Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 6 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

Analysis of Antibiotic Susceptibility and Multidrug Resistance among Clinical Isolates of *Pseudomonas aeruginosa*

Kashif Ali Shar*

Shah Abdul Latif University Khairpur Sindh Pakistan. Corresponding Author Email: sharkashifali@gmail.com

Muhammad Juned Malik

University of Sindh Jamshoro Sindh, Pakistan. junaidkhanmalik4@gmail.com

Prof. Dr. Muhammad Atif Ata

MBBS, PhD (Biochemistry), Suleman Roshan Medical College Tandoadam

atifata@srmc.edu.pk

ABSTRACT

Background: *Pseudomonas aeruginosa* is one of the World Health Organization (WHO) priority pathogens of "greatest public health concern." It does not normally cause invasive infections in normal people but is often linked to severe hospital acquired infections, ventilator-associated pneumonia (VAP), and people who are on broad-spectrum antibiotic therapy, and critically ill intubated patients who have long hospital stays. **Objective**: In this study, the prevalence of *Pseudomonas aeruginosa* and molecular characterization of its antibiotic resistance genes in clinical isolates from blood and pus samples were determined. **Methods**: From December 2025 to March 2026, 20 clinical samples were collected from tertiary care hospitals, Khairpur, Sindh, Pakistan. The samples were processed to isolate and identify *P. aeruginosa* and molecular analysis was carried out for the identification of resistance related genes. **Results**: Out of 20 samples, *Pseudomonas aeruginosa* was isolated in 12 cases (60%). The strains were multidrug-resistant (MDR) with resistance to several classes of antibiotics. **Conclusion:** The study shows high prevalence of MDR *P. aeruginosa* in clinical samples from study area. The results highlight the importance of ongoing surveillance of resistance genes and judicious choice of effective antibiotic classes to treat MDR infections.

Keywords: *Pseudomonas aeruginosa*, Multidrug-resistant (MDR), Gram-negative

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 6 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

bacteria, Antibiotic-resistance

Introduction

Pseudomonas aeruginosa has been identified by the World Health Organization (WHO) as a "species of greatest public health concern. While *P. aeruginosa* seldom causes serious infections in healthy people, it is linked to a number of invasive illnesses in patients who have been hospitalized for an extended period of time. patients with ventilator-associated pneumonia, those receiving cancer chemotherapy or wide spectrum antibiotic therapy, and critically ill intubated patients (World Health Organization, 2017). The prevalence of *P. aeruginosa* resistance to antipseudomonal antibiotics has been increasing with the passage of time in many parts of the world. The incidence of *P. aeruginosa* infections within a few days of starting antimicrobial therapy is greatly influenced by antibiotic resistance in the intensive care unit (ICU). Moreover, the pre-isolation co-infection with one or more microorganisms, total parenteral nutrition, comorbid cerebrovascular disease, history of cerebrovascular disease, admission in intensive care unit, cancer, compromised immune system, and obstruction coronary disease are known risk factors for *P. aeruginosa* infection (Alhussain *et al*, 2019). Additionally, linked include mechanical ventilation, severe respiratory failure, infection sites in the central venous catheter and respiratory tract, intubation, and the use of several invasive devices. However, *P. aeruginosa* has several resistance mechanisms. Thus, antimicrobial therapy is limited (Kollef *et al*, 2014).

Literature Review

Pseudomonas aeruginosa is a Gram-negative, opportunistic pathogen that is commonly found in hospital-acquired infections including bloodstream, ventilator-associated pneumonia, urinary, and wound infections. It has inherent resistance mechanisms, and can quickly become resistant to several classes of antibiotics, therefore treatment can be challenging and is often restricted to last resort antibiotics, which is a particular concern in clinical microbiology. The recent data showed that *P. aeruginosa* is one of the top 5 MDR microorganisms in health-care associated infections (HAIs) worldwide, resulting in substantial morbidity and mortality rates, as well as an extended length of stay (Schwartz et al., 2024).

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 6 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

Global burden of antimicrobial resistance in Pseudomonas aeruginosa

Antimicrobial resistance (AMR) of *P. aeruginosa* is a worldwide problem. The results of systematic reviews indicate that resistance rates are highly variable between geographic regions, hospitals and antibiotic usage practices. MDR *P. aeruginosa* prevalence can be as high as 80% in healthcare-associated-infections in some settings, as reported by a large meta-analysis, thus emphasizing its critical public health importance (Asmare et al., 2024).

The organism is highly adaptable to hospital setting, especially ICU, where there is a lot of antibiotic pressure. The trend of increasing resistance over time has been reported in studies and particularly for the commonly used antibiotics, ceftazidime, ciprofloxacin and gentamicin.

Multidrug-Resistant (MDR) *Pseudomonas Aeruginosa*

Multidrug-resistant (MDR) *Pseudomonas aeruginosa* is defined as resistance to at least one agent in three or more antimicrobial categories, and its prevalence is reported to be alarmingly high in hospital settings worldwide. A systematic review has shown that MDR rates can exceed 70–80% among clinical isolates from healthcare-associated infections, reflecting a serious and growing therapeutic challenge (Asmare et al., 2024). These resistant strains are particularly prevalent in high-risk clinical areas such as intensive care units (ICUs), burn wards, among immunocompromised patients, and in individuals with long-term catheterization. Infections caused by MDR *P. aeruginosa* are strongly associated with increased mortality rates, prolonged illness, and significantly limited treatment options, making them a major concern for modern healthcare systems.

Clinical Implications

The rise of antibiotic resistance in *Pseudomonas aeruginosa* has significant clinical consequences, including increased treatment failure rates, a marked reduction in available therapeutic options, higher healthcare costs, prolonged hospital stays, and increased mortality particularly among critically ill and immunocompromised patients. In many cases, clinicians are forced to rely on last-line therapies such as colistin and combination antibiotic regimens; however, emerging resistance to even these reserve drugs are becoming an additional concern, further complicating the

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 6 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

management of severe infections caused by this pathogen.

Recent Advances and Future Perspectives

Recent research has increasingly focused on alternative treatment strategies to address multidrug-resistant Pseudomonas aeruginosa, including the development of novel antibiotics that target outer membrane proteins, the use of antimicrobial peptides as membrane-disrupting agents, and the application of bacteriophage therapy as a highly specific antibacterial approach. In addition, significant attention has been given to biofilm-disrupting agents aimed at breaking down the protective biofilm matrix that enhances bacterial survival and antibiotic tolerance. Combination antibiotic therapies are also being explored to achieve synergistic effects and reduce the likelihood of resistance development. Furthermore, newer therapeutic candidates such as LptD-targeting compounds have demonstrated promising activity against MDR strains, including those resistant to Carbapenems, highlighting their potential as future treatment options for difficult-to-treat infections (Schwartz *et al.*, 2024).

Pseudomonas is consistently shown in the literature as a high-risk pathogen with ever increasing multidrug resistance around the world. Resistance patterns differ from region to region, but there is a global trend towards a decrease in susceptibility to most commonly used antibiotics. Long-term monitoring, good antibiotic stewardship and the discovery of new antibiotics are necessary to prevent its proliferation and minimize its impact on outcome.

MATERIAL AND METHOD

Sample Collection

Patients with wound, burn, and pus infections were the subjects of sample collection. samples were taken from patients admitted to the District Hospital in Khairpur, Sindh, Pakistan's Intensive Care Unit (ICU), medical ward, Outpatient Department (OPD), and Operation Theatre (OT) under aseptic settings. A thorough foundation for ensuing microbiological analysis and antibiotic susceptibility testing was provided by this varied sampling method, which guaranteed representation across various hospital units and infection types.

Incubation

Samples were inoculated on nutrient brat. Citramide agar plates, incubated at 37c for

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 6 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

24 hours, and examined for morphology and microscopically.

Gram Staining

The microbial isolate ware colonies were spread on a glass slide, smeared, fixed with heat and flame, and then coated with crystal violet ware for 60 seconds before washing with tap water.

Gram's Iodine was applied to a smear for 60 seconds, followed by decolonization with ethanol or acetone for another 60 seconds, and then washed with tap water. The process involves adding safranin for 45 seconds and then washing it with tap water.

The bacteria were observed under a microscope at 400x and 1,000x oil immersion, with purple colonies indicating gram positive bacteria, and red or pink colonies indicating gram negative bacteria.

Phenotypic Identification

Pseudomonas aeruginosa was phenotypically identified using its distinctive colony morphology and specific growth patterns. Colonies looked paler and less defined after the initial inoculation, which was consistent with the organism's usual early growth stage. After additional confirmation they grow on Citramide agar, producing yellow color.

Antibiotic Susceptibility

Pseudomonas aeruginosa isolates were tested for antibiotic susceptibility using the Kirby-Bauer disk diffusion method in accordance with CLSI standards. Commercial antibiotic discs containing Amikacin, Tobramycin, Vancomycin, Imipenem, Fluoroquinolones, Colistin, Tazobactam, Linezolid, and Cefdinir were aseptically applied to the agar surface after pure cultures were inoculated onto Mueller–Hinton agar plates to produce a homogenous bacterial lawn. The plates ware incubated at 37°C for 24 hours. Then put antibiotic to diffuse into the medium and exert their inhibitory effects. After the incubation the diameter of the zone of inhibition of each disc ware measure in millimeters and interpreted as susceptible or resistance according to CLSI method.

Results

The study investigates the prevalence of pseudomonas aeruginosa in tertiary care

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 6 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

hospital in District Khairpur, Sindh, during the period of December 2025 to march 2026. Total 20 samples were collected from the patient across the different hospital wards including ICU, OPD and OT from different infections site such as burns, wound and pus. Out of these 20 samples 12 samples ware showing positive results for pseudomonas aeruginosa indicate a notable presence of this opportunistic pathogen within the hospital environment. Our finding highlight the clinical importance of pseudomonas aeruginosa in hospitalized patients. (shown in Table.1)

Table 1.

	Origin of sample	Nature of a samples	Number of samples	Total No of *P. aeruginosa* isolates
1	OPD	Pus	7	8
2	OT	Pus	8	5
3	ICU	Blood	5	2

Identification of Isolation

Pseudomonas aeruginosa was phenotypically identified using its distinctive colony morphology and specific growth patterns. Colonies looked paler and less defined after the initial inoculation, which was consistent with the organism's usual early growth stage. After additional confirmation they grow on Citramide agar, producing yellow color.

Gram's Staining

After carrying out standard Gram's staining technique the bacteria were observed under a microscope at 40x and 100x oil immersion, where *P. aeruginosa* colonies were observed as pink as shown in (Table 2).

Table 2: Gram staining of *P. aeruginosa*

Total No of isolates	Grams Negative
13	13

Antibiotic Susceptibility

Alarming resistance patterns were found in the antimicrobial susceptibility tests of isolated cultures of *Pseudomonas aeruginosa*, with total resistance against Colistin

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 6 (2026)

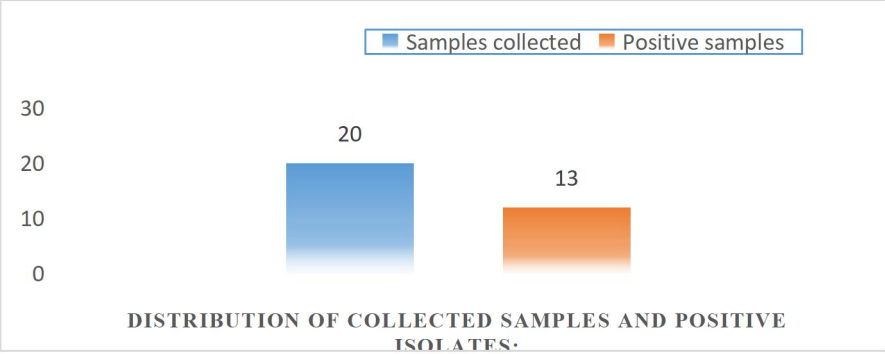
Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

(100%) highlighting the isolates' multidrug resistance. Amikacin (5.8 mm), Vancomycin (5.5 mm), Fluoroquinolones (5.6 mm), Imipenem (3.3 mm), Tobramycin (6.5 mm), Gentamicin (6.0 mm), Tazobactam (6.9 mm), Linezolid (7.5 mm), and Cafdnir (6.8 mm) as (shown in table 3 & Fig 2&3). All had significantly smaller measured zones of inhibition, suggesting poor efficacy. The restricted therapeutic options against these strains are highlighted by the fact that these values are much below the standard interpretative criteria for susceptibility. The results highlight the critical need for ongoing monitoring of resistance patterns, prudent antibiotic usage, and investigation of alternate treatment approaches to counter the escalating threat of multidrug-resistant *P. aeruginosa*.

Table 3: *Antimicrobial resistance of isolated strains against selected antibiotics*

Antibiotic tested	Zone inhibition measured (mm)	Interpretation (Tentative) as per CSLI guiddines
Amikacin (AK) (30 µg)	5.8mm	Resistant
Tobramycin (TOB)(10 µg)	6.5mm	Resistant
Vancomycin (VA)(30 µg)	5.5mm	Resistant
Imipenem (IMP)(10 µg)	3.3mm	Resistant
Fluoroquinolones (F)(300 µg)	0 mm	Completely Resistance

Distribution of Collected and Positive Isolates



Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 6 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

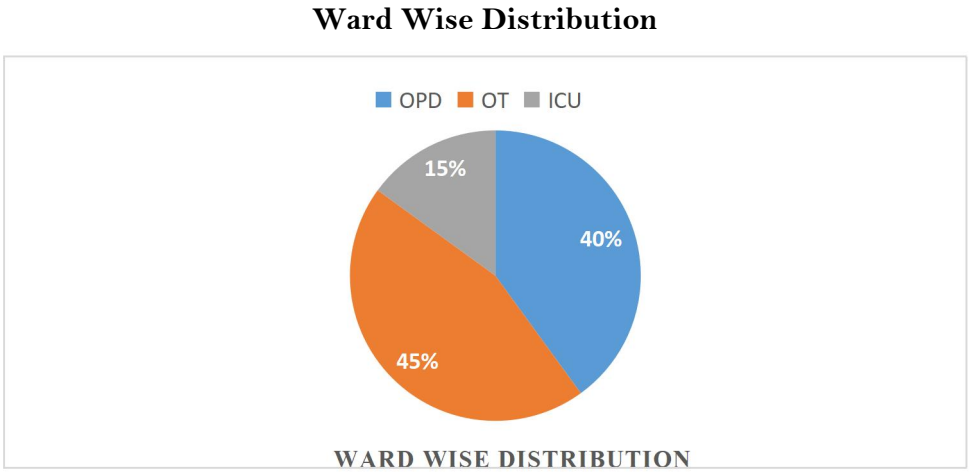
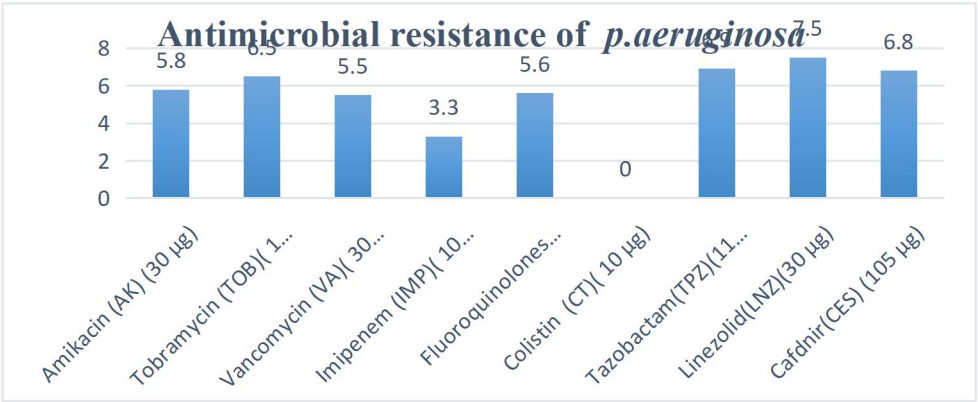


Figure. 1: Antimicrobial resistance of the isolated strains



Discussion

P. aeruginosa is an opportunistic pathogen. It has been reported to infect patients in hospital settings primarily in intensive care units, patients with catheterization and patients with burn and/or chronic diseases. *P. aeruginosa'* capacity to grow at low nutritional needs and tolerate different physical conditions has enabled this organism to survive in both community and hospital environments. However, it rarely infects healthy tissues, but if defenses are lowered. This is the reason why most infections are nosocomial. *P. aeruginosa* inherently resistant to many antimicrobials. It is intrinsically resistant to many antimicrobial agents and this can cause a serious therapeutic problem as it can develop multidrug resistance and mutational acquired resistance to antibiotics via chromosomal mutations. A number of antimicrobial agents, including several Beta–lactams are active against *P. aeruginosa*. Extended-spectrum Penicillin used to treat infection by this bacterium

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 6 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

(Shbaita et al., 2023).

Prevalence of *P. aeruginosa* strain in tertiary hospitals of Khairpur, Sindh, Pakistan was studied. The important of p. aeruginosa, especially in infected patient, is related to their insufficient immune response as well. These *P. aeruginosa* are more difficult, because of the acquisition of mobile genetic element, through which they have acquired both intrinsic resistance and acquired resistance. Based on different study, it is evident that the emergence of resistance *P. aeruginosa* strain is increasing worldwide.

Most Cephalosporins do not have an activity against *P. aeruginosa*, but Ceftazidime (third generation) and Cefepime (4th generation) are highly active and have similar activity. Meropenem is slightly more active against P. aeruginosa than the other Carbapenems, which are a group of broad spectrum β-lactam antibiotics. Ciprofloxacin is the most active of the fluoroquinolones agents on P. aeruginosa. Finally, the Aminoglycosides have been mainstays in the treatment of these infections. But, the adaptability of *P. aeruginosa* has created a challenge in the sensitivity of the microbial identification system and the development of genotype-based characterization systems has now become a necessity which are apt to identifying the bacteria even after phenotypical changes have occurred. So, molecular identification eliminates the problem of variable phenotype and allows for more accurate identification of bacteria (Church et al., 2020).

Conclusion

The study shows high prevalence of MDR *P. aeruginosa* in clinical samples from study area. The results highlight the importance of ongoing surveillance of resistance genes and judicious choice of effective antibiotic classes to treat MDR infections.

Recommendations

The study emphasizes that improved surveillance is needed for *P. aeruginosa* in the clinical setting to track its prevalence and resistance trends. To decrease the use of inappropriate antibiotics and to slow the rise of multidrug resistance, antibiotic stewardship (ASP) programs must be enforced and implemented. Furthermore, strict infection control measures, such as adhering to hygiene protocols,

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

implementing sterilization procedures, and isolating patients as per the need, should be taken to prevent nosocomial transmission. It is also highly recommended that treatment guidelines for empirical treatment be reviewed regularly using local resistance data so that they remain therapeutically effective. Lastly, more studies need to be focused on the discovery of new antimicrobial drugs and alternative therapeutic approaches such as combination therapy to combat the threat of multidrug resistant *P. aeruginosa.*

References

Alhussain F, Yenugadhati N, Al Eidan F, Al Johani S and Badri M (2019) Risk factors, antimicrobial susceptibility pattern and patient outcomes of Pseudomonas aeruginosa infection: A matched case-control study. J. Infect. Publ. Hlth. 14(1), 152- 157.

Asmare, Z., Reta, M. A., Gashaw, Y., Getachew, E., Sisay, A., Gashaw, M., et al. (2024). Antimicrobial resistance profile of Pseudomonas aeruginosa clinical isolates from healthcare-associated infections: A systematic review and meta-analysis. PLOS ONE, 19(8), e0308946. https://doi.org/10.1371/journal.pone.0308946

Church, D. L., Cerutti, L., Gürtler, A., Griener, T., Zelazny, A., & Emler, S. (2020). Performance and application of 16S rRNA gene cycle sequencing for routine identification of bacteria in the clinical microbiology laboratory. Clinical microbiology reviews, 33(4), 10-1128.

Kollef M, Shorr A, Tabak Y, Gupta V, Liu L and Johannes R (2005) Epidemiology and outcomes of health-care-associated pneumonia: results from a large US database of culture-positive pneumonia. Chest 128(6), 3854-3862

Schwartz, B., Klamer, K., Zimmerman, J., Kale-Pradhan, P. B., & Bhargava, A. (2024). Multidrug resistant Pseudomonas aeruginosa in clinical settings: A review of resistance mechanisms and treatment strategies. Pathogens, 13(11), 975. https://doi.org/10.3390/pathogens13110975

Shbaita, S., Abatli, S., Sweileh, M. W., Aiesh, B. M., Sabateen, A., Salameh, H. T., ... & Zyoud, S. E. H. (2023). Antibiotic resistance profiles and associated factors of Pseudomonas infections among patients admitted to large tertiary care

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 6 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

hospital from a developing country. Antimicrobial Resistance & Infection Control, 12(1), 149.

World Health Organization (WHO) (2017) Publisheslist of bacteria for which new antibiotics are urgently needed. Short Summary 25FebET NM WHO.pdf/. Accessed 24 January 2019