ISSN (P&E): 1996-7195, (O): 2957-899X DOI: https://doi.org/10.53350/pjmhs02026202.6

ORIGINAL ARTICLE

Comparative Antibiotic-Resistance Patterns in Environmental vs. Clinical Isolates of *Pseudomonas aeruginosa* in Tertiary Hospitals

MALIK EJAZ AHMED KANHOON[1], AQSA MEHMOOD[2], IBRAR AHMAD[3]
[1]HOD, Department of Peads, Indus Hospital, Chowk Qureshi, Multan
[2]Nishtar Medical University and Hospital, Multan.
[3]Bakhtawar Amin Medical College, Multan and Multan Institute of Kidney Diseases (MIKD), Multan. **Email:** dr.mr.abrarahmad@gmail.com
Correspondence to: Aqsa Mehmood, **Email:** aqsamehmood33@gmail.com

This article may be cited as:
Kanhoon MEA, Mehmood A, Ahmad I; Comparative Antibiotic-Resistance Patterns in Environmental vs. Clinical Isolates of Pseudomonas aeruginosa in Tertiary Hospitals. Pak J Med Health Sci, 2026; 20(02): 28-33.

Received: 02-09-2025
Accepted: 15-02-2026
Published: 28-02-2026

ABSTRACT

Background: *Pseudomonas aeruginosa* is a prominent hospital-acquired infection and is famously known due to its ability to survive damp hospital settings and develop multidrug resistance. Sinks, drainage outlets and medical equipment can also be considered as environmental reservoirs capable of favoring the persistence and spread of resistant strains. The comparison of the resistance profiles among environmental and clinical isolates is essential in terms of the possibility of identifying possible links in the transmission and informing the infection-control measures in the tertiary-care hospitals.

Objectives: The aim of the study was to compare the antibiotic resistance profile of environmental and clinical isolates of P. aeruginosa, establish the prevalence of multidrug resistant (MDR) and extended spectrum 10 -lactamase (ESBL) producing strains, and evaluate the possible role of hospital environments in antimicrobial resistance.

Methods: The study was carried out as a cross-sectional study involving tertiary-care hospitals in Punjab, Pakistan between June 2024 and April 2025. One hundred and 0 isolates were analyzed, including 50 clinical and 50 environmental isolates. The identification was done based on the usual culture features and biochemical tests, which were facilitated by automated systems. The antimicrobial susceptibility testing was performed in the format of Kirby-Bauer disk diffusion based on CLSI 2024. The detection of ESBL was done based on the double-disk synergy test and the MDR classification was done on the basis of internationally accepted definitions.

Results: Clinical isolates were more resistant to carbapenems, cephalosporins, amino-glycosides and fluoroquinolones in comparison with environmental isolates. The resistance levels of carbapenem (imipenem 52; meropenem 48) and ciprofloxacin resistance (62) were high in clinical samples compared with environmental isolates (28, 26 and 38, respectively). The prevalence of MDR was 58 percent in clinical isolates and 32 percent in environmental isolates the production of ESBL was also found in 30 percent of clinical and 14 percent of environmental isolates.

Conclusion: A much higher degree of antibiotic resistance was observed in clinical isolates but the existence of MDR and ESBL-positive environmental isolates demonstrates the significance of environmental reservoirs in maintaining resistant P. aeruginosa in hospitals which requires more aggressive infection-control and stewardship initiatives.

Keywords: *Pseudomonas aeruginosa*, antibiotic resistance, environmental isolates, clinical isolates, MDR, ESBL, tertiary hospitals.

INTRODUCTION

Pseudomonas aeruginosa is a Gram-negative opportunistic pathogen and continues to be the leading cause of healthcare-associated infections, specifically in intensive care units, surgical wards, and in immunocompromised patients[1]. It is highly resilient to its environmental niche and therefore one of the most tenacious in hospital environments especially in moist settings like sinks, water outlets, drainpipes, disinfectant containers, and mechanical ventilator circuits. This environmental strength is a major contributor to cross-contamination, colonization of the susceptible patients and the eruption of outbreaks in tertiary-care units[2].

Clinical challenges have been further augmented by the emergence of antimicrobial resistance (AMR) in P. aeruginosa all over the globe[3]. The organism is intrinsically endowed with various resistance systems such as low outer-membrane permeability, efflux pumps, biofilm production, and has the capacity to obtain other resistance determinants such as carbapenase and ESBL genes. Consequently, treatment choices are becoming smaller, especially in healthcare systems, which have limited resources and where surveillance programs are not well developed. Repeatedly recorded surveillance data in Pakistan show that in spite of extended hospitalization, morbidity, and rising healthcare costs, there is an increasing resistance to cephalosporins, carbapenems, fluoroquinolones, and aminoglycosides across clinical isolates of P. aeruginosa[4].

Clinical isolates have been well-researched whereas the environmental reservoirs in hospital environments have been relatively unexplored regardless of their possible contribution as silent reservoirs of multidrug-resistant (MDR) strains[5]. Environmental contamination may act as a persistent reservoir where resistant strains may survive and be reintroduced into clinical circulation by direct contact, equipment used in a procedure, or infected water routes. The value of comparative analysis of environmental and clinical isolates is therefore critical in determining whether the hospital environments are merely hosting background flora or effectively involved in the epidemiology of antibiotic-resistant P. aeruginosa[6].

The current study aims to address this gap by comparing the patterns of antibiotic resistance between P. aeruginosa isolates collected at the environmental and those collected as clinical isolates obtained in tertiary hospitals in a systematic manner[7]. The knowledge of pattern differences or similarities in resistance patterns will be used to determine possible linkages of transmission, assess the role of the environment in MDR infections, and inform hospital antimicrobial-stewardship policies and infection-control measures[8].

MATERIAL AND METHOD

Study Design and Setting

The study was a cross-sectional study with a laboratory-based comparative study that was carried out in selected tertiary-care hospitals in Punjab, Pakistan. The research was going to be done between June 2024 and April 2025. All microbiological tests, such as isolation, identification and antimicrobial susceptibility tests were done in the respective hospital microbiology laboratories as per standardized clinical procedures.

Sample Size and Sampling Strategy.

The study included 100 *Pseudomonas aeruginosa* isolates, 50 of them being clinical isolates and 50 of them environmental isolates. Patients admitted in intensive care units, surgical wards, medical wards and the outpatient departments had their clinical specimens collected in routine diagnostic sampling techniques which include wound swabs, urine, sputum, endotracheal aspirates and catheter tips. High-risk areas in the hospital environment were also environmental isolates such as sinks, water outlets, drainage pipes, ventilator tubing, suction apparatus, disinfectant containers, and frequently touched surfaces. Sterile and moistened swabs were used to get the environmental samples and they were transferred to the laboratory within one hour in order to preserve the viability.

Isolation and Identification of *Pseudomonas aeruginosa*

The samples obtained were all cultured in selective and differential media which included Cetrimide agar, MacConkey agar and blood agar. Plates were stored at 37 o C between 24 and 48 hours. The colonies with the characteristics of P. aeruginosa were identified according to their typical production of blue-green pigments, grape odor, positive oxidase test, and the capacity to grow at 42 o C. Subsequent biochemical validation, such as oxidative/fermentative reactions, was done as required. The isolates were finally identified with the aid of an automated identification system like VITEK-2 or any other similar platform in order to be accurate.

Antimicrobial Susceptibility Tests.

The KirbyBauer disk diffusion method was used to identify antibiotic susceptibility profiles of all isolates in MuellerHinton agar, as per Clinical and Laboratory Standards Institute (CLSI) 2024 guidelines. The antibiotic panel consisted of carbapenems(imipenem and meropenem), cephalosporins (ceftazidime and cefepime), combinations of 1-2-lactam /1-2-lactamase (piperacillin - tazobactam), aminoglycosides (amikacin and gentamicin), fluoroquinolones (ciprofloxacin and levofloxacin), and

polymyxins (colistin, measured through MIC determination). The zone diameters were analyzed in millimeters, and interpreted as either susceptible, intermediate or resistant based on standardized CLSI breakpoints.

Identification of ESBL Producing Strains.

The phenotypic double-disk synergy test was used to determine the production of extended-spectrum 2-lactamase (ESBL). Ceftazidime and cefotaxime disks were put beside clavulanic-acid combination disks in Mueller-Hinton agar. Any growth of 5 mm or above on the inhibition zone towards the clavulanate disk was considered ESBL positive.

MDR and XDR Strains Classification.

Resistance categories were classified in an international manner. Isolates that were resistant to at least one agent in three or more classes of antimicrobial agents were categorized as multidrug-resistant (MDR). Isolates that were resistant to all the antimicrobial classes that were tested with exception of polymyxins were classified as extensively drug-resistant (XDR). This was done through these classifications in both environmental and clinical isolates.

Quality Control Procedures

Pseudomonas aeruginosa ATCC 27853 was used as a control strain in verifying the antibiotic susceptibility testing through quality control measures. Sterility and expiration check All culture media, reagents and antimicrobial disks were checked prior to use. Standardization of environmental sampling procedures was done to reduce swab collection variability, transport variability and processing variability.

Data Analysis and Statistic.

All the laboratory results were included in Microsoft Excel and then analyzed with the help of SPSS version 25. Resistance frequency and pattern of distributions were determined using descriptive statistics. The chi-square test was used in comparing environmental and clinical isolates. The p-value of less than 0.05 was regarded as statistically significant in all the comparisons.

RESULTS

Overview of Isolates

During the course of the study, 100 isolates of *Pseudomonas aeruginosa* were obtained and these included 50 clinical and 50 environmental isolates. Wound swabs, endotracheal aspirates, urine samples, sputum, and catheter tips were used to acquire clinical isolates, and sinks, drainage outlets, water sources, and ventilator tubing, and frequently touched hospital surfaces were used to obtain environmental isolates.

The P. aeruginosa culture positivity was similar in all the two categories, thus, validating the continued existence of the organism in care areas of patients and in the hospital setting.

There was great resistance difference between clinical and environmental isolates as clinical isolates exhibited greater resistance to most antibiotic classes. The exact distribution of the resistance is presented in Table 1 and the discussion on the interpretation is presented below. The prevalence of carbapenem resistance was significantly greater in clinical isolates where the resistance of imipenem and meropenem were 52 percent and 48 percent respectively against 28 percent and 26 percent in environmental isolates. The same tendency was noted in cephalosporins, the use of β-lactam/ 8-lactamase inhibitor complexes, aminoglycosides and fluoroquinolones which means that patient-derived isolates are subjected to increased pressure of antibiotic selection. Even the environmental isolates, which were less resistant, were highly resistant, which points to the hospital environment as a reservoir of resistant strains.

Table 2 shows the distribution of MDR and XDR isolates between clinical and environmental samples. Most of the clinical isolates (58%) were MDR in contrast to 32% of the environmental isolates. Clinical samples also had more XDR strains (12% vs. 4%). This trend indicates that with repeated exposure of antibiotics in hospitalized patients, there is selective pressure of highly resistant strains, but environmental niches have a smaller share of XDR variants. Extended-spectrum β-lactamase (ESBL) production detection expressed a clear difference between the two groups of isolates. The Table 3 summarizes the results. The production of ESBL was over two times higher in clinical isolates (30%), than in environmental isolates (14%). This is an indication of increased evolutionary pressure among the group of patients and increased exposure to third-generation cephalosporins, which promotes ESBL selection.

The chi-square analysis revealed statistically significant differences ($p < 0.05$) of carbapenems, cephalosporins, aminoglycosides, and fluoroquinolones and combinations of β-lactam/ β-lactamase inhibitors between clinical and environmental isolates. There was no significant difference ($p > 0.05$) in colistin resistance which is in line with its retained efficacy.

These results indicate that environmental isolates moderate resistance whereas clinical isolates are always at a higher resistance level, which proves the effect of selective pressure of antibiotics in patients admitted in hospitals.

In this study, there is a distinct tendency of high resistance to antibiotics among clinical isolates of *Pseudomonas aeruginosa* relative to environmental ones. Nonetheless, the significant occurrence of MDR isolates in hospital setting shows that environmental reservoirs play a role in the maintenance and even propagation of resistant strains in the healthcare facilities. The aggregate outcomes of Tables 1-3 support the essence of regular monitoring of environmental conditions and effective antimicrobial stewardship strategies.

Table 1. Antibiotic Resistance Patterns in Clinical vs. Environmental Isolates of *P. aeruginosa*

Antibiotic Class	Antibiotic	Clinical Isolates Resistant (n=50)	Environmental Isolates Resistant (n=50)
Carbapenems	Imipenem	52%	28%
	Meropenem	48%	26%
Cephalosporins	Ceftazidime	60%	36%
	Cefepime	54%	30%
β-lactam/β-lactamase inhibitor	Piperacillin–Tazobactam	46%	22%
Aminoglycosides	Amikacin	44%	20%
	Gentamicin	56%	32%
Fluoroquinolones	Ciprofloxacin	62%	38%
	Levofloxacin	58%	34%
Polymyxins	Colistin	4%	0%

Table 2. MDR and XDR Classification of *P. aeruginosa* Isolates

Resistance Category	Clinical Isolates (n=50)	Environmental Isolates (n=50)
MDR	58%	32%
XDR	12%	4%

Table 3. ESBL-Producing *Pseudomonas aeruginosa* Isolates

Category	ESBL Positive	ESBL Negative
Clinical isolates (n=50)	30%	70%
Environmental isolates (n=50)	14%	86%

DISUSSION

The current research offers a comparative analysis of the antibiotic-resistance trends in environmental and clinical isolates of *Pseudomonas aeruginosa* collected in tertiary-care hospitals in Punjab, Pakistan[1]. The results have shown a very clear and consistent pattern, the clinical isolates provide significantly higher rates of resistance in most antibiotic classes than environmental ones[2]. Even though it is not unexpected because of the selective pressure caused by antibiotic treatment in hospitalized patients, the presence of moderate to high-resistance of environmental isolates highlights an essential epidemiological connection between environmental reservoirs and patient infections[3].

The high resistance levels of carbapenems, cephalosporins, fluoroquinolones, and aminoglycosides on clinical isolates is ind icative of high usage of these antibiotics in tertiary care units[4]. Carbapenems are most often taken as a last-line agent and their excessive use is closely linked to the development of carbapenem-resistant P. aeruginosa (CRPA). The results of the study with high rates of imipenem and meropenem resistance are similar to the reports of the national surveillance[5], which record the increasing levels of carbapenem resistance in Pakistani hospitals. The processes that lead to this resistance (i.e.: loss of porin-channels (OprD), overexpression of efflux pumps, and expression of carbapenemases) could be more actively selected in patients who receive repeated broad-spectrum antibiotic therapy[6].

The environmental isolates, in contrast, although showing less resistance, also showed a high percentage of MDR strains[7]. The possible causes of environmental contamination are improper disinfection, the existence of biofilm in water outlets, and repeated contact with hospital waste or aerosolized organisms through respiratory equipment. P. aeruginosa is quite famous to develop strong biofilms which provides inherent resistance to antibiotics and disinfectants, which allows the bacterium to endure over a prolonged period in the moist hospital environments like sinks, faucets and drainage pipes[8]. This reservoir is also crucial in reintroducing the resistant strains back into the patient care areas, and so creates a loop of transmission[9].

Of interest is the observation that 14 percent of environmental isolates were ESBL producers[10]. Horizontally transmitted owing to their presence as ESBL genes, which are often plasmid-mediated, the spread of resistance phenotypes to environmental and clinical isolates is also at risk. The relative higher rate of ESBL among clinical isolates

(30) is consistent with exposure of patients to third-generation cephalospin, which implies that treatment pressure increases the rate of selection in favor of ESBL-positive isolates[11].

The fact that both clinical (58%) and environmental (32%) categories of MDR isolates are present means that the hospital environment is not only contaminated, but it actively facilitates the survival of resistant strains[12]. These results indicate the potential bi-directional pathways of transmission: environmental reservoirs can infect patients, and vice versa, clinical isolates can contaminate surfaces due to poor practices of infection control[13].

The findings indicate that there is urgent necessity to develop extensive infection-prevention interventions, such as frequent monitoring of the environment through microbiological tests, observation of cleaning and sterilization practices, and streamlining of antimicrobial stewardship interventions to avoid unjustified exposure to antibiotics[14]. Better surveillance of wet surfaces, drains and respiratory equipment is especially essential since they are always inhabited by P. aeruginosa biofilms[15]. Routine disinfection measures and protocols audit or specific measures, e.g., sink redesigning or anti-biofilm coverage may further reduce the environmental footprint[16].

In general, the current research paper reinforces the thesis that antibiotic resistance in P. aeruginosa should not be viewed solely in the context of clinical-treatment aspect but also in the terms of environmental and infrastructural measures[17]. A two-fold emphasis on the environmental decontamination and the rational use of antibiotics is necessary to reduce the prevalence of MDR and XDR P. aeruginosa in healthcare facilities[18-20].

CONLUSION

This paper has illustrated that *Pseudomonas aeruginosa* isolates that are obtained in clinical specimens in tertiary-care hospitals are much more resistant to antibiotics than those obtained in the environmental setting. The identification of MDR and ESBL-producing strains in hospital settings though, demonstrates that environmental reservoirs are a vital concern in sustaining and possibly transferring of resistant organisms to the patients.

The similarities in the resistance patterns between the two groups of the isolates indicate that there might be an ecological interdependence between the infections of the patients and the contaminated water systems or hospital surfaces. These results indicate that stern infection- control measures, frequent surveillance of the environment, better disinfection routines, and vigorous antimicrobial stewardship initiatives are required to curb the development and dissemination of resistant strains of P. aeruginosa in Punjab, Pakistan tertiary-care facilities.

The integration of interventions at patient level and environmental management will be necessary to minimize the clinical effects of multidrug-resistant P. aeruginosa and to preserve the efficacy rates of the existing antimicrobial agents.

DECLARATION

Funding: None.

Conflicts of interest: None declared

Acknowledgment

The authors gratefully acknowledge the participation of the community members and the support of the data collection and laboratory teams. Appreciation is extended to local authorities and supervising faculty for their guidance throughout the research.

REFERENCES

1. Popovic M, Milosevic I, Vlahovic P, et al. Molecular mechanisms of carbapenem resistance in *Pseudomonas aeruginosa*. J Glob Antimicrob Resist. 2019;17:233-240. doi:10.1016/j.jgar.2019.05.019.
2. Ahmed N, Khan MA, Raza S. Prevalence of multidrug-resistant *Pseudomonas aeruginosa* in tertiary-care hospitals of Pakistan. Infect Drug Resist. 2020;13:341-349. doi:10.2147/IDR.S239841.
3. Levine DP, McGowan JE, Sharma A. Environmental reservoirs of *Pseudomonas aeruginosa* in intensive care units. J Hosp Infect. 2020;104:45-52. doi:10.1016/j.jhin.2019.10.007.
4. Bakhtiari E, Mirzaei R, Jamali A. Biofilm formation and antibiotic resistance in clinical isolates of *Pseudomonas aeruginosa*. Microb Pathog. 2020;149:104506. doi:10.1016/j.micpath.2020.104506.
5. Patel R, Singh H, Rahman S. Antibiotic susceptibility profiles of *Pseudomonas aeruginosa* isolated from hospital water systems. Water Res. 2021;188:116568. doi:10.1016/j.watres.2020.116568.
6. Khurram M, Hanif A, Shahid A. Rising trends in carbapenem-resistant *Pseudomonas aeruginosa* in Pakistan: a multicenter analysis. Pak J Med Sci. 2021;37:776-782. doi:10.12669/pjms.37.3.4290.
7. Otto M, Brown M, Foster T. Environmental contamination and transmission of resistant *Pseudomonas* species. Clin Microbiol Rev. 2021;34:e00221-20. doi:10.1128/CMR.00221-20.
8. Liang Y, Wu L, Li X. ESBL-producing *Pseudomonas aeruginosa*: prevalence, detection and clinical impact. Infect Genet Evol. 2021;89:104727. doi:10.1016/j.meegid.2021.104727.
9. Abbas A, Khan MS, Tahir S. Environmental burden of drug-resistant *Pseudomonas aeruginosa* in hospital intensive care units. J Infect Public Health. 2022;15:182-188. doi:10.1016/j.jiph.2021.11.005.
10. Santos CMA, Lima R, Ferreira J. Comparative resistance analysis of clinical and environmental *Pseudomonas aeruginosa* isolates. J Med Microbiol. 2022;71:001463. doi:10.1099/jmm.0.001463.
11. Malik S, Farooq U, Rizwan H. MDR and XDR profiles of *Pseudomonas aeruginosa* in tertiary hospitals in Lahore. Cureus. 2022;14:e24729. doi:10.7759/cureus.24729.
12. Choudhary R, Gupta A, Kumar N. Prevalence of ESBL-producing *Pseudomonas aeruginosa* in hospital wastewater and environmental surfaces. Int J Environ Res Public Health. 2022;19:7354. doi:10.3390/ijerph19127354.

13. Delfino J, Perez L, Martin R. Hospital sink contamination as a reservoir for *Pseudomonas aeruginosa*: a systematic review. Am J Infect Control. 2023;51:414-423. doi:10.1016/j.ajic.2022.10.010.
14. Imran M, Bashir F, Younas S. Comparative resistance patterns of environmental and clinical pathogens in Pakistani hospitals. J Appl Microbiol. 2023;134:lxad001. doi:10.1093/jambio/lxad001.
15. O'Neil K, Richardson S, Clarke M. Fluoroquinolone resistance mechanisms in *Pseudomonas aeruginosa*. Antibiotics (Basel). 2023;12:512. doi:10.3390/antibiotics12030512.
16. Saeed S, Naz S, Hussain R. Molecular epidemiology of multidrug-resistant *Pseudomonas aeruginosa* in South Asian hospitals. Front Microbiol. 2023;14:1123451. doi:10.3389/fmicb.2023.1123451.
17. Ahmadi A, Hasan A, Murtaza S. Resistance trends in clinical *Pseudomonas aeruginosa* isolates in developing countries. Infect Dis (Lond). 2024;56:45-54. doi:10.1080/23744235.2023.2268445.
18. Zhang R, Wei J, Li Q. Environmental monitoring identifies persistent reservoirs of drug-resistant *Pseudomonas aeruginosa*. Environ Sci Pollut Res. 2024;31:7251-7262. doi:10.1007/s11356-023-30555-2.
19. Fatima H, Javed M, Farhan M. ESBL-producing gram-negative organisms in Pakistan: clinical relevance and environmental sources. J Infect Dev Ctries. 2024;18:169-177. doi:10.3855/jidc.17199.
20. Yousaf A, Khan Z, Riaz M. Antibiotic resistance surveillance of *Pseudomonas aeruginosa* in tertiary-care hospitals: a multicenter evaluation. J Glob Infect Dis. 2025;17:22-30. doi:10.4103/jgid.jgid_221_24.