



Original Research Article

Detection of biofilm production among *Staphylococcus aureus* by Congo red method and tube method

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ABSTRACT

Background: Biofilm is a mode of survival for various microbe by which they form aggregates during unfavourable conditions. *Staphylococcus aureus* is a major cause of nosocomial and community acquired infections.

Materials and Methods: A total of 150 *Staphylococcus* isolates were screened for biofilm production by Congored method and Tube method following standard guidelines.

Results: Of the 150 isolates, 85(56.6%) were MSSA and 65(43.4%) were MRSA. On Congored agar, 63 isolates showed black colonies with dry crystalline consistency indicating biofilm production. Out of 63 isolates 62% of isolates were MRSA and 38% of isolates were MSSA. Biofilm by tube method, 84 isolates showed biofilm production. MSSA were 48.2% and MRSA were 81.53%.

Conclusion: MRSA is the significant biofilm producer when compare to MSSA, Congored method is less accurate when compare to tube method as screening test for the detection of biofilm.

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1. Introduction

Staphylococcus aureus is a commensal bacteria on the human skin and mucosa and also a prominent human pathogen that can cause healthcare associated infections and community associated skin and soft tissue infections.¹ *Staphylococcus* has an ability of acquiring drug resistance and biofilm production in indwelling medical devices making them an important pathogen.¹

Staphylococcal biofilm can develop on various structures such as prosthetic joints, prosthetic heart valves, catheters, contact lenses, cardiac pacemakers and cerebrospinal fluid shunts.² 80% of Nosocomial infections are due to biofilm production. *S. aureus* is one of the frequently found organisms in biofilm associated infections.^{3,4}

Biofilm appear to be the wise move for bacteria to survive to any kind of environmental stress. The response of bacteria needs to be fast enough to survive those stresses. Biofilm productions by *Staphylococcus aureus* begins with adhesion

of bacteria to inert /biotic surface with help of adhesion factor microbial surface components recognizing adhesive matrix molecules (MSCRAMMS).^{5,6}

Bacteria forms monolayer and maturation of cell starts when bacteria aggregate and produce slime layer named as Matrix. Matrix contains exopolysaccharides, protein and extracellular DNA.^{6,7} Cell proliferation takes place from monolayer to micro colony and micro colony to biofilm by Quorum sensing system.^{8,9} Quorum sensing system is a cell to cell communication system to coordinate population density dependent changes. Quorum sensing system of *S. aureus* is autoinducing peptides (AIP) and Agr (Accessory gene regular) induced by an extracellular ligand. Dispersion is the final step of biofilm production. It acts as an important step in expansion of biofilm and also causing systemic dissemination.^{8,9}

Factors that enhances biofilm production in *S. aureus* are high level glucose, NaCl (Sodium chloride), NO (Nitric oxide), Mg^{2+} (Magnesium ion) and in human body, the lack of nutrients (e.g- iron, carbon source) or oxygen.^{10–13}

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Host response towards biofilm production: a) *S. aureus* biofilm secretes specific toxins called leukocidin AB (LukAB) and alpha-toxin (Hla). These toxins facilitate biofilm production by inhibiting macrophage phagocytosis and induce cytotoxicity, promoting macrophage dysfunction.¹⁴ b) Myeloid-derived suppressor cells (MDSCs) inhibit T lymphocyte proliferation and prevent macrophage/monocyte pro-inflammatory activity facilitating biofilm persistence.¹⁵ c) Early Th1 and Th17 inflammatory responses are increased and Th2, Treg responses are decreased.¹⁶ Down regulation of Th2 and Treg responses favor the development of *S. aureus* biofilm infection.¹⁷ Staphylococcus which produces biofilm are more prone to cause disease like endocarditis, urinary tract infections, osteomyelitis, skin and soft tissues infections.^{18,19} Present study was carried out with an interest to isolate the *Staphylococcus aureus* from clinical specimens, detect biofilm production and check for the contribution of Methicillin resistances in biofilm production.

2. Aims and Objectives

1. To study biofilm production among *Staphylococcus aureus* isolates.
2. To know the percentage of biofilm production among MRSA.
3. To compare biofilm detection by Tube and Congored method.

3. Materials and Methods

3.1. Study design

Prospective study.

3.2. Study period

6 Months, October 2018-March 2019.

3.3. Sample size

150.

3.4. Methods of data collection

A total of 150 *Staphylococcus aureus* isolates were collected from various clinical samples like urine, pus, sputum, blood and other body fluid received in Microbiology laboratory, Mandya institute of medical science, Mandya.

First, the isolates were identified as *Staphylococcus* on the basis of colony morphology on Nutrient agar, Blood Agar, Gram's stain and biochemical tests. The yellow coloured, moist, round, glistening opaque colonies with beta hemolysis on blood agar, Gram positive cocci exhibiting positive test result with respective controls to catalase, coagulase (Slide and tube), nitrate reduction,

methyl red, voges proskauer, alkaline phosphatase, urease and fermentative to lactose, mannitol, maltose, mannose, sucrose and trehalose were confirmed as *S. aureus*. Obtained isolates of *S. aureus* were screened for Methicillin resistance by inoculating onto mannitol salt agar and performing antibiotic susceptibility testing using Cefoxitin disc by Kirby-bauer disk diffusion method.²⁰

A total of 150 isolates were detected for biofilm production by Congored method and Tube method.

1. Congored method: it is a qualitative assay for detecting of biofilm. Congored medium was prepared using 37g/L of brain heart infusion agar (BHI), 36g of sucrose and 0.8g of congo red.²¹ Loop full of colonies from agar plate were inoculated and incubated at 37°C for 24 hours, colour change in colonies were recorded. Biofilm producing isolates showed Black colonies with dry crystalline consistency and non biofilm producers were pink in colour.²¹
2. Tube method: a loop full of colonies from agar plate were inoculated into Trypticase soy broth supplemented with 1% glucose and incubated for 24 hours at 37°C. Tubes were decanted and washed with distilled water and dried. Dried tubes were stained with 0.1% crystal violet. Excess stain was removed and washed with deionized water, tubes were dried in an inverted position and observed for biofilm formation. Biofilm formation were considered as visible film lined the wall and bottom of test tube. Negative result was taken as ring formation at the liquid interface. Biofilm formation were determined as weak, moderate and strong.²²

4. Results

4.1. Congored method

A total of 150 isolates were tested for biofilm production by congo red method. Out of 150 isolates, 63 isolates showed black colonies with dry crystalline consistency indicating biofilm production. Out of 63 isolates 39 (62%) isolates were MRSA and 24 (38%) isolates were MSSA as shown in (Graph 1).

4.2. Tube method

A total of 150 isolates were tested by tube method, 84 isolates showed biofilm formation. Out of 84 isolates, 9 isolates were strong biofilm producers, 26 isolates were moderate, and 59 were weak biofilm producers.

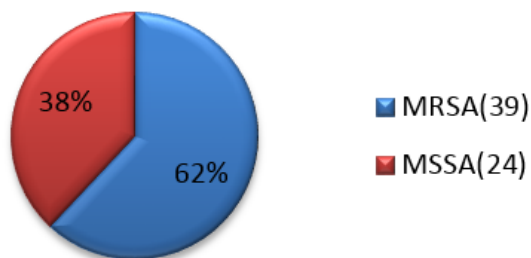
Biofilm producers among MSSA were 48.2% and MRSA were 81.53%. In our study we observed that MRSA isolates were significant biofilm producers when compare to MSSA isolates.

Out of 85 isolates, 51.70% of isolates were non biofilm producer, 27% were weak biofilm producer, 17.60% were

Table 1: Biofilm formation of *S.aureus* by tube method

Total no. of isolates (150)	Biofilm formation tube method			
	Strong (%)	Moderate (%)	Weak (%)	Negative (%)
MSSA (85)	3(3.4%)	15(17.6%)	23(27%)	44(51.7%)
MRSA(65)	6 (9.2%)	11(17.1%)	36 (55.3%)	12(18.4%)

BIOFILM PRODUCTION AMONG STAPHYLOCOCCUS.AUEUS



Graph 1: Biofilm production among *Staphylococcus aureus* by Congored method

moderate producer and 3.40% were strong biofilm producer.

Out of 65 isolates, 18.40% of isolates were non biofilm producer, 55.30% were weak biofilm producer, 17.10% were moderate producer and 9.20% were strong biofilm producer.

5. Discussion

In the present study we included 150 *Staph. aureus* isolates. Out of 150 isolates, 85(56.6%) were MSSA and 65(43.4%) were MRSA. Out of 85 MSSA isolates, 41(48.23%) isolates showed biofilm production by tube method and 24(28.23%) isolates by Congored method. Out of 65 MRSA isolates, 53(81.53%) isolates showed biofilm production by tube method and 39(60%) by Congored method. Our study revealed detection of biofilm by tube method is better than Congored method.

In comparison to our study we found similar type of screening methods used to identify biofilm production. In the study conducted by Malgorzata Piechota et al., out of 130 isolates, 57(43.8%) were MSSA and 73(56.2%) were MRSA. Biofilm producers were about 99.2%. Out of 57 MSSA, 36.8% were strong, 45.6% were moderate and 17.6% were weak biofilm producers. Among 73 MRSA, 39.7% were strong, 47.9% were moderate and 11% were weak biofilm producer.²³

In the study conducted by Afreenish Hassan et al., showed the comparison of biofilm production by tube method and Congored method with respective result.

Screening tube method showed 19% strong, 30% moderate and 51% weak biofilm producers, whereas Congored method showed 3.6% strong, 6.4% moderate and 90% weak.²⁴

In the study conducted by Maria-Guadalupe Avila-Novoa et al observed among 84 isolates of *Staph. aureus*, 90.4% were weak and 7.1% were strong biofilm producer by tube method and 75% were biofilm producers by Congored method.²⁵ Muhammad Sohail et al observed 50% were weak, 27% were moderate and 23% were strong producers.²⁶

6. Conclusion

Biofilms exhibit resistance to antimicrobial agent. Biofilm production among lifesaving devices are untreatable, recurrent and failure of medical devices. *Staphylococcus* is the major pathogen causing biofilm, so study on *Staphylococcus* is important to overcome chronic and recurrent infection. In the present study, based on our observation we found tube method as best screening method in comparison to Congored method.

7. Source of Funding

None.

8. Conflict of Interest

None.

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