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From Attachment to Dispersion: Understanding Protein-Mediated Dynamics in Staphylococcus Aureus Biofilms Via Protein Pathways in Staphylococcus Aureus Biofilm Dispersal: Insights from Lab-Scale Experimental Models

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KEYWORDS

Biofilms, *S aureus,* CAUTI, Implants, antibiotics.

ABSTRACT:

Introduction: By emphasizing the seriousness of medical device associated infections and how Staphylococcus aureus biofilms exacerbate the situation of Catheter associated urinary tract infection (CAUTI) and implant associated osteomyelitis. It highlights the necessity of investigating the features of silicone catheter biofilm formation & implants in order to understand the underlying mechanisms and create practical prevention measures in a lab scale model [1].

Objective: The study aims to investigate in detail the growth and development patterns in *Staphylococcus aureus* biofilms, particularly those found inside silicone catheters and implants. To investigate the specific protein pathways involved in the dispersal of Staphylococcus aureus biofilms using controlled lab-scale experimental models, with the aim of gaining insights into the mechanisms underlying biofilm dispersion and potential targets for intervention strategies

Materials and methods: Biofilm development was observed in silicone urine catheter tubes and stainless steel & titanium orthopedic implants for a particular period. In order to determine if biofilms are developed, it is evaluated using the modified and Congo red methods, scanning electron microscopy (SEM) analysis and antibiotic susceptibility assay to evaluate biofilms against certain antibiotics. Crude protein isolation from the *S aureus* biofilm solution was studied.

Results: The research findings demonstrated the development and existence of *Staphylococcus aureus* biofilms within the walls & surface of silicone urinary catheter & implants. The isolation of the biofilm protein provided evidence of the formation of S aureus biofilm in the lab scale module.

1. Introduction

Two prevalent forms of healthcare-associated infections (HAIs) that, if not properly managed, can have major consequences are implant-associated osteomyelitis and catheter-associated urinary tract infections (CAUTIs). A majority of medical devices implicated in infections associated with implants are orthopedic equipment. Osteomyelitis (OM) refers to a disease caused by microbial pathogen, typically a bacterium that infects bone, causing an inflammatory response that eventually causes bone destruction [2]. Bone sites are accessible to microorganisms as they come in contact with implants, bone becomes susceptible to disease when there is a significant bacterial inoculum or the presence of foreign materials. Some bacteria, including Staphylococcus aureus, attach themselves to the bone by expressing receptors known as adhesins. The certain scenarios in which biofilm are able to form in the implant surgery – [1] High pathogenic bacteria are inoculated initially during surgery [2] Low virulence bacteria are inoculated

during surgery. The equilibrium between the biofilm and host immune system is disrupted as a result of certain local factors [3] Low virulence bacteria are introduced during surgery and they grow because the Introduction 6 human immune system is not competitive [4] hematogenous implant-related infection and bacteraemia [3].

Nosocomial urinary tract infection (UTI), the most frequent illness acquired in hospitals and nursing homes and is typically linked to catheterization [4] . Catheter associated urinary tract infection is one of the common health cares acquired infections. The use of an indwelling urethral catheter is responsible for 70–80% of these infections. According to recent survey, the most prevalent urinary catheter is one of the common indwelling devices, which is present in 17.5% of patients in European hospitals and 23.6% of patients in US hospitals. In the European epidemiology survey, 1.3% of patients accounted for urinary tract infections and the third most common type of illness of all healthcare-acquired infections. In the US epidemiology survey reported that among all health infections urinary tract infections is the fourth most prevalent illness, accounting for 12.9% of all healthcare infections, of these patients, 67.7% had a urinary catheter [5-7]. In any hydrated biologic system, the majority of microorganisms exist primarily as a collaborative group known as a "biofilm".

Staphylococcus aureus can be a deadly, versatile in nature and an opportunist pathogen that might arise from injury or medical intervention. It is a common bacterium that colonizes the epidermis and nostrils of approximately 30% of humans. Once within a host, S. aureus can spread to many different parts of the body, leading to infections of the skin and soft tissues (SSTIs), sepsis, osteomyelitis, peritonitis, pneumonia, and endocarditis [8].

In order to survive, bacteria in the environment create biofilms on surfaces, and the majority of bacteria as well as fungi probably have this ability. An organized group of bacteria called a biofilm is encased in a self-made polymer matrix made of extracellular DNA, proteins, and polysaccharides. Biofilms of bacteria are resistant to antibiotics, chemicals used as disinfectants, phagocytosis, and other elements of the body's innate and adaptive inflammatory defence mechanism [9]. One of the essential biological concepts of the environmental biology is Biofilm formation, which allow microbial cells to adapt to a multicellular lifestyle by immobilizing the cells in an extracellular polymeric matrix [10].

One of the essential biological concepts of the environmental biology is Biofilm formation, which allow microbial cells to adapt to a multicellular lifestyle by immobilizing the cells in an extracellular polymeric matrix [11]. Cell adhesion to solid surfaces, maturation, dispersal, and cell-to-cell adhesion are all intricate steps in the creation of biofilms [12-13]. The formation of biofilms happens in several phases. Bacteria may move in either an active or passive means towards the surface during the first stage. In the second stage, biofilm is developed by reversible attachment of planktonic bacteria to the surface. In this stage, adherence of the cells towards the surface is facilitated by their appendages like pili, fimbriae and flagella. This attachment makes the new beginning of the developmental phase of the microbes. EPS production is one of the important markings of this stage. It shields biofilm against external stresses. The biofilm also exhibits changes including reduced metabolite production, downregulation of genes that produce flagella, and resistance to UV radiation. It is also believed that antibiotic diffusion is impossible at this stage. Subsequently, the biofilm maturation happens which is initiated quorum sensing mechanism. Coordination within the biofilm through a mechanism known as quorum sensing (QS), in which the extracellular environment accumulates signalling chemicals and controls the expression of particular genes. A mature biofilm has, a distinct three-dimensional structure, characteristic water channels and a significant thickness. The microorganisms' separation from the biofilm is its final stage [13-14].

Certain proteins contribute to the production of biofilms via alternative pathways (i.e.- protein pathways). The biofilm-associated protein, or Bap, is a surface protein with 2276 amino acids that is encoded by the S. aureus bap gene. During the production of biofilms, Bap was found to be the primary factor influencing both intercellular and surface adhesion. Through a mechanism independent of extracellular polysaccharides, it facilitates the initial adherence of bacteria to both biological and abiotic surfaces as well as intercellular adhesion. There is a considerable association between the

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ability to create a biofilm on an abiotic surface and the presence of the bap gene in S. aureus strains, as evidenced by their high adhesion and robust biofilm-forming ability [15].

In summary, this article describes the optimization of growth of biofilms in the clinical settings of S. aureus species.

2. Materials & Methods

Bacterial strains: Different strains of Staphylococcus aureus are used for the growth of biofilms in the laboratory model. S aureus was earlier stored in the department as stock culture in the refrigerator. Bacterial strains (named as Subculture1, Subculture 2) from the stock culture was prepared and used, which is held within the Department of Pharmaceutical Biotechnology, JSS College of Pharmacy- Ooty.

Chemicals: Chemicals such as Congo red dye (0.8g – Congo red agar, 0.4g – Modified Congo red agar), Sucrose (36g), Glucose (10g) were used in the different phases of experiments in this research.

Dyes: Reagents such as Crystal violet 0.1% Congo red 0.8gm/l Acridine orange 1% with certain concentrations are used.

Urinary catheters: Medical-grade silicon-based catheter tubes UROBAG were used for this study. Silicon Catheters (some brand) were used for this study.

Orthopedic implants: Two different types of Titanium and steel orthopedic reconstruction distal/ Mandibular plates are used in this project. They have been used as a lab model for biofilm growth.

Isolation and Storage of S aureus: Isolates of S aureus was sub cultured into nutrient broth and nutrient agar incubated aerobically overnight at 37° C and stored.

Growth of biofilms: 10 ml of a Staphylococcus aureus strain in the Nutrient broth media priorly incubated were added to the urinary catheter tubes, and after 48 hours at 37ºC, the formation of biofilm was observed. Stainless steel and titanium Orthopedic implants were submerged in 10 ml of S aureus strain in a cell culture flask to promote the formation of biofilms within the implants and incubated for 48 hours at 37ºC. Implant biofilm development is a result of microbial colonization.

Assay of Biofilm formation:

Congo Red Agar test (CRA): Congo Red Agar is prepared by dissolving suitable ingredients such as Congo red dye, sucrose, Brain heart Infusion agar, water is weighed and transferred into a 200ml flask.

Modified Congo red agar test (MCRA): Modified Congo red agar is prepared by dissolving suitable ingredients such as Congo red dye, glucose, Blood base agar, water are weighed and transferred into a 200ml flask.

Biofilm formation using 96 well plate-based method:

The bacterial suspension of S aureus was added to the microtiter plate and it was then incubated for 48 hours at 37°C. Once the biofilm formation occurs, the plate is washed with phosphate buffer saline followed by the fixation process with sodium acetate. The 96 well plate was dyed with 0.1% crystal violet and left to remain at room temperature for 15 to 20 minutes. The excess stain was then removed using deionized water. Using an ELISA microplate reader set to 570 nm, the plates were dried and then resolubilized using a mixture of methanol and glacial acetic acid. The results were then measured spectrophotometrically [98]. In the 96 well plate, Different concentrations of test organisms (100, 200, and 300 µl) and a control (no organisms) have been introduced to the plate.

Interpretation: Table 1: Interpretation table of Biofilm formation in 96 plate assays

Cut-off value calculation	Mean of OD values results	Biofilm formation abilities
OD > 4×ODc	OD > 0.236	Strong
2×ODc < OD ≤4×ODc	0.118< OD ≤ 0.236	Moderate
ODc< OD ≤ 2×ODc	0.059< OD ≤ 0.118	Weak
OD ≤ 0.059	OD ≤ 0.059	None

Susceptibility assay of antibiotics: The test organism is swabbed on Mueller Hinton agar uniformly at 90° (lawn culture). The disc impregnated with antibacterial agents is placed aseptically with forceps on the medium, and the

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plates were then incubated for 24 hours at 37°C. When an organism is susceptible to a specific antibiotic, a distinct zone known as an inhibitory zone form around it; when the organism is resistant, no zones form around the disc.

Scanning Electron Microscopic (SEM) analysis: To evaluate the results of interaction between nanomaterials like silicon based urinary catheter and Steel & titanium coated orthopedic implants with the bacterial biofilm cells in high resolution electron microscopy.

Isolation of Biofilm proteins:

Biofilm Harvest: Following the extraction of the biofilm from the orthopedic implants and silicon catheter tube. The biofilm solution was collected. The scrapping of biofilm from the surface of clinical devices are also collected.

Cell Debris Removal: Centrifuge the lysate to get rid of any remaining particles of cell debris and gather the supernatant, which contains the extracellular cellular components such as proteins, nucleic acids, and other compounds.

Ammonium sulphate precipitation: Preparation of 70% saturated ammonium sulphate solution. A Fresh desiccated 50 g ammonium sulphate was placed in the hot air oven at 120ºC overnight in a beaker. ¬ Then the ammonium sulphate was grinded to a fine powder. In order to make a 70% saturated solution, the powdered ammonium sulphate was dissolved immediately in 100 ml lab pure water using a magnetic stirrer.

Cell suspension: The suspension of Biofilm was poured in the 20 ml of PBS. The entire mixture was kept overnight and washed using a magnetic stirrer.

Determination of biofilm protein concentration:

Bradfords assay: The Bradford assay was used to measure the protein concentration after it had been soluble. A Materials and methods 59 protein-binding dye, like Coomassie Brilliant Blue, was added to the protein solution for this experiment. As a result of the dye's binding to the proteins, the colour changes in direct proportion to the concentration of proteins. Afterwards, a spectrophotometer was used to measure the solution's absorbance spectrophotometrically at a predetermined wavelength, usually about 595 nm. The protein content in the biofilm sample may be precisely ascertained by comparing the absorbance of the protein solution to a standard curve created from known concentrations of a protein standard. Distilled water is used to dilute Bradford dye in a 1:4 ratio (1 millilitre of dye to 4 millilitres of water). The dye solution undergoes filtration. For almost two weeks, the diluted reagent was utilized. To identify the unknown sample, a standard curve was required. To create a standard curve, six cuvettes with a volume of one millilitre each are filled with the following concentrations of BSA: 0 (Baseline), 2, 5, 8, 10, and 15 micrograms. 1 ml of Bradford dye was prepared in this case. Following a thorough vortex, the cuvettes were incubated at room temperature for about five minutes. As absorbance rises with time, the length of time samples is incubated prior to spectrometric measurement should also be constant. The OD was determined at 595 nm using a spectrometer. To eliminate the extraction buffer's contribution to the total protein, the OD of the unidentified samples and the extraction buffer (blank) were assessed. A standard curve was produced by plotting the BSA data. The protein concentration of the unidentified sample was established using the curve.

Protein analysis:

SDS- PAGE was used to analyze the isolated proteins. SDS-PAGE requires a series of steps: The resolving and stacking gel are prepared and combined acrylamide, bis-acrylamide, Tris-HCl buffer, SDS, and water; then, the mixture is polymerized in the gel cassette. Subsequently, protein samples are prepared by combining them with loading buffer with SDS and a reducing agent in it, and then it was denatured by heating. After loading the samples into the wells and the comb was removed, the gel was run at a constant voltage in a buffer-filled device. Following electrophoresis, imaging equipment was used to observe the protein bands on the gel by staining it with Coomassie Blue and destained. Examine the bands and determine the size and quantity of the proteins by comparing them to a molecular weight marker.

3. Results

Biofilm formation:

Urinary catheter: The Planktonic organisms intended to form biofilms attach to the surface of catheter and spread

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as a thin slimy layer. After 48 hours of incubation at 37° C, (Figure 1) the biofilm layer is visible through naked eye.

Figure 1: Formation of biofilm as a thin layer on the surface walls of the catheter.

Orthopedic Implants: After incubation at 37° C for 48 hours, the media in the cell culture flask appears turbid. Once withdrawn from the flask, the implant acquires on a slimy texture.

Figure 2: Formation of biofilm on the stainless-steel implant.

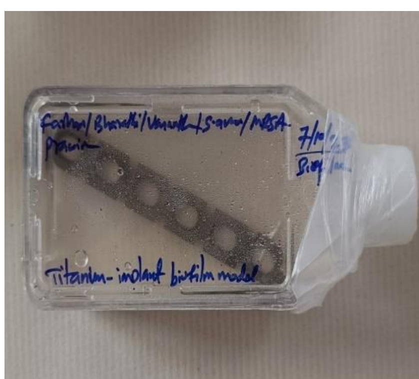
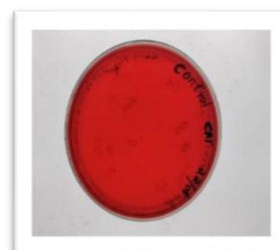


Figure 3: Formation of biofilm on the Titanium implant.

Congo red agar test (CRA):

Using this technique, colonies can be directly analyzed to distinguish between bacteria that create slime (black colonies on Congo red agar) and strains that do not form biofilms create red-coloured colonies. ¬ The presence of black colonies indicates that the bacterium can form biofilms. The presence of non-black colonies indicates that the bacterium cannot form biofilms. This results from Congo red molecules interacting with the exopolysaccharides of biofilm bacteria.

A)



B)

Figure 4: A) Control of CRA. B) Black crystalline colonies of biofilm formed on the CRA medium depicts that the organism is strong biofilm producer

Modified Congo red agar test (CRA)

Modified Congo red agar test is the modification of CRA test. Using this technique, colonies can be directly analysed to distinguish between bacteria that create slime (dry crystalline black colonies on modified Congo red agar) and strains that do not form biofilms create red-coloured colonies on modified Congo red agar. The presence of dry crystalline black colonies indicates that the bacterium can form biofilms. The presence of non-

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black colonies indicates that the bacterium cannot form biofilms.

C)

D)

Figure 5: C) Control of MCRA. D) Dry crystalline black colonies of biofilm formed on the MCRA medium depicts that the organism is strong biofilm producer.

Biofilm formation using 96 well plate-based method.

Plate: 100µl, 200µl, and 300µl of the organism that had been infected in TSB medium were put onto the plates. Following two days of incubation at 37°C, the plates were decanted, cleaned with phosphate buffer saline, and stained with 1% crystal violet for 15 to 20 minutes. Acetic acid was then used to decolorize the plates, and the results were measured spectrophotometrically at 570 nm.

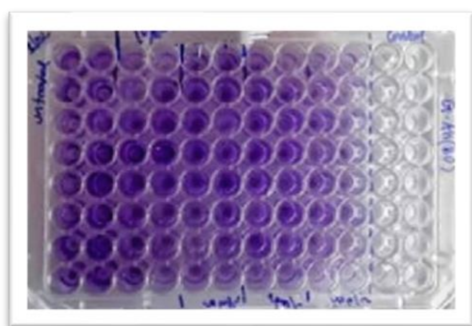

Figure 6: 96 wells Microtiter plates are populated with organisms that have varying antibiotic concentrations. There was significant biofilm buildup in the untreated wells. In contrast, a well that had a high concentration of antibiotics in it displayed a modest biofilm producer.

Table 2: Biofilm formation determined by OD value in biofilm 96 plate assay

Load of organism	OD value at 570nm	Inference
100µL Organism	0.2312	Moderate biofilm formation
200µl Organism	0.2478	Strong biofilm formation
300µL Organism	0.3524	Strong biofilm formation
Control (only TSB)	0.1133	Weak biofilm formation/none

Susceptibility assay of antibiotics

Certain antibiotics like Ciprofloxacin, Kanamycin, Norfloxacin, Ofloxacin, Chloramphenicol, Gentamycin, Erythromycin shows sensitive reaction to S aureus biofilms. Antibiotics like Metronidazole, Cephotoxime, Penicillin-G, Cefixime, Clindamycin shows resistance reaction to S aureus. One factor contributing to the chronicity of infections linked to implanted medical devices is biofilm resistance to antibiotics. The response of antibiotics to S. aureus biofilms is complex and consists of multiple mechanisms that diminish the effectiveness of antibiotics in eliminating germs associated with biofilms.

Figure 8, Antibiotic susceptibility assay of antibiotics against biofilms.

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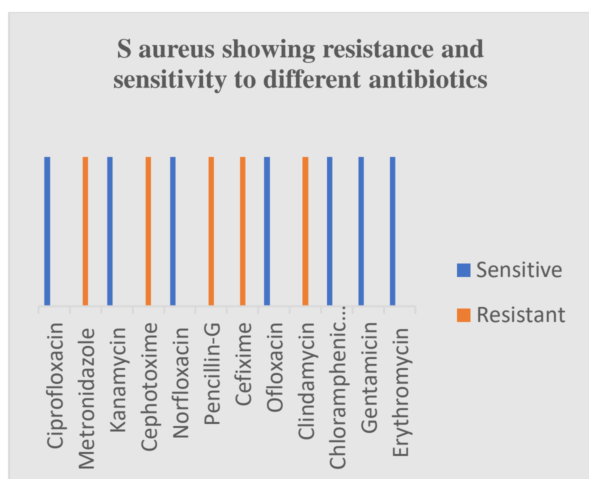

Figure 9, Antibiotic showing resistance & sensitive reactions to biofilms based on assay.

Scanning electron microscopy

The biofilm formation on the clinical lab scale models is observed through scanning electron microscopy (SEM) analysis. The technique of scanning electron microscopy (SEM) is extensively employed for imaging biofilms owing to its high magnification and resolution.

Urinary catheter:

The biofilm formation of S aureus is well identified as it is spread on the wall of the urinary catheter. The layer of the biofilm is spread over the catheter.

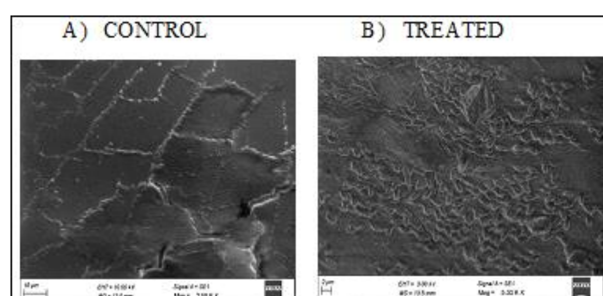

Figure 10: Scanning electron microscopy analysis of the biofilm formation on the walls of the urinary catheter.

A) Control: Catheter inoculated with media shows no formation of biofilms
B) Treated: Catheter inoculated with culture organism shows a formation of layer of complex organisms after incubation of 48 hours at 37° C

Orthopedic implants:

S aureus biofilms are easily identifiable as they grow on the orthopedic implants. Representative SEM images of biofilm formed on stainless steel (A+B) and on Titanium (C+D) implants with bacterial cell to cell attachments.

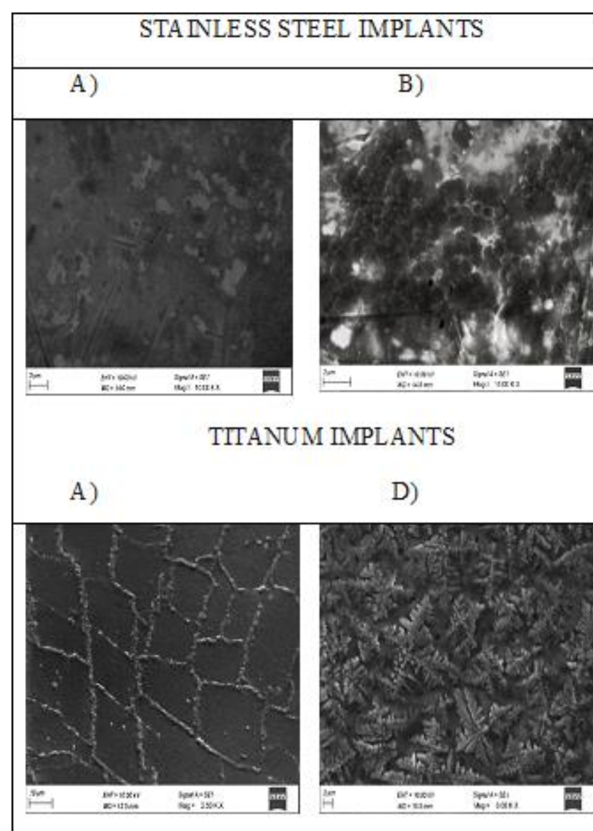

Figure 11: Scanning electron microscopy analysis of the biofilm formation on the walls of the Stainless steel & titanium orthopedic implants.

A) Control of stainless-steel implant placed in cell culture flask inoculated with nutrient broth.
B) Treated stainless steel implant with S aureus with shows biofilm formation.
C) Control of Titanium implant placed in cell culture flask inoculated with nutrient broth.
D) Treated titanium implant which identifies biofilm formation of the walls of implant.

Isolation of Biofilm proteins:

The organism's biofilm protein is extracted to determine whether phage treatment on interaction with protein influences biofilm dispersal. The extraction of proteins

from the biofilm solution (which is obtained from the inoculation solution and the biofilm scrape of the catheter) has been carried out according to established techniques. The picture below depicts the protein isolation that follows centrifugation at the very end of the process. The supernatant from the centrifuge tube was collected to isolate the extracellular proteins of biofilm.

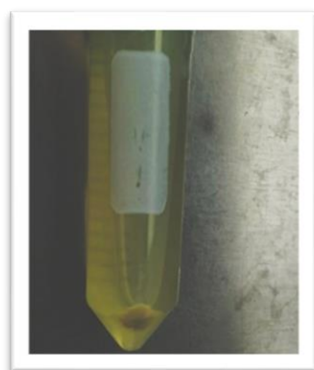

Figure 12: Biofilm protein of organism is isolated.

Ammonium sulphate precipitation:

Protein separation and purification are frequently accomplished using ammonium sulphate precipitation.It involves adding ammonium sulphate into a supernatant solution, which, because of its decreased solubility, causes the proteins to precipitate out.

$$G = 43.6^* (S_2 – S_1) / 100 – 0.3\ S_2.$$

G = Grams of ammonium sulphate required for 70% saturation.
S_1 = Starting Concentration.
S_2 = Final Concentration.

Amount of ammonium sulphate for 70% saturation:
= 533* 70 / 100 – 21
= 472.27 g for 1000 ml.
= 23.6 g for 50 ml

Protein precipitation of cell suspension:

The 70% saturated ammonium sulphate solution was mixed with the 45 ml cell suspension placing in the magnetic stirrer. This solution was allowed to precipitate for around 30 minutes on continuous stirring. Centrifugation was done at 4000 rpm for 30 mins in order to get the extracellular protein. The precipitated supernatant was carefully removed.

Cell Suspension of salted buffer:

Phosphate buffered saline, or PBS, is frequently used to dissolve proteins because it maintains pH levels and stabilizes proteins. The 20 ml protein suspension was added to the 20 ml PBS tube. Using a magnetic stirrer, the entire mixture was maintained overnight and purified.

Figure 13, Suspension of PBS and precipitated extracellular biofilm protein.

Determination of biofilm protein concentration: The Bradford protein test is a colorimetric technique that quantifies the amount of protein present in a solution by monitoring the change in Coomassie Brilliant Blue G 250 dye's absorbance upon binding to denatured proteins.

Table 3-Standard graph of Bovine serum albumin

Concentration (μg/ ml)	Absorbance
0	0.04
2	0.450
5	0.670
8	0.830
10	1.23

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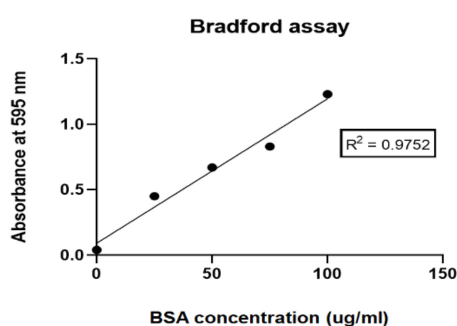
Table 4- Standard graph of Bovine serum albumin

Test sample amount	Absorbance	Concentration (µg/ ml)
1 µl/ ml	0.940	7.83 µg/ mL

Graph 1: Bradford assay of biofilm proteins.



The 1 µg/ ml of test sample was taken and experiment was done. The Concentration of biofilm protein was determined by calibration as sample absorbance with standard graph (by Bradford assay). It is reported that 1 µl/ ml of sample contain µg/ ml of protein.

Protein analysis:

12 % SDS- PAGE was used to analyse the isolated proteins. By using sodium dodecyl sulphate (SDS), a powerful cationic detergent, to complicate proteins and separate them on sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), one can calculate the apparent molecular weight (MW) of a protein.

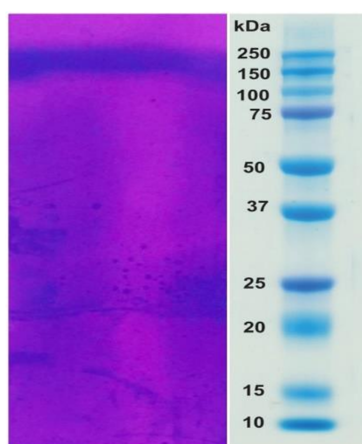


Figure 14, 12% SDS- PAGE gel showing bands

DISCUSSION:

The article Effectively encapsulates the essence of the study while leaving room for further specificity regarding the Bap protein's role in triggering biofilm dispersion. underscores the focus on understanding the molecular pathways involved in biofilm dispersal, suggesting a comprehensive investigation into the underlying mechanisms. By emphasizing the use of lab-scale experimental models, it highlights the meticulous approach taken to dissect these pathways in a controlled environment, ensuring reliable and detailed insights. The Bap protein of S aureus, a particular molecular actor that is recognized to be essential for the dispersal of biofilms. By concentrating on a well-characterized protein with established consequences in the biofilm dynamics of Staphylococcus aureus, this selectivity deepens the investigation. Additionally, it indicates the subject that the study probably explores the mechanisms of Bap-mediated dispersion. Mentioning that the biofilm dispersion is triggered by the Bap protein adds a layer of complexity to the research. It suggests that the study investigates the conditions or signals that activate Bap-mediated dispersal, which could have significant implications for understanding the regulatory mechanisms controlling biofilm formation and dispersal in S. aureus. This information is critical for developing targeted therapeutic strategies aimed at modulating biofilm behavior. The use of lab-scale experimental models implies a controlled environment conducive to detailed analysis of Bap protein pathways and their effects on biofilm dispersal. This allows for precise manipulation and observation of variables, leading to a deeper understanding of the underlying mechanisms. The inclusion of this aspect in the title reinforces the scientific rigor and methodological sophistication of the study.

CONCLUSION:

Research has demonstrated that the ability to produce biofilms serves as essential for bacteria to grow in a variety of harsh conditions. Biofilm production is a unique growth pattern that microorganisms adopt in response to varied environmental challenges. Medical equipment frequently develops microbial adhesion and biofilm formation, which can cause life-threatening diseases or even death. There is uncertainty over the pathogenicity and biofilm generation process of S.

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aureus infections in the urinary tract and bone. The outcome of the biofilm study showed the growth of community of microorganisms that adhere to the surface of clinical lab module.

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CONFLICT OF INTEREST:

The author has confirmed the absence of any conflicts of interest

References

[1] Wang Y, Cheng LI, Helfer DR, et al (2017) Proc Natl Acad Sci U S A. Mouse model of hematogenous implant-related Staphylococcus aureus biofilm infection reveals therapeutic targets

[2] Jason A. Inzana, Edward M. Schwarz, Stephen L. Kates, Hani A. Awad, (2016) Biomaterials approaches to treating implant-associated osteomyelitis, Biomaterials, Volume 81, Pages 58-71, ISSN 0142-9612

[3] Glage S, Paret S, Winkel A, Stiesch M, Bleich A, Krauss JK, Schwabe K. Acta Neurochir (Wien) [2017] A new model for biofilm formation and inflammatory tissue reaction: intraoperative infection of a cranial implant with Staphylococcus aureus in rats

[4] John W. Warren (2001),Catheter-associated urinary tract infections,International Journal of Antimicrobial Agents,Volume 17, Issue 4,Pages 299-303,ISSN 0924-8579

[5] Nicolle LE. (2014) Catheter associated urinary tract infections. Antimicrob Resist Infect Control;3:23.doi: 10.1186/2047-2994-3-23

[6] Zarb P, Coignard B, Griskevicienne J, Muller A et al (2012) The European Centre for Disease Prevention and Control (ECDC) pilot point prevalence survey of healthcare-associated infections and antimicrobial use. Euro Surveill.

[7] Magill SS, Edwards JR, Bamberg W, Beldaus ZG (2014), Multistate point-prevalence survey of health care-associated infections. N Engl J Med.

[8] Høiby, N., Ciofu, O., Johansen, H. et al. (2011) The clinical impact of bacterial biofilms. Int J Oral Sci 3, 55–65

[9] Kotakonda Arunasri, S. Venkata Mohan, (2019) Chapter 2.3 - Biofilms: Microbial Life on the Electrode Surface, Editor(s): S. Venkata Mohan, Sunita Varjani, Ashok Pandey, In Biomass, Bibliography 91 Biofuels and Biochemicals, Microbial Electrochemical Technology, Elsevier.

[10] Kotakonda Arunasri, S. Venkata Mohan, 2019 Chapter 2.3 - Biofilms: Microbial Life on the Electrode Surface, Editor(s): S. Venkata Mohan, Sunita Varjani, Ashok Pandey, In Biomass, Biofuels and Biochemicals, Microbial Electrochemical Technology, Elsevier.

[11] Ahmad Amiri, Hadi Zare-Zardini, Mehdi Shanbedi, Salim Newaz Kazi, Asghar TaheriKafrani, Bee Teng Chew, Ali Zarrabi (2016) Chapter 2 - Microbial toxicity of different functional groups-treated carbon nanotubes, Editor(s): Alexandru Mihai Grumezescu, Surface Chemistry of Nanobiomaterials, William Andrew Publishing

[12] K.K. Jefferson, (2004) What drives bacteria to produce a biofilm? FEMS Microbiol. Lett. 236

[13] Ramya Veerubhotla, Jhansi L. Varanasi, Debabrata Das, (2018) Chapter 12 - Biofilm Formation Within Microbial Fuel Cells, Editor(s): Patit P. Kundu, Kingshuk Dutta, Progress and Recent Trends in Microbial Fuel Cells, Elsevier.

[14] G.O. Toole, H.B. Kaplan, R. Kolter, (2005) Biofilm formation as microbial development, Annu. Rev. Microbial. 54

[15] Peng Q, Tang X, Dong W, Sun N, Yuan W. A (2022) Review of Biofilm Formation of Staphylococcus aureus and Its Regulation Mechanism. Antibiotics (Basel); 12(1):12.