth (TSB) and brain heart infusion (BHI) broth with different sugar supplements for detection of biofilm formation in 128 ica-positive *S. aureus* isolates, in this study 57.1% of *S. aureus* displayed a biofilm-positive phenotype under optimized conditions in the TCP test and the Tube test correlated well with the TCP test for strongly biofilm-producing<sup>(8)</sup> In this study Comparision between TM and TCP for *S.aureus* showed sensitivity of 97.30% and specificity of 100%. Similar comparision in CONS showed a high sensitivity of 100%, but a low specificity of 62.5%. Positive Predictive values will actually tell us what is the chance that a positive test truly has the condition tested,

biofilm formation in this study. Sensitivity and specificity are characteristics of the test, with high sensitivity and specificity TM is a reliable test. Where as predictive values are influenced by the prevalence, since biofilm-associated infections are most frequently caused by *Staphylococci* the predictive values of present study may be different in organisms which are not good biofilm producers. Tube method has the advantage of not requiring spectrophotometer to read results, in resource limited laboratories which are devoid of spectrophotometer, Tube method can still be used as a reliable method for biofilm detection. Results of Tube method suffer the limitation of subjective interpretation.

### Conclusion

Nosocomial strains of MRSA should be routinely screened for biofilm formation and treated accordingly. As biofilm formation has an important role in pathogenicity of infections, its detection should be mandatory in a laboratory set up. Since Tube method is more qualitative and reliable method to detect biofilm producing organism, it has to be used as a general screening method for detection of biofilm producing bacteria in laboratories.

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### References

1. Mathur T, Singhal S, Khan S, Upadhyay DJ, Fatma T, Rattan A. Detection of biofilm formation among the clinical isolates of *Staphylococci*: an evaluation of three different screening methods. Indian J Med Microbiol. 2006 Jan;24(1):25-9.
2. Robert D. W, Kamila O, Malgorzata K, Danuta I, Arkadiusz D, Tomaz M, Michal K, Maria M, Tomasz J.W. Biofilm formation and Antimicrobial Susceptibility of *Staphylococcus epidermidis* Strains from a hospital Environment. Int. J. Environ. Res. Public Health. 2014,11,4619-4633.
3. Oliveira A, Lourdes M, Cunha RS. Comparison of methods for detection of biofilm production in coagulase-negative *Staphylococci*. BMC research notes. 2010.3:260.
4. Freeman DJ, Falkner FR, Keane CT. New method for detecting slime production by coagulase-negative *Staphylococci*. J Clin Pathol. 1989;42:872–874. doi:10.1136/jcp.42.8.872.
5. Srinivasa Rao R, Uma Karthika R, Singh KP, Shashikala P, Kanungo R, Jayachandran S, Prashanth K. Correlation between biofilm production and multiple drug resistance in Imipenem resistant clinical isolates of *Acinetobacter baumannii*. Indian Journal of Medical Microbiology. 2008;26(4):333-7.
6. Christensen GD, Bisno AL, Simpsom WA, Beachey EH. Adherence of slime producing strains of *Staphylococcus epidermidis* to smooth surfaces. Infect Immun. 1982;37:318–326.
7. Rewatkar AR, Wadher BJ. *Staphylococcus aureus* and *Pseudomonas aeruginosa*-biofilm formation methods.

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2013;Nov 8(5):36-40.

8. Knobloch JK, Horstkotte MA, Rohde H, Mack D. Evaluation of different detection methods of biofilm formation in *Staphylococcus aureus*. Med Microbiol Immunol. 2002 Oct;191(2):101-6. Epub 2002 Jun 29.