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ORIGINAL ARTICLE

Antibiogram and molecular profiling of non-respiratory isolates of *Pseudomonas aeruginosa* using ERIC-PCR in a tertiary care hospital

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ABSTRACT

Background and Objectives: *Pseudomonas aeruginosa* is a major nosocomial pathogen implicated in a wide range of hospital-acquired infections. Prompt detection and effective control of outbreaks caused by this organism are essential to minimize associated morbidity and mortality. Multiple strains may circulate within healthcare settings due to cross-transmission and persistent environmental reservoirs. Various molecular typing techniques have proven valuable in tracing the sources and transmission dynamics of outbreaks. The present study aimed to investigate the antibiogram profiles and enterobacterial repetitive intergenic consensus–polymerase chain reaction (ERIC-PCR) patterns of *P. aeruginosa* isolates recovered from non-respiratory clinical samples at a tertiary care hospital.

Materials and Methods: A total of 157 clinical isolates phenotypically identified as *P. aeruginosa* were subjected to 16S rDNA-PCR for species confirmation. Among these, 150 isolates were confirmed as *P. aeruginosa* and subsequently analysed for antimicrobial susceptibility and molecular typing. Antimicrobial susceptibility testing was performed according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Molecular typing was carried out using ERIC-PCR. The resulting ERIC-PCR profiles were analysed using PAST version 4.03 software, and a dendrogram was constructed based on Dice similarity coefficients to assess genetic relatedness among isolates. Fisher's exact test was employed to determine associations between specific ERIC types and antibiotic resistance profiles.

Results: Overall, 84% of the *P. aeruginosa* isolates were identified as multidrug-resistant (MDR), with widespread distribution among both inpatients and outpatients. The highest resistance rate was observed against ticarcillin/clavulanic acid, followed by ceftazidime, whereas no resistance was detected against colistin sulphate. A total of 54 distinct antibiotypes were identified, indicating substantial variability in antimicrobial resistance patterns. All 150 isolates were successfully typed using ERIC-PCR, which revealed 41 distinct clusters at an approximate similarity coefficient of 70%, demonstrating considerable genetic diversity among the isolates.

Conclusion: This study enhances the understanding of the epidemiology of *P. aeruginosa* infections in healthcare settings by characterizing both the molecular diversity and antimicrobial resistance patterns of clinical isolates. The high prevalence of MDR strains and the circulation of multiple genetically diverse strains among hospital patients highlight the ongoing risk of transmission within the healthcare environment. Notably, the findings suggest a comparatively higher rate of cross-infection among burn patients. These observations underscore the urgent need for stringent infection prevention and control measures, particularly in burn units, to limit the spread of MDR *P. aeruginosa.*

Keywords: *Pseudomonas aeruginosa*; Polymerase chain reaction (PCR); Multi drug resistance; Molecular typing (ERIC); 16S Ribosomal gene (16S rRNA gene); Antibiogram; Microbial sensitivity testing

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INTRODUCTION

Pseudomonas comprises a group of approximately 120 closely related species collectively referred to as pseudomonads. Among them, *Pseudomonas aeruginosa* has been recognized as a major species based on phylogenetic analyses conducted in 2020 (1). It is ubiquitously distributed in nature and demonstrates remarkable adaptability, enabling its survival in domestic habitats as well as hospital environments. Although it is rarely a part of the normal microbiota of healthy individuals, *P. aeruginosa* is an important opportunistic pathogen. Transmission may occur through ingestion of contaminated food or water, exposure to contaminated medical devices or solutions, penetrating injuries such as burn wounds, and the use of contaminated needles among intravenous drug users (2). In healthcare settings, *P. aeruginosa* is commonly associated with ventilator-associated pneumonia, catheter-associated urinary tract infections; burn wound infections, and septicemia, with its pathogenicity being multifactorial in nature (3).

Antimicrobial susceptibility testing (AST) of *P. aeruginosa* is particularly important because of the increasing emergence of multidrug-resistant (MDR) strains. The Kirby–Bauer disk diffusion method remains a simple, cost-effective, and widely used technique that does not require automated or sophisticated instrumentation. Treatment of *P. aeruginosa* infections has become increasingly challenging due to its intrinsic resistance to multiple antimicrobial agents and the rapid acquisition of additional resistance mechanisms. Consequently, therapeutic options are becoming progressively limited. Of particular concern is the recent emergence of carbapenemase-producing strains of *P. aeruginosa* (2).

Infections caused by MDR isolates are associated with prolonged hospitalization, increased healthcare costs, and higher morbidity and mortality rates. Antimicrobial resistance may arise through chromosomal mutations or via horizontal transfer of resistance determinants mediated by extra chromosomal genetic elements such as plasmids and transposons, often under selective antimicrobial pressure (4).

At any given time, multiple strains of *P. aeruginosa* may circulate within a healthcare setting. Molecular typing of these strains is essential for outbreak investigation, identification of unusual phenotypes, detection of clonal spread, epidemiological surveillance, and implementation of effective infection control measures (5). Among the available typing techniques, Enterobacterial Repetitive Intergenic Consensus polymerase chain reaction (ERIC-PCR) is considered a rapid, simple, cost-effective, reproducible, and discriminatory genotyping method. ERIC sequences are 127-bp imperfect palindromic repetitive elements present in multiple copies throughout bacterial genomes. ERIC-PCR amplifies genomic regions located between ERIC sequences, enabling differentiation of bacterial strains based on variations in the distribution of these elements within the genome. This technique has been successfully applied for molecular typing of several bacterial pathogens, including *Escherichia coli*, *Staphylococcus aureus*, *Acinetobacter baumannii*, and *Helicobacter pylori* (6).

The present study aimed to investigate the antibiotypes and ERIC-PCR patterns of *P. aeruginosa* isolates recovered from non-respiratory clinical samples at a tertiary care hospital.

MATERIALS AND METHODS

The study period extended over ten months from January to October 2022 in the Department of Microbiology at SKIMS, Soura, a tertiary care hospital. Collection and isolation of samples was done in first three months and followed by seven months of molecular testing. This research protocol was reviewed and approved by the Institutional Ethical committee (IEC/SKIMS Protocol #085/2022, dated 16-06-2022), ensuring highest ethical standards for human research.

Bacterial strains. Consecutive, non-repeat, clinical isolates of *P. aeruginosa* (n=157) isolated from different clinical samples received in our laboratory were identified by phenotypic methods including gram-staining, catalase, oxidase, growth on MacConkey agar (HiMedia, Mumbai, India) and other biochemical tests. A confirmed bacterial strain of *P. aeruginosa* obtained from PGI, Chandigarh and ATCC 27853 strain was also included in the study as control strains.

DNA extraction. Genomic DNA was extracted from 157 isolates using alternate boiling and freezing method (7). Briefly, a colony from nutrient agar were transferred into Luria Bertani (LB) broth (HiMedia, Mumbai India) and incubated at 37°C for 48 hours. After brief vortexing, 1.5ml of the broth was trans-

ferred into a 2ml microfuge tube and centrifuged at 10,000 rpm (10,864 × g) for 10 min using a Heraeus Megafuge 16R (Thermo Fisher Scientific Inc.). After the supernatant was discarded, 1.5ml of RNase free water was added to the pellet and vortexed. Tube was then subjected to alternate cycles of boiling (at 100°C 10 min in water bath) and freezing (at -20°C for 10 min) thrice. Finally, the tube was centrifuged at 10,000 rpm (10,864 × g) for 10 min. The final supernatant was assessed for purity and concentration using NanoDrop spectrophotometer (Multiskan Sky; Thermo Fisher). DNA samples with a A260/A280 ratio of approximately 1.8 were considered pure and stored at -20°C for PCR analysis.

***P. aeruginosa* confirmation by 16s rRNA.** All the 157 isolates identified phenotypically were processed for species-specific 16s rDNA gene using a primer pair as described by Hematzadeh A et al (2021) (4), F: (5'-GGGGGATCTTCGGACCTCA-3') and R: (5'-TCCTTAGAGTGCCCACCCG-3') which yielded a 956 bp band (4). The extracted DNA was subjected to PCR using specific primers synthesized by Sigma Aldrich Chemicals Pvt. Ltd. and amplification was done by Applied Biosystems thermocycler (Model 2720). The reaction mixture consisted of 25µl volume containing 4µl of DNA template and 21µl of master-mix (18.25 µL of distilled water, 2.5µL of 10X buffer, 0.5 µL of 10 mM dNTPs, 0.25 µL of 10 mM of each primer, and 0.25 µL of 5 U/µL Taq polymerase). A three-room setup was used to prevent amplicon contamination. Sterile nuclease water was used as a negative control which showed no amplification.

The thermocycler PCR amplification conditions were set at an initial denaturation at 95°C for 2 minutes; followed by 30 cycles of denaturation at 94°C for 20 seconds, annealing at 58°C for 20 seconds and extension at 72°C for 40 seconds and a final extension at 72°C for 7 minutes. PCR products were further separated by electrophoresis on a 2% agarose gel (LE Quick Puregene) stained with ethidium bromide at 80V/cm^2 for 45 min and detected in comparison to 100bp DNA ladder from Thermo Fisher as molecular weight marker in UV gel documentation system (AlphaImager, USA). ATCC 27853 strain of *P. aeruginosa* obtained from PGI, Chandigarh was used a positive control.

Of the 157 isolates, 150 isolates and the control strain, were found to be positive for the 956 bp band on PCR (Fig. 1). The 150 confirmed isolates were thus included in this study. These 150 isolates were recovered from body fluids 21 (14%), wound swab 95 (63%), urinary samples 26 (17%), tissue 1 (0.6%), bone marrow 1 (0.6%), catheter tips 3 (2%) and blood cultures 3 (2%).

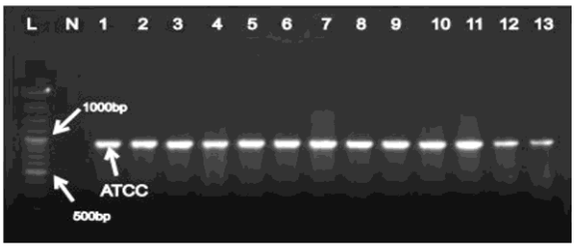

Fig. 1. Gel electrophoresis confirmation of *P. aeruginosa* by 16r DNA-PCR. Lane L: 100 bp DNA ladder, Lane N: Negative control (Sterile nuclease free water), Lane 1 ATCC Control strain, Lanes 2-13 *P. aeruginosa* isolates

Microbial sensitivity testing. Microbial sensitivity testing was done in accordance to the CLSI 2022, 32nd edition using Kirby-Bauer disc diffusion method on Mueller-Hinton agar (HiMedia Pvt. Ltd. Mumbai, India), except for colistin sulphate (Potency ≥15000 IU/µg from Sigma-Aldrich Chemicals Pvt. Ltd) which was tested by broth microdilution (BMD) using cation-adjusted Mueller–Hinton broth (CAMHB), which is the reference method for testing colistin MIC. MIC values ≤ 2 µg/mL are considered intermediate and those with MIC ≥ 4 µg/mL are considered resistant (7).

All the 150 isolates were tested for their susceptibility against seven class of antimicrobials, which included cephalosporins: cefepime (30 µg), ceftazidime (30 µg); beta lactams: aztreonam (30 µg); aminoglycosides: amikacin (30 µg), gentamicin (10 µg), carbapenems: meropenem (10 µg), imipenem (10 µg), doripenem (10 µg); beta lactam-beta lactamase inhibitor (BL/BLI) combinations: piperacillin-tazobactam (100/10 µg), cefoperazone/sulbactam (75/30 µg), ticarcillin/clavulanic acid (75/10 µg); quinolones: ciprofloxacin (5 µg), levofloxacin (5 µg); polymyxins: colistin sulphate (potency ≥15000 IU/mg); tigecycline (30 µg). ATCC 27853 strain of *P. aeruginosa* was used as quality control strain for both disc diffusion as well as BMD for determination of susceptibility and colistin MIC respectively.

ERIC-PCR genotyping of *P. aeruginosa* isolates. All the confirmed 150 isolates of *P. aeruginosa* were subjected to ERIC-PCR using protocol followed by

Nitz et al. (2021) (8), ERIC-1 (5' -ATGTAAGCTCCT-GGGGATTCAC-3') and ERIC-2 (5' - AAGTAAGT-GACTGGGGTGAGCG-3') primers (Sigma Aldrich Chemical Pvt. Ltd). ATCC 27853 strain of *P. aeruginosa* was used as positive control for PCR efficiency and banding patterns. A gradient PCR was performed in triplicate using DNA from the control strain to determine the optimal annealing temperature for ERIC-PCR. The annealing temperature gradient ranged from 30°C to 45°C. The amplification was done using Applied Biosystems thermocycler, in a 25 µl volume containing 3 µl of DNA template and 22 µl of master-mix (19.25 µL of distilled water, 2.5 µL of 10X buffer, 0.5 µL of 10 mM dNTPs, 0.25 µL of 10 mM of each primer, and 0.25 µL of 5 U/µL Taq polymerase). The PCR conditions were set at an initial denaturation at 94°C for 3 minutes; followed by 40 cycles of 94°C for 50 seconds, 30 to 45°C gradient temperatures for 1 minute and 72°C for 2 minutes and followed by final extension at 72°C for 7 minutes. The PCR products were further analysed by electrophoresis gel on 2.5% agarose gel (LE Quick, Puregene) stained with ethidium bromide at 85V/cm^2 for 1 hour. Multiple bands obtained (Fig. 2) were analysed in comparison to a 100bp DNA ladder as molecular weight marker in UV gel documentation system (AlphaImager, USA). An annealing temperature of 42°C was found to be optimal.

To evaluate the precision and reproducibility of our standardized method, the protocol was repeated in triplicate for a batch of ten isolates using a conventional thermocycler (Applied Biosystems 2720). Visual comparison of band patterns showed no discernible differences for all the batches, indicating good reproducibility of the method.

Data analysis. Microbial sensitivity data were summarized as frequencies and percentages for each drug tested.

The association between MDR status and patient category (inpatients versus outpatients) was evaluated using the Chi-square test. The analysis yielded a p-value of 0.811, indicating no statistically significant association between MDR status and patient category.

Similarly, the association between MDR status and ward distribution (burn ward versus other wards) was analyzed using the Chi-square test, which also demonstrated no statistically significant relationship between the variables ($p = 0.697$).

For the analysis of the association between ERIC-PCR clusters and MDR status, several expected cell frequencies were less than 5, violating the assumptions of the Chi-square test. Therefore, Fisher's Exact test was applied instead. The resulting p-value was 0.458, indicating no statistically significant association between ERIC-PCR cluster distribution and MDR status.

The ERIC-PCR products were analysed using a UV gel documentation system, considering all visible bands. Band intensity variations among isolates were not considered significant. The sizes of the ERIC-PCR products were determined by comparison with a 100 bp DNA ladder (Thermo Fisher), serving as a molecular weight marker.

The ERIC-PCR fingerprints of the amplified DNA fragments of 150 isolates obtained from agarose gel electrophoresis were documented and analysed. ERIC fingerprints of all 150 isolates were converted into a binary matrix, where '1' indicated the presence and '0' indicated the absence of bands.

For evaluation of clustering, each isolate was considered an operational taxonomic unit (OTU). The clustering correlation was based on the Dice similarity coefficient clustering using ERIC-PCR based on the unweighted pair group method with arithmetic averages (UPGMA). To facilitate interpretation and reduce the number of OTUs in the dendrogram, iso-

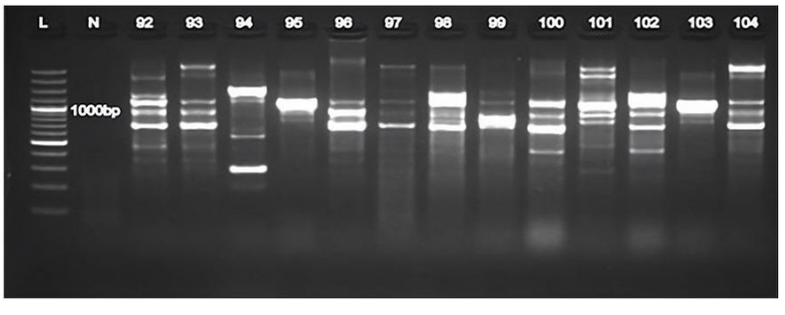

Fig. 2. ERIC-PCR profiles of *P. aeruginosa* isolates. Lane L: 100bp DNA ladder, N: Negative control (Sterile nuclease free water), Lanes 92-104 strains of *P. aeruginosa*.

lates exhibiting a similarity coefficient at a position tolerance of 70% (Dice similarity coefficient at 0.70) were categorized as a single isolate or ERIC type. The banding pattern generated by ERIC-PCR was analysed using the numerical taxonomy and multivariate analysis software PAST (Version 4.03) (9).

RESULTS

Demographic characteristics. Among the 150 confirmed *P. aeruginosa* isolates, 94 (62.6%) isolates were from in-patients and 56 (37.4%) were from out-patients. The isolates were collected from 150 patients, of whom 74 (49.4%) were female and 76 (50.6%) were male (Table 1). Highest number of isolates were recovered from burn ward 33 (22%), followed by colorectal ward 11 (7.3%).

Table 1. Demographic characteristics of patients

Gender Distribution of Samples (n=150)	
Male	Female
76 (50.6%)	74 (49.4%)
Location Distribution of Samples	
Outpatients	Inpatients
55 (36.7%)	95 (63.3%)

Microbial sensitivity testing. All the 150 isolates were tested for their susceptibility against 15 antibiotics (Table 2).

Isolates exhibiting resistance to at least one antimicrobial agent in three or more antimicrobial categories were considered to be multi-drug resistant (MDR) (10). Overall, 125 (83.3%) of the isolates displayed resistance to at least three antibiotic classes, highlighting the high prevalence of multidrug resistance among our study population. Antibiotic resistance rates varied widely (Table 2), ranging from 0% for colistin sulphate to 96.7% for Ticarcillin/Clavulanic acid. All the isolates had MIC less than 2µg/ml by broth microdilution method (BMD) for colistin sulphate while as 19 (12.7%) isolates were non-MDR and 6 (4%) isolates were pan-susceptible (susceptible to all 15 antibiotics tested). Among the 95 isolates recovered from inpatients, 78 (82.1%) isolates were MDR and 17 (17.9%) isolates were non-MDR, while 55 isolates recovered from outpatients, 46 (83.6%) were MDR and 6 (16.4%) were non-MDR isolates.

Table 2. Overall percentage resistance to tested antibiotics

S. No.	Antibiotic tested (n=15)	Observed Resistance in *Pseudomonas aeruginosa* isolates (n=150) (%)
1	Amikacin	70 (46.7%)
2	Aztreonam	77 (51.3%)
3	Cefepime	115 (76.7%)
4	Cefoperazone/sulbactam	111 (74%)
5	Ceftazidime	132 (88%)
6	Ciprofloxacin	83 (55.3%)
7	Doripenem	107 (71.3%)
8	Gentamicin	79 (52.7%)
9	Imipenem	94 (62.7%)
10	Levofloxacin	90 (60%)
11	Meropenem	71 (47.3%)
12	Piperacillin/tazobactam	106 (70.7%)
13	Colistin sulphate	0 (%)
14	Ticarcillin/clavulanic acid	145 (96.7%)
15	Tigecycline	114 (76%)

The 150 resistant isolates exhibited wide range of diversity in their antibiotic resistance profiles, yielding 54 distinct antibiograms. Each unique antibiogram was considered a distinct antibiotype (Table 3) taking into consideration a single antibiotic from each class of tested antimicrobials (AMK-LVX-ATM-CAZ-CSL-TZP-MEM-CS) as its representative antibiotic.

The most prevalent antibiotypes were antibiotype 54 (AMK-LVX-ATM-CAZ-CSL-TZP-MEM), antibiotype 50 (AMK-LVX-CAZ-CSL-TZP-MEM), and antibiotype 22 (CAZ-CSL-TZP-MEM), with 21 (14%), 12 (8%), 8 (5.3%) isolates, respectively. Notably, 25 of the 54 antibiotypes (46.3%) were represented by a single isolate, indicating a relatively higher degree of strain diversity within this collection. Isolates belonging to antibiotype 1 included isolates susceptible to all antimicrobials while antibiotype 54 constituted pan resistant isolates (except colistin sulphate). All the 125 MDR strains were distributed across all 54 antibiotypes, highlighting the complexity of resistance patterns acquired by *P. aeruginosa.*

Genetic diversity by 16s RNA and ERIC PCR analysis. All the 150 isolates showed an expected result on 16s RNA PCR analysis. All 150 isolates and positive control exhibited an expected band size of 956bp for 16s RNA PCR (Fig. 1).

All the 150 isolates were typeable by ERIC-PCR,

Table 3. Observed antibiograms of resistant *P. aeruginosa* isolates.

Antibiotype	Isolate No.	Antibiotic Resistance Profile (Representing six antimicrobial classes)	No.(%)
1	PSA56, PSA70, PSA71, PSA104	Sensitive to all tested antimicrobials	4 (2.7)
2	PSA102, PSA161	TZP	2 (1.3)
3	PSA55, PSA77	CAZ	2 (1.3)
4	PSA13, PSA14, PSA29, PSA86, PSA169	ATM	5 (3.3)
5	PSA196	CSL-TZP	1 (0.7)
6	PSA20, PSA44, PSA101, PSA160	CAZ-TZP	4 (2.7)
7	PSA37, PSA52, PSA82, PSA90, PSA91, PSA95	CAZ-CSL	6 (4)
8	PSA68	ATM-CSL	1 (0.7)
9	PSA34, PSA50, PSA61	ATM-CAZ	3 (2)
10	PSA182	CIP-TZP	1 (0.7)
11	PSA67	CIP-CAZ	1 (0.7)
12	PSA58, PSA162	CAZ-CSL-MEM	2 (1.3)
13	PSA3, PSA5, PSA31	CAZ-CSL-TZP	3 (2)
14	PSA80	ATM-CAZ-TZP	1 (0.7)
15	PSA135, PSA140	ATM-CAZ-CSL	2 (1.3)
16	PSA115	CIP-CSL-TZP	1 (0.7)
17	PSA139	CIP-CAZ-CSL	1 (0.7)
18	PSA6	AMK-CAZ-TZP	1 (0.7)
19	PSA40	AMK-ATM-CSL	1 (0.7)
20	PSA154	AMK-ATM-CAZ	1 (0.7)
21	PSA51	AMK-CIP-CAZ	1(0.7)
22	PSA110, PSA118, PSA123, PSA125, PSA126, PSA129, PSA131, PSA133	CAZ-CSL-TZP-MEM	8 (5.3)
23	PSA32, PSA83	ATM-CAZ-TZP-MEM	2 (1.3)
24	PSA10, PSA39, PSA41, PSA46	ATM-CAZ-CSL-TZP	4 (2.7)
25	PSA137	CIP-CAZ-CSL-MEM	1 (0.7)
26	PSA146, PSA148, PSA166, PSA175, PSA179, PSA185	CIP-CAZ-CSL-TZP	6 (4)
27	PSA36, PSA38, PSA49	CIP-ATM-CAZ-TZP	3 (2)
28	PSA99	AMK-CAZ-CSL-MEM	1 (0.7)
29	PSA158	AMK-CAZ-CSL-TZP	1 (0.7)
30	PSA8	AMK-ATM-CAZ-TZP	1 (0.7)
31	PSA84	AMK-CIP-CSL-MEM	1 (0.7)
32	PSA63	AMK-CIP-CAZ-TZP	1 (0.7)
33	PSA64	AMK-CIP-ATM-CAZ	1 (0.7)
34	PSA59, PSA96, PSA128	ATM-CAZ-CSL-TZP-MEM	3 (2)
35	PSA109, PSA164, PSA184	CIP-CAZ-CSL-TZP-MEM	3 (2)
36	PSA85, PSA87, PSA89	CIP-ATM-CAZ-CSL-MEM	3 (2)
37	PSA23, PSA178, PSA191	CIP-ATM-CAZ-CSL-TZP	4 (2.7)
38	PSA134	AMK-CAZ-CSL-TZP-MEM	1 (0.7)
39	PSA151	AMK-ATM-CAZ-TZP-MEM	1 (0.7)
40	PSA92, PSA108	AMK-ATM-CAZ-CSL-MEM	2 (1.3)
41	PSA1, PSA57	AMK-ATM-CAZ-CSL-TZP	2 (1.3)
42	PSA79	AMK-CIP-CAZ-TZP-MEM	1 (0.7)
43	PSA88	AMK-CIP-CAZ-CSL-MEM	1 (0.7)
44	PSA145, PSA149, PSA163, PSA168, PSA187,	AMK-CIP-CAZ-CSL-TZP	6 (4)

Table 3. Continuing ...

	PSA198		
45	PSA35	AMK-CIP-ATM-CSL-MEM	1 (0.7)
46	PSA62, PSA100	AMK-CIP-ATM-CAZ-TZP	2 (1.3)
47	PSA105, PSA136	AMK-CIP-ATM-CAZ-CSL	2 (1.3)
48	PSA127, PSA130, PSA165	CIP-ATM-CAZ-CSL-TZP--MEM	3 (2)
49	PSA75, PSA122	AMK-ATM-CAZ-CSL-TZP-MEM	2 (1.3)
50	PSA7, PSA112, PSA124, PSA138, PSA144, PSA150, PSA167, PSA171, PSA173, PSA176, PSA189, PSA194	AMK-CIP-CAZ-CSL-TZP-MEM	12 (8)
51	PSA199	AMK-CIP-ATM-CAZ-TZP-MEM	1 (0.7)
52	PSA103	AMK-CIP-ATM-CAZ-CSL-MEM	1 (0.7)
53	PSA24, PSA152, PSA156, PSA197	AMK-CIP-ATM-CAZ-CSL-TZP	4 (2.7)
54	PSA12, PSSA21, PSA26, PSA28, PSA33, PSA48, PSA94, PSA106, PSA117, PSA141, PSA142, PSA143, PSA159, PSA170, PSA172, PSA174, PSA181, PSA190, PSA193, PSA195, PSA200	AMK-CIP-ATM-CAZ-CSL-TZP-MEM	21 (14)

AMK=Amikacin, CIP=Ciprofloxacin, ATM= Aztreonam, CAZ=Ceftazidime,
CSL= Cefaperazone-Sulbactum, TZP= Piperacillin-Tazobactam, MEM=Meropenem.

producing multiple molecular weight bands ranging from 55-1577 bp in size indicating wide range of genetic diversity among the isolates (Fig. 2).

The dendrogram at similarity coefficient of 70% was obtained for all the 150 isolates (Fig. 3).

127 ERIC types (P1 to P127) were obtained (Table 4). Strikingly, 109 isolates were genetically non-identical had consisted of single isolates indicating high prevalence of genetic diversity. However, ERIC types P14, P18, P20, P40, P44, P48, P51, P57, P66, P71, P75, P99, P108, P119 comprised of two isolates each, while ERIC types P19, P88 and P12 consisted of three isolates each. Interestingly ERIC type P50 had four isolates. All the 150 isolates were further grouped into 41 clusters (I to XLI) at a 70% similarity coefficient, again showing high genetic diversity among our isolates. Clusters II, IX, XI, XIII, XXII, XXIV, XXXIV, XXXVIII consisted of single isolates belonging to different ERIC types thus, depicting very high genetic diversity with rest of the isolates.

DISCUSSION

Pseudomonas aeruginosa is an opportunistic pathogen commonly encountered in hospital environments, where it frequently exists as a colonizer. It has the ability to colonize the hands of healthcare workers, invasive medical devices, endotracheal tubes, suction tubing, and hospital surfaces, particularly in high dependency units (HDUs) (2). Owing to its intrinsic and acquired resistance mechanisms, therapeutic options for *P. aeruginosa* infections are limited. The rapid emergence and dissemination of resistance genes have further complicated the control and eradication of infections caused by this organism. Intensive care unit admission, mechanical ventilation, acute respiratory failure, and central venous catheterization are major risk factors associated with increased mortality in patients with *P. aeruginosa* bacteremia (11). Despite advances in diagnostic techniques, implementation of surveillance programs, and development of newer antimicrobial agents, multidrug-resistant (MDR) *P. aeruginosa* continues to pose a major challenge to clinicians and microbiologists, particularly in nosocomial settings (12).

Consistent with previous reports, our findings demonstrated a high prevalence of *P. aeruginosa* infections among burn patients and in HDUs, along with a high burden of MDR strains circulating within the hospital environment (1-3). The species-specific 16S rDNA primers used in this study successfully amplified the target gene, confirming the accurate identification of *P. aeruginosa*, as previously de-

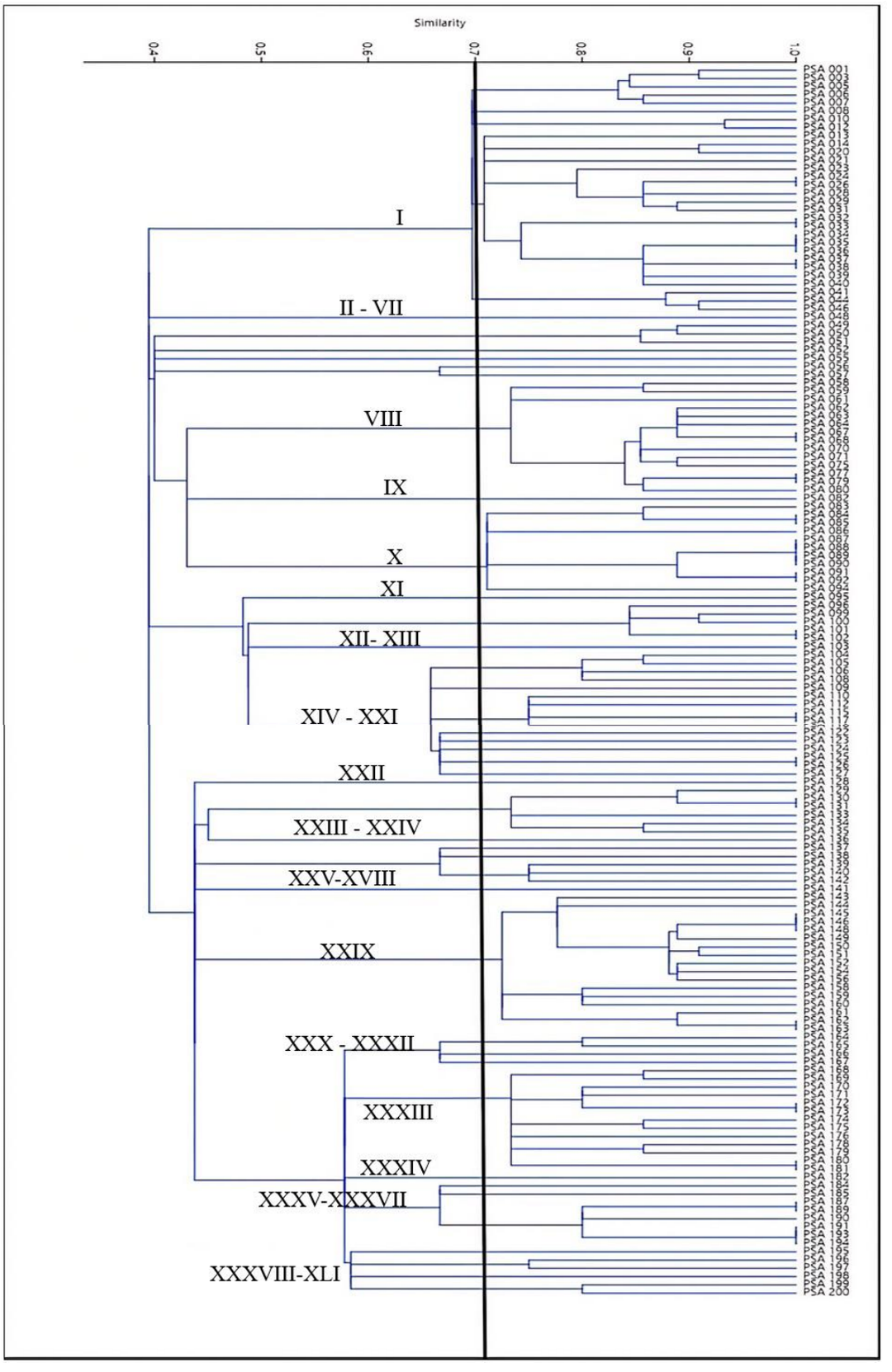

Fig. 3. Dendrogram depicting the genetic relatedness of antibiotic-resistant *P. aeruginosa* isolates based on ERIC-PCR profiles.

Table 4. ERIC-PCR typing and characteristics of antibiotic-resistant of *P. aeruginosa* isolates.

S. No.	ERIC Types	Isolates ID	Specimen	Gender	Location	MDR Status
1	P1	1	Wound swab	Male	inpatient	MDR
2	P2	3	Urine	Male	outpatient	MDR
3	P3	5	Wound swab	Female	inpatient	NON-MDR
4	P4	6	Wound swab	Female	inpatient	MDR
5	P5	7	Urine	Female	outpatient	MDR
6	P6	8	Wound swab	Male	inpatient	MDR
7	P7	10	Wound swab	Male	inpatient	MDR
8	P8	12	Body fluid	Male	inpatient	MDR
9	P9	13	Wound swab	Male	outpatient	NON-MDR
10	P10	14	Body fluid	Female	outpatient	NON-MDR
11	P11	20	Body fluid	Female	inpatient	NON-MDR
12	P12	21	Wound swab	Female	inpatient	MDR
13	P13	23	Wound swab	Male	inpatient	MDR
14	P 14	24	Wound swab	Male	inpatient	MDR
15		26	Wound swab	Male	inpatient	MDR
16	P 15	28	Wound swab	Male	inpatient	MDR
17	P 16	29	Blood	Female	inpatient	NON-MDR
18	P 17	31	Body fluid	Male	inpatient	MDR
19	P 18	32	Body fluid	Male	inpatient	MDR
20		33	Wound swab	Female	inpatient	MDR
21	P 19	34	Wound swab	Female	inpatient	MDR
22		35	Wound swab	Female	inpatient	MDR
23		36	Wound swab	Female	outpatient	MDR
24	P 20	37	Wound swab	Male	inpatient	MDR
25		38	Body fluid	Female	inpatient	MDR
26	P 21	39	Body fluid	Male	inpatient	MDR
27	P 22	40	Wound swab	Female	inpatient	MDR
28	P 23	41	Body fluid	Female	inpatient	MDR
29	P 24	44	Wound swab	Male	outpatient	NON-MDR
30	P 25	46	Wound swab	Female	outpatient	MDR
31	P 26	48	Wound swab	Male	inpatient	MDR
32	P 27	49	Body fluid	Female	inpatient	MDR
33	P 28	50	Wound swab	Male	outpatient	MDR
34	P 29	51	Bone marrow	Male	inpatient	MDR
35	P 30	52	Body fluid	Male	inpatient	MDR
36	P 31	55	Wound swab	Female	outpatient	NON-MDR
37	P 32	56	Wound swab	Male	inpatient	NON-MDR
38	P 33	57	Wound swab	Male	inpatient	MDR
39	P 34	58	Wound swab	Female	inpatient	NON-MDR
40	P 35	59	Wound swab	Male	inpatient	MDR
41	P 36	61	Wound swab	Female	outpatient	MDR
42	P 37	62	Wound swab	Male	inpatient	MDR
43	P 38	63	Wound swab	Female	inpatient	MDR
44	P 39	64	Urine	Male	outpatient	MDR
45	P 40	67	Wound swab	Female	inpatient	NON-MDR
46		68	Wound swab	Male	inpatient	NON-MDR
47	P 41	70	Wound swab	Male	inpatient	NON-MDR

Table 4. Continuing ...

48	P 42	71	Wound swab	Male	inpatient	NON-MDR
49	P 43	75	Body fluid	Female	inpatient	MDR
50	P 44	77	Wound swab	Female	outpatient	NON-MDR
51		79	Urine	Female	outpatient	MDR
52	P 45	80	Wound swab	Female	inpatient	NON-MDR
53	P 46	82	Wound swab	Female	inpatient	MDR
54	P 47	83	Body fluid	Male	inpatient	MDR
55	P 48	84	Urine	Female	outpatient	MDR
56		85	Central line tip	Male	inpatient	MDR
57	P 49	86	Blood	Female	inpatient	NON-MDR
58	P 50	87	Wound swab	Female	outpatient	MDR
59		88	Wound swab	Male	inpatient	MDR
60		89	Wound swab	Male	inpatient	MDR
61		90	Wound swab	Male	inpatient	MDR
62	P 51	91	Wound swab	Male	inpatient	MDR
63		92	Wound swab	Female	inpatient	MDR
64	P 52	94	Body fluid	Male	outpatient	MDR
65	P 53	95	Wound swab	Male	inpatient	MDR
66	P 54	96	Body fluid	Male	outpatient	MDR
67	P 55	99	Wound swab	Female	inpatient	MDR
68	P 56	100	Urine	Male	outpatient	MDR
69	P 57	101	Wound swab	Female	outpatient	NON-MDR
70		102	Wound swab	Male	outpatient	NON-MDR
71	P 58	103	Urine	Female	outpatient	MDR
72	P 59	104	Wound swab	Male	inpatient	NON-MDR
73	P 60	105	Urine	Male	outpatient	MDR
74	P 61	106	Body fluid	Male	inpatient	MDR
75	P 62	108	Wound swab	Female	outpatient	MDR
76	P 63	109	Wound swab	Female	inpatient	MDR
77	P 64	110	Body fluid	Male	inpatient	NON-MDR
78	P 65	112	Urine	Male	inpatient	MDR
79	P 66	115	Wound swab	Female	inpatient	MDR
80		117	Wound swab	Male	inpatient	MDR
81	P 67	118	Wound swab	Male	inpatient	NON-MDR
82	P 68	122	Wound swab	Male	inpatient	MDR
83	P 69	123	Wound swab	Male	outpatient	MDR
84	P 70	124	Wound swab	Female	outpatient	MDR
85	P 71	125	Wound swab	Male	outpatient	MDR
86		126	Wound swab	Female	outpatient	MDR
87	P 72	127	Urine	Female	outpatient	MDR
88	P 73	128	Urine	Female	outpatient	MDR
89	P 74	129	Wound swab	Female	inpatient	MDR
90	P 75	130	Wound swab	Male	inpatient	MDR
91		131	Wound swab	Female	outpatient	MDR
92	P 76	133	Body fluid	Female	inpatient	MDR
93	P 77	134	Wound swab	Female	inpatient	MDR
94	P 78	135	Urine	Female	outpatient	MDR
95	P 79	136	Urine	Female	outpatient	MDR

Table 4. Continuing ...

96	P 80	137	Wound swab	Male	inpatient	MDR
97	P 81	138	Urine	Female	outpatient	MDR
98	P 82	139	Wound swab	Male	inpatient	MDR
99	P 83	140	Central line tip	Female	inpatient	MDR
100	P 84	141	Wound swab	Male	outpatient	MDR
101	P 85	142	Wound swab	Female	inpatient	MDR
102	P 86	143	Urine	Male	outpatient	MDR
103	P 87	144	Urine	Female	outpatient	MDR
104	P 88	145	Urine	Female	outpatient	MDR
105		146	Central line tip	Male	inpatient	MDR
106		148	Tissue	Female	inpatient	MDR
107	P 89	149	Wound swab	Female	outpatient	MDR
108	P 90	150	Wound swab	Male	inpatient	MDR
109	P 91	151	Body fluid	Female	inpatient	MDR
110	P 92	152	Wound swab	Male	outpatient	MDR
111	P 93	154	Body fluid	Female	inpatient	MDR
112	P 94	156	Wound swab	Male	outpatient	MDR
113	P 95	158	Wound swab	Female	inpatient	MDR
114	P 96	159	Wound swab	Male	outpatient	MDR
115	P 97	160	Wound swab	Female	outpatient	NON-MDR
116	P 98	161	Wound swab	Male	inpatient	NON-MDR
117	P 99	162	Wound swab	Male	outpatient	MDR
118		163	Urine	Female	outpatient	MDR
119	P100	164	Body fluid	Female	inpatient	MDR
120	P101	165	Urine	Female	outpatient	MDR
121	P102	166	Wound swab	Female	outpatient	MDR
122	P103	167	Urine	Female	outpatient	MDR
123	P104	168	Urine	Female	outpatient	MDR
124	P105	169	Blood	Male	inpatient	NON-MDR
125	P106	170	Body fluid	Male	inpatient	MDR
126	P107	171	Urine	Male	outpatient	MDR
127	P108	172	Wound swab	Female	inpatient	MDR
128		173	Wound swab	Male	inpatient	MDR
129	P 109	174	Wound swab	Male	outpatient	MDR
130	P 110	175	Wound swab	Male	inpatient	MDR
131	P 111	176	Wound swab	Male	inpatient	MDR
132	P 112	178	Urine	Female	outpatient	MDR
133	P 113	179	Wound swab	Male	inpatient	MDR
134	P 114	180	Wound swab	Female	outpatient	MDR
135	P 115	181	Wound swab	Male	inpatient	MDR
136	P 116	182	Wound swab	Female	outpatient	NON-MDR
137	P 117	184	Wound swab	Male	inpatient	MDR
138	P 118	185	Wound swab	Male	inpatient	MDR
139	P 119	187	Wound swab	Male	inpatient	MDR
140		189	Urine	Female	outpatient	MDR
141	P 120	190	Wound swab	Male	inpatient	MDR
142	P 121	191	Wound swab	Female	inpatient	MDR
143		193	Wound swab	Female	inpatient	MDR

Table 4. Continuing ...

144		194	Urine	Female	outpatient	MDR
145	P 122	195	Wound swab	Male	inpatient	MDR
146	P 123	196	Wound swab	Female	inpatient	NON-MDR
147	P 124	197	Urine	Male	inpatient	MDR
148	P 125	198	Wound swab	Female	inpatient	MDR
149	P 126	199	Wound swab	Male	inpatient	MDR
150	P 127	200	Wound swab	Female	inpatient	MDR

scribed by other investigators (4). The expected 956-bp amplicon was also detected in the reference strain *P. aeruginosa* ATCC 27853, a well-characterized control strain extensively used in molecular studies, including ERIC-PCR analysis (4, 12, 13).

In the present study, 95.6% (150/157) concordance was observed between routine biochemical identification and species-specific PCR, supporting the reliability of conventional biochemical methods for identification of *P. aeruginosa*. However, variations in laboratory protocols, atypical phenotypes, and the emergence of novel strains may affect identification accuracy, emphasizing the need for confirmatory molecular techniques, particularly in samples obtained from critical care settings.

P. aeruginosa exhibits intrinsic resistance to several antimicrobial agents because of the low permeability of its outer membrane, overexpression of efflux pumps, and production of antibiotic-inactivating enzymes such as inducible cephalosporinases. First-line antipseudomonal β-lactam agents include β-lactam/β-lactamase inhibitor (BL/BLI) combinations such as piperacillin–tazobactam and ticarcillin–clavulanate, as well as antipseudomonal cephalosporins including ceftazidime, cefepime, and cefoperazone. Cefepime remains one of the most commonly used β-lactam antibiotics for treatment of *P. aeruginosa* infections. Fluoroquinolones, particularly ciprofloxacin and levofloxacin, are currently the only oral therapeutic options available for susceptible strains. However, ciprofloxacin is generally preferred over levofloxacin due to the higher risk of emergence of quinolone-resistant strains associated with levofloxacin use. Additionally, older fluoroquinolones demonstrate reduced efficacy in acidic environments such as the urinary tract, whereas newer agents like finafloxacin and delafloxacin show enhanced activity under acidic conditions, although their availability remains limited (14).

During the course of this study, markedly high resistance rates were observed against several first-line antipseudomonal antibiotics, posing a significant therapeutic challenge. Resistance rates were highest for ticarcillin–clavulanate (96.7%), followed by ceftazidime (88%), cefepime (76.7%), cefoperazone (74%), and piperacillin–tazobactam (70.7%). Comparatively lower resistance rates were observed for ciprofloxacin (55%), levofloxacin (60%), and meropenem (47.3%). Similar resistance patterns have been reported by Khosravi et al. (13), Ranjbar et al. (15), and Eladawy et al. (16). The high resistance rates observed in this study may be attributed to chromosomal mutations, horizontal transfer of resistance determinants via plasmids and transposons, and selective antimicrobial pressure (4). Furthermore, the emergence of quinolone resistance mediated through efflux mechanisms may contribute to cross-resistance with other antibiotic classes. These findings highlight the urgent need for continuous antimicrobial resistance surveillance and targeted infection control interventions.

All isolates in the present study remained susceptible to colistin sulphate, consistent with previous reports (7, 16, 17). Nevertheless, the global prevalence of MDR *P. aeruginosa*, including strains producing extended-spectrum β-lactamases (ESBLs) and carbapenemases, continues to rise, although significant geographical variation exists (18). A substantial proportion of MDR strains also fulfil the criteria for extensively drug-resistant (XDR) phenotypes, thereby further limiting available therapeutic options.

Among the 150 confirmed *P. aeruginosa* isolates, 125 (84%) were identified as MDR, consistent with findings reported by Khosravi et al. (13). Similarly, a meta-analysis by Horcajada et al. (18) documented high resistance rates to cephalosporins, monobactams, aminoglycosides, carbapenems, BL/BLI combinations, and quinolones. The high prevalence of MDR strains may be associated with irrational and indiscriminate antibiotic use in both hospital and

community settings. In addition, several epidemic outbreaks involving MDR and XDR "high-risk" clones have been documented worldwide, underscoring the strong association between antimicrobial usage and development of resistance (4).

A large multicentre study conducted across 51 hospitals in Spain reported that 26% of *P. aeruginosa* isolates were MDR, and 65% of these fulfilled criteria for XDR phenotypes, with most isolates remaining susceptible only to colistin, either alone or in combination with aminoglycosides. Colistin-only-sensitive profiles and even pan-drug-resistant strains have now been documented in several healthcare settings globally, findings that are in agreement with our observations.

ERIC-PCR analysis in our study demonstrated considerable genetic diversity among the isolates, with 41 clusters identified at a similarity coefficient of 70%. These findings are consistent with reports by Hematzadeh A et al. (4), Eladawy et al. (16), and Abdelaziz et al. (19), all of whom reported marked heterogeneity among *P. aeruginosa* isolates. For example, Hematzadeh A et al. identified 38 clones among 96 isolates, while Eladawy et al. reported 99 ERIC clones among 103 isolates (4, 16). The high number of clones observed in our study suggests substantial genetic heterogeneity and indicates the circulation of multiple subtypes of *P. aeruginosa* within our hospital environment. Similar findings have been reported by Khosravi et al. (13) and Sener et al. (20). Such heterogeneity likely reflects differences in environmental conditions, infection control practices, and hospital hygiene standards.

Despite the observed heterogeneity, several isolates exhibited identical or highly similar ERIC-PCR profiles, suggesting clonal dissemination. For instance, isolates PSA34, PSA35, and PSA36 shared ERIC type P19; PSA145, PSA146, and PSA148 shared type P88; PSA191, PSA193, and PSA194 shared type P121; and isolates PSA87–PSA90 shared type P50. Additionally, fourteen ERIC types were each shared by two isolates. These findings indicate the presence of clonal spread within the hospital setting.

Compared with phenotypic typing methods, molecular typing techniques offer superior discriminatory power and reproducibility (21). Various genotypic methods, including pulsed-field gel electrophoresis (PFGE), ribotyping, random amplified polymorphic DNA (RAPD), and ERIC-PCR, have been used to assess relatedness among clinical isolates (22). Among these, ERIC-PCR is a rapid, inexpensive, and technically straightforward method that has been widely used in routine epidemiological investigations of *P. aeruginosa* (19).

In our study, the highest number of isolates (n = 33) originated from the burn ward and clustered into only two major groups, suggesting possible cross-infection among burn patients. This finding contrasts with the study by Khosravi et al. (13), who reported greater heterogeneity among burn unit isolates. The relatively limited clustering observed in our burn ward isolates may reflect transmission of closely related strains within the unit.

Traditionally, infection control strategies have focused primarily on periodic revision of antibiotic prescribing policies. However, incorporation of molecular typing methods into routine surveillance programs is increasingly important for early outbreak detection and identification of infection sources. Although next-generation sequencing (NGS) and whole-genome sequencing (WGS) provide superior resolution for molecular epidemiology studies, their high cost, technical complexity, and prolonged turnaround time limit their routine use in many clinical microbiology laboratories. In contrast, ERIC-PCR represents a cost-effective and relatively simple alternative suitable for routine epidemiological surveillance.

The present study demonstrated that the majority of *P. aeruginosa* strains circulating within our hospital were MDR, which is highly concerning. Although significant genetic heterogeneity was observed, clustering of similar strains in the burn ward suggests possible cross-transmission. These findings underscore the need for stringent infection prevention and control measures, particularly in burn units.

While ERIC-PCR proved useful for assessing genetic relatedness among isolates, it is important to recognize its limitations, including lower reproducibility and discriminatory power compared with PFGE and WGS. Consequently, the true genetic diversity and clonal relationships among isolates may have been underestimated. Nevertheless, ERIC-PCR provided valuable preliminary epidemiological insights and established a foundation for future investigations using higher-resolution molecular typing techniques. To the best of our knowledge, this study represents one of the first investigations from our region employing a combined antibiotyping and molecular typing approach for characterization of *P.*

aeruginosa isolates. Future studies utilizing WGS are warranted to better understand the clonality, evolutionary dynamics, and transmission patterns of MDR *P. aeruginosa*. Continuous surveillance using combined phenotypic and molecular approaches remains essential for monitoring the emergence and spread of resistance, guiding infection control strategies, and strengthening antimicrobial stewardship programs tailored to local epidemiological needs.

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