Biomedical and Biopharmaceutical Research

Abbreviation: Biomed. Biopharm. Res.
Volume: 22: Issue: 02 |Year: 2025
Page Number: 723-728

Isolation of Pseudomonas Aeruginosa from various clinical samples and its Correlation with Biofilm and Antimicrobial Susceptibility Pattern at Tertiary Care Centre

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Article Received:28-06-2025

Article Accepted:30-07-2025

Abstract

INTRODUCTION: *Pseudomonas aeruginosa* can cause community acquired or hospital acquired infections and has potential to develop antimicrobial resistance. Biofilm is one of the factors that help in the establishment of the organism on different host tissues. The current study aims to identify the multidrug resistant *P.aeruginosa* and correlate the relationship of drug resistance with biofilm production.

MATERIALS AND METHODS: In this cross-sectional study, a total of 1440 clinical specimens were included. All clinical samples including sputum, urine, pus, wound swabs, ET secretions, high vaginal swabs were processed for culture and sensitivity as per the standard guidelines. Biofilm testing of *Pseudomonas* isolates was done by Tissue Culture Plate Method (TCPM). Categorical variables were tested by chi square analysis, the p value <0.05 is considered as statistically significant.

RESULTS: Out of 168 isolates, maximum number were from sputum 58 (34.5%) followed by pus 44(26.1%) urine 42 (25%), wound swabs 15 (8.9%), high vaginal swabs (HVS) 6 (3.5%) and ET secretions of 3 (1.7%) isolates. MDR percentage among *Pseudomonas* isolates noted was 48.2%. Out of 168 *Pseudomonas* isolates 42 (25%) were Biofilm producers and remaining 126 (75%) were non biofilm producers. Among 42 biofilm producers, 66.6% (28/42) were weakly adherent, 10 (23.8%) were moderate adherent and remaining 4 (9.5%) were strongly adherent. Biofilm producers and non biofilm producers in relation to multidrug resistance pattern was found statistically significant.

CONCLUSION: To combat the drug resistant of *P.aeruginosa* a stringent measures of infection control policies and microbiological screening to start accurate therapy during the critical time will definitely help. Regular antibiogram plotting in the hospital and early screening microbiological screening methods implementation will aid clinicians to give the prompt and accurate treatment to patients.

KEY WORDS: *Pseudomonas aeruginosa*, Biofilm, antibiotics.

INTRODUCTION

Pseudomonas aeruguniosa is a ubiquitous, non fermentative, aerobic, motile, gram negative bacilli belongs to the family *Pseudomonadaceae*. It is an opportunist pathogen which has capability to infect the immunocompromised individuals, while the emergence of the *Pseudomonas* pathogens has been increasing globally from the recent years. This emergence could be due to increase in the population, migration of population, increase in the life longevity of immunocompromised population, patient comorbidities, steroid usage, prolonged length of hospital stay, frequent hospital visits, bacterial pathogenicity change and surge in advance medical technologies resulting in high medical intervention [1]. *Pseudomonas aeruginosa* is associated with nosocomial infections such as ventilator-associated pneumonia, urinary tract infections, and bloodstream infections. *Pseudomonas aeruginosa* can cause community acquired or hospital acquired infections either by direct transmission from patient to susceptible person or by indirect transmission from reservoir to objects or contaminated surfaces without direct human to human contact [2]. *Pseudomonas aeruginosa* has various factors that help to adhere and damage cells, and mucosal tissues of the host, elicit inflammation, and impair defense mechanisms [3]. Biofilm is one of the factors that help in the establishment of the organism on different host tissues. Biofilm formation

occurs by *P.aeruginosa*, particularly in the case of pulmonary infections in patients with cystic fibrosis, contribute to its resistance to antimicrobial agent [3].
US Centers for Disease Control and Prevention and the National Nosocomial Infection Surveillance System is a good network of hospital infection control surveillance where the data base has infection rates of all hospitals in US. They observed the *P. aeruginosa* is the second most common cause of nosocomial pneumonia (17%), the third most common cause of urinary tract infection (7%), the fourth most common cause of surgical site infection (8%), the seventh most frequently isolated pathogen from the bloodstream (2%) and the fifth most common isolate (9%) overall from all sites [4].
Pseudomonas aeruginosa has potential to develop antimicrobial resistance by various mechanisms including developing efflux pumps, producing target enzymes, acquisition of drug resistant genes by plasmids or transposons [5]. This pathogen can form biofilm on different host tissues as a survival factor. It is an alert to microbiologists and clinicians on early bacterial identification at microbiology laboratory and knowing the resistance pattern of pathogens. Screening for biofilm producing pathogens can help us to know the epidemiology in the particular community and acquisition of knowledge on antibiotic resistance pattern of biofilm producers will also help to frame the empirical antibiotics.

AIM & OBJECTIVES
The current study aims to identify the multidrug resistant *P.aeruginosa* and correlate the relationship of drug resistance with biofilm production.
1. To Identify the *P.aeruginosa* in clinical samples.
2. To determine the correlation between biofilm formation & antimicrobial susceptibility testing

MATERIALS AND METHODS
Study Design & Settings: A cross-sectional study was conducted at a department of Microbiology, Government Medical College, Anantapur, Andhra Pradesh. A total number of 1440 clinical samples were collected at Microbiology department from patients attending Government General Hospital from July 2024 to December 2024.

Sample Collection: As per central laboratory standard institute guidelines all the samples were collected. Clinical samples (e.g., sputum, pus, urine, swabs from wounds, high vaginal swabs, endotracheal secretions) were collected from patients suspected of having bacterial infections. Samples were collected aseptically as per the laboratory instructions and transported immediately to the microbiology laboratory for analysis.

Inclusion criteria:
1. All clinical samples including sputum, urine, pus, wound swabs, ET secretions, high vaginal swabs
2. Patients of all age groups and both sexes.

Exclusion criteria:
Mixed growth of 3 or more types (probably contaminated sample).

Bacterial Identification:
All samples were processed for microscopic examination, culture and antibiotic susceptibility testing according to CLSI protocols. Specimens were inoculated onto nutrient agar, 5% sheep blood agar, Macconkey agar and chocolate agar. After incubation at 37C for 24-48 hours, pathogen identification up to species was performed by colony characterization, biochemical reactions and inoculation on special media. Pseudomonas isolates were identified based on colony morphology, Gram staining, special biochemical tests (e.g., oxidase test, OF test, TSI) and special media (eg.,cetrimide agar).

Antimicrobial Susceptibility Testing:
Antimicrobial susceptibility of the isolated Pseudomonas aeruginosa was determined using the Modified Kirby-Bauer disk diffusion method. The results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Pseudomonas isolates antibiotics were: aztreonam (30 µg), piperacillin+tazobactum (100/10 µg), ceftazidime (30 µg), cefaperazone+sulbactum (75/30 µg), imipenem (10 µg), ciprofloxacin (5 µg), meropenem (10 µg), amikacin (30 µg), gentamicin (10 µg) and cefipime (30 µg).

Biofilm Testing:
Biofilm testing of *Pseudomonas* isolates was done by Tissue Culture Plate Method (TCPM), as described by Christensen et al [6], which is considered as a gold standard method for biofilm detection. A single colony of bacteria was emulsified in normal saline and adjusted to 0.5 Mc Farland standards. After the dilution of this suspension (1:100) using a tryptic soy broth medium, inoculated into microtitre plate (200 ul in each well). The microtitre plate was incubated for 24 hours at 37 C, later washed for 3 times with phosphate buffered saline (PBS) (pH7.2). Gently tapped and inverted the microtitre plate at room temperature which is followed with fixation using 2% sodium acetate. Later on, it is stained with 0.1% crystal violet solution for about 10-15 minutes. The microtire plate is again washed 3 times with PBS. At this point bacteria could be seen macroscopically. Finally, the bounded CV dye was resolubilized in 30% acetic acid for 30 minutes and measured in an ELISA reader at 570 nm to determine the optical density (OD). The cut-off OD (ODc) was defined as equivalent to three standard deviations above the mean OD of the negative control (sterile broth). Three categories of isolates were identified (Table 1). The assays were performed in triplicate.

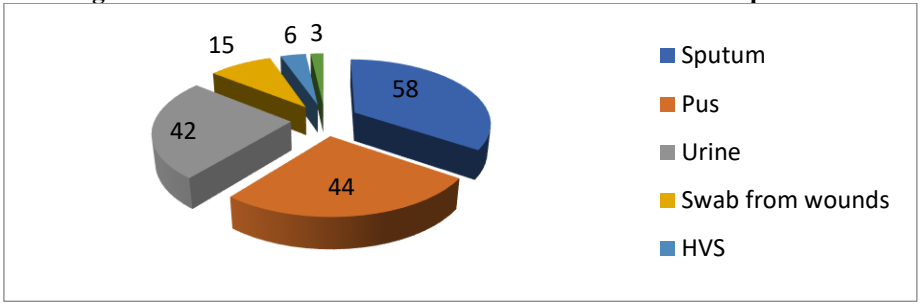
Table 1. Grading of biofilm formation

S.n	**Optical densities**	Rule	**Biofilm formation**
1.	< 0.494	ODtest < ODc	None/weak
2.	0.494–0.986	ODc < ODtest < 2×ODc	Moderate
3.	> 0.986	2×ODc < ODtest < 4×ODc	Strong

Data Collection:
All the data was collected and entered into spread Microsoft excel sheet for analysis. Calculation of Frequency, percentages, and odds ratio would be done for qualitative data. Categorical variables were tested by chi square analysis, the p value <0.05 is considered as statistically significant.

RESULTS
In this study a total of 1440 clinical isolates were studied. Among 1440 examined samples. A total 168 clinical isolates of *Pseudomonas aeruginosa* were isolated and studied further to evaluate the antibiotic sensitivity testing and biofilm production.
Out of 168 isolates, maximum number were from sputum 58 (34.5%) followed by pus 44(26.1%) urine 42 (25%), wound swabs 15 (8.9%), high vaginal swabs (HVS) 6 (3.5%) and ET secretions of 3 (1.7%) isolates (Fig 1).

Fig 1. Pseudomonas isolates distribution in various clinical specimens



Out of 168 *Pseudomonas* isolates 42 (25%) were Biofilm producers and remaining 126 (75%) were non biofilm producers (Table 2). In almost all the samples 15-30% of biofilm producers were observed.

Table 2. Biofilm producers in various clinical samples

Clinical samples	**Total no of isolates (n=168)**	**Biofilm producers (n=42)**	**Non biofilm producers (n=126)**
Sputum	58(34%)	18(31%)	40(69%)
Pus	44(26%)	11(25%)	33(75%)
Urine	42(25%)	10(24%)	32(76%)
Wound swabs	15(8.9%)	2(13.3%)	13(86.6%)
HVS	6(3.5%)	1(16.6%)	5(83.3%)
ET secretions	3(1.7%)	0	3(100%)

On assessment of antimicrobial susceptibility testing, above 90% of isolates were sensitive to amikacin, gentamicin, 80% of sensitivity shown towards meropenem, around 75% of isolates were sensitive to imipenem. MDR percentage among Pseudomonas isolates noted was 48.2% (Table 3).
Biofilm producers showed higher antibiotic resistance pattern to various antibiotics tested when compared to non biofilm producers (Table 3).

Table 3. Antibiotic susceptibility pattern of both biofilm and non biofilm producers.

Antimicrobial	Biofilm Producers sensitivity no. (%) (n=42)	Biofilm non producers sensitivity no. (%) (n=126)	Total no. of sensitive isolates (%) (n=168)
Amikacin	35 (83.3%)	124 (98.4%)	159 (94.6%)
Gentamicin	31 (73.8%)	123 (97.6%)	154 (91.6%)
Ciprofloxacin	11 (26.1%)	101 (80.1%)	112 (66.6%)
Ceftazidime	9 (21.4%)	69 (54.7%)	78 (46.4%)
Cefepime	14 (33.3%)	78 (61.9%)	92 (54.7%)
Aztreonam	11 (26.1%)	54 (42.8%)	65 (38.6%)
Imipenem	35 (42.2%)	90 (71.4%)	125 (74.4%)
Meropenem	24 (57.1%)	112 (88.8%)	136(80.9%)
Piperacillin/ tazobactam	14 (33.3%)	78 (61.9%)	98 (58.3%)
Cefaperazone/sulbactum	16 (38.09%)	84 (66.6%)	100 (59.5%)

Biofilm producers showed high multidrug resistance than non biofilm producers. Among 42 biofilm producers, 66.6% (28/42) were weakly adherent, 10 (23.8%) were moderate adherent and remaining 4 (9.5%) were strongly adherent. Categorical variables of biofilm producers and non biofilm producers in relation to multidrug resistance pattern found the chi-square statistic is 17.5541. The p-value is 0.000028 which is statistically significant (Table 4).

Table 4. Susceptibility pattern of Biofilm and non biofilm producers.

Susceptibility pattern	Biofilm producer (n=42)	Non biofilm producer (n=126)
Sensitive isolates - 87(51.7%)	10 (11.4%)	77 (88.5%)
MDR isolates - 81(48.2%)	32 (39.5%)	49 (60.4%)

DISCUSSION

Pseudomonas aeruginosa are posing a major threat to health care settings as they have high potential for antimicrobial resistance. Most of these pathogens are multidrug resistant particularly in debilitating and immunocompromised hosts [7]. Antimicrobials are the only efficient choice to treat the infections, but the resistance against antimicrobials of various pathogens is increasing since many decades. Antimicrobial resistance in clinical settings poses a significant challenge to health care providers. Addition to these Pathogens like *Pseudomonas aeruginosa*, *Acinetobacter baumanii, Stenotrophomonas maltophila* inherent resistant patterns are different and significant when compared to other pathogens. The increase in prevalence of such pathogens are often associated with severe infections, particularly in immunocompromised patients, and can lead to prolonged hospital stays, increased healthcare costs, and higher morbidity and mortality rates [8].
In this study a total of 1440 clinical isolates were studied. Among 1440 examined samples. A total 168 (11.6%) clinical isolates of *Pseudomonas aeruginosa* were isolated. Similar to this study, a tertiary care centre study *Pseudomonas aeruginosa* isolates among 1049 clinical samples observed 68 (6.48%) (4.99-7.97, 95% CI) *Pseudomonas aeruginosa [9]. Shidiki A et al [10] noted 4.15% of prevalence rate of Pseudomonas isolation. Shrestha M [11] et al observed 8.59% of Pseudomonas isolates in various clinical specimens. Saroj Golia et al [12] noted high prevalence rate of Pseudomonas aeruginosa from their clinical isolates, it was 24%.*
Out of 168 isolates, maximum number were from sputum 58 (34.5%) followed by pus 44(26.1%) urine 42 (25%), wound swabs 15 (8.9%), high vaginal swabs (HVS) 6 (3.5%) and ET secretions of 3 (1.7%) isolates. Maharjan N et al [9] studied that urine (29.41%) and pus (19.11%) specimens has majority of Pseudomonas aeruginosa isolates, followed by stone (11.76%), blood (11.76%), sputum (10.29%), high vaginal swabs (10.29%), and ET secretions (7.36%). Agarwal S [13] and Bezalwar PM et al [14] noted significant isolation of *Pseudomonas* in urine and pus specimens. Koirala A et al [15] and Shidiki A et al [10] mentioned the highest isolation was from pus samples. Saroj Golia et al [12] found wound/pus (55.83%), sputum (20.83%), and tracheal aspirates (8.33%) were the predominant sources of specimens of *P. aeruginosa*.

Out of 168 Pseudomonas isolates 42 (25%) were Biofilm producers and remaining 126 (75%) were non biofilm producers. Among 42 biofilm producers, 66.6% (28/42) were weakly adherent, 10 (23.8%) were moderate adherent and remaining 4 (9.5%) were strongly adherent. A study from Brazil [16] did biofilm detection by tissue culture plate method documented that 77.5% (31/40) of the isolates were considered biofilm producers, being distributed in the following categories: 42.5% (17/40) weakly adherent, 27.5% (11/40) moderately adherent, and 7.5% (3/40) strongly adherent. Perez et al [17] noted biofilm production was present in 93.4% (85/91), being distributed in the following categories: 60% (51/85) poorly adherent, 25.9% (22/85) moderately adherent, and 14.1% (12/85) strongly adherent. Lima JLDC et al [18] study chosen both qualitative and quantitative methods for biofilm production, the qualitative technique showed that only 15% of the isolates were considered biofilm producers, while the quantitative technique showed that 75% of the isolates were biofilm producers. The quantitative technique was more effective than the qualitative technique for the detection of biofilm production. Kamali E et al [19] observed Biofilm phenotypes accounted for 83.75% (n = 67), being distributed in the following categories: 16.25% (n = 13) produced strong biofilm; 33.75% (n = 27) produced moderate biofilm; 33.75% (n = 27) produced weak biofilm, whilst 16.25% of isolates (n = 13) were identified as non-biofilm producer.

On assessment of antimicrobial susceptibility testing, above 90% of isolates were sensitive to amikacin, gentamicin, 80% of sensitivity shown towards meropenem, around 75% of isolates were sensitive to imipenem. MDR percentage among *Pseudomonas* isolates noted was 48.2%, that were resistant to more than 3 antibiotics belonging to different classes. Maharajan N et al noted high sensitivity to Polymyxin B 63 (92.64%) followed by imipenem 61 (89.70%) for *Pseudomonas aeruginosa.* Ceftazidime was found to be the least effective drug showing 44.1% sensitivity. 21 (30.9%) of isolated *Pseudomonas aeruginosa* were multidrug-resistant. Koirala A et al [15] did a study on ESBL and MBL mediated resistance of non fermenters isolated from clinical samples, in which 69.1% were MDR pathogens. Another study on Pseudomonas antibiotic susceptibility pattern noted 63.3% of multidrug resistant isolates [20]. Saroj Golia et al [12] documented among 112 isolated pathogens 35(31.25%) showed resistance to aminoglycosides, 30(26.78%) to cephotaxime. Resistance rates to Piperacilin/ tazobactam, ceftazidime, ofloxacin varied from 10-15(8.92% to 13.39%).10/112(8.92%) isolates were multidrug resistant. All strains were found to be sensitive to imipenam, colistin (100%). Srinivasan et al [21] found *P. aeruginosa* was resistant to beta lactams viz. cephalothin, carbeniciilin, ceftazidime (100%), and cephalexin (98%) respectively. Saha et al [22] stated *Pseudomonas* is most sensitive to beta lactams - imipenem (98.72%), followed by aztreonam (33.44%) and ceftazidime (38.32%). They reported the combination of antibiotics will yield better results due to synergistic effect, promising result against MDR *P.aeruginosa* was observed by using the combination of carbapenem, cefepime, or piperacillin+tazobactam, amikacin or tobramycin.

Biofilm producers showed higher antibiotic resistance pattern to various antibiotics tested when compared to non biofilm producers, the p-value is 0.000028 which is statistically significant. In line with our study, Rifa Parveen aimed at *Pseudomonas aeruginosa* biofilm and antimicrobial susceptibility pattern noted among 110 MDR isolates, 53 (48.2%) were biofilm producers and among 115 sensitive isolates, only 9 (7.82%) were biofilm producers [23]. Biofilms create a microenvironment that enhances survival under antimicrobial pressure, contributing to the persistence of infections. [24].

CONCLUSION

In this study we concluded *Pseudomonas aeruginosa* is a most commonly isolated from respiratory secretions, urine and pus. These isolates were highly sensitive to colistin, gentamcin, amikacin, meropenem and imipenem and around 50% of isolates are sensitive to ciprofloxacin, piperacillin+tazoabactum and cefaperazone+sulbactum. A combination of beta lactams and aminoglycosides may yield promising results to fight against MDR *P.aeruginosa*. Biofilm producers are highly resistant to antimicrobials when compared to non biofilm producers. To combat the drug resistant of *P.aeruginosa* a stringent measures of infection control policies and microbiological screening to start accurate therapy during the critical time will definitely help. Regular anitbiogram plotting in the hospital and early screening microbiological screening methods implementation will aid clinicians to give the prompt and accurate treatment to patients.

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