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ORIGINAL RESEARCH

Evaluation of Antibiotic Resistance Patterns and Biofilm Formation among the Clinical Isolates of *Pseudomonas aeruginosa*

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ABSTRACT

Objectives: To evaluate the antibiotic resistance pattern of *Pseudomonas aeruginosa* isolated from clinical specimen and to detect Metallo beta lactamase producers as well as to accesses their biofilm forming capacity by both qualitative and quantitative analysis.

Methods: The study was conducted in Shree Birendra Hospital, Chhauni, from June to August 2025. The total of 6444 specimens was cultured and isolates of *P. aeruginosa* were subjected to antibiotic susceptibility tests. Metallo beta lactamase producers were identified by modified Hodge and EDTA synergy tests. Biofilm was detected by the Congo Red Agar and Microtiter Plate Assay method.

Results: Out of 671 positive isolates (15.05%) from pus, urine and wound, 101 isolates of *P. aeruginosa* were obtained. The highest rate of distribution was observed in in-patients as well as in the age group of 61-70 years. Among the isolates, high resistance was observed against Aztreonam (65.59%) whereas isolates were most sensitive against Tobramycin (76%). 37 were found to produce Metallo beta lactamase enzyme and almost 46% were MDR. The biofilm isolates accounted for 34 by CRA but MPA detected 100 biofilm producers. The biofilm producers showed high resistance against Aztreonam (59.41%) and Levofloxacin (56.44%). Furthermore, the MBLS were the most resistant against Levofloxacin (28.7%) followed by Aztreonam (27.7%), Cefepime (27.7%), Ceftazidime (25.7%), Imipenem (25.7%) and Meropenem (25.7%). Out of all the isolates, 36 biofilm isolates were highlighted to produce MBL enzyme as well.

Conclusion: *Pseudomonas aeruginosa* was most frequent in sputum and pus samples from inpatients and older patients, with rising resistance to monobactams, fourth-generation cephalosporins, and fluoroquinolones. High rates of MBL production and biofilm formation contributed to marked β-lactam resistance, emphasizing the need for alternative therapeutic strategies.

Keywords: *Pseudomonas aeruginosa*, Metallo beta lactamase, Microtitre plate, Biofilm

INTRODUCTION

Pseudomonas aeruginosa is one of the opportunistic pathogens, recognized for its significance in clinical settings and thrive most in wet surfaces. It is responsible for number of cases of nosocomial and systemic infections including urinary tract infections, respiratory tract infection, dermatitis, bacteremia, soft tissue, bone and joint infection (Mahaseth et al., 2020, Shrestha et al., 2019). Their remarkable ability to resist antibiotics makes infection proliferate especially

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among inpatients with weakened immune systems. Natural resistance to antibiotics by mechanisms like outer membrane permeability, efflux pumps, antibiotic inactivating enzymes, biofilm production, acquisition of resistance genes makes the entry of antibiotics way more critical (Mahaseth et al., 2020, Pang, et al., 2019). The ability of its genome to encode large amounts of regulatory enzymes causes high morbidity rate in cystic fibrosis patients. MDR Gram negative bacteria are generally resistant to broad spectrum antibiotics which make it burdensome to reduce the relapse of infection (Chaudhary et al., 2024).

Metallo Beta Lactamase (MBL) producing *P. aeruginosa* has been clinically significant with a high mortality rate. MBL enzyme inhibits the action of Carbapenem drugs before the drug reaches the PBP targets. The outer membrane porin protein OprD is responsible for quick uptake of Carbapenems whereas in absence, it is resistant to drug (Pang et al., 2019). Its mechanism is followed by the usage of divalent cation like zinc as cofactor that activates the enzyme to hydrolyze the β-lactam rings (Farajzadeh Sheikh et al., 2014). *IMP, VIM, SPM, GIM, NDM* and *FIM* genes encode enzymes capable of hydrolyzing the β-lactam antibiotics. It is usually inhibited by chelating agents like EDTA but not inhibited by clavulanic acid, sulbactam, or by developmental penicillanic acid sulfones and diazabicyclooctanes (Boyd et al., 2020).

The alarming threat of antibiotic resistant microorganisms poses a significant challenge to public health, as these pathogens can easily spread within the population, doubling the risk for treatment. Infection caused by *P. aeruginosa* is a formidable challenge to treat. MBL enzyme heavily involves the changing of the structural compound of Carbapenems which is usually used as Tier 3-4 drugs. Current key players to treat MBL producers is Colistin (>97%susceptibility) and Aztreonam though its activity weakens if the isolates are known to coproduce extended-spectrum β-lactamases (ESBLs) or AmpC enzymes (Boyd et al., 2020). Though as of currently there are strategies to control MBL producers which includes combination of Carbapenem drugs with Monobactams or direct MBL inhibitors.

Biofilm formation is a multi-step complex process involving the transition of bacteria from free-swimming planktonic to sessile form (Rather et al., 2021). The maturation of biofilm after multiplication and formation of microcolony induces antimicrobial resistance alongside acting as a protection from harsh conditions. Dispersion of biofilm further increases the accumulation of biofilm resulting in severity of the infection. The resistance developed by them at the early stage is less but in the later stage, it is prominent which is about 1000 times foldmore (Krishnasamy & Velmurugan, 2024).

This study addresses that gap by systematically evaluating clinical isolates for MBL production and biofilm formation and correlating these phenotypes with multidrug-resistance profiles and specimen/ clinical source. Understanding the prevalence and co-association of MBLs and biofilms will (1) clarify the microbiological drivers of treatment failure, (2) identify high-risk isolate phenotypes that warrant enhanced infection control and stewardship measures, and (3) inform therapeutic strategies—such as targeted combination therapy, use of MBL inhibitors, or biofilm-disrupting adjuncts. By linking phenotypic resistance mechanisms with clinical specimen data and antibiotic susceptibility patterns, this research work has generated actionable knowledge to improve diagnostics, guide empiric therapy, and reduce relapse and mortality from *P. aeruginosa* infections in healthcare settings.

METHODS

Study design, duration and site

A hospital based descriptive cross-sectional study was conducted in the Microbiology laboratory of Shree Birendra Hospital from June to August 2025. Sample collection, processing and biofilm assessment were done in the hospital laboratory.

Inclusion and exclusion criteria

All age groups of both sexes from inpatient and outpatient departments including immunocompromised patients who gave written consent were enrolled in the study. All kinds of samples were included in the study.

Sample types and size

Non-probability consecutive sampling techniques were used for sample collection. Different clinical samples; sputum, pus, wound swabs, urine, fluids, blood, etc, were taken and processed in the laboratory.

Sample collection and processing

A total of 6444 samples were processed including sputum, pus, wound swabs, urine, aspirates, fluids and blood. All samples were collected aseptically in

sterile containers and sent immediately for processing. They were inoculated aseptically on Mac-Conkey agar, Blood agar and Chocolate agar. For urine samples, Cysteine–Lactose–Electrolyte-Deficient (CLED) agar was used. The isolates were identified by standard Microbiological procedure including colony morphology, Gram staining, and Biochemical tests. Only *P. aeruginosa* were taken in the study. Cetrimide agar was used as a selective media for *P. aeruginosa* (Cheesbrough, 2006).

Antibiotic susceptibility test

Antibiotic susceptibility test was carried out by Modified Kirby Bauer disk diffusion method on Mueller Hinton agar and interpretation was done following Clinical and Laboratory Standards Institute (CLSI) guideline (CLSI, 2024). Antibiotics included in the test were Piperacillin/tazobactam (100/10 μg), Ceftazidime (30 μg), Cefepime (30 μg), Imipenem (10 μg), Meropenem (10 μg), Amikacin (30 μg), Tobramycin (10 μg), Ciprofloxacin (5 μg), Levofloxacin (5 μg), Aztreonam (30 μg). Isolates that showed resistance to at least 3 or more antibiotic categories were considered Multi Drug Resistance (MDR) (Magiorakos et al., 2012).

Detection of Metallo Beta Lactamase (MBL) producers

Metallo beta lactamase (MBL) producers were determined by Imipenem-EDTA Disk Method as described by Yong et al., (2002) with modification. Solution of 0.5 M EDTA was prepared by dissolving 186.1 g of disodium EDTA in 1,000 ml of distilled water and adjusting it to pH 8.0 by using NaOH. One Imipenem disc was taken and 10 μL of the prepared solution was added to the disc. The disc was air-dried. On a lawn culture on Mueller Hinton Agar, 2 Imipenem discs, one with 10 μL of EDTA (750 μg) and the other disc without EDTA were placed and incubated overnight. Zone size of ≥7mm in IMP+EDTA was considered an MBL producer (Shukla et al., 2022).

Biofilm detection by Congo Red Agar method

The screening of biofilm production was done by Congo Red Agar method (Freeman et al., 1989). Congo red was prepared as a concentration solution and autoclaved. It was added to the medium when agar is cooled to 55˚C and poured into petri plates (Harika et al., 2020). The isolates were inoculated in the prepared agar and incubated at 37˚C up to 72 hr. Dark black crystalline colonies were considered as strong biofilm strains, darker colonies without dry and crystalline structure were considered as weak positive and pink or red colonies were considered negative respectively (Abdulhaq et al., 2020, Bhatta et al., 2019).

Bioflm detection by Microtiter Plate Assay

The isolates were quantitatively evaluated by Microtiter plate assay (MPA). The isolate was grown overnight in 2 ml of LB broth and diluted in 1:100 in sterile fresh broth. In a 96-well flat-bottomed plate, 200μl of the diluted culture was inoculated and incubated for 24hr at 37˚C. After incubation, the cells were aspirated out and washed with sterile saline to remove free- floating bacteria. This step was repeated 2 to 3 times. The plate was air-dried for 20-30 min, favoring the fixation of the biofilm. Each well was stained by adding 125μl of crystal violet (0.1%) and incubated at room temperature for 10-15 mins. The plate was rinsed and dried. 200μl of 95% ethanol was added to solubilize the crystal violet. The plate was covered with the lid to minimize evaporation and incubated at room temperature for 15-30 mins. The absorbance or the optical density (OD) was measured at 570 nm using 95% ethanol in water as negative control using an ELISA reader (O'Toole 2011, Stepanović et al., 2007).

To evaluate biofilm formation, the average optical density (OD) of each isolate was calculated, and a cut-off value (ODc) was determined. The ODc was defined as the mean OD of the negative control plus three standard deviations, ensuring a reliable threshold for distinguishing true biofilm production from background noise. Isolates with OD values lower than the ODc were classified as non-biofilm producers. Those with OD values between the ODc and twice the ODc were categorized as weak biofilm producers, while values between two and four times the ODc indicated moderate biofilm formation. Isolates with OD values greater than four times the ODc were identified as strong biofilm producers. For the purpose of this study, all isolates classified as weak, moderate, or strong producers were considered biofilm-positive.

Quality Control

*Pseudomonas aeruginos*a ATCC 27853 was used as a routine quality control for MBL producing strains.

Data analysis

All the analysis of the data was done using Microsoft Excel 2016.

Ethical consideration

The study was approved by the Institutional Review

Committee (IRC) of Nepal Army Institute of Health Science (NAIHS) (Reg. No. 1355, 2025).

RESULTS

Bacterial growth in clinical samples:

Out of 6444 samples processed, 671 samples were growth positive whereas 101 (15.1%) were positive for *P. aeruginosa*. The maximum number of *P. aeruginosa* was isolated from sputum (n=49), followed by pus (n=19), wound swab and urine (n=9) and other specimens (Table 1).

Table 1: Distribution of *P. aeruginosa* among the clinical specimens

Clinical Specimens	Positive growth	*P. aeruginosa* growth no. (%)
Urine	279	9 (3.2)
Pus	63	17 (27)
Blood	31	1 (3.2)
Sputum	170	49(28.8)
Wound swab	60	9 (15)
BAL fluid	4	1 (25)
Throat swab	1	1 (100)
Tissue	5	2 (40)
Bed sore	5	3 (60)
Aspirated fuid	36	1 (2.8)
Tracheal fluid		7 (19.4)
Intra articular fluid	3	1 (33.3)
EVD tip	4	0
CVP tip	1	0
CSF fluid	1	0
High Vaginal Swab	7	0
Placenta	1	0
Grand Total	**671**	**101(15.1)**

Demographic and Department-Wise Distribution of *P. aeruginosa* among Inpatients and Outpatients

The higher number of *P. aeruginosa* was isolated from inpatients (62.4%) in comparison to outpatients (37.6%). *P. aeruginosa* was isolated more from male inpatients (n=37) than female inpatients (n=26). However, female inpatients of the aged group 61-70 were the largest proportion of all age groups and genders (Figure 1). The statistical analysis demonstrated a significant association in the distribution of isolates between specimen and type of patients ($p<0.05$).

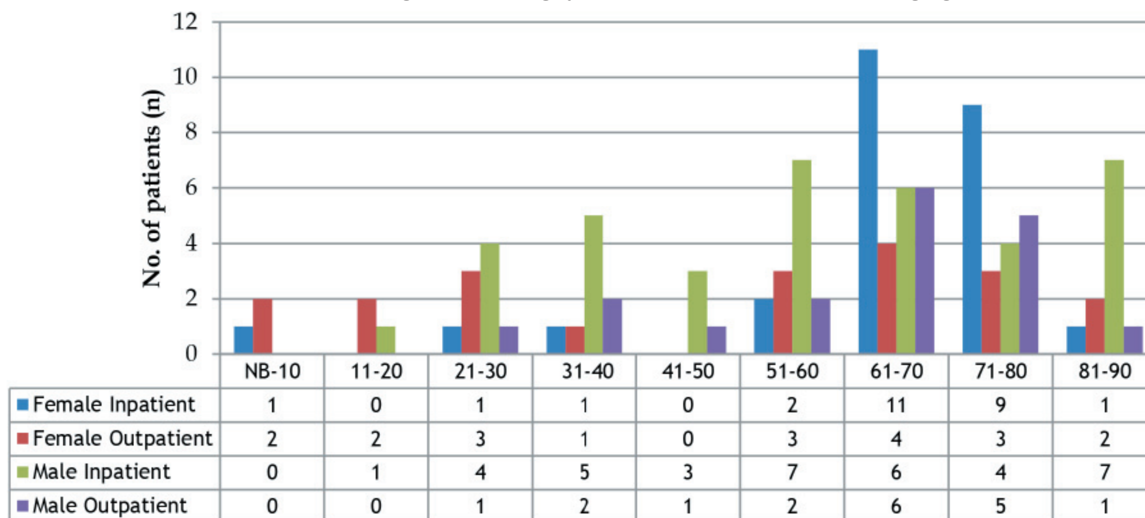


Figure 1: Distribution of *P. aeurginosa* among patients of different age groups

Antibiotic susceptibility testing of *P. aeurginosa*

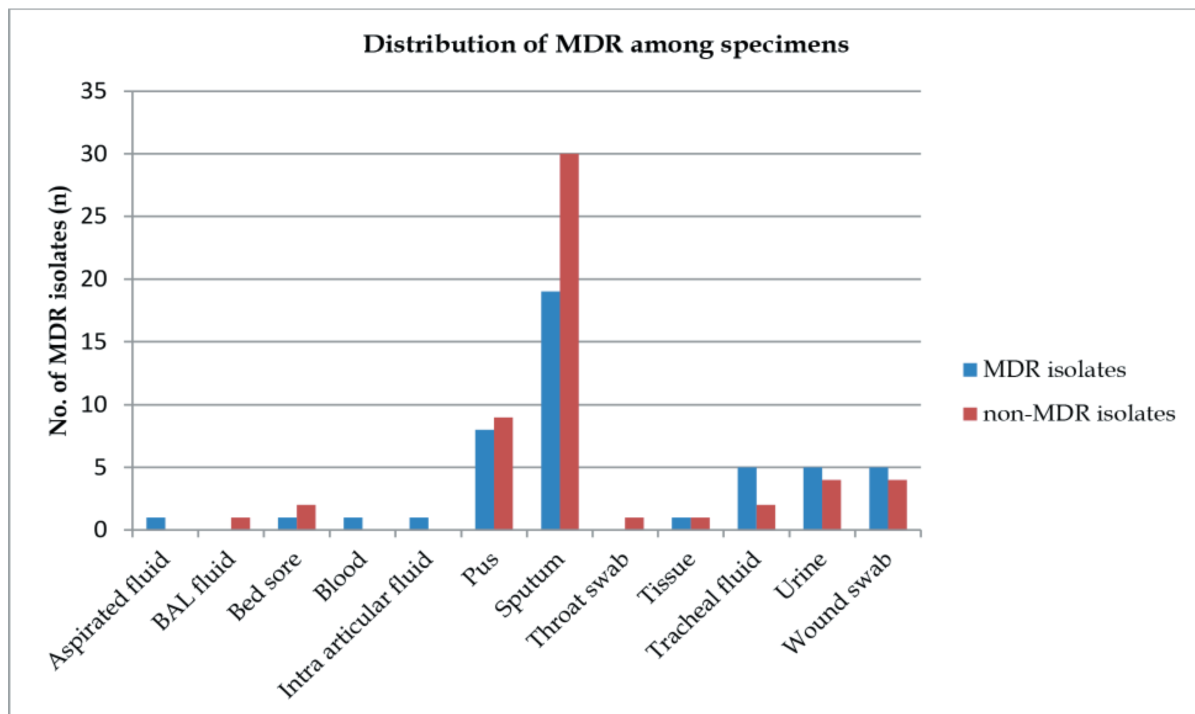
The bacterium was found to be resistant to most of the antibiotics used. Majority of isolates were resistant against Aztreonam, followed by Levofloxacin, Cefepime, Ciprofloxacin and Ceftazidime. However, isolates were sensitive to Tobramycin followed by Imipenem, Meropenem Piperacillin/Tazobactam and Ceftazidime (Table 2).

Table 2: Antibiotic Susceptibility Pattern of *Pseudomonas aeruginosa* (n=101)

Group of Antibiotics	Name of Antibiotics	Antibiotic Susceptibility Pattern	
		Resistant no.(%)	Sensitive no.(%)
Carbapenem	Imipenem	31 (30.7)	70 (69.3)
	Meropenem	38 (37.6)	63 (62.4)
Aminoglycoside	Tobramycin	24 (24)	76 (76)
	Amikacin	23 (31.1)	51 (68.9)
Monobactam	Aztreonam	60 (63.8)	34 (36.2)
B-lactams	Piperacillin + Tazobactam	38 (38.7)	60 (61.2)
Cephalosporins	Cefepime	49 (50)	49 (50)
	Ceftazidime	41 (40.6)	60 (59.4)
Fluoroquinolone	Levofloxacin	54 (54.5)	45 (45.5)
	Ciprofloxacin	42 (42)	58 (58)

Distribution of MDR among specimens

In the study, out of 101 isolates of *P. aeruginosa*, 47 isolates were of MDR strains. The higher number of MDR isolates were obtained from inpatients (32.7%) as compare to outpatients (13.9%). MDR strains were isolated mostly from sputum samples (n=19) followed by pus (n=8), tracheal fluid (n=5), urine (n=5), wound swabs (n=5). (Figure 2).



*MDR= Multi Drug Resistance

Figure 2: Distribution of MDR among different clinical samples

Out of 101 isolates of *P. aeruginosa*, 37 (36.6%) were MBL producers and 64 (63.4%) isolates were MBL negative.

Among the 101 isolates examined, the Congo Red Agar (CRA) method identified 15.8% as strong biofilm producers, 6.9% as moderate producers, and 10.9% as weak producers, while the remaining 66.3% were classified as non-biofilm producers. In contrast, the Microtiter Plate Assay (MPA) demonstrated a substantially higher detection of biofilm formation: 40.6% of isolates were categorized as strong biofilm producers, 48.5% as moderate producers, and 9.9% as weak producers. Only a single isolate (0.99%)

was identified as a non-biofilm producer using this method (Table 3). These findings highlight a marked discrepancy between the two techniques, with MPA showing greater sensitivity in detecting biofilm-producing phenotypes.

Table 3: Biofilm producers by Congo Red Agar Method (CRA) and Microtiter Plate Assay (MPA) method

Isolate of *Pseudomonas aeruginosa*	Congo Red Agar Method (CRA) no.(%)	Microtiter Plate Assay (MPA) no.(%)
Strong Biofilm producer	16 (15.8)	41 (40.6)
Moderate Biofilm producer	7 (6.9)	49 (48.5)
Weak Biofilm producer	11 (10.9)	10 (9.9)
Non-biofilm producer	67 (66.3)	1 (0.9)

(Note: MPA evaluation: Non-biofilm producer = OD < ODc; Weak biofilm producer = ODc < OD < 2×ODc; Moderate biofilm producer = 2×ODc < OD < 4×ODc; Strong biofilm producer = 4×ODc < OD. CRA detection: Non-biofilm producer = pink or red colony; Weak biofilm producer = only dark colony without dryness; Moderate biofilm producer = dark and dry but without crystalline colony; and Strong biofilm producer = dark black dry crystalline colony)

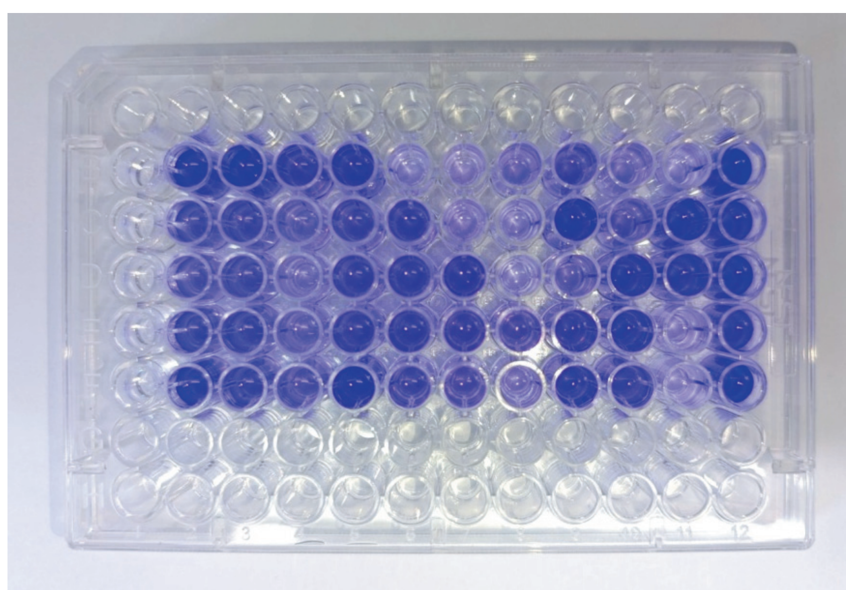


Figure 3: Biofilm formation in 96 well plate

Biofilm detection by CRA and MPA methods:

The CRA method correctly identified 34 biofilm isolates but missed 66 true biofilm producers. This meant that CRA was poor at detecting true biofilm producers. CRA is very specific but poorly sensitive for detecting biofilm in *P. aeruginosa*. The test demonstrated sensitivity of 34%, specificity of 100%, positive predictive value (PPV) of 100%, and negative predictive value (NPV) of 1.49% for CRA method compare with MPA method, indicating low reliability for biofilm detection (Table 4).

Table 7: Comparative study of CRA and MPA method

Biofilm detection techniques / Biofilm formation		CRA no. (%)		Total
		Biofilm Producer	Non-producers	
MPA method (Gold standard)	Biofilm	34	66	100
	Non-Biofilm producer	0	1	1
Total		**34**	**67**	**101**
Sensitivity of CRA		0.34 (34%)		
Specificity of CRA		1 (100%)		
Positive predictive value		1 (100%)		
Negative predictive value		0.0149 (1.49%)		

Comparison of antibiotic susceptibility patterns between biofilm and MBL procedures with non-producers:

Likewise, the investigation also revealed that a higher number of biofilm producers are resistant to antibiotics in comparison to non-biofilm producers. The maximum resistance by the biofilm producers was recorded by Aztreonam (59.4%) in succession with Levofloxacin (56.4%), Cefepime (47.5%), Ceftazidime and Ciprofloxacin (40.6%).

Furthermore, the MBL producers showed maximum resistance towards Levofloxacin (28.7%) followed by Aztreonam (27.7%), Cefepime (27.7%), Ceftazidime (25.7%), Imipenem (25.7%) and Meropenem (25.7%) (Table 5).

Table 5: Antibiotic resistance pattern of biofilm producers and non-biofilm producers

Antibiotic	Resistance Pattern			
	Biofilm producer no.(%)	Non-biofilm producer no.(%)	MBL producer no.(%)	Non-MBL producer no. (%)
Imipenem	29 (28.7)	1 (0.9)	26 (25.7)	4 (3.9)
Meropenem	37 (36.6)	1 (0.9)	26 (25.7)	12 (11.8)
Tobramycin	23 (22.8)	1 (0.9)	18 (17.8)	6 (5.9)
Amikacin	22 (21.8)	1 (0.9)	15 (14.9)	7 (6.9)
Piperacillin/tazobactam	36 (35.6)	1 (0.9)	24 (23.8)	13 (12.8)
Aztreonam	60 (59.4)	1 (0.9)	28 (27.7)	33 (32.7)
Cefepime	48 (47.5)	1 (0.9)	28 (27.7)	21 (20.8)
Ceftazidime	41 (40.6)	1 (0.9)	26 (25.7)	16 (15.8)
Ciprofloxacin	41 (40.6)	1 (0.9)	25 (24.8)	17 (16.8)
Levofloxacin	57 (56.4)	1 (0.9)	29 (28.7)	29 (28.7)

Comparison between biofilm and MBL strains:

The results provided insights into the biofilm producers with comparison to MBL strains. 36 biofilm isolates were determined to produce the MBL enzyme and only one non-biofilm isolate produced the enzyme. The association between biofilm producers and MBL strains were not statistically significant ($p<0.05$) (Table 6).

Table 6: Comparative study between biofilm and MBL strains

Biofilm (MPA)	MBL producers no. (%)	Non-MBL producers no. (%)	Total
Strong biofilm	11 (10.9)	30 (29.7)	41 (40.6)
Moderate biofilm	22 (21.8)	27 (26.7)	49 (48.5)
Weak biofilm	3 (2.9)	7 (6.9)	10 (9.9)
Non-biofilm	1 (0.9)	0	1 (0.9)
Total	37 (36.6)	64 (63.8)	101 (100)

DISCUSSION

Findings from this study showed the increase in distribution of *P. aeruginosa* among clinical samples in comparison to past researches. Bhatta et al., (2019) reported the prevalence rate of 7% which in comparison to this study was less in proportion. The prior publications had observed the less percentage in the prevalence rate of the pathogen in contrast to the present work. The majority of isolates obtained from sputum of patients indicated that *P. aeruginosa* as one of the major bacterium in lower respiratory tract infection. Likewise, the isolates from pus, wound and bed sore samples indicated that it is a common pathogen in hospital or community acquired infections. The sole isolate from a blood sample acquired from inpatient refl ected sepsis, including the severity of the pathogenesis and necessity for proper hospital care. The largest proportion of positive isolates among urine samples of outpatients possibly implies community-acquired infections. The samples from inpatients of both genders (36.6% from male and 25.7% from female) had the highest number of culture positives in comparison to that of male and female outpatients (17.8% and 19.8% respectively) which supported the fact that the occurrence of the pathogen in hospital environment is more common than usual. Furthermore, the study conducted by Chaudhary et al., (2024) from the same hospital earlier reported that had the highest number isolated from sputum samples (33.3%). The comparison of two data shows an increment in the prevalence of the pathogen from the same type of sample. Likewise, Bhatta et al., (2020) reported the highest number of the pathogen was isolated from sputum (n=93), followed by wound (n=35) and pus (n=29) out of 200 isolates over a one-

year study period.

On the basis of this study, bacterium showed maximum resistance against Monobactam followed by Cephalosporin and Fluoroquinolone. Kamali et al., (2020) also reported that *P. aeruginosa* isolated from ICU samples showed resistance against Amikacin and Piperacillin/tazobactam (12.5%) to Levofloxacin (23.7%). However, our study revealed higher rate of resistance indicating the progressive increase in resistance over time. In the contrary, the data presented by Soni et al., (2024) revealed that the bacterium exhibit the significant resistance (93.3%) against Ceftazidime. The present study has concordant fi nding with the study of Krishnasamy &Velmurugan (2024) in terms of antibiotic resistance against classes of antibiotics. Likewise, isolates of *P. aeruginosa* were highly sensitive towards Tobramycin followed by Imipenem and Meropenem. However, sensitivity towards Amikacin and Meropenem was high in the similar study performed by Bhatta et al., (2020). This can be interpreted as the change in the sensitivity of drugs over time which requires thorough investigation. Overall, the resistance pattern seemed to vary along the years among different researches indicating demand for in-depth evaluations.

The rate of MDR has exceeded as reported in the past that was 34.5% from Bhatta et al., (2020). Sharma et al., (2021) reported the pus sample contained the highest MDR (n=18) which is a similar to this study (n=17). Likewise, many papers have drawn attention to a higher number of ICU isolates containing possible MDR strains. This might be due to the prolonged stay in hospital rooms which might have proliferated the survival and the resistance pattern. The trend of the MDR seemed to be progressively rising with almost no sign of possible downward trend if there is no availability of standard control of antibiotic distribution (Kamali et al., 2020, Soni et al., 2024).

In our study, 36.6% (37/101) of *P. aeruginosa* isolates were MBL producers. Maharjan (2022) stated that only 6 out of 68 were MBL producers, Shukla et al., (2022) depicted 22 out of 115 and Yadav et al., (2024) reports 58 out of 205 (28%) produced this enzyme. A similar study conducted in Brazil reported crucial prevalence of MBLs among Imipenem resistant *P. aeruginosa* (30.4%) obtained from blood samples (Franco et al., 2010). Such Beta-lactamase enzyme inactivates Carbapenems, Cephalosporins and Penicillins, and very often not effective by use of Beta-lactamase inhibitors. As of now, the phenotypic method for its detection has not been standardized nationally and internationally but the number of the resistance caused by the enzyme is still prevalent (Farajzadeh Sheikh et al., 2014). Hence, requiring a guideline for proper diagnosis is vital in clinical laboratories. The manual technique in lab detection usually involved the use of chelating agent like EDTA in combination with Imipenem disc (Shukla et al., 2022, Yong et al., 2002).

Congo Red Agar method is a qualitative method for biofilm detection where the presence of dark crystalline colonies is considered a certain level of biofilm producers. A past research paper detected a total 57 biofilm positive isolates from UTI patients in which out of 72 isolates of *P. aeruginosa* produced biofilm (Bhatta et al., 2019). While the results were comparable, the analysis done by Baniya et al., (2017) revealed only 13 out of 85 were biofi lm producers from the CRA method. It is worth mentioning that a lot of past literatures in CRA method had ruled out false-negative as biofilm producer as well. As for the data observed by the Microtiter Plate Assay, 100 isolates were calculated to produce a certain level of biofi lm out of 101 isolates in our study. The data was taken and analyzed after the absorbance reading of Crystal Violet at 570 nm where visually the biofi lm that stained darker was corresponding to the biofi lm isolates. The ratio of biofilm to non-biofilm isolates were found to be parallel to Kamali et al., (2020) where 70/80 isolates were related to biofilm related genes. Superior outcomes highlighted the relation of biofi lm phenotype and genotype where 176 detected as biofi lm formers while 29 did not form biofilm. Other literature also highlights closely related findings similar to the trend of the above data. The current study detected a higher rate of biofilm production from the Microtiter Plate Assay cancelling out false positive results from the CRA method. A similar trend was observed as CRA detected 44.2% while MPA detected 94.2% biofilm formers (Abdulhaq et al., 2020) and likewise, from findings of Harika et al., (2020). This investigation differed from the fi ndings of Bhatta et al., (2019) where their findings contained a lesser proportion of biofi lm producers. The CRA method correctly identified 34 biofilm isolates but missed 66 true biofilm producers in this study. This meant that CRA was poor at detecting

true biofi lm producers. CRA is very specific but poorly sensitive (sensitivity 34%) for detecting biofi lm in *P. aeruginosa*.

Maximum number of the biofilm isolates was resistant to the antibiotics as opposed to non-biofilm isolates. While almost all isolates were resistant to Aztreonam, Levofloxacin, Cefepime and Ciprofloxacin, Tobramycin and Amikacin were the least resistant. This ratio of resistance by biofi lm producers was consistent with those reported by Chhunju et al., (2021) whereas Imipenem and Meropenem resistant isolates were more resistant from findings of Saha et al., (2018). The fi ndings suggest that the ineffective treatment with antibiotics alone was due to the result of biofi lm secretion leading to promotion of chronic and recurrent infections.

This study has also shown correlation between antibiotic resistance and Metallo beta lactamase (MBL) producers, especially against Carbapenem drugs where each 26 isolates were resistant to Imipenem and Carbapenem. The MBL production were shown to be directly associated with Carbapenem resistance. The findings suggest that the MBL enzyme were able to break down the β-lactam antibiotics making them unaffected against pathogen. MBL genes are often carried on plasmids that also carry additional resistance genes leading towards MDR. Overall, the prevalent number of MBL isolates displaying resistance to various antibiotics showed MBL enzyme as one of the crucial factor behind antibiotic resistance against the broad spectrum β-lactam drugs. The study by Baniya et al., (2017) was not able to statistically associate biofilm with MBL producers which is in agreement with our findings. However, there have been reports where the isolates coproduced strong biofilms as well as MBL (Heydari & Eftekhar, 2015, Singhai et al., 2013).

CONCLUSION

This study shows a higher prevalence of *Pseudomonas aeruginosa* infections in older individuals, with MDR isolates occurring more frequently in inpatients, likely due to prolonged hospital exposure and cross-contamination. The strong biofilm-forming ability of the isolates may contribute to persistent or recurrent infections, especially in immunocompromised patients. Resistance was highest to monobactams, cephalosporins, and fluoroquinolones, indicating these antibiotics are unsuitable for treatment in this setting. The prominence of biofilm and MBL producers suggests the presence of related resistance genes, supporting the need for molecular confirmation through methods such as PCR. Given the increasing carbapenem resistance, routine phenotypic detection of MBLs and improved diagnostic strategies are essential for effective management and control of *P. aeruginosa* infections.

ABBREVIATIONS

AMR: Antimicrobial Resistance, AST: Antibiotic Susceptibility Pattern, BHI: Brain Heart Infusion, CFU/ml: Colony Forming Unit per Milliliter, CIP:, CLED: Cysteine Lactose Electrolyte Deficient, CLSI: Clinical and Laboratory Standards Institute, CSF: Cerebrospinal Fluid, EDTA: Ethylenediamine tetraacetic acid, ELISA= Enzyme linked Immunosorbent Assay, LB: Luria Bertani/ Lysogeny Broth, MBL: Metallo Beta Lactamase, MDR: Multi Drug Resistance, MHA: Mueller Hinton Agar, MPA: Microtiter Plate Assay, OD: Optical Density, ODc: cut-off value of Optical Density, PBP: Penicillin- binding protein, PBS: phosphate-buffered saline, XDR: Extensively established drug resistance

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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