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ISOLATION AND PHENOTYPIC CHARACTERIZATION OF MULTI-DRUG-RESISTANT PSEUDOMONAS AERUGINOSA FROM HEALTHCARE SETTING

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Abstract

Multidrug-resistant (MDR) *Pseudomonas aeruginosa* is an important cause of healthcare-associated infections, particularly among critically ill and immunocompromised patients. The development of resistance to multiple antimicrobial classes creates a serious challenge for effective infection control. A total of 50 clinical and hospital environmental samples were obtained from the Bahria International Hospital, Lahore. The isolates were identified as *P. aeruginosa* using conventional microbiological procedures, including growth on selective culture media, Gram staining, and biochemical tests. Antibiotic susceptibility was evaluated by the Kirby-Bauer disc diffusion method using commonly prescribed antibiotics, and isolates resistant to three or more classes of antimicrobial agents were classified as multidrug resistant according to the established guidelines. *P. aeruginosa* was detected in 11 (22%) out of 50 samples analyzed. Antibiotic susceptibility testing showed that isolates were resistant to several antibiotics. Resistance was 100% to Cefepime, Imipenem, and Levofloxacin; 90.9% to Polymyxin B and Colistin; 72.7% to Meropenem and Tobramycin; 63.6% to Gentamicin; 36.4% to Piperacillin and 27.3% to Ciprofloxacin and Aztreonam. These findings showed the prevalence of MDR *P. aeruginosa* in both clinical and hospital environmental samples. The high level of antimicrobial resistance observed among the isolates highlights an

increasing threat of MDR pathogens in healthcare settings. Regular microbiological surveillance, antimicrobial susceptibility testing, strict infection control practices, and antimicrobial stewardship programs are important to reduce the spread of MDR *P. aeruginosa*, improve patient care, and support appropriate empirical antibiotic therapy.

INTRODUCTION

Pseudomonas aeruginosa is a motile, rod-shaped, Gram–negative opportunistic bacterium that is commonly found in a wide range of natural and man-made environments. It is frequently present in soil, water, plants, rivers, swimming pools, hot tubs, sewage treatment facilities, and hospital wastewater. The broad metabolic capabilities of *P. aeruginosa*, together with its remarkable adaptability, enable the organisms to colonize diverse ecological niches. In healthcare settings and treated wastewater systems, biofilm formation enhances its survival by reducing the effectiveness of conventional disinfection practices (Grobe et al., 2001). Hospital wastewater and clinical settings serve as an important reservoir of multidrug-resistant *P. aeruginosa,* facilitating the persistence and transmission of resistant strains throughout healthcare facilities (Roulová et al., 2022). Through its exceptional adaptive capacity and continuous acquisition of antimicrobial resistance, *P. aeruginosa* has become a significant healthcare-associated pathogen. These infections often lead to increased disease severity, high mortality rates, extended hospitalization, and substantial economic burden on the healthcare system (Tabak et al., 2019). Owing to its increased prevalence, the Centers for Disease Control (CDC) and Prevention has designated multidrug-resistant *P. aeruginosa*, a serious threat to public health (Yang et al., 2023). *P. aeruginosa* is responsible for nearly 10% of hospital-acquired infections worldwide and is considered the fourth most common cause of healthcare-associated infection (Sawant et al., 2026). Compared with antimicrobial susceptible isolates, MDR *P. aeruginosa* infections are associated with nearly 20% higher mortality rates, prolonged hospitalization, increased readmissions, and greater healthcare expenditure (Nelson et al., 2022; Tabak et al., 2019). Individuals with chronic lung diseases, cystic fibrosis, burn injuries, immunocompromised conditions, and intravenous drug use are particularly vulnerable to infection (Schwartz et al., 2024). The clinical burden of *P. aeruginosa* is particularly evident in intensive care units (ICUs), where it is a frequent cause of ventilator-associated pneumonia (VAP), bloodstream infections, urinary tract infections, and catheter-associated urinary tract infections (Weber et al., 2007; Weiner-Lastinger et al., 2020). Approximately one third of adult ICU patients with VAP are infected with multidrug-resistant (MDR) *P. aeruginosa*, although considerable geographical variation exists (Li et al., 2024). These infections are associated with poor clinical outcomes, with nearly 37.3% of affected ICU patients dying within 30 days, while bloodstream infections associated with MDR *P. aeruginosa* have mortality rates ranging from 43.2% to 58.8% (Thaden et al., 2017; Zavascki et al., 2006). The likelihood of MDR *P. aeruginosa* infection is increased by previous hospitalization, residence in nursing homes,

tracheostomy, severe underlying illness, recent exposure to broad-spectrum antibiotics, bronchiectasis, chronic obstructive pulmonary disease (COPD), and previous colonization or infection with *P. aeruginosa* (Recio et al., 2020). The persistence of *P. aeruginosa* in healthcare settings is largely driven by its ability to form biofilms and express phenotypic resistance mechanisms that enhance survival on medical devices and hospital surfaces (Cole et al., 2014; Jiang et al., 2023). The type III secretion system (T3SS), through the delivery of *ExoU*, *ExoS*, *ExoT*, and *ExoY* effector proteins, contributes directly to host cell damage, while pigments secreted through the type II secretion system interfere with host cellular functions and immune responses (Galle et al., 2012). The intrinsic resistance of *P. aeruginosa* is mainly associated with multidrug resistance efflux systems, limited outer membrane permeability, and production of antimicrobial inactivating enzymes. Conversely, acquired resistance develops through chromosomal mutations and horizontal transfer of resistance genes (Yin et al., 2022). The active transport of antimicrobial agents out of bacterial cells by efflux pumps reduces membrane permeability, limits antibiotic entry, and chromosomally encoded AmpC β-lactamases confer resistance to penicillins and cephalosporins (Ghai & Ghai, 2018; Causapé et al., 2017). Resistance to aminoglycosides is primarily mediated by aminoglycoside-modifying enzymes, whereas resistance to chloramphenicol is mainly associated with chloramphenicol acetyltransferases (Feldgarden et al., 2019). In addition, β-lactam resistance is significantly enhanced by increased production of chromosomal AmpC β-lactamases resulting from dacB suppression, as well as horizontal acquisition of resistance genes (Zamorano et al., 2010). Biofilm formation enhances bacterial survival by restricting the penetration of antimicrobial agents, lowering metabolic activity, and shielding the cells from host immune defenses, thereby contributing to chronic and recurrent infection (Agata, 2004). Multi-drug-resistant *P. aeruginosa* is widely reported worldwide; however, its occurrence and antimicrobial susceptibility patterns differ among healthcare facilities and geographical regions. Continuous monitoring and phenotypic characterization of *P. aeruginosa* isolated from clinical and hospital environmental samples are essential for identifying emerging antimicrobial resistance trends, supporting appropriate empirical antibiotic therapy, and improving infection prevention and control practices. In view of these concerns, the present study was designed to isolate MDR *P. aeruginosa* from healthcare-associated clinical and environmental samples. The recovered isolates were identified and phenotypically characterized using conventional microbiological and biochemical methods, followed by antimicrobial susceptibility testing to assess the burden of multidrug resistance in the healthcare setting.

Methodology:

Study Area and Sample Collection:

A total of 50 clinical and environmental samples were collected from Bahria International Hospital, Lahore, according to the suspected infection site. Clinical samples included blood collected from patients with suspected systemic infections, urine specimens collected from patients with urinary tract infection using an appropriate cleansing procedure to minimize contamination, ear swabs, and throat swabs obtained from patients with localized infections. Environmental samples were collected using sterile swabs from suction machines, door knobs, and wash basins. All the samples were appropriately labelled according to the specimen source and transported to the Microbiology and Molecular Genetics Lab, University of Okara, at 4 ˚C for further processing within 24 hours of collection. The distribution of collected samples by specimen type is shown in Table 1.

Table 1: Distribution of collected samples by specimen type

Sample Category	Specimen Type	Number of Samples
Clinical Samples	Blood (B)	10
	Urine (U)	11
	Ear Swabs (ES)	13
	Throat Swabs (TS)	9
Environmental Samples	Suction Machine Swabs (SMS)	2
	Door Knob Swabs (DMS)	2
	Wash Basin Swabs (WBS)	3
Total		50

Isolation and Identification of *Pseudomonas aeruginosa*:

For isolation of *Pseudomonas aeruginosa*, the collected samples were given pre-enrichment by inoculation in 10 mL of tryptone soya broth (Oxoid CM0129B) followed by aerobic incubation at 37 ˚C for 24 hrs. For selective isolation of *P. aeruginosa,* 0.1 mL of pre-enriched culture was streaked on MacConkey agar (Oxoid CM0115) and blood agar (5% sheep blood in blood agar base) (Oxoid CM0055). MacConkey agar was incubated at 35- 37 °C for 18 to 24 h in an aerobic environment, while blood agar was incubated for 24 h at 37 ˚C. Colony morphology and β-hemolysis on blood agar were observed. Gram staining was performed on isolated

colonies by preparing a thin smear on a clean glass slide, followed by staining with crystal violet, Gram's iodine, 70% acetone-alcohol, and safranin. Microscopic examination of stained slides was performed under oil immersion (100x objective) to identify the Gram staining characteristics and cellular morphology of bacterial isolates (Forbes et al., 2007).

Biochemical Confirmation of *Pseudomonas aeruginosa*:

Biochemical tests included Catalase, Citrate utilization, Oxidase, Indole, and Triple sugar iron tests were performed to confirm *P. aeruginosa.* 3% hydrogen peroxide was used to perform the catalase test. Immediate formation of oxygen bubbles was interpreted as a positive reaction, whereas the absence of bubbles formation was considered a negative result. Citrate utilization was determined using Simmons citrate agar (Oxoid CM0155) slants following incubation at 37 ˚C for 24-48 hours where growth with colour change from green to Prussian blue considered a positive result, while absence of growth and no colour change was interpreted as a negative result (Cappuccino & Sherman, 2011). The oxidase test was performed using freshly prepared 1% N, N, N', N', tetramethyl-p-phenylenediamine dihydrochloride reagent (Sigma-Aldrich T3134). A small amount of bacterial growth was rubbed on the reagent-soaked filter paper; and the appearance of dark purple colour within 10-30 seconds was interpreted as a positive result. No colour change and delayed colour interpreted as negative. For the indole test, a loop full of culture was inoculated into sterile tryptophan broth (Liofilchem 610197) and incubated at 37 ˚C for 24 to 48 hours, after which 4 to 5 drops of Kovac's reagent (Liofilchem 80271) were added; appearance of a red or pink ring was considered a positive result, whereas yellow ring formation or absence of any colour indicates a negative reaction. For the Triple Sugar Iron (TSI) test, bacterial isolates were stabbed in the butt and streaked on the slant of the TSI agar (Oxoid CM0277B) tubes. The inoculated tubes were then incubated at 37 ˚C for 18 to 24 hrs. After incubation, colour changes and gas production were recorded; a yellow butt with red slant was indicative of glucose fermentation; only the butt and slant turning yellow was indicating lactose/sucrose fermentation; gas production was interpreted by cracks, bubbles formation, lifting of the agar medium; and a black precipitate indicated H_2S production (Su et al., 2018).

Antimicrobial Susceptibility Testing:

Antimicrobial susceptibility of biochemically confirmed *P. aeruginosa* isolates was evaluated using the Kirby-Bauer disk diffusion method in accordance with Clinical and Laboratory Standards Institute (CLSI, 2024) guidelines. Bacterial suspension with the turbidity equivalent

to 0.5 McFarland standard (1.5 × 10^8 CFU/mL) was prepared by transferring isolated colonies from pure culture to sterile normal saline. The standardized bacterial suspension was evenly spread onto Mueller-Hinton agar (Oxoid CM0337B) using sterile cotton swabs. The antibiotic discs were then aseptically placed on agar surface with sterile forceps. Following incubation at 35-37 ˚C for 16 to 18 hrs, the diameter of each inhibition zone was measured in millimeters by using transparent ruler. The isolates were categorized as susceptible, intermediate or resistant based on CLSI 2024 interpretive criteria (Yang et al., 2023). The antibiotic panel included Piperacillin (PRL), Aztreonam (ATM), Cefepime (FEP), Imipenem (IPM), Meropenem (MEM), Ciprofloxacin (CIP), Levofloxacin (LEV), Tobramycin (TOB), Gentamicin (CN), Polymyxin B (PB), and Colistin (CS), representing Penicillin, Monobactams, Cephalosporins, Carbapenems, Fluoroquinolones, Aminoglycosides, and Polymyxins, respectively.

Multi Drug Resistant Profiling:

The tested antimicrobial agents were classified into five groups, β-lactams, carbapenems, fluroquinolones, aminoglycosides and polymyxins. Multi Drug resistance was defined as resistance to at least one microbial agent in three or more antimicrobial classes. Isolates meeting the criterion was classified as Multi Drug Resistant (MDR) (Magiorakos et al., 2012).

Results:

Isolation and Identification of *Pseudomonas aeruginosa*:

A total of 50 clinical and environmental samples were analyzed to determine the occurrence of *P. aeruginosa*. Based on microscopic examination and biochemical confirmation, 11 (22%) samples were tested positive, whereas remaining 39 samples were negative. Among the clinical samples, *P. aeruginosa* was recovered from blood (n=1/10), Urine (n=1/11), Ear Swabs (ES, n=3/13), and Throat Swabs (TS, n=3/9). Environmental isolates were obtained from Suction Machine Swabs (SMS, n=1/2), Door Knob Swabs (DKS, n=1/2) and Wash Basin Swabs (WBS, n=1/3) as shown in Table 2. The isolates appeared colorless to pale yellowish translucent colonies, consistent with their non-lactose fermenting nature. The colonies were generally round and flat, while some clinical isolates displayed slightly raised center with flat margins. On 5% sheep blood agar, colonies appeared large, opaque, convex, and β-hemolytic with slightly round margins and light tan pigmentation after 16- 24 hours of incubation. Gram

staining confirmed that the isolates were Gram-negative bacilli, appearing as pink to red rod-shaped cells following safranin counterstaining.

Table 2: Distribution of *Pseudomonas aeruginosa* isolates by sample source and specimen type

Sample Category	Specimen Type	Number of Samples	+ve, (n, %)	-ve, (n)
Clinical Samples	Blood (B)	10	1 (10)	9
	Urine (U)	11	1 (9.1)	10
	Ear Swabs (ES)	13	3 (23.1)	10
	Throat Swabs (TS)	9	3 (33.1)	6
Environmental Samples	Suction Machine Swabs (SMS)	2	1(50)	1
	Door Knob Swabs (SKS))	2	1(50)	1
	Wash Basin Swabs (WBS)	3	1(42.9)	2
Total		50	11(22)	39

Biochemical Identification of *Pseudomonas aeruginosa*:

Microscopically identified Gram-negative rods were further subjected to biochemical confirmation to confirm the identity of *P. aeruginosa.* The isolates were catalase positive, exhibiting immediate bubble formation, citrate positive, showing visible growth on the slant with color changes from green to Prussian blue, and oxidase positive, producing the purple blue color following the oxidase test. All the isolates were indole negative, with no red or pink ring observed after the addition of Kovac's reagent, indicating the absence of tryptophan hydrolysis by the enzyme tryptophanase, consequently no indole production. On TSI agar, isolates produced an alkaline slant and alkaline butt (K/K), with no fermentation of glucose, lactose, or sucrose, no hydrogen sulfide production, and no visible gas production. These findings were consistent with the biochemical characteristics of *P. aeruginosa* as shown in Table 3.

Table 3: Microscopic Examination and Biochemical Confirmation of *Pseudomonas aeruginosa* isolate

Sample ID	Gram Staining & Microscopy	Confirmation Tests				
		Catalase	Citrate	Oxidase	Indole	TSI Agar
S-2-B	-ve rods (Pink)	+ve	+ve	+ve	-ve	K/K, Gas-ve, H_2S-ve
S-8-U	-ve rods (Pink)	+ve	+ve	+ve	-ve	K/K, Gas-ve, H_2S-ve
S-2-ES	-ve rods (Pink)	+ve	+ve	+ve	-ve	K/K, Gas-ve, H_2S-ve
S-5-ES	-ve rods (Pink)	+ve	+ve	+ve	-ve	K/K, Gas-ve, H_2S-ve
S-6-ES	-ve rods (Pink)	+ve	+ve	+ve	-ve	K/K, Gas-ve, H_2S-ve
S-1-TS	-ve rods (Pink)	+ve	+ve	+ve	-ve	K/K, Gas-ve, H_2S-ve
S-8-TS	-ve rods (Pink)	+ve	+ve	+ve	-ve	K/K, Gas-ve, H_2S-ve
S-9-TS	-ve rods (Pink)	+ve	+ve	+ve	-ve	K/K, Gas-ve, H_2S-ve
S-2-SMS	-ve rods (Pink)	+ve	+ve	+ve	-ve	K/K, Gas-ve, H_2S-ve
S-2-DKS	-ve rods (Pink)	+ve	+ve	+ve	-ve	K/K, Gas-ve, H_2S-ve
S-1-WBS	-ve rods (Pink)	+ve	+ve	+ve	-ve	K/K, Gas-ve, H_2S-ve

S-Sample, B-Blood, U-Urine, ES-Ear Swab, TS-Throat Swab, SMS-Suction Machine Swab, DKS-Door Knob Swab, WBS-Wash Basin Swab, (+ve) Positive test, (-ve) Negative Test, K/K (Alkaline butt/Alkaline slant), Gas-ve (No gas Production), H_2S-ve (No Hydrogen sulfide production)

Antimicrobial Susceptibility Testing:

Antimicrobial susceptibility of positive *P. aeruginosa* isolates (n=1) was evaluated against 11 antibiotics representing six antimicrobial classes as per CLSI interpretive breakpoints. The isolates with a resistant profile have high levels of resistance to β-lactams, carbapenems, fluoroquinolones, aminoglycosides, and polymyxins, as shown in Figure 1. Complete resistance (100%) was observed against cefepime, levofloxacin, and imipenem, whereas aztreonam (72.7%), piperacillin (63.6%), ciprofloxacin (45.4%), and gentamicin (36.4%) showed the highest susceptibility rates. The detailed antimicrobial susceptibility profile is presented in Table 4.

Table 4: Antimicrobial Susceptibility Profile of *Pseudomonas aeruginosa*

Class	Antibiotic	Symbol	(µg)	CLSI Breakpoints (R/I/S, mm)	S (n, %)	I (n, %)	R (n, %)
β-lactam (Penicillin)	Piperacillin	PRL	100	≤14 / 15–20 / ≥21	7 (63.6)	0	4 (36.4)
β-lactam (Cephalosporin)	Cefepime	FEP	30	≤14 / 15–17 / ≥18	0 (0)	0	11 (100)
β-lactam (Monobactams)	Aztreonam	ATM	30	≤15 / 16–21 / ≥22	8 (72.7)	0	3 (27.3)
Carbapenem	Imipenem	IPM	10	≤15 / 16–18 / ≥19	0 (0)	0	11 (100)
	Meropenem	MEM	10	≤15 / 16–18 / ≥19	2 (18.2)	1 (9.1)	8 (72.7)
Fluoroquinolones	Ciprofloxacin	CIP	5	≤18 / 19–24 / ≥25	5 (45.4)	3 (27.3)	3 (27.3)
	Levofloxacin	LEV	5	≤14 / 15–21 / ≥22	0 (0)	0 (0)	11 (100)
Aminoglycoside	Tobramycin	TOB	10	≤12 / 13–14 / ≥15	1 (9.1)	2 (18.2)	8 (72.7)
	Gentamicin	CN	10	≤12 / 13–14 / ≥15	4 (36.4)	0 (0)	7 (63.6)

Polymyxin	Polymyxin B	PB	300	≤14 / 15–16 / ≥17	1 (9.1)	0 (0)	10 (90.9)
	Colistin	CS	10	≤2 / 2–3 / ≥4	1 (9.1)	0 (0)	10 (90.9)

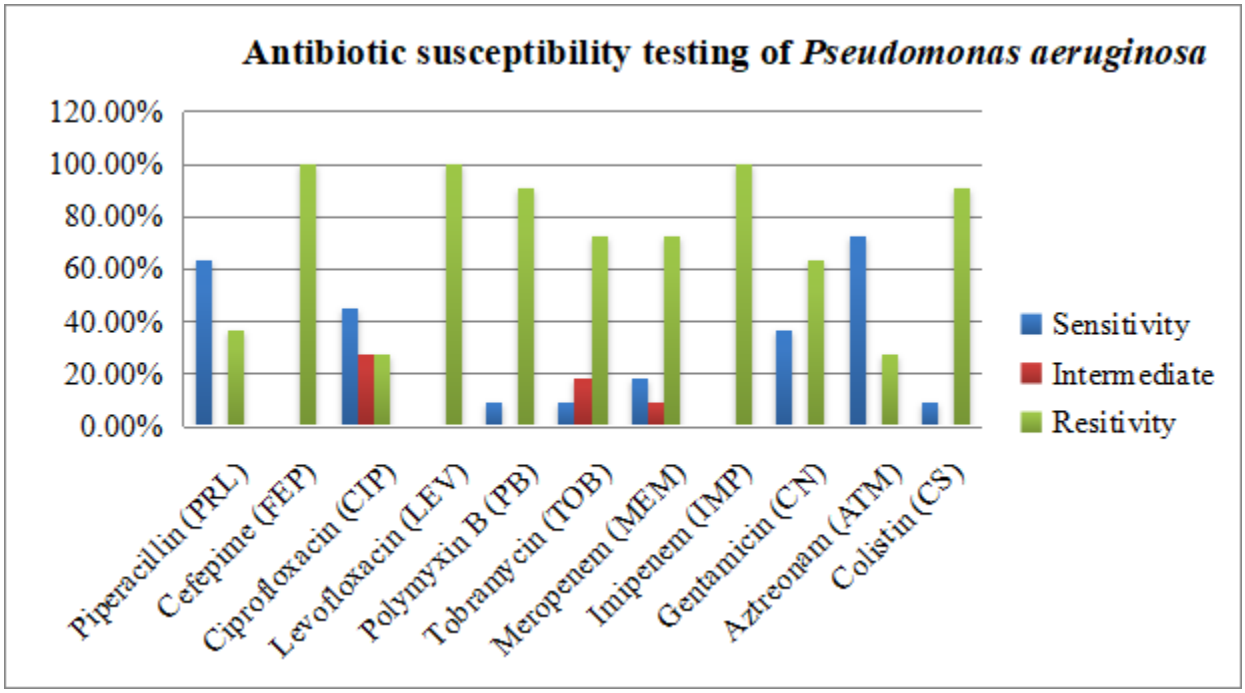


Figure 1: Antibiotic susceptibility profile of *Pseudomonas aeruginosa*.

MDR Classification of *Pseudomonas aeruginosa* Isolates:

The multidrug-resistant profile of *P. aeruginosa* isolates was determined by using Antimicrobial Susceptibility Test results obtained from the Kirby-Bauer Disc diffusion method. The tested antibiotics were grouped into five major categories: β-lactams (PRL, FP, ATM), carbapenems (MEM, IPM), fluoroquinolones (CIP, LEV), aminoglycosides (TOB, CN), and polymyxins (PB, CS). Based on resistance to at least one antimicrobial agent in each category, all 11 isolates exhibited resistance to 4 or more antimicrobial categories. Consequently, all isolates (n=11/11) were classified as multidrug resistant (MDR), as presented in Table 5.

Table 5: Multi-Drug resistant (MDR) profile of *Pseudomonas aeruginosa* Isolates

Sample ID	β-lactams	Carbapenems	Fluoroquinolones	Aminoglycosides	Polymyxins	*(n/5), MDR*
S-2-B	R(PRL,FP,ATM)	R (MEM, IPM)	R (CIP, LEV)	R (TOB)	R (PB)	(5/5) *Yes*
S-8-U	R (FEP, ATM)	R (MEM, IPM)	R (CIP, LEV)	R (CN)	R (PB)	(5/5) *Yes*
S-2-ES	R (FEP, ATM)	R (MEM, IPM)	R (CIP)	R (CN)	*S*	(4/5) *Yes*
S-5-ES	R (FEP, ATM)	R (MEM, IPM)	R (CIP, LEV)	*S*	R (PB)	(4/5) *Yes*
S-6-ES	R(PRL,FEP,ATM)	R (MEM, IPM)	R (CIP, LEV)	R (TOB, CN)	R (PB)	(5/5) *Yes*
S-1-TS	R(PRL,FEP,ATM)	R (MEM, IPM)	R (CIP, LEV)	R (TOB, CN)	R (PB)	(5/5) *Yes*
S-8-TS	R (FEP, ATM)	R (MEM, IPM)	R (CIP, LEV)	R (TOB)	*S*	(4/5) *Yes*
S-9-TS	R (FEP, ATM)	R (MEM, IPM)	R (CIP, LEV)	R (TOB)	R (PB)	(5/5) *Yes*
S-2-SMS	R (PRL, ATM)	R (MEM, IPM)	R (CIP, LEV)	R (TOB, CN)	*R*	(5/5) *Yes*
S-2-DKS	R (FEP, ATM)	R (MEM, IPM)	R (CIP, LEV)	R (TOB, CN)	R (PB)	(5/5) *Yes*
S-1-WBS	R (FEP, ATM)	R (MEM, IPM)	R (CIP, LEV)	R (TOB, CN)	R (PB)	(5/5) *Yes*

Discussion:

P. aeruginosa is an opportunistic pathogen with intrinsic and acquired resistance mechanisms against multiple antimicrobial agents, making its treatment increasingly challenging in

healthcare settings. It is a leading cause of hospital-acquired infections (HAIs), particularly in immunocompromised individuals and patients admitted to surgical wards, burn units, and intensive care units. The rising prevalence of multidrug-resistant (MDR) *P. aeruginosa* has become a major challenge in clinical microbiology, as it significantly reduces available therapeutic options and is associated with poor clinical outcomes. In previous studies, it has been reported that the widespread dissemination of MDR *P. aeruginosa* in hospitals worldwide highlights its emergence as an important healthcare-associated pathogen (Livermore, 2002; McGrath & Asmar, 2011; Cachón et al., 2019). The persistence and multidrug resistance of *P. aeruginosa* are associated with several well-established mechanisms of resistance. Biofilm formation is a major virulence characteristic that enables bacterial persistence on medical devices, including catheters, ventilators, and other indwelling equipment, thereby reducing antibiotic penetration and promoting recurrent infections (Mah & O'Toole, 2001). In addition, reduced metabolic activity, differential gene expression, and increased alginate production further enhance biofilm-associated resistance (Li et al., 2023). Overexpression of multidrug efflux pumps, particularly MexAB-OprM, and reduced expression or loss of the OprD porin have also been identified as major contributors to resistance against β-lactams, fluoroquinolones, and carbapenems (Poole, 2004; Schärer et al., 2023; Xie et al., 2024). Furthermore, horizontal gene transfer facilitates the dissemination of resistance determinants, including metallo-β-lactamase genes such as IMP, VIM, and NDM, which are frequently associated with plasmids and integrons in clinical isolates (Botelho et al., 2019). The global dissemination of these resistance determinants has been particularly reported in intensive care units; however, MDR *P. aeruginosa* remains comparatively less common in community-acquired infections because of reduced exposure to resistant reservoirs (Horcajada et al., 2019). In the present study, all *Pseudomonas aeruginosa* isolates were identified as multidrug resistant as each isolate exhibited resistance to four or more antimicrobial classes. Among β-lactam antibiotics, complete resistance was observed to cefepime, being consistent with the previous reports highlighting the increasing resistance of hospital associated *P. aeruginosa* isolates to cephalosporins (Cachón et al., 2019). Conversely, piperacillin exhibited 36.4% resistance and 63.6% susceptibility, comparatively showing better activity than cefepime against the isolates. This finding is different from the Qader et al. (2022), who reported higher level of resistance to piperacillin, primarily associated with the production of extended spectrum β-lactamases (ESBL) and AmpC β-lactamase. This discrepancy may reflect the regional variations in antimicrobial use and distribution of resistant genes (Qader et al., 2022). Within the β-lactam group, Aztreonam showed the highest susceptibility rate of 72.7%, in contrast with the

international reports that have reported widespread resistance of antimicrobial agents (Torrens et al., 2022). A high level of carbapenem resistance was observed in the current study, with all the *P. aeruginosa* isolates 100% resistant to imipenem and 72.7% resistant to meropenem. These findings are in line with the previous reports, which link carbapenem resistance to the production of metallo-β-lactamases and loss of OprD porin, which substantially reduce susceptibility to carbapenem antibiotics (Poole, 2004; Schwartz et al., 2024). A comparable resistance pattern has been reported in *P. aeruginosa* isolates obtained from ICU burn centers. This comparison highlights the diminishing effectiveness of carbapenems as a last-line treatment option. Among the fluoroquinolones evaluated, ciprofloxacin showed 27.3% resistance, 27.3% intermediate susceptibility, and 45.4% complete susceptibility, whereas all isolates were completely resistant to levofloxacin. These findings are consistent with Horcajada et al. (2019), who attributed fluoroquinolone resistance to mutated *gyr*A and *par*C genes along with the overproduction of multidrug efflux pumps (Horcajada et al., 2019). In the present study, ciprofloxacin demonstrated greater resistance activity than levofloxacin, in contrast to different international findings that have reported similar resistance patterns for both fluoroquinolones. Reduced susceptibility among the aminoglycosides was also reported, as gentamycin was 63.6% resistant and 36.4% susceptible, while tobramycin exhibited 72.1% resistance and 9.1% susceptibility. These findings are in agreement with the previous reports, which have identified multidrug efflux pumps and aminoglycosides modifying enzymes as primary mechanisms of aminoglycoside resistance in *P. aeruginosa* (Husejnagić et al., 2025). Across South Asian hospitals, similar resistance rates have also been reported, highlighting the growing burden of MDR *P. aeruginosa* isolates (Jiménez et al., 2025; Parmanik et al., 2022). The polymyxins also demonstrated limited antimicrobial activity in the current study. Both polymyxins showed only 9.1% susceptibility, reflecting a higher level of resistance among the isolates. These findings are in line with recent reports showing increasing prevalence of polymyxin resistance by modifying the bacterial outer membrane that reduces the binding affinity of polymyxin (Olaitan et al., 2014). Overall, these findings are consistent with the international reports highlighting the increasing prevalence of multidrug-resistant *P. aeruginosa*, although some variations in the local resistance pattern were observed. The high level of resistance to carbapenems and polymyxins aligns with the global trends and highlights the urgent need for effective antibiotic stewardship programs, continuous resistance surveillance, and stringent infection prevention and control measures to limit the spread of these resistant pathogens.

Conclusion:

The current study demonstrated a high level of MDR *P. aeruginosa* burden in local health care settings, with all isolates exhibiting resistance to at least three antimicrobial classes. These highlight the diminishing effectiveness of several key antimicrobial agents and emphasize the need for proactive measures, including continuous antimicrobial resistance surveillance, rational use of antibiotics, and effective infection prevention and control practices to limit the emergence and spread of multidrug-resistant strains of *P. aeruginosa.* Overall, this study provides important baseline data on local resistance trends that can support evidence-based treatment decisions and antimicrobial stewardship programs.

References:

Botelho, J., Grosso, F., & Peixe, L. (2019). Antibiotic resistance in *Pseudomonas aeruginosa*–Mechanisms, epidemiology and evolution. Drug Resistance Updates, 44, 100640.

Cadenas-Jiménez, I., Badía-Tejero, A. M., López-Causapé, C., Morosini, M. I., Portillo-Calderón, I., Machado, M., Larrosa, N., Dávila, P. M., Palacios-Baena, Z., Puig-Albasanz, A., Tubau, F., Oliver, A., Sastre, E., Martí, S., & Gudiol, C. (2025). Molecular epidemiology and antimicrobial resistance profiles of *Pseudomonas aeruginosa* causing bloodstream infections in neutropenic cancer patients. Frontiers in Microbiology, 16, 1681506. https://doi.org/10.3389/fmicb.2025.1681506

Cappuccino, J. G., & Sherman, N. (2011). Microbiology: a laboratory manual. San Francisco: Pearson Benjamin Cummings.

Cole, S. J., Records, A. R., Orr, M. W., Linden, S. B., & Lee, V. T. (2014). Catheter-associated urinary tract infection by *Pseudomonas aeruginosa* is mediated by exopolysaccharide-independent biofilms. Infection and Immunity, 82(5), 2048-2058. https://doi.org/10.1128/iai.01652-14

D'Agata, E. M. (2004). Rapidly rising prevalence of nosocomial multidrug-resistant, Gram-negative bacilli: a 9-year surveillance study. Infection Control & Hospital Epidemiology, 25(10), 842-846.

Feldgarden, M., Brover, V., Haft, D. H., Prasad, A. B., Slotta, D. J., Tolstoy, I., Tyson, G. H., Zhao, S., Hsu, C.-H., & McDermott, P. F. (2019). Validating the AMRFinder tool and resistance gene database by using antimicrobial resistance genotype-phenotype

correlations in a collection of isolates. Antimicrobial agents and chemotherapy, 63(11), 10.1128/aac. 00483-00419.

Forbes, B. A., Sahm, D. F., & Weissfeld, A. S. (2007). Diagnostic microbiology. Mosby St Louis.

Galle, M., Carpentier, I., & Beyaert, R. (2012). Structure and function of the Type III secretion system of *Pseudomonas aeruginosa*. Current Protein and Peptide Science, 13(8), 831-842. https://doi.org/10.2174/138920312804871210

Ghai, I., & Ghai, S. (2018). Understanding antibiotic resistance via outer membrane permeability. Infection and Drug Resistance, 523-530.

Grobe, S., Wingender, J., & Flemming, H. C. (2001). Capability of mucoid *Pseudomonas aeruginosa* to survive in chlorinated water. International Journal of Hygiene and Environmental Health, 204(2-3), 139-142. https://doi.org/10.1078/1438-4639-00085

Horcajada, J. P., Montero, M., Oliver, A., Sorlí, L., Luque, S., Gómez-Zorrilla, S., Benito, N., & Grau, S. (2019). Epidemiology and treatment of multidrug-resistant and extensively drug-resistant *Pseudomonas aeruginosa* infections. Clinical Microbiology Reviews, 32(4), 10.1128/cmr. 00031-00019.

Husejnagić, D., Avdić, A., Hercegovac, A., Širanović, S., & Tihić, N. (2025). Detection of aminoglycoside-modifying enzyme genes in *Pseudomonas aeruginosa* clinical isolates. Genetics & Applications, 9(2).

Jiang, S., Wei, Y., Ke, H., Song, C., Liao, W., Meng, L., Sun, C., Zhou, J., Wang, C., & Su, X. (2023). Clinical impact of fast platforms and laboratory automation for the rapid diagnosis of infectious diseases and detection of antimicrobial resistance determinants. Frontiers in Cellular and Infection Microbiology, p.6.

Li, X., Gu, N., Huang, T. Y., Zhong, F., & Peng, G. (2023). *Pseudomonas aeruginosa*: A typical biofilm forming pathogen and an emerging but underestimated pathogen in food processing. Frontiers in Microbiology, 13, 1114199.

Li, Y., Roberts, J. A., Walker, M. M., Aslan, A. T., Harris, P. N., & Sime, F. B. (2024). The global epidemiology of ventilator-associated pneumonia caused by multi-drug resistant *Pseudomonas aeruginosa*: A systematic review and meta-analysis. International Journal of Infectious Diseases, 139, 78-85.

Livermore, D. M. (2002). Multiple mechanisms of antimicrobial resistance in *Pseudomonas aeruginosa*: our worst nightmare. Clinical Infectious Diseases, 34(5), 634-640.

López-Causapé, C., Sommer, L. M., Cabot, G., Rubio, R., Ocampo-Sosa, A. A., Johansen, H. K., Figuerola, J., Cantón, R., Kidd, T. J., & Molin, S. (2017). Evolution of the

Pseudomonas aeruginosa mutational resistome in an international cystic fibrosis clone. Scientific reports, 7(1), 5555.

Magiorakos, A.-P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M., Giske, C., Harbarth, S., Hindler, J., Kahlmeter, G., & Olsson-Liljequist, B. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clinical Microbiology and Infection, 18(3), 268-281.

Mah, T.-F. C., & O'Toole, G. A. (2001). Mechanisms of biofilm resistance to antimicrobial agents. Trends in Microbiology, 9(1), 34-39.

McGrath, E. J., & Asmar, B. I. (2011). Nosocomial infections and multidrug-resistant bacterial organisms in the pediatric intensive care unit. Indian Journal of Pediatrics, 78(2), 176-184. https://doi.org/10.1007/s12098-010-0253-4

Nelson, R. E., Hyun, D., Jezek, A., & Samore, M. H. (2022). Mortality, length of stay, and healthcare costs associated with multidrug-resistant bacterial infections among elderly hospitalized patients in the United States. Clinical Infectious Diseases, 74(6), 1070-1080.

Olaitan, A. O., Morand, S., & Rolain, J.-M. (2014). Mechanisms of polymyxin resistance: acquired and intrinsic resistance in bacteria. Frontiers in Microbiology, 5, 643.

Parmanik, A., Das, S., Kar, B., Bose, A., Dwivedi, G. R., & Pandey, M. M. (2022). Current Treatment Strategies Against Multidrug-Resistant Bacteria: A Review. Current Microbiology, 79(12), 388. https://doi.org/10.1007/s00284-022-03061-7

Poole, K. (2004). Efflux-mediated multiresistance in Gram-negative bacteria. Clinical Microbiology and Infection, 10(1), 12-26.

Qader, G. M., Jarjees, K. K., & Jarjees, R. K. (2022). Molecular detection of Metallo-Beta-Lactamase and alginate in multidrug resistance *Pseudomonas aeruginosa* isolated from the clinical specimen. Journal of Medicine and Life, 15(9), 1105.

Recio, R., Sánchez-Diener, I., Viedma, E., Meléndez-Carmona, M. Á., Villa, J., Orellana, M. Á., Mancheño, M., Juan, C., Zamorano, L., & Lora-Tamayo, J. (2020). Pathogenic characteristics of *Pseudomonas aeruginosa* bacteraemia isolates in a high-endemicity setting for ST175 and ST235 high-risk clones. European Journal of Clinical Microbiology & Infectious Diseases, 39(4), 671-678.

Roulová, N., Mot'ková, P., Brožková, I., & Pejchalová, M. (2022). Antibiotic resistance of *Pseudomonas aeruginosa* isolated from hospital wastewater in the Czech Republic. Journal of Water and Health, 20(4), 692-701.

Sawant, S., Shaikh, S., Baldwin, T. C., Karakoula, A., & Rahman, A. (2026). Pseudomonas aeruginosa: associated pathogenesis, epidemiology, resistance mechanisms, host response and emerging treatment strategies. Archives of Microbiology, 208(7). https://doi.org/10.1007/s00203-026-04839-0

Schärer, V., Meier, M.-T., Schuepbach, R. A., Zinkernagel, A. S., Boumasmoud, M., Chakrakodi, B., Brugger, S. D., Fröhlich, M. R., Wolfensberger, A., & Sax, H. (2023). An intensive care unit outbreak with multi-drug-resistant *Pseudomonas aeruginosa*–spotlight on sinks. Journal of Hospital Infection, 139, 161-167.

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). https://doi.org/10.3390/pathogens13110975

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.

Su, S. S., Lae, K. Z. W., & Ngwe, H. (2018). Isolation and Identification of *Pseudomonas aeruginosa* from the Clinical Soil. University of Yangon Research Journal, 8, 271-275.

Tabak, Y., Merchant, S., Ye, G., Vankeepuram, L., Gupta, V., Kurtz, S., & Puzniak, L. (2019). Incremental clinical and economic burden of suspected respiratory infections due to multi-drug-resistant *Pseudomonas aeruginosa* in the United States. Journal of Hospital Infection, 103(2), 134-141.

Thaden, J. T., Park, L. P., Maskarinec, S. A., Ruffin, F., Fowler Jr, V. G., & Van Duin, D. (2017). Results from a 13-year prospective cohort study show increased mortality associated with bloodstream infections caused by *Pseudomonas aeruginosa* compared to other bacteria. Antimicrobial Agents and Chemotherapy, 61(6), 10.1128/aac. 02671-02616.

Torrens, G., van der Schalk, T. E., Cortes-Lara, S., Timbermont, L., Del Barrio-Tofiño, E., Xavier, B. B., Zamorano, L., Lammens, C., Ali, O., Ruzin, A., Goossens, H., Kumar-Singh, S., Kluytmans, J., Paling, F., MacLean, R. C., Köhler, T., López-Causapé, C., Malhotra-Kumar, S., & Oliver, A. (2022). Susceptibility profiles and resistance genomics of *Pseudomonas aeruginosa* isolates from European ICUs participating in the ASPIRE-ICU trial. Journal of Antimicrobial Chemotherapy, 77(7), 1862-1872. https://doi.org/10.1093/jac/dkac122

Uc-Cachón, A. H., Gracida-Osorno, C., Luna-Chi, I. G., Jiménez-Guillermo, J. G., & Molina-Salinas, G. M. (2019). High prevalence of antimicrobial resistance among gram-negative isolated bacilli in intensive care units at a tertiary-care hospital in Yucatán Mexico. Medicina, 55(9), 588.

Weber, D. J., Rutala, W. A., Sickbert-Bennett, E. E., Samsa, G. P., Brown, V., & Niederman, M. S. (2007). Microbiology of ventilator–associated pneumonia compared with that of hospital-acquired pneumonia. Infection Control & Hospital Epidemiology, 28(7), 825-831.

Weiner-Lastinger, L. M., Abner, S., Edwards, J. R., Kallen, A. J., Karlsson, M., Magill, S. S., Pollock, D., See, I., Soe, M. M., & Walters, M. S. (2020). Antimicrobial-resistant pathogens associated with adult healthcare-associated infections: summary of data reported to the National Healthcare Safety Network, 2015–2017. Infection Control & Hospital Epidemiology, 41(1), 1-18.

Xie, X., Liu, Z., Huang, J., Wang, X., Tian, Y., Xu, P., & Zheng, G. (2024). Molecular epidemiology and carbapenem resistance mechanisms of *Pseudomonas aeruginosa* isolated from a hospital in Fujian, China. Frontiers in Microbiology, 15, 1431154.

Yang, A. F., Huang, V., Samaroo-Campbell, J., & Augenbraun, M. (2023). Multi-drug resistant *Pseudomonas aeruginosa:* a 2019–2020 single center retrospective case control study. Infection Prevention in Practice, 5(3), 100296.

Yin, R., Cheng, J., Wang, J., Li, P., & Lin, J. (2022). Treatment of *Pseudomonas aeruginosa* infectious biofilms: Challenges and strategies [Mini Review]. Frontiers in Microbiology, Volume 13 - 2022. https://doi.org/10.3389/fmicb.2022.955286

Zamorano, L., Moyá, B., Juan, C., & Oliver, A. (2010). Differential beta-lactam resistance response driven by ampD or dacB (PBP4) inactivation in genetically diverse *Pseudomonas aeruginosa* strains. Journal of Antimicrobial Chemotherapy, 65(7), 1540-1542. https://doi.org/10.1093/jac/dkq142

Zavascki, A. P., Barth, A. L., Fernandes, J. F., Moro, A. L. D., Gonçalves, A. L. S., & Goldani, L. Z. (2006). Reappraisal of *Pseudomonas aeruginosa* hospital-acquired pneumonia mortality in the era of metallo-β-lactamase-mediated multidrug resistance: a prospective observational study. Critical care, 10(4), R114.