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Antibiotic Sensitivity and Antibiotic Resistance Genes in *Pseudomonas aeruginosa* Isolates Recovered from Human Patients

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BP: Conceptualization, performed the experimental work, data analysis and prepared the manuscript. SHK: Provided supervision and reviewed the manuscript. SFHR: Provided guidance for the study and assisted in manuscript editing. MA: Helped in data analysis and manuscript write-up. ZA: Facilitated sample provision and laboratory support. All authors read and approved the final manuscript.

Key words
***Pseudomonas aeruginosa*, Antimicrobial resistance genes, Antibiotic sensitivity, β lactam resistance genes, ESBL genes, Co-trimoxazole resistance**

ABSTRACT

Pseudomonas aeruginosa, an important bacterial pathogen, is frequently linked to hospital acquired infections, especially in immunocompromised individuals, necessitating prompt detection for control and prevention. The study aimed to examine the expression of antibiotic sensitivity and resistance genes in local *P. aeruginosa* isolates. The study was carried out between August 2023 to July 2024 at Islamabad Diagnostic centre (IDC), in the Department of Zoology, Quaid-i-Azam University, Islamabad. After being isolated from a variety of clinical specimens and processed in IDC, *P. aeruginosa* was identified and its sensitivity assessed. Genes for antibiotic resistance were identified in *P. aeruginosa*. From a total of 105 samples from pus, urine, sputum, BAL, tissues, tips, blood, wound and BW, 50 *P. aeruginosa* isolates were recovered. Antibiotics sensitivity results of these isolates showed that colistin and polymyxin B were found to be effective due to their 100% sensitivity. Moreover, amikacin, tazobactam piperacillin, tobramycin and meropenem showed 76%, 74%, 72%, and 72% effectiveness against *P. aeruginosa,* respectively. Bacteria showed highest resistance against co-trimoxazole (100%) and 32-44% resistance was also observed against imipenem and ciprofloxacin, levofloxacin, piperacillin. β lactam resistance genes (*DHA*) and ESBL genes (*CTX-M*) were present in 2% and 16% of isolates, respectively. Similarly, quinolones resistance genes *qnrA, qnrB,* and *qnrS* genes were detected in 2%, 6% and 2% of isolates respectively. The genes, *oqX, MOX, ACC, EBC, FOX, bla TEM, CIT*, and *bla SHV* did not exist in any of the isolate. It is critical to avoid using antibiotics against which *P. aeruginosa* has shown high resistance, as these isolates may have spread from person to person.

INTRODUCTION

The rod-shaped, Gram-negative bacterium *Pseudomonas aeruginosa* is well-known for producing green pigment (Peix *et al*., 2009). It is a member of a genus containing more than 120 species that are harmful to people, plants, and animals and are usually found in damp areas. *P. aeruginosa* has a high degree of genetic plasticity, which allows it to transform from a commensal to an opportunistic infection, particularly in people with weakened immune systems (Ramos, 2011). Immunocompromised patients are primarily affected by *P. aeruginosa*, which is primary cause of infections obtained in critical care facilities. It is associated with pneumonia, bacteraemia, cystic fibrosis, and urinary tract infections. Up to 23% of ICU infections are caused by this bacterium, which mostly affects the respiratory system (Gales *et al*., 2001).

The genome of *P. aeruginosa* consists of a single circular chromosome and plasmids that contain a high percentage of (65–67%) G+C (Winsor *et al*., 2016) and 5.5–7 Mbp genome size (Schmidt *et al*., 1996). All strains share a core genome that houses virulence factors, enable this bacterium to proliferate in diverse settings and infect humans (Römling *et al*., 1995).

This study aimed to examine the involvement of local *P. aeruginosa* strains in various infections by recovery from various types of infections along with its antibiotic susceptibility pattern and genes for antibiotic resistance in local *P. aeruginosa* strains via antibiotic sensitivity testing and PCRs.

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MATERIALS AND METHODS

Sample collection

One hundred and five samples of pus, urine, sputum, ear swab, blood, bronchoalveolar lavage (BAL), tissue, tips (tracheal tips, catheter tips, stent, foley catheter), bronchial washes (BW), wound, were collected at Islamabad Diagnostic Centre, Islamabad under Clinical and Laboratory Standard Institute (CLSI) guidelines. Isolates were recovered by culturing on MacConkey, Cled agar, and blood agar and incubated at 37 ºC temperature for 24 h. The isolates identification was confirmed by using microbiological conventional methods, which include Gram's staining response, the morphological appearance of the colonies, and other conventional biochemical tests such the oxidase test. MacConkey agar was used to differentiate between lactose vs non lactose fermenters, Cled agar for urinary microbe's differentiation while haemolysis was observed on blood agar. Further, strain was identified with the VITEK MS system (bioMérieux) and MALDI-TOF MS. A *P. aeruginosa* species-specific gene *ecfX PCR* was done to verify the isolate's identity.

Antibiotic susceptibility testing

Using the disc diffusion method, the antibiotic sensitivity of 50 positive *P. aeruginosa isolates* was assessed. Using a 0.5 McFarland standard, colonies were suspended in saline for inoculum preparation. Three orientations with 60° rotations were used to streak the suspension on Muller Hinton agar. Filter paper discs containing different antibiotics were positioned and incubated at 37ºC temperature for 24 h after being allowed to air dry (Bauer *et al*., 1966). Using a Vernier calliper, inhibition zones were calculated, and the results were classified as sensitive, intermediate, and resistant using CLSI recommendations (CLSI, 2015) (Table I).

PCR for detection of antibiotic resistance genes

Fifty *P. aeruginosa* samples were tested for antibiotic resistance genes using PCR. The resistant genes for quinolone, ESBL, and β-lactamase (AmpC) genes were identified with results confirmed through gel electrophoresis.

The boiling procedure was used to extract genomic and plasmid DNA. 4 to 5 colonies were transferred from the overnight culture and suspended in 200 µl of nuclease-free PCR water. In heating block, the tubes were heated for 10 min, for 5 min immediately cooled the tubes in ice and later centrifuged at 14,000 rpm for 10 min. Using the supernatant as template DNA.

Table I. List of antimicrobials tested.

S. No	Class	Name of antibiotics	Code	Conc. (ug)
1	Aminoglyco-sides	Amikacin	AK	30
		Gentamycin	CN	10
		Tobramycin	TOB	10
2	Cephalosporins	Ceftazidime	CAZ	10
		Cefoperazone+sulbactam	SCF	105
		Cefepime	FEP	30
3	Sulphonamides	Co-trimoxazole (Septran)	SXT	25
4	Quinolones	Levofloxacin	LEV	5
		Ciprofloxacin	CIP	5
5	Fluoroquinolo-nes	Moxifloxacin	MXF	5
6	Penicillin	Piperacillin	PRL	100
		Tazobactam piperacillin	TZP	110
7	Polymyxins	Colistin	CT	10
8	Carbapenems	Imipenem	IPM	10
		Meropenem	MEM	10
9	Polypeptide	Polymyxin B	PB	300

For PCR amplification AmpC *bla* genes, the primers shown in Table II were used. The final volume of 25 µl. Reaction contained 2.5 µl 10X KCl, 1.5 µl $MgCl_2$, 0.5 µl of 10mM dNTPs, 0.75 µl primers MOXF, MOXR, DHA-F, DHA-R, CIT-F, CIT-R, 0.63 µl primers ACC- F, ACC-R, EBC-F, EBC-R, 0.5 µl primers FOX-F, FOX-R, 0.5 µl Taq DNA polymerase (Thermo Scientific), 7 µl PCR water, and 5 µl template DNA. The thermal cycle comprised initial denaturation at 94 for 3 min, followed by 25 cycles each of denaturation at 94 for 30 sec, annealing at 64 for 30 sec and extension at 72 for 1 min. Then final extension at 72 for 7 min (Pérez-Pérez and Hanson, 2002).

For PCR amplification of ESBLs Genes (*bla TEM, bla SHV, bla CTX*), the 25 µl final volume of reaction mixture contained 0.8 µl $MgCl_2$, 0.5 µl of 10mM dNTPs, 2.5 µl of 10X NH_4SO_4 buffer, 0.25 µl Taq DNA polymerase (Thermo Scientific), 1 µl primers ESBL-TEM-F, ESBL-TEM-R, ESBL- SHV-F, ESBL- SHV-R, ESBL-CTX-M-F, ESBL-CTX-M-R, and 13.95 µl PCR water 5 µl template DNA. *ESBL* gene PCRs were conducted individually; they were not multiplex PCRs. The protocol involved an initial 5 min of denaturation at 94°C, 30 cycles each of denaturation at 94°C for 30 sec, annealing for bla TEM at 56°C, bla SHV at 58°C, and for bla CTXM at 54°C for 30 sec, and an extension at 72°C for 2 min. Finally, there was a single, final extension at 72°C for 10 min and a hold at 22°C in the PCR machine for an infinite number of cycles (Chen *et al*., 2010).

Table II. Primers used in the PCRs.

Gene/Sequence (5′ → 3′)	Product size(bp)
β-Lactamases (Pérez-Pérez and Hanson, 2002)	
AmpC-MOX F= GCTGCTCAAGGAGCACAGGAT R= CACATTGACATAGGTGTGGTGC	520
AmpC-CIT F= TGGCCAGAACTGACAGGCAAA R= TTTCTCCTGAACGTGGCTGGC	462
AmpC-DHA F= AACTTTCACAGGTGTGCTGGGT R= CCGTACGCATACTGGCTTTGC	405
AmpC-ACC F= AACAGCCTCAGCAGCCGGTTA R= TTCGCCGCAATCATCCCTAGC	346
AmpC-EBC F= TCGGTAAAGCCGATGTTGCGG R= CTTCCACTGCGGCTGCCAGTT	302
AmpC-FOX F= AACATGGGGTATCAGGGAGATG R= CAAAGCGCGTAACCGGATTGG	190
Extended Spectrum β-Lactamases (Chen *et al*., 2010)	
ESBL-TEM F= ATAAAATTCTTGAAGACGAAA R= GACAGTTACCAATGCTTAATC	1086
ESBL-SHV F= GGGTTATTCTTATTTGTCGC R= TTAGCGTTGCCAGTGCTC	567
ESBL-CTXM F= CGCTTTGCGATGTGCAG R= ACCGCGATATCGTTGGT	550
Plasmid-mediated quinolones (Robicsek *et al*., 2006; Qiu *et al*., 2019)	
oqxA F= GATCAGTCAGTGGGATAGTTT R= TACTCGGCGTTAACTGATTA	671
qnrA F= ATTTCTCA CGCCAGGATTTG R= GATCGGCAAAGGTTAGGTCA	516
qnrB F= GATCGTGAAAGCCAG AAAGG R= ACGATGCCTGGTAGTTGTCC	469
qnrS F= ACGACATTCGTCAACT GCAA R= TAAATTGGCACCCTGTAGGC	417

For PCR amplification of Qnr Genes (*qnrA, qnrB, qnrS*), the final volume of 25 µl of reaction mixture contained 2.5 µl of 10X KCl, 0.5 µl of 10mM dNTPs, 1.5 µl $MgCl_2$, 0.25 µl Taq DNA polymerase (Thermo Scientific), 1 µl primers qnrA-F, qnrA-R, qnrB-F, qnrB-R, qnrS-F, qnrS-R, 9.25 µl PCR water and 5 µl template DNA. The PCR conditions were performed with certain modifications including, denaturation at 94°C for 45 sec, annealing at 52°C for 45 sec, and extension at 72°C for 60 sec, with a cycle number of 32 (Robicsek *et al*., 2006).

For PCR amplification r *oqxA* gene, the 25 µl final volume contained 2.5 µl of 10X KCl, 0.5 µl of 10mM dNTPs, 1.5 µl $MgCl_2$, 0.25 µl Taq DNA polymerase (Thermo Scientific), 1 µl each primers oqxA-F and oqxA-R, 13.25 µl PCR water, and 5 µl template DNA. The PCR conditions were performed with certain modifications. For example, 5 min of initial denaturation at 94°C, followed by 30 cycles each of denaturation at 94°C for 60 sec, 55 sec of annealing at 56°C, and 6 min of extension at 68°C. Additionally, a final 10-min extension at 72°C (Qiu *et al*., 2019). Above PCR products were analysed on 1% agarose gel using ethidium bromide. A 50 bp DNA ladder was used and bands were visualised through gel documentation system.

RESULTS

Out of 105 samples cultured on MacConkey, Cled, and blood agar, 48% were identified as *P. aeruginosa*, displaying reddish or pale-yellow colonies. All 50 isolates tested positive for the oxidase test after gram staining showed pink rods. Samples presenting a green index for *P. aeruginosa* species with a confidence value of 95- 99.9% were considered to be positive in MALDI-TOF VITEK MS. A *P. aeruginosa* species-specific gene *ecfX* PCR verified the isolate's identity. Maximum isolates were found in pus, followed by urine, sputum, tissue, BAL, wound, blood, ear swab, catheter tips, and BW (Table III). The study found that 54% of the recovered strains were male, and 46% were female, with a median age of 50 years (Table IV).

Table III. Samples tested positive for *P. aeruginosa* from different sources.

S. no	Type of sample	Total samples taken	Sample +ve for *P. aeruginosa*
1	Pus	21	14
2	Urine	20	12
3	Sputum	11	9
4	Tissue	8	5
5	BAL	9	3
6	Wound	10	3
7	Blood	6	1
8	Ear swab	5	1
9	Tips	9	1
10	BW	6	1
	Total	105	50

Table IV. Age-wise distribution of samples.

Age group	Sample size
0-10	3
11-20	5
21-30	2
31-40	2
41-50	5
51-60	9
61-70	14
71-80	7
81-90	3

Table V. Antimicrobial sensitivity results for *P. aeruginosa.*

Antibiotic tested	Total isolates tested	Number of sensitive isolates (%)	Number of intermediate isolates (%)	Number of resistant isolates (%)
Amikacin (AK)	50	38 (76%)	1 (2%)	11 (22%)
Ceftazidime (CAZ)	50	34 (68%)	2 (4%)	14 (28%)
Co-trimoxazole (Septran) (SXT)	50	0(0%)	0(0%	50(100%)
Gentamycin (CN)	50	35 (70%)	1 (2%)	14 (28%)
Levofloxacin (LEV)	50	34 (68%)	0 (0%)	16 (32%)
Moxifloxacin (MXF)	50	33 (66%)	0 (0%)	15 (30%)
Piperacillin (PRL)	50	33 (66%)	0 (0%)	16 (32%)
Cefoperazone + sulbactam (SCF)	50	35 (70%)	1 (2%)	12 (24%)
Cefepime (FEP)	50	34 (68%)	1 (2%)	15 (30%)
Ciprofloxacin (CIP)	50	34 (68%)	0 (0%)	16 (32%)
Colistin (CT)	50	50(100%)	0(0%)	0(0%)
Imipenem (IPM)	50	27 (54%)	1 (2%)	22 (44%)
Meropenem (MEM)	50	36 (72%)	1 (2%)	13 (26%)
Tazobactam piperacillin (TZP)	50	37 (74%)	0(0%)	13 (26%)
Polymyxin B (PB)	50	49 (98%)	1 (2%)	0(0%)
Tobramycin (TOB)	50	36 (72%)	0 (0%)	14 (28%)

P. aeruginosa have shown highest resistance against Co-trimoxazole as 100%, followed by imipenem (44%), levofloxacin, ciprofloxacin, piperacillin (32%). colistin (100%) and polymyxin (100%) were more effective than other antimicrobials. Similarly, amikacin (76%), tazobactam piperacillin (74%), meropenem and tobramycin (72%) were also reported sensitive drugs (Table V).

Among β lactam resistance genes, only (DHA) gene and among *ESBL-CTXM, TEM, SHV* genes, only *CTX-M* were present in 2% and 16% of isolates, respectively. The study found that quinolones resistance genes *qnrA, qnrB*, and *qnrS* were detected in 2%, 6%, and 2% of isolates respectively, while *oqxA* gene was not detected.

DISCUSSION

P. aeruginosa, due to its widespread presence and resistance to antibiotics, poses a significant threat to healthcare professionals and the public. The decreased antibiotic uptake, efflux pump overexpression, overproduction of β-lactamases, and changed drug targets are among the mutations that render *P. aeruginosa* challenging to eliminate as compared to other pathogens (Breidenstein *et al*., 2011). Mutations in *mexR* and *nfxB* genes derepress the efflux pumps MexAB–OprM and MexCD–OprJ (Stickland *et al*., 2010), while *mexZ* gene mutation causes overexpression of MexXY–OprM, causing resistance to aminoglycosides, cefepime and fluoroquinolones (Muller *et al*., 2011).

This six-month study was directed to assess antibiotic sensitivity and resistant genes in hospital *P. aeruginosa* isolates, finding 48% MDR *P. aeruginosa* resistance against 16 antibiotics. A frequency of 36.5% was reported in Karachi hospitals in 2015 (Mansoor *et al*., 2015).

The study found 50 *P. aeruginosa* isolates in 105 clinical samples, with 54% from male and 46% from female patients. The patients median age of 50 years indicates that older persons may be more vulnerable to *Pseudomonas* infections. This study's findings align with a 2022 survey in Palestine, involving 54.6% males and 45.4% females, with a median age of 53 years (Shbaita *et al*., 2023). *P. aeruginosa* was primarily isolated from pus (28%), urine (24%), and sputum (18%) specimens, consistent with a study in Rawalpindi, Pakistan in 2010 (Gill *et al*., 2011) (Table VI).

Oue research found polymyxin B and colistin are 100% effective against *Pseudomonas* infections, with colistin being a 100% sensitive antimicrobial in Pakistan, according to Farooq *et al*. (2019) and Ladadweh *et al*. (2021). The study found amikacin (76%), piperacillin-tazobactam (74%), tobramycin (72%), and meropenem (72%), which were similar to results from a Peshawar 2014 study with amikacin showing the highest sensitivity (92.86%) (Samad *et al*., 2017). Pokharel *et al*. (2019) reported comparable findings with piperacillin/tazobactam (76.0%),

Table VI. Number of genes detected and source of isolates.

S. No	Source of isolates	Number of isolates	Antibiotic resistance pattern	Genes detected
1	Pus	5	SXT	Nil
2	Pus	3	IPM, SXT	Nil
3	Pus	4	AK, CAZ, IPM, MEM, SXT, TZP, FEP, TOB, CIP, CN, LEV, SCF MXF, PRL	Nil
4	Pus	1	AK, SXT, IPM, MEM, TZP, TOB, FEP, CIP, CN, LEV, PRL	Nil
5	Pus	1	SXT	*CTX, qnrS*
6	Urine	3	SXT	Nil
7	Urine	2	SXT, IPM	Nil
8	Urine	1	CAZ, SXT, MEM, TZP, TOB, SCF, PRL, FEP, CIP, CN LEV, MXF	Nil
9	Urine	1	AK, CAZ, SXT, IPM, MEM, TZP, TOB, FEP, CIP, CN, LEV, MXF, PRL, SCF	Nil
10	Urine	1	SXT, IPM	*qnrB*
	Urine	1	SXT, IPM	Nil
11	Urine	1	SXT	*DHA, qnrA*
12	Urine	1	SXT, IPM	*CTX*
13	Urine	1	CAZ, SXT, IPM, MEM, TZP, TOB, FEP, CIP, LEV, MXF, PRL, SCF	*CTX*
14	Sputum	2	SXT	*CTX*
15	Sputum	5	SXT	Nil
16	Sputum	1	SXT, PRL	Nil
17	Sputum	1	AK, CAZ, SXT, IPM, MEM, TZP, TOB, FEP, CIP, CN, LEV, MXF PRL, SCF	*CTX, qnrB*
18	Tissue (Hip)	3	SXT	Nil
19	Tissue (Hip)	1	SXT	*CTX*
20	Tissue (Hip)	1	AK, CAZ, SXT, IPM, FEP, CIP, CN, LEV, MXF, PRL,	*DHA*
21	BAL	2	SXT	Nil
22	BAL	1	SXT	*CTX*
23	Wound	2	AK, CAZ, SXT, IPM, MEM, TZP, TOB, FEP, CIP, CN, LEV, MXF, PRL, SCF	Nil
24	Wound	1	CAZ, SXT, IPM, PRL, FEP, CIP, LEV, MXF, PRL	*qnrB*
25	Blood	1	SXT	Nil
	Ear swab	1	SXT, IPM, TOB, CIP, CN, LEV, MXF, PRL, SCF	Nil
26	Tips	1	SXT	Nil
27	BW	1	CAZ, SXT, IPM, MEM, TOB, FEP, CIP, CN, LEV, MXF	Nil

AK, Amikacin; CAZ, Ceftazidime, CIP, Ciprofloxacin; FEP, Cefoperazone; GEN, Gentamicin; LEV, Levofloxacin; IMP, Imipenem; MEM, Meropenem; BPB, Polymyxin; TZP, Tazobactam piperacillin; TOB, Tobramycin; MXF, Moxifloxacin; PRL, Piperacillin; SCF, Cefoperazone + sulbactam; CT, Colistin; SXT, Co-trimoxazole.

amikacin (71.7%), tobramycin (71.7%) and meropenem 65.2% sensitive against *Pseudomonas* (Pokharel *et al*., 2019). Of all the antibiotics examined in this study, amikacin was the most effective medication of choice for treating MDRPA, followed by colistin and polymyxin.

This study found 100% cotrimoxazole resistance against *Pseudomonas* infections, consistent with previous studies with imipenem being the most resistant antibiotic (44%) (Aneela *et al*., 2019).

In Gujrat India, bacteria showed higher resistance against imipenem (Javiya *et al*., 2008), followed by β-lactams and quinolones, with ciprofloxacin, levofloxacin, and piperacillin showing 32% and 32%, respectively.

Data from a literature review reported that imipenem resistance in *P. aeruginosa* is caused by mutations in the protein present in outer membrane and deactivation of the *oprD* gene. Resistance against Imipenem and other antibiotics are caused by mutations in the *mexT* or *mexS* genes, which decrease OprD and increase MexEF-OprN pump activity. Furthermore, the acquisition of carbapenemase genes (CEGs) leads to chromosomal changes that cause carbapenem resistance, the hydrolysis of carbapenems by β-lactamases leads to resistance to most β-lactam antibiotics. These carbapenemases are characterized by the presence of heterologous genes obtained through HGT (Botelho *et al*., 2015, 2017, 2018;

Breidenstein *et al*., 2011). AmpC β-lactamase induction is influenced by ampG (PA4393), and mutation in this gene significantly reduces sensitivity to β-lactams. This suggests that *P. aeruginosa* uses defensive mechanisms in an adaptive manner to fight against the antibiotics' inhibitory effects (Zhang *et al*., 2010). It has been demonstrated that ampC target mutations promote cephalosporins's resistance (Berrazeg *et al*., 2015; Rodríguez-Martínez *et al*., 2009).

Different surveys reported that *P. aeruginosa* usually develop resistance against cephalosporins and penicillin such as piperacillin dur to overexpression of AmpC via certain mutations in genes involved in peptidoglycan-recycling, including *dacB, ampD, ampDh2, ampDh3* and *ampR* genes (Cabot *et al*., 2011; Juan *et al*., 2006; Moya *et al*., 2009). Mutations in the ftsK gene for cell division have been linked to inherent resistance to β-lactam and ciprofloxacin (Alvarez-Ortega *et al*., 2010; Breidenstein *et al*., 2008). *P. aeruginosa*'s resistance to FQs is caused by mutations in *gyrA, gyrB, parC,* and *parE* genes, which encode DNA gyrase and topoisomerase IV (Bruchmann *et al*., 2013).

In this study, PCR analysis on 50 strains isolated DNA revealed varying percentages of specific genes. Among the six *AmpC* genes, the study detected only *blaDHAM* gene in 4% of isolates, and the other five AmpC resistance genes were not found even in single amplicon. The study examined three genes *TEM, SHV,* and *CTX-M*, revealing the highest prevalence of the *blaCTX-M* gene at 16%. The most common ESBL gene in Brazil was blaCTX-M-2 (19.6%) (Polotto *et al*., 2012) Which is somehow close to our study.

The study identified quinolones resistance genes *qnrA* (2%), *qnrB* (6%), *qnrS* (2%), and *oqxA* (0%) in *Pseudomonas* isolates. *qnrB* was common among *qnr* genes. Our results were in accordance with study where *qnrB* found to be high prevalent gene (Saki *et al*., 2022).

Antimicrobial resistance is influenced by HGT of resistance genes, bacterial mutations, and spontaneous evolution. Healthcare settings are hotspots for AMR, with longer hospital stays and increased antimicrobial costs. The development of cheap, novel antimicrobials, avoiding longer stays, and low healthcare costs are crucial for combating this problem.

Hand hygiene is crucial in reducing healthcare-associated infections. Promoting frequent hand decontamination and enhancing hand wash basin availability can improve compliance. Decontaminating hospital floor and equipment is essential for *Pseudomonas* infections. Enforcing a law prohibiting medication sales without a physician's prescription is necessary to prevent self-medication.

CONCLUSION

In conclusion, antimicrobial susceptibility testing (AST) results revealed that a significant percentage of *P. aeruginosa* isolates had β-lactam, quinolones and fluoroquinolones resistance. The most effective antibiotics according to the results are, colistin, polymyxin B, amikacin, piperacillin/tazobactam and tobramycin. *P. aeruginosa* shown high resistance to antibiotics such as co-trimoxazole, ciprofloxacin, imipenem, levofloxacin, and piperacillin. Antibiotics like colistin and polymyxin B will be helpful if the strain of *P. aeruginosa* is highly drugs resistant. Therefore, physicians must provide rational treatments to prevent the emergence of *P. aeruginosa* strain antibiotic resistance. As a result, it is critical to avoid using antibiotics against which *P. aeruginosa* has shown high resistance, as these isolates may have spread from person to person. In our study, the AMR genes that have been found were *DHA, CTX-M, qnrA, qnrB,* and *qnrS*. Therefore, it is critical to carry out additional research focusing on resistance genes other than those examined in this study, as limited AR genes have been found in *P. aeruginosa* in the present study.

DECLARATIONS

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Ethical statement and IRB approval

This study was approved by the Institutional Bioethics Committees of Quaid-i-Azam University.

Generative AI and AI-assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

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