

Original Research Article

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Detection of Biofilm Formation in Multi-Drug Resistant *Staphylococcus aureus* from Rural Tertiary Care Hospital in North India

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ABSTRACT

One of the hallmark characteristic of *Staphylococcus aureus* infection is its inherent ability to form biofilm on abiotic and abiotic surfaces. Biofilm producing Organisms are notoriously hard to treat owing to their high resistance against commonly used antibiotics. This study aimed at Detection of biofilm formation by Multi-drug resistant *Staphylococcus aureus* strain resistant to methicillin and Ciprofloxacin from clinical samples. The objectives of the study were: To detect biofilm formation by multi-drug resistant *Staphylococcus aureus* isolated from clinical specimens by three different methods: Tissue Culture Plate (TCP), Tube Adherence Method (TAM) and Modified Congo Red Agar (MCRA) method. The main objective is to determine the sensitivity, specificity, positive predictive value and negative predictive value of MCRA and TAM against TCP method. A total of 50 *Staphylococcus aureus* strains resistance to methicillin and Ciprofloxacin from all clinical isolates from April 2015 to March 2016 was subjected to biofilm detection methods. Out of 50 *S. aureus* resistant to methicillin and ciprofloxacin, 26(52%) were found to be biofilm producers. Rate of Detection of biofilm was highest by Modified Congo red agar (42%) and Tube Adherence method (38%) followed by Tissue Culture plate method (32%). Rate of detection by three different methods in combination was 42.3% and decreased as per number of tests. In the present study the rate of biofilm producers detected by all three methods was 26(52%). Among which, Modified Congo Red Agar Method was best 21(42%) followed by Tube adherence method 19(38%) and Tissue Culture Plate Method 16 (32%). Rate of detection by combination of all three methods was 52%. Hence, this study endorses for frequent detection of Biofilm production by *S. aureus* especially by multi-drug resistant organisms.

Keywords

Biofilm,
Multi-drug resistant
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Introduction

Prevalence of *Staphylococcus aureus* infection is the one that worries many countries, especially when it becomes a human life threatening disease, currently this pathogen is endemic in many countries worldwide.¹ the hallmark characteristic of *S. aureus* is its inherent ability to form biofilm on biotic and abiotic surface consequently reducing the antibiotic diffusion rates leading

to antibiotic resistance and making them notoriously hard to treat.² As the entrance of certain types of immune barricades like macrophages, shows incomplete penetration into the biofilm medium organisms growing in it easily continue to exist.³ Therefore, it is essential to detect biofilm as early as possible as it may help in appropriate treatment. Various approaches to detect biofilm

comprise assessing visually by electron microscopy and genotypic methods such as Polymerase chain reaction which is costly and beyond the reach of poor settings laboratories. The phenotypic techniques such as Tissue culture plate (TCP) method which is quantitative test, which is considered as a gold standard method, Tube method (TM), Congo red agar method, Modified Congo Red Agar method (MCRA) can be used in routine laboratories test.

Materials and Methods

A cross sectional study was conducted on 294 clinical specimens in the Department of Microbiology M. M. Institute of Medical Sciences and research Mullana, AMBALA from April 2015 to March 2016. Multi-drug resistant (resistant to methicillin and ciprofloxacin) *S. aureus* strains were selected and biofilm formation was detected by:

Tissue Culture Plate Method (TCPM)

Test strains were inoculated in brain heart infusion broth (BHI) with 2% sucrose, incubated for 24hrs at 37⁰C. On the next day 200µl inoculum from BHI broth was filled in microtiter well and again incubated for 24 hrs at 37⁰ C. The microtiter plate was washed four times with 0.2ml of phosphate buffer saline (PBS pH 7.2) and fixed with sodium acetate (2%). It was stained with crystal violet (0.1%w/v) for one minute. Then rinsed off with deionized water and dried.

The biofilm if present on all side wells were uniformly stained with crystal violet. Optical Density (OD) of stained adherent bacteria was determined with a micro ELISA auto analyser at wavelength of 570 nm (OD 570nm). These OD values was considered as index of bacteria adhering to surface and forming biofilms. The experiment was repeated three times and the data was then averaged to

calculate the standard deviation. The biofilm formation, due to adherence of bacteria was classified based on OD values as follows:

Tube Adherence Method (TAM)

Test strains were inoculated in brain heart infusion broth (BHI) with 2% sucrose and incubated for 24hrs at 37⁰C in a test tube. The tube was washed for four times with 0.2ml of phosphate buffer saline (PBS pH 7.2) and stained with crystal violet (0.1%w/v) for one minute. The excess stain was removed by washing it with distilled water for three times. Dry tubes were kept in inverted position, the biofilm was seen at bottom of the tube and along the side of walls of test tube.

Modified Congo Red Agar method (MCRA)

Test strains were inoculated on Modified Congo red agar, Incubated for 24-48 hours at 37⁰C. Black colored colonies with dry crystalline consistency were interpreted as positive biofilm producing strains whereas red colored colonies as negative.

Results and Discussion

A total of 294 samples including blood, pus, urine, wound, sputum, ear discharge HVS and Foley tip were taken in which the rate of *Staphylococcus aureus* isolates was 25.85% out of which 65.7% showed multi-drug resistance. 52% strains were biofilm producers by all the three phenotypic methods (Table 1).

The detection of biofilm production was highest by Modified Congo red agar (42%) followed by Tube Adherence method (38%) and Tissue Culture plate method (32%) (Table 2 and Figs. 1–3). Interestingly, the biofilm formation detected was highest by combination of all three methods 42.3% while

MCRA+TAM detected 15.4%, 11.5% by MCRA+TCP and 7.6% by TAM+TCP (Table 3). Multidrug resistant *S. aureus* is still one of the five most common causes of nosocomial infections conferring it almost impossible to be eradicated if associated with biofilm production. The prevalence of biofilm formation varies from hospital to hospital ⁴.

On the same grounds, In the present study, rate of biofilm producing Multi-drug resistant *Staphylococcus aureus* came out to be 52%.(Table 1) which was in well accordance with the studies done by Jayashri Apurva *et al.*, ⁵, Sibabrata *et al.*, ⁶ and Richa Gupta *et al.*, ⁷ 52.63%, 53.2% and 54.5% respectively.

Fig.1 Modified Congo Agar method for detection of biofilm

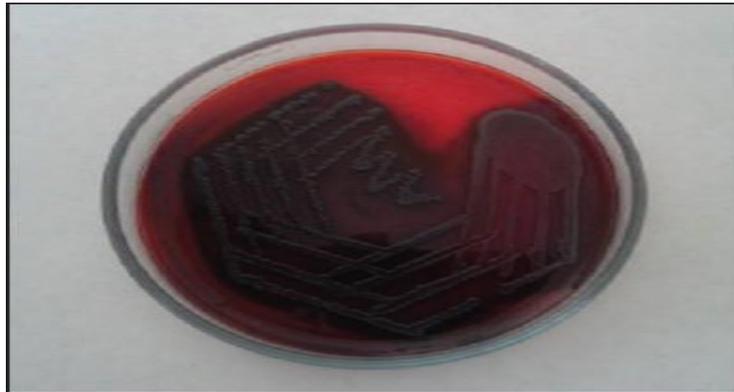


Fig.2 Tube Adherence method for detection of biofilm



Fig.3 Tissue Culture Plate method for detection of biofilm



Table.1 Rate of biofilm producing multidrug resistance *Staphylococcus aureus*

Multi drug resistance <i>Staphylococcus aureus</i>	Biofilm producers (by all three methods)
50	26(52%)

Table.2 Rate of Detection of biofilm production by individual phenotypic methods

Methods	Number of biofilm producers	Percentage of biofilm producers
Tissue culture plate method	16	32%
Modified Congo red agar method	21	42%
Tube adherence method	19	38%

Table.3 Rate of biofilm production by three methods in combination

Total Number of Positive Strains By N=26	Total number of isolates	Total percentage of isolates
MCRA + TAM + TCP	11	42.3%
MCRA + TAM	4	15.4%
MCRA + TCP	3	11.5%
TAM + TCP	2	7.6%

Tissue Culture Plate Method (TCPM)

Mean OD Value	Adherence	Biofilm formation
<0.120	Non	Non/Weak
0.120-0.240	Moderate	Moderate
0.240	Strong	High

The rate of detection of multidrug resistant *Staphylococcus aureus* in current study was maximum by Modified Congo red agar Method, 42%, followed by tube adherence method 38% and lowest positivity 32%, was rendered by TCP method (Table 2). The sensitivity, specificity and overall accuracy of MCRA was 82.35%, 75.75%, 78% and of Tube method 82.35%, 84.84%, 84%.

It was further supported by the studies done by Rewatkar *et al.*,⁸ and Wenbo Hou *et al.*,⁹ by Congo Red Agar Method, 45% and 55.56% respectively. The current study was totally contradictory to the study of Neetu Peedikayil John *et al.*,¹⁰ in which Congo Red Agar gives the lowest positivity, 4.30%. Many researchers have reported that tissue

culture plate method is considered to be the gold standard for the biofilm detection as it provides only true positive results due to hydrophobic interaction between two surfaces, bacterial surface and substratum. Despite this fact, the current study showed the maximum detection of biofilm producers by MCRA since this method is fast, reproducible, and evaluation criteria is based on visual analysis of the color of colony.

Furthermore, in TAM, strong biofilm producers could be easily detected but it was difficult to discriminate between weak and biofilm negative isolates as results may vary by different observer. The best result observed was by combination of all three methods than a single one. In the present

study, the results with combination of all three methods showed 42.3% (Table 3) positivity followed by the rate was decreasing as the less number of tests were evaluated. As far as our knowledge is concerned this is the first study which has shown the results in combination.

To conclude Study was conducted in the department of microbiology on 294 various clinical samples. The rate of biofilm production was 52%. The biofilm detection was maximum by modified Congo red agar method (42%), followed by Tube adherence method (38%) and Tissue culture plate method (32%). Detection of biofilm by combination of all three methods showed 42.3%. Therefore early detection of Biofilm production of multi-drug resistant *Staphylococcus aureus* by these tests should be included in routine to minimized the risk of infection especially among the patients who are admitted in hospital, thereby enabling quick treatment and reducing the mortality and morbidity rate effectively. For the optimum result the analysis should always be done by combination of all three methods.

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