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Detection of biofilm formation among *Pseudomonas aeruginosa* isolated from patients in a tertiary care hospital.

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Abstract:

Introduction: *Pseudomonas aeruginosa* is a gram negative bacterium which causes opportunistic infections. It has major role in healthcare associated infections (HAI) like ventilator associated pneumonia (VAP), burns, surgical site infections. Biofilm formation among the isolates has led to poor prognosis which aggravates the infections and increase in antibiotic resistance causing increase in morbidity and mortality.

Aims and objectives: 1. Isolation and identification of *Pseudomonas aeruginosa* 2. To detect biofilm formation among *Pseudomonas aeruginosa* isolates

Methodology: A prospective cross sectional study was conducted on all the pus samples received at Department of Microbiology, SIMS, Shimoga between January 2018 to June 2018. The samples were processed, identification and isolation of *Pseudomonas aeruginosa*, detection of biofilm formation was done as per Standard microbiological methods.

Results: Of 182 pus samples received 124(85.5%) were positive cultures and 58(31.86%) were culture negative samples. *Staphylococcus aureus* 44(35.48%) was most common organisms isolated followed by *Pseudomonas spp.* 35(28.22%), *Klebsiella species* 28(22.58%), Gram negative non fermenters (GNNF) 14(11.29%), *Coagulase negative Staphylococcus aureus* (CoNS) 03(2.41%). Out of 35 *Pseudomonas aeruginosa* isolates 19(54.28%) were biofilm positive by tube method and 26(74.28%) by Microtitreplate method.

Conclusion: *Pseudomonas aeruginosa* was common organism isolated from pus next to *Staphylococcus aureus*. Most of the *Pseudomonas aeruginosa* isolates were biofilm producers.

Key words: *Pseudomonas aeruginosa*, biofilm, pus

Introduction

Surgical site infections are most common healthcare associated infection. Most important recognisable sign of infection is pus.¹ *Pseudomonas aeruginosa* is gram negative bacteria which is saprophytic and frequently colonise hospital environments contributing to causation of healthcare associated infections(HAI) mainly in intensive care units.^{1,2} It is opportunistic bacteria which has intrinsic resistance to antibiotics because of its metabolic versatility, adaptability to changing environments, and ability to survive in challenging habitats.³ Multiple drug resistance has led to longer duration of hospital stay which causes high morbidity and mortality.⁴ *Pseudomonas aeruginosa* is commonly involved in immunocompromised patients or patients with chronic infections (e.g.cancer, AIDS,cystic fibrosis, bronchiectasis, chronic wounds, urinary tract infections).^{3,4} It has ability to cause infection if it enters the devitalised tissue through disrupted skin and mucous membrane.⁵ Burn wounds are most chronic, non-healing, and difficult to treat due to evolving and changing antimicrobial profiles of the related pathogens.⁶ There are various virulence factors involved like Exotoxin A, Pili, Slime layer and alginate glycoalyx causing biofilm formation, extracellular enzymes, enterotoxin, pigment production like pyorubin, pyomelanin, fluorescein, pyocyanin.^{6,7} Biofilm is important virulence factor involved in pathogenesis. It is considered pathogenic determinant which allows strains to persist a long time and interfere with bacterial eradication.⁷ Biofilm is a group of microorganisms encased in an exopolymer coat which are like intracellular pods that allow bacteria to outlast a strong host immune response to establish dormant reservoir of pathogens.⁸ Re-emergence of bacteria from this reservoir causes recurrent infections. This also causes antibiotic resistance.^{7,8,9}

Material and methods

A cross sectional prospective study was conducted in Department of Microbiology attached to McGann hospital, Shimoga institute of medical sciences, Shimoga. The study was conducted during the period May 2018 to October 2018 from the pus samples of both in-patient and out-patient department

Isolation and Identification of *Pseudomonas aeruginosa*

Under aseptic precautions wound area cleaned with normal saline and pus specimens were collected using sterile swabs or by aspiration of pus in sterile syringes and sample transported to the microbiology laboratory. Two sterile cotton swabs of pus sample collected for each patient. One sterile cotton swab was used to perform gram stain smear another pus swab was

sent for culture. All pus samples were processed on MacConkey agar, blood agar and incubated at 37° c under aerobic conditions for 24 hours. Identification of the bacterial colony followed by gram stain smear and subjecting the culture growth for, biochemical reactions as per standard conventional microbiological methods. Biochemical tests Indole test, urease test, citrate test, Triple sugar iron test, Mannitol motility agar tests were done. Quality control used in the study is *Pseudomonas aeruginosa* ATCC 27853 strain.¹⁰

Detection of Biofilm formation by *Tube method*

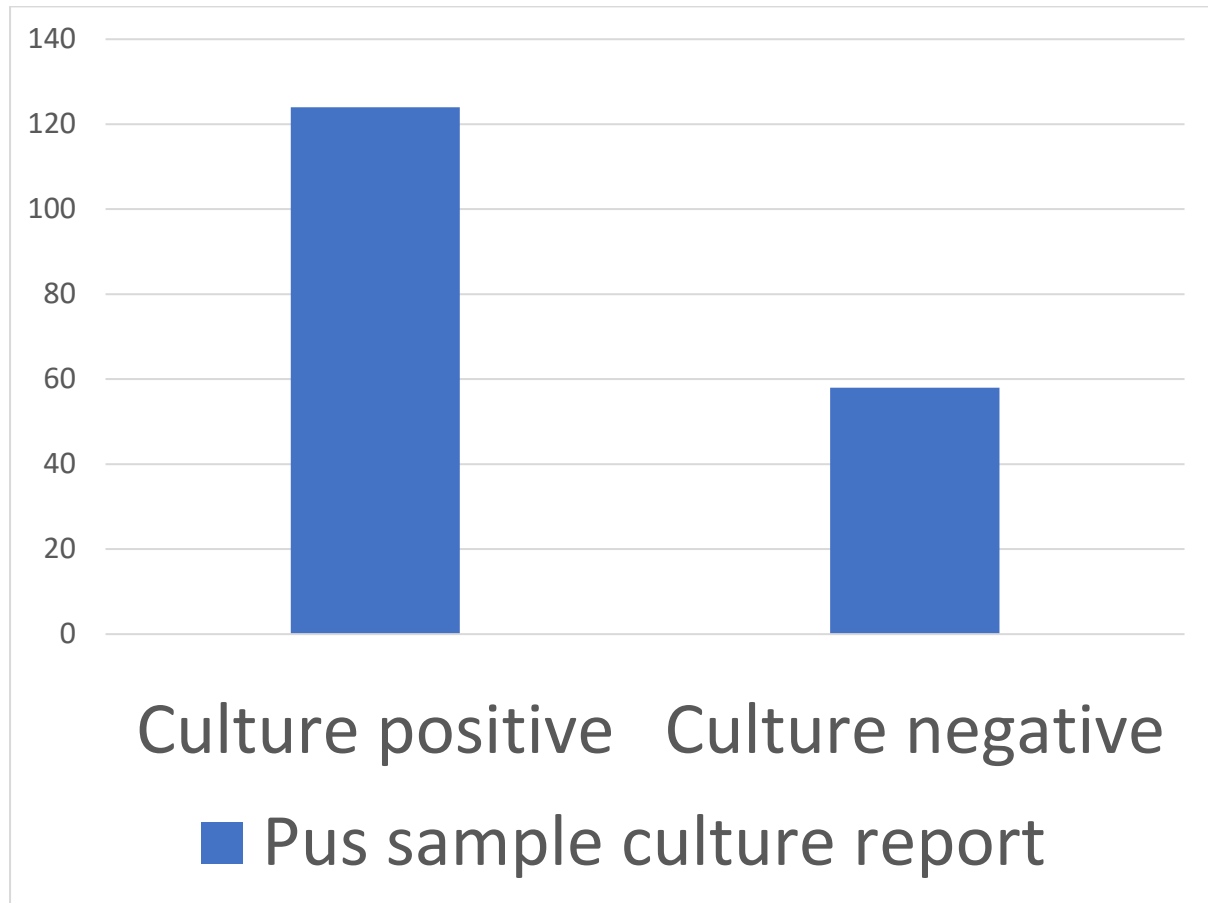
Biofilm production was estimated qualitatively for *Pseudomonas aeruginosa* isolates by tube method as described by Christensen et al. Procedure: Glass test tube containing Brain Heart Infusion broth was inoculated with a loopful of pure culture of *Pseudomonas aeruginosa* isolate and was incubated aerobically at a temperature of 35° C for a period of 2 days After incubation for 48hrs, the supernatant was discarded and the glass tube was stained by 1% safranin solution for 7 minute. The glass tube was then washed with distilled water for 3 times and dried. Result: A positive result was defined as the presence of a layer of stained material adhered to the inner walls of the tube.^{9,10}

Detection of biofilm formation by *Microtitre-plate method(MTP)*

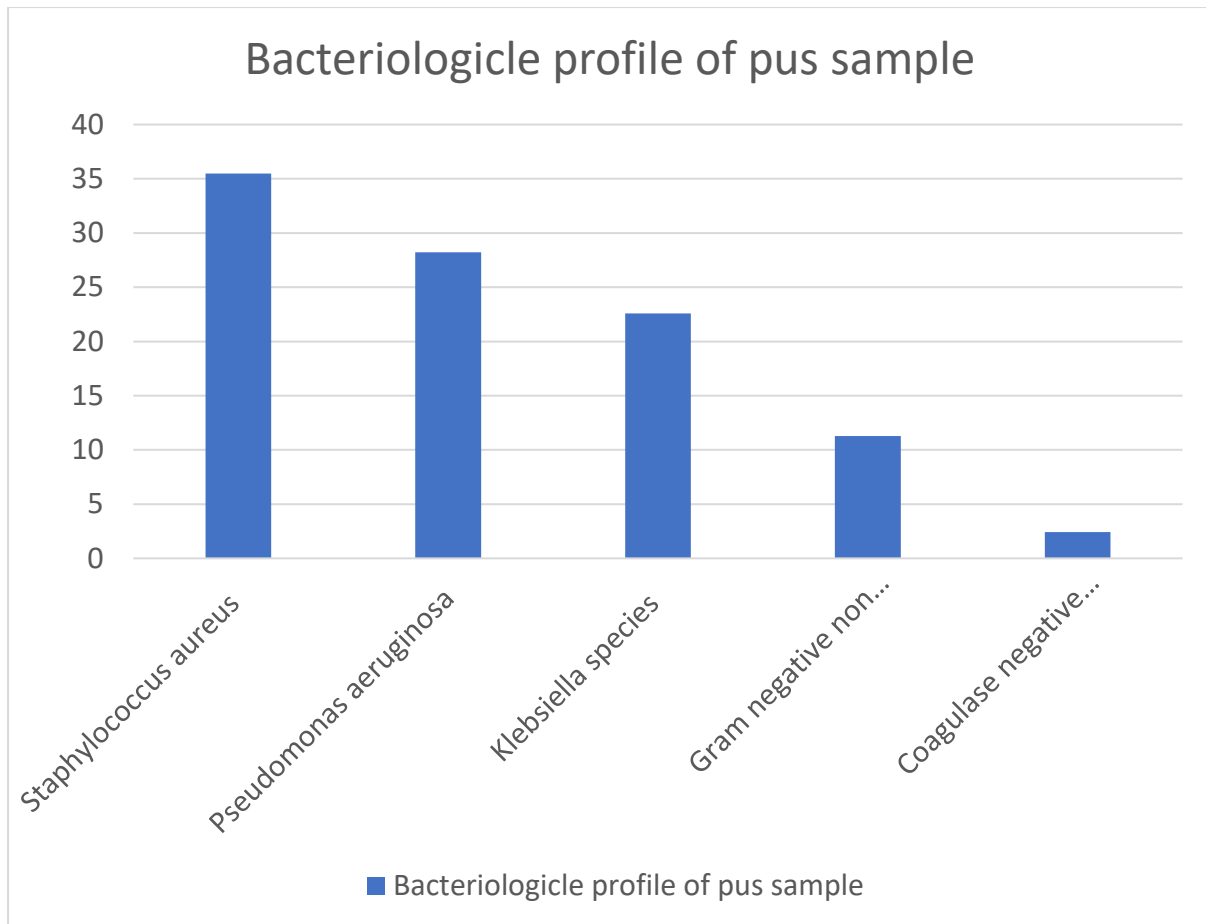
Pseudomonas aeruginosa isolates were grown overnight in Brain heart infusion broth(BHI) at 37°C. Culture suspension of about 200 µl was used to inoculate the wells of a sterile 96-well flat bottomed polystyrene microtitre plate. Negative control well was filled with BHI broth only. Microtitre plate was covered, incubated aerobically at 37°C for 24hrs. After 24hr of incubation, the content of each well was washed three times with 250 µl of sterile physiological saline. Then the plate was dried in inverted position. Plate was then stained for 5 min with 1% safranin. Excess stain was rinsed off by placing the plate under running tap water. The OD of each well was measured at 578 nm using ELISA reader The cut-off optical density (ODc) for the microtitre-plate is defined as three standard deviations above the mean OD of the negative controls. For the purpose of comparative analysis of test results, the adherence capabilities of the test strains were classified into the following four categories: non-adherent (0), weakly(+), moderately (++) , or strongly (+++) adherent, based upon the ODs of bacterial films. All the tests were carried three times and the results were averaged. Strains were classified as follows $OD \leq ODc$ -non-adherent, $ODc < OD \leq 2 \times ODc$ weakly adherent, $2 \times ODc < OD \leq 4 \times ODc$ - moderately adherent, $4 \times ODc < OD$ –strongly adherent.^{9,10}

Results:

A prospective cross sectional study was conducted on all the pus samples received at laboratory of Department of Microbiology, Shimoga institute of medical sciences and hospital, Shimoga between June 2018 to December 2018. Of 182 pus samples received 124(85.5%) were positive cultures and 58(31.86%) were culture negative samples.

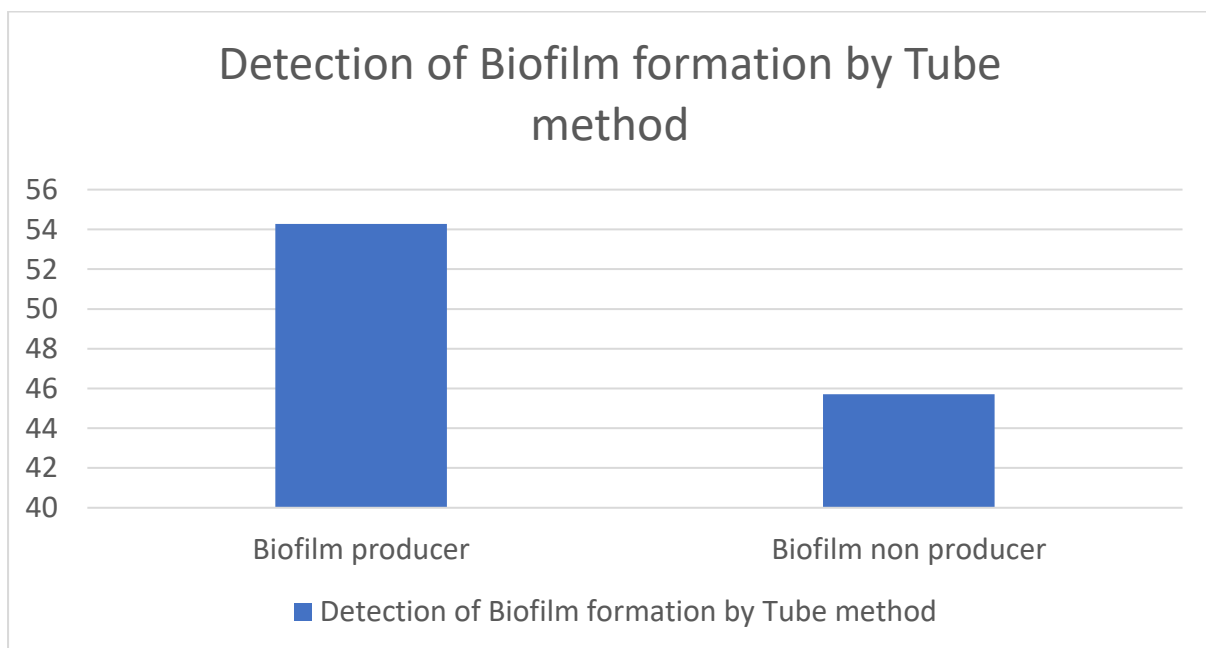


Staphylococcus aureus 44(35.48%) was most common organisms isolated followed by *Pseudomonas spp.* 35(28.22%),*Klebsiella species* 28(22.58%),Gram negative non fermenters(GNNF) 14(11.29%) , *Coagulase negative Staphylococcus aureus* (CoNS) 03(2.41%).



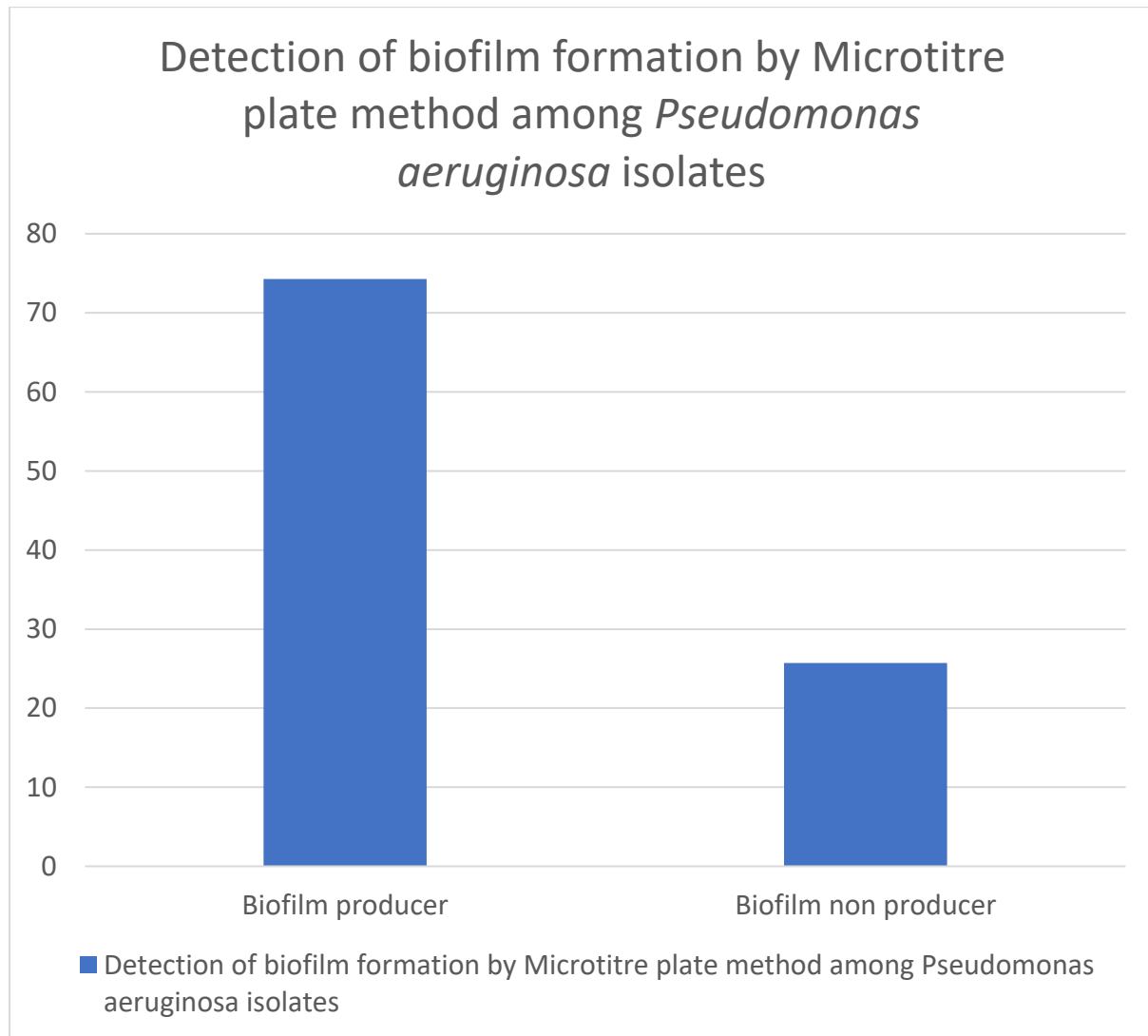
Detection of Biofilm formation by Tube method among *Pseudomonas aeruginosa* isolates

Out of 35 *Pseudomonas aeruginosa* isolates 19(54.28%) were biofilm producer by tube method and 16(45.71%) were biofilm non producer.



Detection of Biofilm formation by Microtitre plate(MTP) method among *Pseudomonas aeruginosa* isolates

Out of 35 *Pseudomonas aeruginosa* isolates 26(74.28%) were biofilm producer and 9(25.17%) were biofilm non producers by Microtitre plate method(MTP)



Discussion

Pseudomonas aeruginosa causes healthcare associated infections leading to high mortality and morbidity. According to our study out of 182 pus samples received 124(85.5%) were positive cultures and 58(31.86%) were culture negative samples and rate of gram negative organisms was 77(62.09%) and rate of gram positive organism was 47(37.90%). Similar study done by Asati S et al total of 190 bacterial isolates were obtained from 133 pus samples rate of isolation of gram-negative bacteria was found to be 76% while gram-positive

microorganisms were only 24%.³ Similar study done by Patil P et al shows out of the 50 samples analysed, 86% were culture positive and 14% were culture negative for bacteria.¹¹

According to our study *Staphylococcus aureus* 44(35.48%) was most common organisms isolated followed by *Pseudomonas spp.* 35(28.22%),*Klebsiella species* 28(22.58%),Gram negative non fermenters(GNNF) 14(11.29%) , *Coagulase negative Staphylococcus aureus* (CoNS) 03(2.41%). Similar study done by Asati et al shows *P. aeruginosa* (32.1%) was the most commonly isolated bacteria followed by *Klebsiella spp.* (16.3%) and *Staphylococcus aureus* (14.7%).³ Similar study done by Ramakrishna et al shows *Pseudomonas aeruginosa* was the predominant isolate (33.3%) followed by *Acinetobacter* (23.3%) and *Staphylococcus aureus* (16.6%). Similar study done by Patil P et al shows *Pseudomonas sp.* (30.2%) and *Acinetobacter sp.* (20.9%). *Proteus mirabilis* (2.3%) and *Staphylococcus aureus* (2.3%) were the least frequently isolated bacteria.¹¹

Pseudomonas aeruginosa is most commonly isolated because of its adaptability to different environment, intrinsic resistance to multiple antibiotics and metabolic versatility.

According to our study out of 35 *Pseudomonas aeruginosa* isolates 19(54.28%) were biofilm producer 16(45.71%) were biofilm non producer by tube method and 26(74.28%) were biofilm producer , 9(25.17) were biofilm non producers by Microtitreplate method(MTP) Similar study done by Baniya et al shows among 85 *P. aeruginosa* isolates, 23 (27.05%) were biofilm producers based on tube adherence test and 13 (15.29%) were biofilm producers as per Congo red agar method.² Similar study done by Asati et al *P. aeruginosa* (73.8%) followed by *S. aureus* (71.4%) by modified tissue culture plate method.³

Similar study done by Ramakrishna et al shows among the Gram-negative bacterial isolates, 44% of *Pseudomonas aeruginosa* isolates were biofilm producers by Microtitre plate method where (43%) were biofilm producers (moderate/strong) and 17 (57%) isolates were either weak or non-biofilm producers. Four (31%) were strong biofilm producers, and 9 (69%) were moderate biofilm producers.⁶

Similar study done by Cho HH et al shows among 82 carbapenem resistant *P. aeruginosa* isolates, 76 (92.7%) were biofilm producers and 6 (7.3%) were biofilm non-producers.¹² Similar study done by Emami et al shows among 50 isolates, 35 (70%) were biofilm producers and 15 (30%) were non-biofilm producers and 70% of the *P. aeruginosa* isolates were biofilm producers.¹³

Biofilm detection was more with microtitre plate method in comparison with tube adherence method in contrast with similar studies done by Baniya et al tube adherence method was better than congo red agar method.

Conclusion:

In our study *Pseudomonas aeruginosa* was second most common organism isolated from pus sample. Most of the *Pseudomonas aeruginosa* isolates were biofilm producers. Biofilm detection was better with Microtitreplate method compared to tube adherence method. Biofilm producing *Pseudomonas aeruginosa* isolates cause healthcare associated infections with high morbidity and mortality and produce multiple drug resistance strains.

References:

1. Freiberg JA. The mythos of laudable pus along with an explanation for its origin. *J Community Hosp Intern Med Perspect.* 2017;13(3):196–8
2. Baniya B, Pant ND, Neupane S, Khatiwada S, Yadav UN, Bhandari N, Khadka R, Bhatta S, Chaudhary R. Biofilm and metallo beta-lactamase production among the strains of *Pseudomonas aeruginosa* and *Acinetobacter* spp. at a Tertiary Care Hospital in Kathmandu, Nepal. *Ann Clin Microbiol Antimicrob.* 2017 Nov 2;16(1):70. doi: 10.1186/s12941-017-0245-6.
3. Asati S, Chaudhary U. Prevalence of biofilm producing aerobic bacterial isolates in burn wound infections at a tertiary care hospital in northern India. *Ann Burns Fire Disasters.* 2017 Mar 31;30(1):39-42.
4. Topley WWC, Wilson SGS. *Topley and Wilson's Microbiology and Microbial Infections: 8 Volume.* Wiley–Blackwell; 2007.
5. Winn W, Janda AS, Koneman W, Procop E, Schreckenberger G, Woods P, et al. *Koneman's color atlas and textbook of diagnostic Microbiology.* Lippincott, New York: Philadelphia; 2006. p. 1443– 535
6. Ramakrishnan M, PutliBai S, Babu M. Study on biofilm formation in burn wound infection in a pediatric hospital in Chennai, India. *Ann Burns Fire Disasters.* 2016;29(4):276–280.
7. Stepanovic S, Vukovic D, Hola V, Bonaventura GD, Djukic S, Cirkovic I, et al. Quantification of biofilm in microtitre plates: Overview for assessment of biofilm production by staphylococci. *APIMS.* 2007;115(8):891–9. doi:10.1111/j.1600-0463.2007.apm_630.x

8. Tsiry Rasamiravaka,¹ Quentin Labtani, ¹ Pierre Duez,² and Mondher El Jaziri¹ The Formation of Biofilms by *Pseudomonas aeruginosa*: A Review of the Natural and Synthetic Compounds Interfering with Control Mechanisms. *BioMed Res Intl* 2014;15
9. Christensen GD, Simpson WA, Bisno AL, Beachey EH. Adherence of slime producing strains of *Staphylococcus epidermidis* to smooth surfaces. *Infect Immun.* 1982;37(1):318–26. doi:10.1128/iai.37.1.318- 326.1982
10. Collee JG, Marr W. Culture of bacteria. In: Collee J, Fraser A, Marmion B, Simmons A, editors. *Mackie and McCartney Practical Medical Microbiology*. 14 edn. New York: Churchill Livingstone; 1996. p. 113–29.
11. Patil P, Joshi S, Bharadwaj R. Aerobic bacterial infections in a burns unit of Sassoon General Hospital, Pune. *Int J. of Healthcare and Biomedical Research*. 2015;3(3):106–112.
12. Cho HH, Kwon KC, Kim S, Park Y, Koo SH. Association between Biofilm Formation and Antimicrobial Resistance in Carbapenem-Resistant *Pseudomonas Aeruginosa*. *Ann Clin Lab Sci*. 2018 May;48(3):363-368.
13. Emami S, Nikokar I, Ghasemi Y, Ebrahimpour M, Sedigh Ebrahim-Saraie H, Araghian A, Faezi S, Farahbakhsh M, Rajabi A. Antibiotic Resistance Pattern and Distribution of *pslA* Gene Among Biofilm Producing *Pseudomonas aeruginosa* Isolated From Waste Water of a Burn Center. *Jundishapur J Microbiol*. 2015 Nov 14;8(11):e23669. doi: 10.5812/jjm.23669.