Content available at: https://www.ipinnovative.com/open-access-journals

IP International Journal of Medical Microbiology and Tropical Diseases

Journal homepage: https://www.ijmmtd.org/

Original Research Article

Detection of biofilm formation and their correlation with Imipenem resistance among clinical isolates of *Pseudomonas aeruginosa*

Vijetha Sajjanar[1]*, D E Premalatha[2]

[1]Dept. of Microbiology, KLE JGMM Medical College (KAHER'S University), Hubballi, Karnataka, India
[2]Dept. of Microbiology, Shimoga Institute of Medical Sciences and Hospital, Shivamogga, Karnataka, India

Abstracts

Background: Pseudomonas aeruginosa causes life threatening infections especially hospital acquired infections or nosocomial infections. Biofilm producing Pseudomonas aeruginosa isolates causes multidrug resistance pattern causing increase morbidity and mortality in a tertiary care hospital.
Aim's and Objectives: 1. Isolation and identification of Pseudomonas aeruginosa; 2. To detect biofilm formation among Pseudomonas aeruginosa isolates; 3. To detect antibiotic resistance pattern among Pseudomonas aeruginosa isolates; 4. To study correlation between biofilm formation and Imipenem resistance among Pseudomonas aeruginosa isolates
Materials and Methods: A prospective cross sectional study was conducted on all the clinical samples received from ICU (intensive care unit) at Department of Microbiology, SIMS, Shimoga between January 2018 to December 2018. All the clinical samples were processed, identification and isolation of Pseudomonas aeruginosa, detection of biofilm formation was done as per Standard microbiological methods. Antibiotic susceptibility test was done following Kirby bauer disk diffusion test (DDT) as per CLSI 2017 guidelines.
Results: Out of 383 clinical samples received 294(76.76%) were positive cultures and 89(23.23%) were culture negative samples. Klebsiella species 106(36.05%) was most common organisms isolated followed by Staphylococcus aureus 70(23.80%), Pseudomonas spp. 66(22.44%), Escherichia coli 21(7.14%), Gram negative non fermenters (GNNF) 19(6.46%), Coagulase negative Staphylococcus aureus (CoNS) 12(4.08%). Out of 66 Pseudomonas aeruginosa isolates 48(72.72%) were biofilm producers and 18(27.27%) were biofilm non producers. Among 48 biofilm positive Pseudomonas aeruginosa isolates 17(54.28%) were biofilm positive by tube method, 31(74.28%) by Microtiterplate method (MTP). Out of 66 Pseudomonas aeruginosa isolates 47(71.21%), were Imipenem sensitive and 19(28.78%) were Imipenem resistant. All the 19(100%) Imipenem resistant Pseudomonas aeruginosa isolates were biofilm producers.
Conclusion: Majority of Pseudomonas aeruginosa isolates were biofilm producers. Correlation between biofilm production and Imipenem resistance contributes to morbidity and mortality and poor prognosis of patients.

Keywords: Biofilm, Pseudomonas aeruginosa, Imipenem resistance, ICU, Antimicrobial resistance

Received: 21-04-2025; **Accepted:** 19-05-2025; **Available Online**: 28-05-2025

1. Introduction

Pseudomonas aeruginosa is pigment producing gram negative bacilli,oxidase positive and major pathogen isolated from hospital acquired infections(nosocomial infections).[1] It is opportunistic pathogen involved in causation of outbreak of post operative wound infections in ICU(intensive care units), urinary tract infections ,burn injury infections, cystic fibrosis.[1,2] Biofilm producing *Pseudomonas aeruginosa* isolates causes multidrug resistance pattern leading to increase morbidity and mortality in a tertiary care hospital.[2]

Pseudomonas aeruginosa form biofilm where its exopolysaccharide component impairs the antimicrobial penetration and also restrict phagocytosis by the host immune system.[3,4] This is one of the major contributing factors for its long term colonization of Pseudomonas spp leading to persistence of infection in intensive care unit(ICU) patients causing chronic infections. Biofilms can also act as diffusion barriers, restricting the entry of antibiotics into bacterial cells causing multidrug resistance.[4,5] Three exopolysaccharides Psl, Pel, and alginate genetically synthesized by Pseudomonas aeruginosa helps in maintenance of the biofilm

*Corresponding author: Vijetha Sajjanar
Email: drvijethasajjanar@gmail.com

http://doi.org/10.18231/j.ijmmtd.2025.033

matrix. Psl has important role in initial steps of biofilm formation, Pel has an important role in surface attachment,cell-to-cell interactions. Alginate important virulence factor in *Pseudomonas aeruginosa*.[5,6]

Antimicrobial agents like Cefepime, Ceftazidime, Carbapenems are effective against Pseudomonas aeruginosa isolates. Carbapenems like Imipenem effective for treating multidrug resistant Pseudomonas aeruginosa. Imipenem is a broad-spectrum beta-lactam antibiotic which belongs to the carbapenem class. It targets the PBPs(penicillin binding proteins), exerts its antibacterial effect primarily through the inhibition of bacterial cell wall synthesis.[7] Distinguishing features of imipenem is its resistance to beta-lactamases produced by resistant bacteria like *Pseudomonas aeruginosa* and Enterobacteriaceae. This makes imipenem a valuable option in treating infections caused by beta-lactamase-producing organisms. Carbapenem resistant strains has decreased the efficacy of Imipenem in treating *Pseudomonas aeruginosa* isolates because of biofilm formation and lack of OprD due to OprD gene mutation. This has impact on patient outcome,less antimicrobial therapy options and poor patient prognosis.[7,8]

2. Materials and Methods

A prospective cross sectional study was conducted on all the clinical samples received from ICU (intensive care unit) patients at Department of Microbiology, SIMS, Shivamogga between January 2018 to December 2018.Ehical comitee approval received from the instituition to conduct the study.

2.1. Detection of Pseudomonas aeruginosa: Phenotypic method

All the clinical samples were processed as per standard microbiological methods. Samples were inoculated on MacConkey agar and blood agar plates. Plates were incubated at 37^0 C aerobically for 24hrs.Culture plate colony identification was done for colony morphology and colony characteristics, Gram stain smear done for Gram negative bacilli .Culture growth was subjected for biochemical reactions which includes Indole test, urease test, citrate test, Triple sugar iron(TSI) test, Mannitol motility agar tests were done. All the tests were performed as per standard conventional microbiological methods.[9,10,11] Quality control for Antimicrobial susceptibility testing(AST) used in the study is *Pseudomonas aeruginosa* ATCC 27853 strain.[12]

2.2. 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: Inoculate a pure culture of *Pseudomonas aeruginosa* into brain heart infusion broth (glass test tube). Aerobic incubation at a temperature of of 35◦ C for 48hrs.Post 48hrs Supernatant fluid discarded and tube was stained by 1% safranine solution for 7 minute. Washed the glass tube 3 times with distilled water and dried. Results read: A positive result was defined as the presence of a layer of stained material adhered to the inner walls of the tube.[13,14]

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

Inoculated a pure culture of *Pseudomonas aeruginosa* into brain heart infusion broth. *Incubated for 24hrs at* at 37^0C.Inoculated the wells of a sterile 96-well flat bottomed polystyrene microtitre plate with culture suspension of about 200 µl. BHI broth added to Negative control well. Microtitre plate was covered. MTP pate was incubated aerobically at 37^0C for 24hrs.Post 24hr of incubation, Washed each well content for three times with 250 µl of sterile physiological saline. Then the plate was dried.1% safranine was used to stain Plate for 5 min. Excess stain was rinsed off by placing the plate under running tap water. ELISA reader was used to measure the optical density(OD) of each well at 578nm.Definition of cut off optical density (ODc) for microtiter plate is three standard deviations above the mean OD of the negative controls.Classification of adherence capabilities of test strains based upon the ODs of bacterial films: non-adherent (0), weakly(+),moderately (++), or strongly (+++) adherent. 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.[13,14]

2.4. Antibiotic susceptibility testing (AST)

The antimicrobial susceptibility tests for *Pseudomonas aeruginosa* isolates from clinical samples was done by Kirby–Bauer's Disk Diffusion method.

Bacterial suspensions were matched with McFarland StandardBacterial suspensions was inoculated into Mueller–Hinton Agar using lawn culture. The AST test report was interpreted as per clinical laboratory standard institution guidelines (CLSI 2017 guidelines).12

Following antibiotics discs were used for *Pseudomonas aeruginosa* (Gram negative organisms).

Ampicillin (Amp)-10µg, Ampicillin sulbactam (20µg), Amoxyclavulanic acid(20/10µg), Amikacin (30µg), Piperacillin-Tazobactam(100/10µg) Ceftazidime clavulanic acid (30/10µg),Ceftazidime (30µg),Cefotaxim (30µg) Ciprofloxacin (5µg), Levofloxacin (5µg), Norfloxacin (5µg)Gentamicin (10µg) , Fosfomycin (200µg), Nitrofurantoin (300µg), Imipenem (10µg),Meropenem (10µg),[9,12] Chi squared test was performed including biofilm characteristics and antimicrobial resistance using SPSS software, 18.0 (SPSS Inc., Chicago, IL, USA). A p value < 0.05 was considered as statistically significant.

3. Results

Out of 383 clinical samples received 294(76.76%) were positive cultures and 89(23.23%) were culture negative samples as shown in **Figure 2**. *Klebsiella species* 106(36.05%) was most common organisms isolated followed by *Staphylococcus aureus* 70(23.80%), *Pseudomonas spp*. 66(22.44%), *Escherichia coli* 21(7.14%), Gram negative non fermenters (GNNF) 19(6.46%), *Coagulase negative Staphylococcus aureus* (*CoNS*) 12(4.08%) as shown in **Figure 1**. Out of 66 *Pseudomonas aeruginosa* isolates 48(72.72%) were biofilm positive and 18(27.27%) were biofilm negative as shown in **Figure 3**. Among 48 biofilm positive *Pseudomonas aeruginosa* isolates 17(54.28%) were biofilm positive by tube method, 31(74.28%) by Microtiterplate method (MTP).17(54.28%) *Pseudomonas aeruginosa* isolates were positive by both tube and microtitre plate method as shown in **Figure 4**.

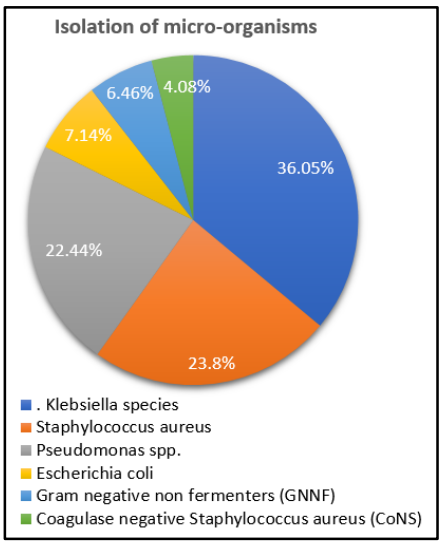

Figure 1: Isolation of microorganism

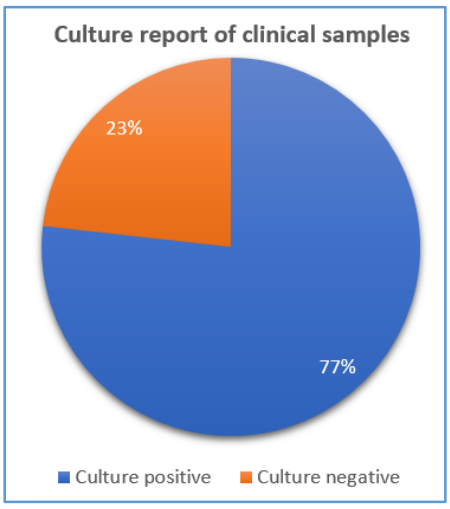

Figure 2: Culture report of clinical samples

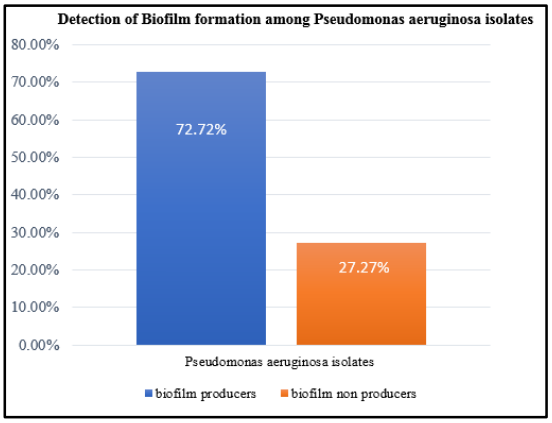

Figure 3: Detection of Biofilm formation among *Pseudomonas aeruginosa* isolates

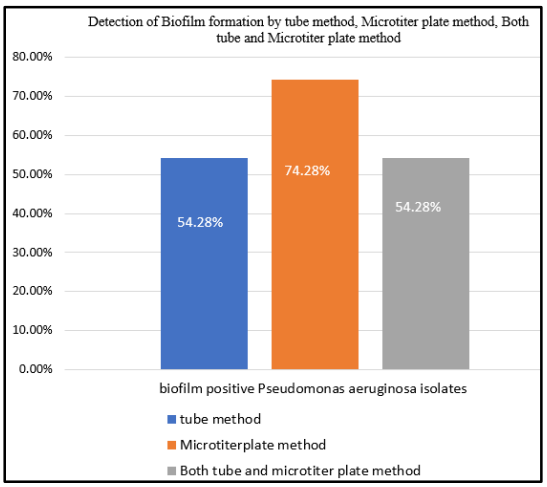

Figure 4: Detection of Biofilm formation among *Pseudomonas aeruginosa* isolates

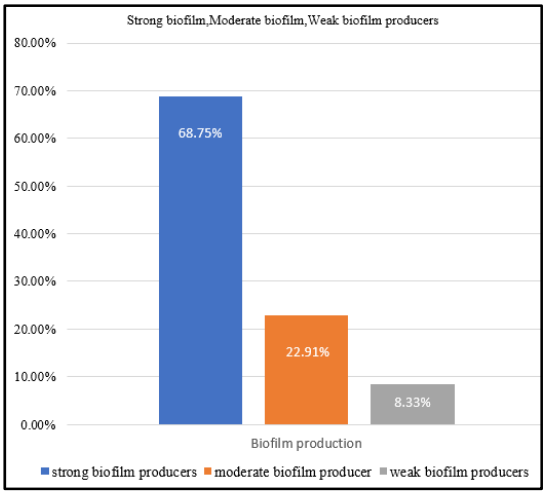

Figure 5: Strong biofilm, Moderate biofilm, Weak biofilm producers

Among 48 biofilm positive *Pseudomonas aeruginosa* isolates 33(68.75%) were strong biofilm producers, 11(22.91%) were moderate biofilm producer, 4(8.33%) were weak biofilm producers as shown in **Figure 5**. Out of 66 *Pseudomonas aeruginosa* isolates 47(71.21%), were Imipenem sensitive and 19(28.78%) were Imipenem

resistant. All the 19((100%) Imipenem resistant Pseudomonas aeruginosa isolates were strong biofilm producers as shown in **Table 1** and **Figure 7.** Chi-square test was performed and P value was <0.05 and is statistically significant

3.1. Antibiotic sensitivity pattern

Imipenem 47(71.21%), Meropenem 47(71.21%), Piperacillin-Tazobactam 56(84.44) Ceftazidime clavulanic acid 34(51.51%), Ceftazidime 31(46.96%), Cefotaxim 31(46.96%) Ciprofloxacin 24(36.36%), Levofloxacin 26(39.39%), Norfloxacin 28(42.24%), Gentamicin 24(36.66%), Amikacin 53(80.30%), Nitrofurantoin 59(89.39%), Fosfomycin 63(95.45%), Ampicillin 37(56.06%), Amoxyclavulanic 56(84.84%), Ampicillin sulbactam 34(51.51%) as shown in **Figure 6.**

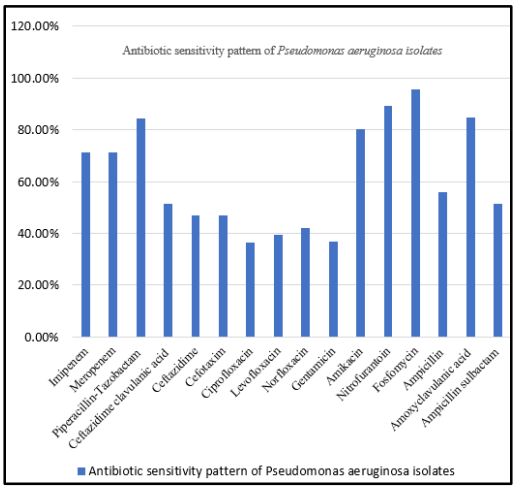

Figure 6: Antibiotic sensitivity pattern of *Pseudomonas aeruginosa* isolates

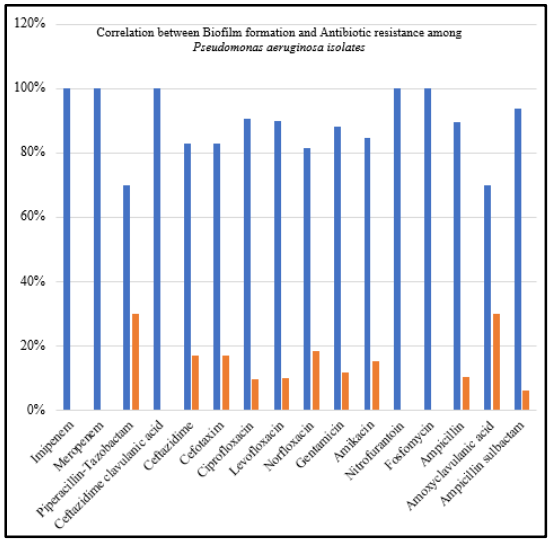

Figure 7: Correlation between Biofilm formation and Antibiotic resistance among *Pseudomonas aeruginosa isolates*

Table 1: Correlation between biofilm formation and antibiotic resistance

Antibiotic resistance isolates	Biofilm producers (%)	Biofilm non producers (%)
Imipenem(19)	19(100%)	0(0)
Meropenem(19)	19(100%)	0(0)
Piperacillin-Tazobactam(10)	07(70%)	03(30%)
Ceftazidime-Clavulanic acid(32)	19(59.37%)	13(40.62%)
Ceftazidime(35)	29(82.85%)	06(17.14%)
Cefotaxim(35)	29(82.85%)	06(17.14%)
Ciprofloxacin(42)	38(90.47%)	04(9.52%)
Levofloxacin(40)	36(90%)	04(10%)
Norfloxacin(38)	31(81.57%)	07(18.42%)
Gentamicin(42)	37(88.09%)	05(11.90%)
Amikacin(13)	11(84.61%)	02(15.38%)
Nitrofurantoin*(07)	07(100%)	0(0)
Fosfomycin(03)	03(100%)	0(0)
Ampicillin(29)	26(89.65%)	03(10.34%)
Amoxyclavulanic acid(10)	07(70%)	03(30%)
Ampicillin sulbactam(32)	30(93.75%)	02(6.25%)

*Nirofurantoin for all urine samples only

4. Discussion

According to our study Out of 383 clinical samples received 294(76.76%) were positive cultures and 89(23.23%) were culture negative samples. Similar study done by Gill et al shows 597 pathogenic bacteria with culture positivity rate of 31.2%.[15] Similar study done by Patil P et al shows out of the 50 samples, 86% were culture positive, 14% were culture negative for bacteria.[17]

In our study *Pseudomonas spp*. 66(22.44%) species isolated and *Klebsiella species* 106(36.05%) was isolated and only *Pseudomonas aeruginosa* isolates were tested for biofilm formation. Research study by Chika EO et al reports that *Pseudomonas aeruginosa* organisms (22) from both clinical and environmental samples. Research by Gill et al *shows Klebsiella (43%), Acinetobacter* (22%), and *P. aeruginosa* (15%) isolation.[15,16]

In comparison with above research study, Ramakrishna et al study reports *Pseudomonas aeruginosa* (33.3%) as the common organism in their samples. Similar study done by Patil P et al shows Pseudomonas sp. (30.2%) and Acinetobacter sp. (20.9%).[17,18]

Pseudomonas aeruginosa is ubiquitous organism in environment and therefore causes opportunistic infections in immunocompromised patients. All the above research study

reports indicate the increase in the prevalence of *P. aeruginosa* isolation from the samples leading to increase rate of nosocomial infection.

In our study among 66 *Pseudomonas aeruginosa* isolates 48(72.72%) were biofilm producers and 18(27.27%) were biofilm non producers. Among 48 biofilm positive *Pseudomonas aeruginosa* isolates 17(54.28%) were biofilm positive by tube method, 31(74.28%) by Microtiterplate method (MTP). 17(54.28%) *Pseudomonas aeruginosa* isolates were positive by both tube method and microtitre plate .Microtiter plate (MTP) method is the gold standard method for detection of biofilm formation which has high sensitivity and specificity in comparison with tube method. Among 66 *Pseudomonas aeruginosa* isolates 47(71.21%) were Imipenem sensitive and 19(28.78%) were Imipenem resistant. All the 19(100%) Imipenem resistant *Pseudomonas aeruginosa* isolates were biofilm producers.

Study done by Saha et al, by Congo red agar method 39 (29.1%) *Pseudomonas aeruginosa* isolates were positive for biofilm formation. The resistant rates of Imipenem was 13.5% and Meropenem was 21.6%. Biofilm producing Imipenem resistant strains were 13(72.2%) and biofilm non-producing imipenem resistant strains were 5(27.8%) and Biofilm producing Meropenem resistant strains were 24 (82.8%)and Biofilm non-producing Meropenem resistant strains 5(17.2%).[7]

Study done by Ramakrishna et al shows reports 44% of Pseudomonas aeruginosa isolates were biofilm producers by Microtitre plate method (43%) were moderate/strong biofilm producers and 17 (57%) isolates were either weak or non-biofilm producers.[18]

Similar study done by Emami et al shows among 70 % were positive for biofilm formation, 15 (30%) were non-biofilm producers, 70% of the *P. aeruginosa* isolates were biofilm producers.[6]

According to study by Ghorbani et al by microtiter plate assay, 26 isolates (72.2%) positive for biofilm formation.2 isolates (5.6%) were strong biofilm producers and high resistance was against gentamicin, 86%, Resistance rates 50% for imipenem, 44.4% for meropenem.[20]

According to Sayad et al biofilm production was 70% for clinical samples. gentamicin (74%) and meropenem (70%) shows high resistance.[8]

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. In this study, 92.7%of the carbapenem-resistant P. aeruginosa isolates studied formed biofilms and showed high levels of antimicrobial resistanc.[19]

Study done by Chika et al P. aeruginosa had highest resistance rates to the cephalosporins (ceftazidime, cefuroxime, cefotaxime, cefepime) at a rate of 100%. The least amount of resistance was observed in amikacin (13.6%) and no resistance was seen against imipenem.[16]

Biofilm detection had high sensitivity and specificity with microtitre plate method in comparison with tube adherence method.There is increase in carbapenem resistant strains like imipenem resistance and exixtence of correlation between biofilm production and Imipenem resistance.

5. Conclusion

Imipenem a valuable option in treating infections caused by beta-lactamase-producing organisms like Pseudomonas aeruginosa. Biofilm producing Pseudomonas aeruginosa isolates which are Imipenem resistant cause healthcare associated infections with high morbidity and mortality and produce multiple drug resistance strains. Misuse of antibiotics causing multidrug resistance is restricted by implementation of antibiotic policies at tertiary care hospitals and also formation of HICC (hospital infection control comitee) to prevent nosocomial infection

6. Conflict of Interest

None.

7. Source of Funding

None.

References

1. Tsiry R, Quentin L, Pierre D, Mondher EJ. The Formation of Biofilms by Pseudomonas aeruginosa: A Review of the Natural and Synthetic Compounds Interfering with Control Mechanisms. *BioMed Res Int*. 2014;15:759348
2. Hoiby N, Bjarnsholt T, Givskov M, Molin S, Ciofu O. Antibiotic resistance of bacterial biofilms. *Int J Antimicrob Agents*. 2010;35(4):322–32.
3. Hu X, Huang YY, Wang Y, Wang X, Hamblin MR. Antimicrobial photodynamic therapy to control clinically relevant biofilm infections. *Front Microbiol*. 2018;9:1299.
4. Baniya B, Pant ND, Neupane S, Khatiwada S, Yadav UN, Bhandari N, Khadka R, et al. 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;16(1):70.
5. 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;30(1):39-42
6. Emami S, Nikokar I, Ghasemi Y, Ebrahimpour M, Sedigh Ebrahim-Saraie H, Araghian A, et al. 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;8(11):e23669.
7. Saha S, Devi KM, Damrolien S, Devi KS, Krossnunpuii, Sharma KT. Biofilm production and its correlation with antibiotic resistance pattern among clinical isolates of Pseudomonas aeruginosa in a tertiary care hospital in north-east India. *Int J Adv Med*. 2018;5(4):964–8.
8. Sayyad G, Mohammad T, Nasrollah S. Comparison of biofilm formation and antibiotic resistance pattern of Pseudomonas aeruginosa in human and environmental isolates. *Microb Pathog*. 2017:109:94–8.

9. 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; 1996:113–29.
10. Topley WWC, Wilson SGS. Topley and Wilson′s Microbiology and Microbial Infections: 8 Volume. Wiley–Blackwell; 2007.
11. 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; 2006:1443–535
12. Clinical and laboratory institute. Performance standards for antimicrobial susceptibility testing. 27th informational supplement. M100. Available from: https://clsi.org/media/1469/m100s27_sample. pdf.
13. Christensen GD, Simpson WA, Bisno AL, Beachey EH. Adherence of slime producing strains of Staphylococcus epidermidis to smooth surfaces. *Infec Immu*. 1982;37(1):318–26.
14. 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.
15. Gill JS, Arora S, Khanna SP, Kumar KH. Prevalence of Multidrug-resistant, Extensively Drug-resistant, and Pandrug-resistant Pseudomonas aeruginosa from a Tertiary Level Intensive Care Unit. *J Glob Infect Dis*. 2016;8(4):155–9.
16. Chika EO, Nneka AR, Dorothy ON, Chika E. Multi-Drug Resistant Pseudomonas aeruginosa Isolated from Hospitals in Onitsha, South-Eastern Nigeria. *Int Arch BioMed Clin Res*. 2017;3(3):17–21.
17. Patil P, Joshi S, Bharadwaj R. Aerobic bacterial infections in a burns unit of Sassoon General Hospital, Pune. *Int J Healthcare Biomed Res*. 2015;3(3):106–12.
18. 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):276–80.
19. 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;48(3):363-8.
20. Ghorbani H, Memar MY, Sefidan FY, Yekani M, Ghotaslou R. In vitro synergy of antibiotic combinations against planktonic and biofilm Pseudomonas aeruginosa. *GMS Hyg Infect Control*. 2017;12:Doc17.

Cite this article: Sajjanar V, Dindare P. Detection of biofilm formation and their correlation with Imipenem resistance among clinical isolates of *Pseudomonas aeruginosa*. *IP Int J Med Microbiol Trop Dis.* 2025;11(2):208-213.