Journal of Applied Biology & Biotechnology Vol. 14(6), pp. 107-118, November-December, 2026
Available online at http://www.jabonline.in
DOI: 10.7324/jabb.2026.315277

Isolation and genomic characterization of drug-resistant *Pseudomonas aeruginosa* and its phage from Iraqi hospital wastewater

Ban Oday Abdulsattar[1]*, Abdulrahman Ali Abdulhussein[2], Jaleel N. Samanje[3], Munim Radwan Ali[1], Jwan Oday Abdulsattar[4]

[1]Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq.
[2]College of Medicine, Aliraqia University, Baghdad, Iraq.
[3]Technical Medical Institute- Al-Mansur, Middle Technical University, Baghdad, Iraq.
[4]Department of Chemistry, College of Science, Mustansiriyah University, Baghdad, Iraq.

ARTICLE INFO

Article history:
Received on: 26/05/2026
Accepted on: 09/08/2026
Available Online: 25/09/2026

Key words:
Pseudomonas aeruginosa, Bacteriophage, Characterization, Genome comparison

ABSTRACT

Pseudomonas aeruginosa can develop resistance to antibiotics and tolerance to antimicrobials. Bacteriophages have been proposed as an alternative strategy for eliminating pathogens, but they require further genetic studies. Therefore, this study aimed to isolate a bacteriophage from hospital wastewater, examine its biological properties, and analyze the genomes of both the host bacterium and bacteriophage. A bacteriophage was isolated from hospital wastewater, purified, and amplified. The optimal temperature range was 20°C–37°C, with an optimal pH of 7 and multiplicity of infection of 1. The bacteriophage exhibited growth inhibition against 24% of *P. aeruginosa* clinical isolates. The host bacterium genome spanned 104 contigs, with an estimated total length of 6,455,470 bp and GC content of 66.27% and contained five prophage regions. Draft genome assembly of the phage consisted of nine contigs totalling 94,292 bp of double-stranded DNA. It contained 158 open reading frames, none of which encoded virulence or antibiotic resistance genes, but one encoded a transfer RNA. Whole-genome sequence comparison using BLASTn showed approximately 99% nucleotide identity with Pseudomonas phage *Samunavirus* SM1. Our study represents the first genomic report of an SM1-like bacteriophage and its host isolated from hospital wastewater in Iraq.

1. INTRODUCTION

Pseudomonas aeruginosa is a common, motile Gram-negative opportunistic pathogen that is receiving increasing attention because it causes severe nosocomial infections that often result in death. It is widely distributed across different environments, including soil, animal farms, hospital wastewater, and aquatic environments [1]. It is one of the primary pathogens causing nosocomial infections, bacteremia, urinary tract infections (UTIs), chronic wound infections, and lung infections in patients with cystic fibrosis [2].

Although antibiotics are considered an effective approach to treating bacterial infections, their overuse and misuse have reduced their effectiveness. *Pseudomonas aeruginosa* has developed resistance to the available treatment options through its ability to form a biofilm and produce hemolysins and other virulence factors [3]. In Iraq, infections with multidrug-resistant (MDR) and extremely drug-resistant strains of *P. aeruginosa* have been recognized as major contributors to nosocomial infections at Basra Hospital [4]. In addition, *P. aeruginosa* clinical isolates from Al-Diwaniyah Hospital showed increased resistance to aminoglycosides [5], while those from Al-Hillah Al-Sadiq Hospital often showed MDR [6].

Global health systems, including those in Iraq, face a serious threat from the spread of drug-resistant strains of *P. aeruginosa*. Therefore, a new and effective alternative approach for treating MDR strains of *P. aeruginosa* is required. Bacteriophage therapy has received renewed research interest as an alternative to existing antibiotics. In any environment, the presence of bacteriophages depends on that of their bacterial hosts, with bacterial species often susceptible to one or more bacteriophages [7]. Bacteriophages are known for their ubiquity and diversity, which play a significant role in the diversity and evolution of bacterial species [8]. Notably, bacteriophages have recently shown promising efficacy in experimental models in treating MDR strains of *P. aeruginosa* [9], and combining an isolated bacteriophage with ciprofloxacin or meropenem significantly decreased biofilm formation by *P. aeruginosa* clinical isolates *in vitro* [10]. However, there remains a lack of isolated bacteriophages targeting *Pseudomonas* spp., as well

*Corresponding Author
Ban Oday Abdulsattar, Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq. E-mail: banoday @ uomustansiriyah.edu.iq

as research focused on the genomic characterization of both bacterial hosts and bacteriophages.

Although most prior studies have employed phenotypic assays to characterize bacteriophages, genomic sequencing and bioinformatics analysis can more accurately ensure bacteriophage safety. Therefore, this study aimed to isolate a bacteriophage from hospital wastewater samples and evaluate its inhibitory activity against pathogenic *P. aeruginosa*. It examines the bacterial host and bacteriophage genomes via whole-genome sequencing (WGS) to assess the bacteriophage's suitability for therapeutic applications and to explore its evolutionary relationships.

2. MATERIALS AND METHODS

2.1. Study area and physicochemical analysis

To comprehensively assess water quality at baseline, wastewater samples collected at Ghazi Al-Hariri Hospital (Baghdad City, Iraq) in November 2025 were analyzed for 18 physicochemical parameters. Temperature, pH, and electrical conductivity (EC) were measured *in situ*. Biochemical oxygen demand (BOD), oxygen demand (OD), total dissolved solids (TDS), sulfate (SO_4^{2-}), alkalinity (Alk.), total hardness (TH), oil and grease (O&G), turbidity (Turb.), chloride (Cl), nitrate (NO_3^-), phosphate (PO_4), calcium (Ca), magnesium (Mg), potassium (K), and sodium (Na) were analyzed in a laboratory according to standard procedures [11].

2.2. Isolation of the host bacterium and bacteriophage

Two wastewater samples were collected at Ghazi Al-Hariri Hospital, stored in a cooler at 4°C, transported to the laboratory, and processed on the same day. First, they were centrifuged at 6,000 rpm for 20 minutes to remove cell debris, as previously described [10], with slight modification. Next, a portion of the supernatant was cultured on nutrient agar and MacConkey agar at 37°C for 24 hours (Himedia, India), while the remainder was passed through 0.45-µm and 0.22-µm filter membranes (Merck Millipore, France) for bacteriophage isolation. The next day, colonies suspected to be *P. aeruginosa* were replated onto nutrient agar and MacConkey agar and incubated at 37°C for 24 hours for purification. Then, five suspected *P. aeruginosa* isolates were identified at the species level using the VITEK® 2 compact system (bioMérieux, France).

All environmental *P. aeruginosa* isolates were grown on nutrient agar plates for 24 hours at 37°C. The next day, each isolate (100 µl) in the logarithmic phase (2×10^8) was mixed with 4 ml of melted soft agar (0.7%) and left to solidify for 10 minutes. Then, wastewater supernatant was spotted on top of the agar for each *P. aeruginosa* isolate and left at room temperature for 10 minutes. All plates were then incubated at 37°C for 24 hours. Then, those isolates showing a lysis zone were considered the host bacterium. Finally, the bacteriophage was transferred into 4 ml phosphate-buffered saline (PBS) and stored in a refrigerator.

2.3. Bacteriophage purification

The bacteriophage was purified using the previously described double-layer agar overlay method [12]. Briefly, the host *P. aeruginosa* isolate in logarithmic phase was mixed with 100 µl of bacteriophage lysate, incubated at room temperature for 10 minutes, then mixed with 4 ml of melted soft agar (0.7%), poured onto nutrient agar plates, and incubated at 37°C for 24 hours. Then, a single clear plaque was chosen and transferred into 5 ml of PBS. The purification cycle was repeated four times.

Bacteriophage plaque morphology was examined the following day using the agar overlay method, and plaque morphology and diameters were recorded. Each plate was incubated for an additional 48 hours, and changes in plaque morphology were recorded and imaged.

2.4. Examination of bacteriophage morphology

A uniform plaque plate was immersed in PBS (5 ml) and left at room temperature for 24 hours. Next, the PBS was collected into a sterile Eppendorf tube, centrifuged at 2,000 rpm for 5 minutes, and the supernatant was passed through a 0.22-µm membrane filter. Then, the filtrate was concentrated using 100 kDa Amicon Ultra centrifugal filter units (Merck Millipore, France) to a final volume of 0.5 ml by centrifugation at 3,000 rpm and 4°C for 5–10 minutes. Next, the bacteriophage lysate titer was measured, and 10 µl of the lysate was placed onto carbon-coated copper grids (Precise, China) and left at room temperature to dry for 10 minutes. Filter paper was used to absorb excessive bacteriophage suspension, and the grid was then negatively stained with uranyl acetate (2%). Finally, bacteriophage morphology was observed using a Supra 55vp field emission scanning electron microscope (FESEM; Zeiss, Germany) in the College of Pharmacy at Basra University (Basra, Iraq).

2.5. Assessment of temperature and pH stability

Bacteriophage stability at different temperatures (−20°C, 4°C, 20°C, 37°C, 50°C, 65°C, and 75°C) was assessed by incubating 300 µl of bacteriophage lysate at the required temperature for 1 hour, and then determining the bacteriophage titer using the double-layer agar overlay method. Bacteriophage stability at different pHs (pH 2, 4, 6, 7, 8, and 10) was assessed by adding 300 µl of bacteriophage lysate to 1 ml of PBS at the required pH, incubating the solution at room temperature for 1 hour, and then determining the bacteriophage titer using the double-layer agar overlay method [13].

2.6. Assessment of multiplicity of infection (MOI)

Briefly, 100 µl of bacteriophage suspension at different titers was mixed with a suspension of the host bacterium (~108 colony-forming units [CFU]/ml) to achieve different MOI values (10, 1, 0.1, 0.01, and 0.001). Serial dilutions were prepared for each mixture, spread onto a fresh nutrient agar plate, and incubated at 37°C for 24 hours. After incubation, the bacteriophage titer was determined. The experiment was performed in triplicate, and the MOI that produced the highest titer was considered the optimal MOI [14].

2.7. Identification of bacterial species

In November and December 2025, all clinical samples for the host range test were collected from the Microbiology Laboratory at Ghazi Al-Hariri Hospital. They originated from various sources, including back mass, blood, bronchial wash, ear swab, endotracheal tube (ETT) tip, Foley tip, pus, sputum, and urine. All isolates were further phenotypically identified using the VITEK® 2 compact system (bioMérieux, France) and based on their antimicrobial profile. Stock cultures of all isolates were prepared by adding 20% glycerol to brain heart infusion broth and storing them at −80°C.

2.8. Assessment of bacterial host range

The lytic activity of the isolated bacteriophage against 61 different pathogenic bacteria (25 *P. aeruginosa* strains, 16 *Escherichia coli* strains, 12 *Klebsiella* spp., and 8 *Staphylococcus aureus* strains) was evaluated using a standard spot assay. Briefly, 100 µl of each tested

bacterium at the mid-log phase was added to semi-solid nutrient agar (4 ml) and poured onto a fresh nutrient agar plate. All plates were left to solidify, spotted with 3 µl of bacteriophage suspension ($10^{9–10}$ plaque-forming units [PFU]/ml), left to dry for 10 minutes, and then incubated at 37°C for 24 hours. After incubation, the presence of clear spots indicated bacteriophage growth inhibition activity [15].

2.9. Isolation of genomic DNA from the host bacterium and bacteriophage

Genomic DNA was isolated from the *P. aeruginosa* host using the Wizard® Genomic DNA Purification Kit (Promega, USA) according to the manufacturer's instructions. Bacteriophage DNA was extracted using the Phage DNA Isolation Kit (Norgen Biotek, Canada) according to the manufacturer's instructions. Briefly, bacteriophage lysate (10^9 PFU/ml) was incubated with 10 µl of RNase-free DNase I at room temperature for 15 minutes. Next, 500 µl of lysis solution was added, and the mixture was vortexed for 10 seconds. Then, 4 µl of proteinase K was added to the mixture, and it was incubated at 55°C for 15 minutes to increase DNA yield. The mixture was then incubated in a water bath at 65°C for 15 minutes, with thorough mixing performed 2–3 times during incubation by inverting the tube. Next, the tube was cooled and centrifuged at 14,000 rpm for 1 minute. Then, 1 ml of the supernatant was transferred to a new sterile microfuge tube, mixed with binding buffer (400 µl), and vortexed. Isopropanol (320 µl) was then added, and the mixture was vortexed for few seconds. Next, the lysate (650 µl) was transferred to a spin column and centrifuged at 8,000 rpm for 1 minute; this step was repeated with the remaining lysate. Then, washing buffer (400 µl) was added, and the column was centrifuged at 8,000 rpm for 1 minute; this step was repeated twice, followed by an extra centrifugation at 8,000 rpm for 2 minutes to dry the resin. Next, the elution buffer (75 µl) was added, and the column was centrifuged at 8,000 rpm for 1 minute. To obtain additional DNA, the elution step was repeated twice. The obtained DNA was stored at −20°C. Bacteriophage genomic DNA fragment size was estimated by electrophoresis on a 1% agarose gel at 85 V for 1 hour with a DNA ladder (SMOBIO Technology, Taiwan) [10].

2.10. Determination and screening of the bacteriophage genome sequence

The bacteriophage genome sequence was determined by WGS using the NovaSeq X Plus platform (Illumina, USA) and annotated using version 4 of the Viral Genome ORF Reader (VIGOR4) and PHANOTATE. The bacteriophage genome sequencing data were deposited in the National Center for Biotechnology Information Sequence Read Archive under BioProject (accession number PRJNA1332805).

The bacteriophage genome was screened against the Comprehensive Antibiotic Resistance Database (CARD) and the Virulence Factor Database. Match detection was considered statistically significant when the sequence identity was ≥90% and query coverage was ≥80%.

2.11. Bioinformatics characterization of the host and bacteriophage genome

Paired-end Illumina sequencing (2 × 150 bp) was used after extracting the genomic DNA of the bacterial host. Raw reads were quality-filtered using Trim Galore v 0.6.5, BBNorm, and assembled using Unicycler v 0.4.8. Pilon was used to enhance the assembly. The completeness of the genome assembly was confirmed, and the average genome assembly reached a depth of 295x, and genome coverage was adequate for further analyses. QUAST v 5.2.0 was used to examine the quality of the genomes.

The bacteriophage genomic sequence data were assembled, functionally annotated, and subjected to variant calling. For *de novo* assembly, the sequencing reads first underwent quality control and were then organized into contiguous sequences. The draft genome was then revised and refined to high quality. Genome diversity and evolutionary adaptation were assessed through polymorphisms and structural variations captured during variant calling. The genome was annotated using the PHANOTATE pipeline to systematically identify coding sequences (CDS), structural components, replication-associated genes, and accessory elements. This task included predicting the biological functions of the elements that comprise the various genes in the bacteriophage genome and describing the functional elements that determine host recognition, lysis, and horizontal gene transfer. The combined analyses of assembly, variant calling, and annotation using the PHANOTATE pipeline established a useful basis for genomic characterization. Statistical analyses were performed using R (version 4.5.2).

3. RESULTS

3.1. Bacteriophage isolation

The bacteriophage was initially isolated by spot assay screening of *P. aeruginosa* environmental isolates. A clear zone indicated the presence of a bacteriophage that could lyse *P. aeruginosa*, which was further purified and designated Pa25IQ. The bacteriophage formed a plaque with a clear center in a double agar layer. The plaques had diameters of 1–2 mm, with regular, clear dots that remained constant after incubation for 48 hours. The FESEM examination showed that the isolated bacteriophage exhibited a typical icosahedral head with a contractile tail (Fig. 1A and B; Fig. 2).

3.2. Characteristics of bacteriophage Pa25IQ

Bacteriophage Pa25IQ maintained high infectivity over 1 hour across the temperature range of 20°C–37°C (Fig. 3A). However, its titer decreased rapidly at temperatures exceeding 50°C. It also maintained high infectivity over 1 hour across the pH range of 4–8, peaking at pH 7 (Fig. 3B). However, no titer was detected at pH below 4 or above 8.

These laboratory thresholds align with *in situ* measurements of wastewater from Ghazi Al-Hariri Hospital, which had an average pH

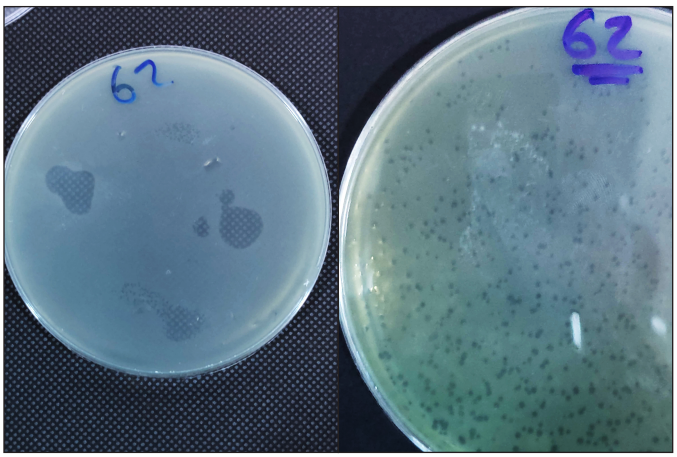


Figure 1. Isolation and plaque morphology of bacteriophage Pa25IQ. (A) Lytic zones produced by bacteriophage Pa25IQ on a Pseudomonas aeruginosa lawn using the spot assay; (B) clear plaques formed by bacteriophage Pa25IQ using the double-layer agar overlay assay.

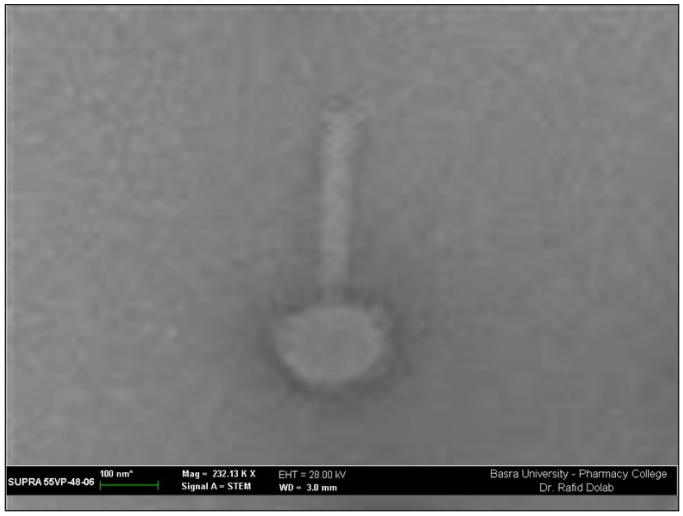
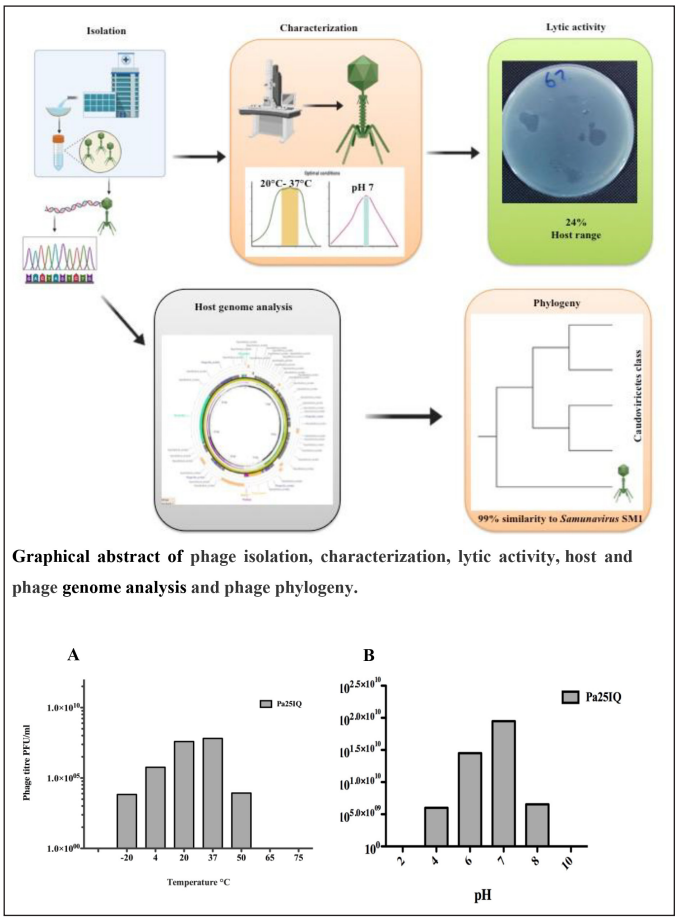


Figure 2. Morphology of phage Pa25IQ. The bar indicates 100 nm.



Graphical abstract of phage isolation, characterization, lytic activity, host and phage genome analysis and phage phylogeny.

Figure 3. Phage Pa25IQ temperature and pH stability experiment. (A) Phage titer was measured via double agar overlay after phage lysate incubation at different temperatures. (B) Phage titer was measured via double agar overlay after phage lysate incubation at different pH values. Error bars represent the standard deviation of three independent replicates.

of 7.4, temperature of 33.2°C, and alkalinity of 167.85 mg/l. Its ionic strength characteristics, including EC of 678 µs/cm, TDS of 420.3 mg/l, and TH of 222 mg/l, along with the presence of specific cations (Na^+, K^+, Mg^{2+}, and Ca^{2+}) and anions (Cl^-, SO_4^{2-}, NO_3^{-1}, and PO_4^-), provided satisfactory conditions for bacteriophage structural stability. In addition, the low O&G level (0.065 mg/l) and turbidity (64 NTU) facilitated bacteriophage–host interactions, while the OD (9.5 mg/l) and BOD (5 mg/l) further characterized the baseline water quality during the isolation period (Table S1).

3.3. MOI of bacteriophage Pa25IQ

Bacteriophage lysis activity is influenced by the MOI (ratio of bacteriophage to bacteria). With the decrease in MOI, bacterial growth increases, however, MOI cannot entirely reduce bacterial cell growth [16]. For bacteriophage Pa25IQ, an MOI of 1 appeared optimal (Fig. 4).

3.4. Sources and antimicrobial sensitivity of the P. aeruginosa *clinical isolates*

A total of 25 *P. aeruginosa* clinical isolates were collected from the Microbiology Laboratory at Ghazi Al-Hariri Hospital. Of these isolates, 76% were obtained from hospitalized male patients and 24% from hospitalized female patients (Fig. 5A). Most were from urine (28%), followed by bronchial wash (24%), back mass (4%), ear swab (4%), ETT tip (4%), and pus (4%, Fig. 5B). The susceptibility of the *P. aeruginosa* clinical isolates to 10 different antibiotics was assessed (Table 1). While many (44%) showed resistance to gentamicin, tobramycin, and imipenem, most showed susceptibility to meropenem (64%) and cefepime (60%).

3.5. Target species of bacteriophage Pa25IQ

Bacteriophage Pa25IQ demonstrated growth inhibition against 6 of the 25 (24%) *P. aeruginosa* clinical isolates, but showed no lysis activity against other tested Gram-positive (8 *S. aureus strains*) and Gram-negative (16 *E. coli* and 12 *Klebsiella* spp. strains) clinical isolates. The results indicate a narrow host range (Table 2).

3.6. Construction of the P. aeruginosa genome

The genome of the *P. aeruginosa* host was sequenced to investigate pathogenicity, virulence, and host resistance to bacteriophage infection. It comprised 104 contigs, with an estimated genome length of 6,455,470 bp, and an average GC content of 66.27%. The N_{50} length, the shortest sequence length at 50% of the genome length, was 124,016 bp. The L_{50} count, the smallest number of contigs whose length sum produces the N_{50}, was 15. The *P. aeruginosa* genome was annotated using the RAST toolkit and assigned the unique genome identifier 287.45055. The *P. aeruginosa* genome was found to contain 6,163 protein CDS, 55 transfer RNA (tRNA) genes, and 3 ribosomal RNA (rRNA) genes (Fig. 6).

The CARD analysis indicated that the host *P. aeruginosa* strain carries a highly complex, dense, and essential set of antimicrobial resistance genes distributed throughout its entire chromosome (Fig. 7), which is known to contribute to antibiotic resistance. For example, it contains the efflux pumps MexAB-OprM, MexCD-OprJ, MexEF-OprN, and MexXY, as well as the beta-lactamases PDC-5 (*Pseudomonas*-derived cephalosporinase) and OXA-396 (an oxacillinase). The presence of cprS/cprR, parS/parR, and basS/basR indicates the existence of regulatory systems that sense environmental stressors, such as antibiotics and activate resistance genes.

Table S1. Alignment of laboratory data with in situ field physicochemical parameters for Pa25IQ phage stability.

Parameter	Laboratory data	*In situ* (field) data	Alignment	Comment
pH	7.0	7.4	Optimal	Maintains high phage stability range
Temperature	37°C	33.2°C	Optimal	Correspond to the peak infectivity range.
Alkalinity	Required for pH stabilization	167.85 mg/l	High	Provides strong buffering capacity
Divalent cations	Essential for host attachment	Ca^{2+}(62 mg/l, and Mg^{2+} (20 mg/l)	Ideal	Neutralize electrostatic charges
Monovalent cations	Regulates particles aggregation	Na^{+}(36 mg/l), and K^{+}(1.25 mg/l)	Sufficient	Ensures particles stability and prevents aggregation
TDS	Influences host attachment	420.3 mg/l	Favorable	Support efficient infection kinetic
EC		678 µs/cm		
Oil and Grease	Inhibits adsorption	0.065 mg/l	Excellent	Low concentration prevent trapping
Turbidity	Affect phage- host interaction	64 NTU	Good	Enhances contact frequency in suspension.

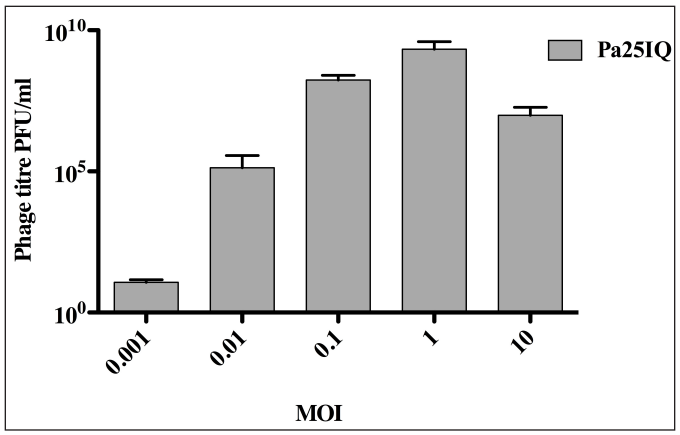


Figure 4. The optimal MOI experiment results. Error bars represent the standard deviation of three independent replicates.

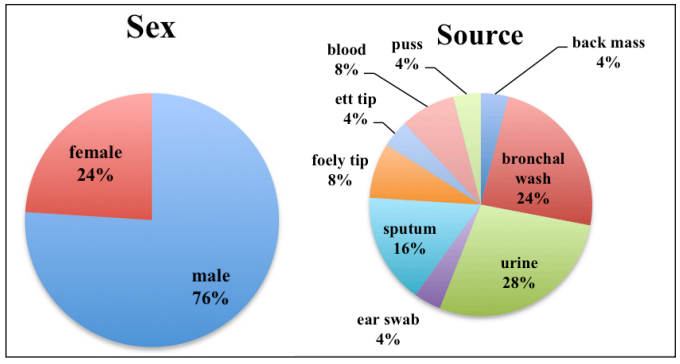


Figure 5. The distribution of clinical *P. aeruginosa* samples according to sex and source. (A) The distribution of *P. aeruginosa* clinical isolates according to sex; (B) the distribution of *P. aeruginosa* clinical isolates according to source.

Table 1. Antibiotic antibiogram profile against *P. aeruginosa* clinical isolates. Percentages of antimicrobial susceptibility rates of *P. aeruginosa* isolates.

Antibiotics	R	I	S
Amikacin (AK)	9 (36.0%)	3 (12.0%)	13 (52.0%)
Cefepime (FEP)	8 (32.0%)	2 (8.0%)	15 (60.0%)
Ceftazidime (CAZ)	10 (40.0%)	1 (4.0%)	14 (56.0%)
Ciprofloxacin (CIP)	9 (36.0%)	9 (36.0%)	7 (28.0%)
Gentamicin (GM)	11 (44.0%)	1 (4.0%)	13 (52.0%)
Imipenem (IMI)	11 (44.0%)	1 (4.0%)	13 (52.0%)
Levofloxacin (LVX)	9 (36.0%)	6 (24.0%)	10 (40.0%)
Meropenem (MEM)	9 (36.0%)	0 (0.0%)	16 (64.0%)
Piperacillin/tazobactam (TZP)	10 (40.0%)	2 (8.0%)	13 (52.0%)
Tobramycin (TOB)	11 (44.0%)	1 (4.0%)	13 (52.0%)

Amikacin (AK), Cefepime (FEP), Ceftazidime (CAZ), Ciprofloxacin (CIP), Gentamicin (GM), Imipenem (IMI), Levofloxacin (LVX), Meropenem (MEM), Piperacillin/tazobactam (TZP), Tobramycin (TOB). R: Resistance; I: Intermediate; S: Sensitive.

Table 2. Spot assay results.

Phage	Strains	Effectiveness of phage via spot assay	
		Positive (Lysis)	Negative (No lysis)
Phage	*Pseudomonas aeruginosa* strains (25)	6 (24%)	19 (76%)
	Escherichia coli strains (16)	0	16 (100%)
	Klebsiella spp. strains (12)	0	12 (100%)
	Staphylococcus aureus strains (8)	0	8 (100%)

3.7. Bacteriophage infection history of the host P. aeruginosa strain

Screening of the host P. aeruginosa genome sequence identified five prophage regions, of which four were intact and one was suspect (Table 3; Fig. 10). The prophage regions ranged in length from 6 to 94 kb. The first intact region showed similarity to *Pseudomonas* bacteriophage phiCTX (NCBI GenBank ID: NC_003278), the second intact region to *Pseudomonas* bacteriophage SM1 (NCBI GenBank ID: NC_041877), the third intact region to *Pseudomonas* bacteriophage vB_PaeS_PM105 (NCBI GenBank ID: NC_028667), and the fourth (intact) and fifth (Questionable) regions to *Pseudomonas* bacteriophage phiCTX (NCBI GenBank ID: NC_003278). These regions indicate prior bacteriophage infection that may have influenced the evolution of this *P. aeruginosa* strain.

3.8. Bacteriophage defense mechanisms in the host *P. aeruginosa*

Bacteria usually use clustered regularly interspaced short palindromic repeats (CRISPRs) as defense mechanisms against bacteriophage

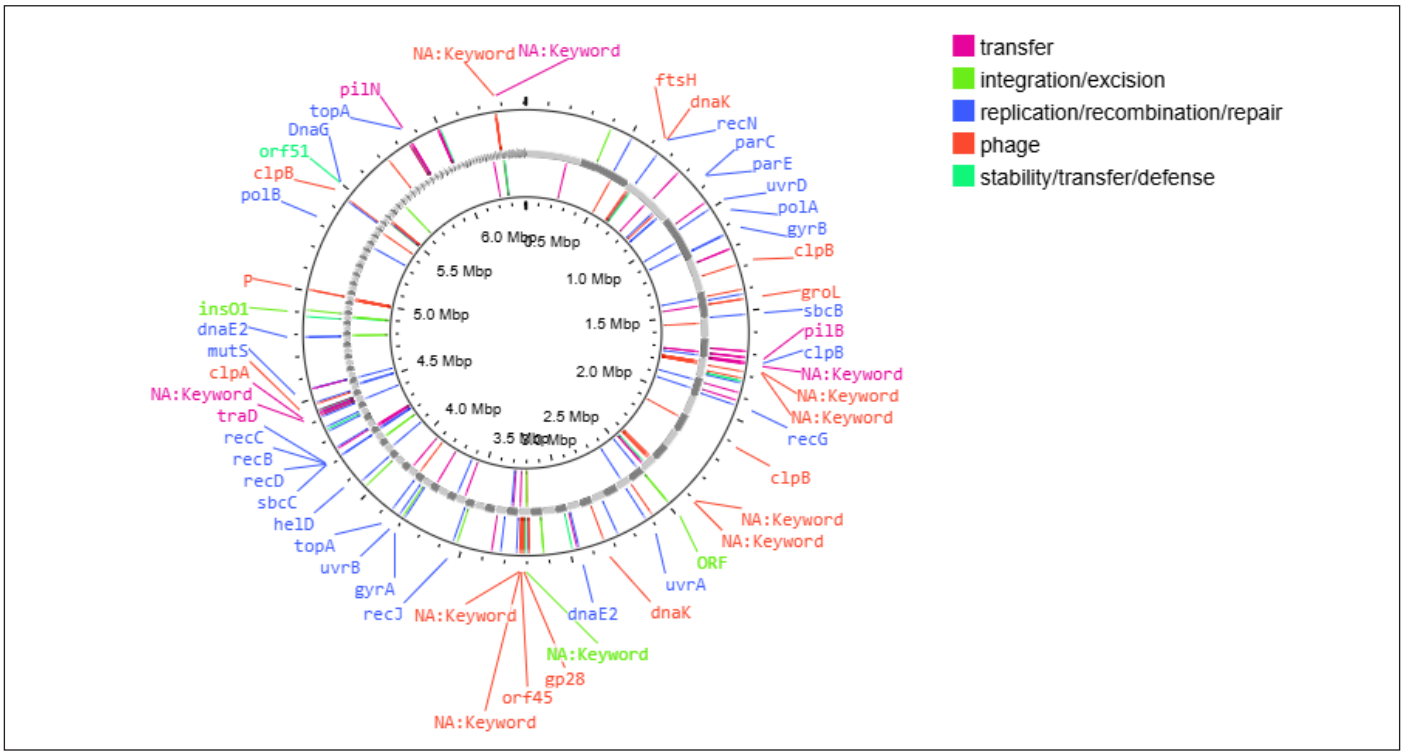


Figure 6. Genome map of host *P. aeruginosa*.

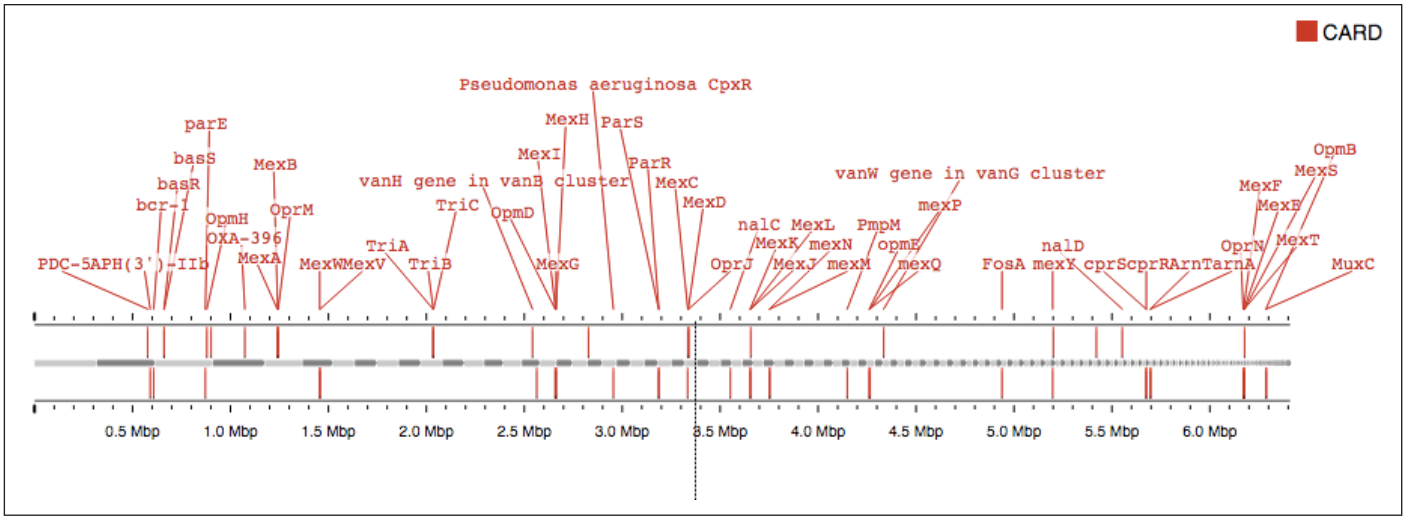


Figure 7. Map of identified AMR genes and regulatory systems loci. Red bars indicate the locations of specific resistance genes or regulatory elements on the host genome.

infections. The host *P. aeruginosa* genome sequence was found to contain one CRISPR array comprising 18 direct repeats and 17 spacer sequences, 6 CRISPR-associated genes (Cas), and 11 putative antibiotic-resistance genes in the CARD database (Fig. 8).

Two CRISPR-Cas sequences were also identified in the host genome. The first was A-12-contig-89-1 at the CRISPR location (19,961–20,060 bp), with a 1048 bp CRISPR length, one repeat, 28 bp repeat length, and 17 spacers. The second was A-12-contig-7-1 at the CRISPR location (67,055–67,168 bp), with a 113 bp CRISPR length, one repeat, 18 bp repeat length, and one spacer).

3.9. Characterization of the bacteriophage Pa25IQ genome

Following its isolation, the size and purity of the bacteriophage genomic DNA were assessed by gel electrophoresis. The gel electrophoresis confirmed the extraction of intact, high-molecular-weight (HMW) genomic DNA, which migrated above the used marker band (10kb) of the ladder (Fig. 9). The bacteriophage genome was sent for WGS for further analysis.

The draft bacteriophage Pa25IQ genome assembly consisted of nine contigs with a total assembled length of 94,292 bp and an average GC content of 55.14%. Because the genome was not completely

Table 3. Predicted prophage in *P. aeruginosa* host genome.

Region	Length	Completeness	Most common phage
1	18.4Kb	intact	(130) PHAGE_Pseudo_phiCTX_NC_003278 [6]
2	94Kb	intact	(144) PHAGE_Pseudo_SM1_NC_041877 (124)
3	45Kb	intact	(150) PHAGE_Pseudo_vB_PaeS_PM105_NC_028667 [17]
4	12.5Kb	intact	(130) PHAGE_Pseudo_phiCTX_NC_003278 [15]
5	6Kb	Questionable	(80) PHAGE_Pseudo_phiCTX_NC_003278 [7]

Intact (score > 90) Questionable (score 70–90) Incomplete (score < 70)

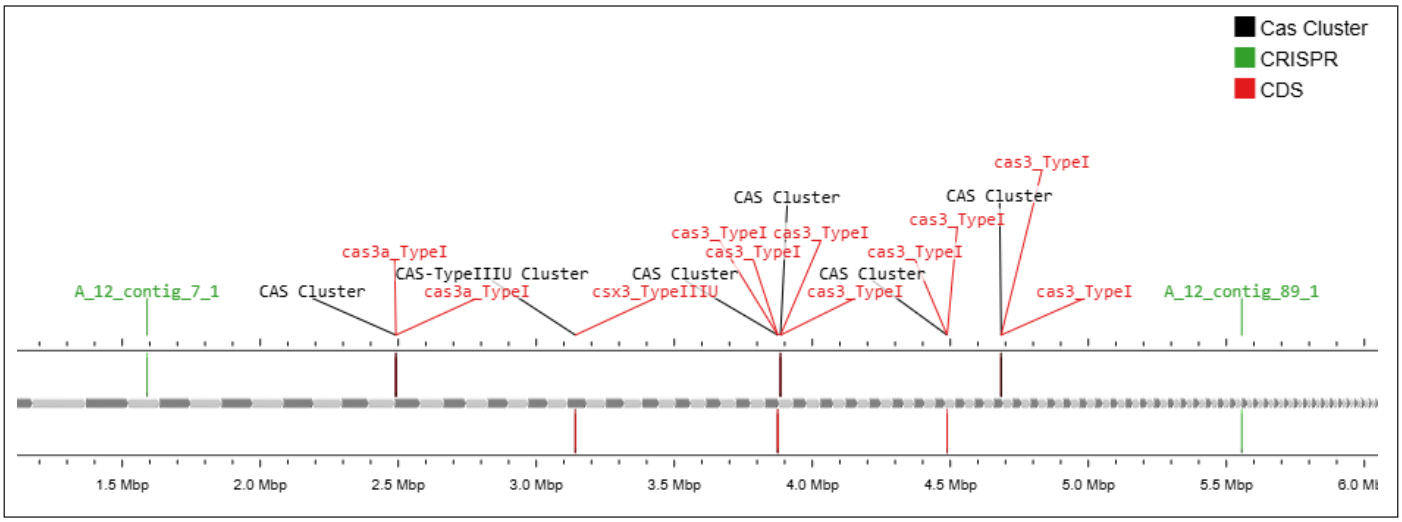


Figure 8. Host genome mapping. Black color: Cas system in six loci; green color: CRISPR in two loci; and red color indicating resistant gene islands by CARD (red color).

assembled, genome topology and terminal structures could not be determined. The N_{50} length was 31,418 bp.

3.10. Genomic comparison of bacteriophage Pa25IQ with bacteriophage SM1

Whole-genome synteny comparison analysis of *Pseudomonas* bacteriophages Pa25IQ and SM1 revealed highly conserved synteny and overall structural organization, with minor genetic variations suggestive of adaptive divergence (Fig. 11).

The synteny map shows strong colinearity between the SM1 and Pa25IQ genomes. Conserved homologous regions (gray ribbons) indicate high amino acid identity (70%–99%) across most of the genome. Several gene inversions and rearrangements are evident, particularly near the terminal regions, indicating the modular exchange typical of *Pseudomonas* bacteriophages. The central region (~45–65 kb) showed the highest conservation, corresponding to structural and replication modules, such as the major capsid, tail fiber, and portal proteins. Notably, the *Pseudomonas* bacteriophages SM1 and Pa25IQ shared a highly conserved genomic backbone, which is typical of *Pseudomonas*-infecting bacteriophages (Tables 4 and 5).

The additional open reading frames (ORFs) observed in the bacteriophage Pa25IQ genome likely resulted from recombination or horizontal gene transfer, leading to minor genome expansion. These genetic differences may contribute to differences in host ranges, tail

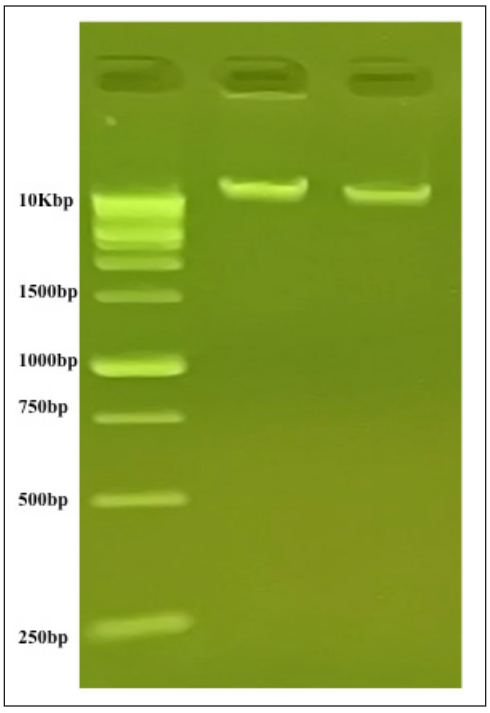


Figure 9. Gel electrophoresis of phage DNA. Lane 1: Marker (1Kb DNA Ladder); lanes 2 and 3 phage DNA.

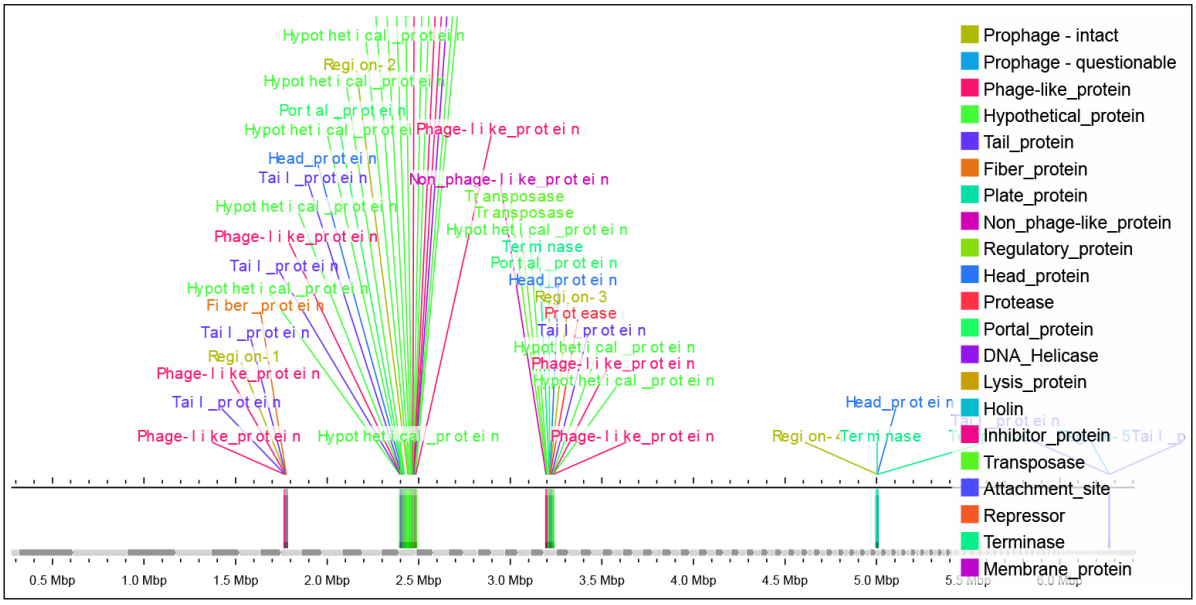


Figure 10. Visualization of prophage regions identified in the genome of the Pseudomonas host counted by PHASTER. Regions were marked with colors representing intact, questionable, and incomplete prophage elements predicted within the bacterial chromosome.

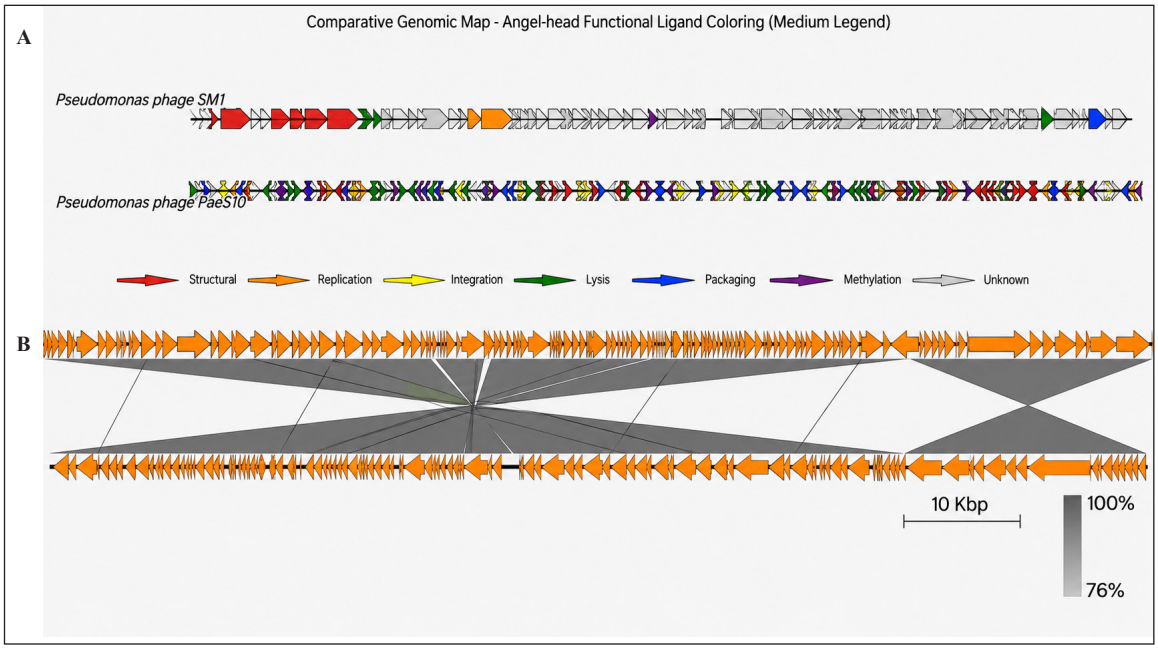


Figure 11. Comparative genomic analysis of *Pseudomonas* phages SM1 and Pa25IQ. (A) Comparative functional genome map showing the distribution of predicted gene functional categories in both phages. Gene colors indicate functional categories as shown in the legend. (B) EasyFig whole-genome comparison between Pa25IQ and SM1. Arrows represent predicted CDSs and their transcriptional orientation, while gray ribbons indicate homologous genomic regions identified by sequence comparison, with shading proportional to sequence similarity.

Table 4. Comparison of SM1 phage with Pa25IQ phage general features.

Parameter	SM1	Pa25IQ	Interpretation
Genome length (bp)	93,191	94,292	~1.1 kb increase in Pa25IQ—possible gene acquisition
GC content (%)	55.24	55.14	Essentially identical—same GC equilibrium
Predicted CDS/ ORFs	129	158	~22% more ORFs in Pa25IQ (insertions or extended regions)
tBLASTx identity range (%)	70–99	70–99	Strong conservation; minor local divergence
Shared core genes (%)	~85	~85	Typical for closely related myoviruses
Unique ORFs (%)	~15	~15	Mosaicism and evolutionary flexibility

Table 5. Comparison of phage SM1 with phage Pa25IQ.

Function	SM1 (%)	Pa25IQ (%)
Structural	25	24
Replication	15	14
Integration/excision	5	5
Lysis	8	8
Packaging	5	5
Methyl/acetyltransferase	7	10
Unknown	35	34

fiber variability, or adaptation to environmental pressures. Despite these insertions, the overall GC content and genome length remained similar, reflecting strong evolutionary constraints preserving essential bacteriophage functions.

4. DISCUSSION

The incidence of *P. aeruginosa* infections is increasing rapidly, posing a significant global threat. Interest in bacteriophages has been renewed due to their potential as candidates for treating infectious diseases [18]. Hospital wastewater is a rich source of bacteriophages, as it harbors a high diversity of them. In addition, close contact facilitates the coevolution of both the bacteriophage and host bacteria. One advantage of bacteriophage therapy over antibiotic therapy is that bacteriophages do not infect mammalian cells [19]. However, identifying a bacteriophage that fits therapeutic applications requires deep genetic analysis of both hosts and bacteriophages.

This study isolated a P. aeruginosa bacteriophage, designated Pa25IQ, from hospital wastewater, a known good source for bacteriophage isolation [20]. Bacteriophage Pa25IQ produced a small, clear plaque, and morphological characteristics observed by FESEM identified it as belonging to the Caudoviricetes class, according to the International Committee on Taxonomy of Viruses. Previous studies have reported isolation of diverse bacteriophages from sewage water [21–24]. The stability of bacteriophage Pa25IQ was assessed across different temperatures and pHs using the double-layer agar overlay method, revealing that it was relatively stable across temperatures of −20°C to 50°C and pHs of 4–8, peaking at pH 7, consistent with the bacteriophage isolated in a previous study [25,26].

The comparison of laboratory stability data and *in situ* measurement data during the isolation period indicates that the physical and chemical characteristics of wastewater at Ghazi Al-Hariri Hospital fell within the tolerances of bacteriophage Pa25IQ. The wastewater had an average temperature of 33.2°C and a pH of 7.4. Its alkalinity of 167.85 mg/l indicates a buffering capacity that likely minimizes pH fluctuations capable of inducing capsid deactivation. Along with monovalent cations (i.e., Na^+ and K^+) and background anions (i.e., Cl^-, NO_3^-, SO_4^{2-}, and PO_4^-), this matrix is hypothesized to provide a favorable electrostatic environment that may facilitate Pa25IQ attachment [27]. Finally, its DO (9.5 mg/l) and BOD (5 mg/l) serve strictly as descriptive indicators of organic and oxygen baselines of the wastewater matrix as defined by standard water-quality protocols [11]. Altogether, these baseline data indicate that the properties of Ghazi Al-Hariri Hospital's wastewater during the isolation period in November 2025 remained within the tolerances of bacteriophage Pa25IQ.

To determine the host range of bacteriophage Pa25IQ, *P. aeruginosa* clinical isolates were obtained from the Microbiology Laboratory at Ghazi Al-Hariri Hospital and identified using the VITEK® 2 compact system. Different studies conducted locally have reported varying prevalence of *P. aeruginosa* among samples from male and female patients [28,29]. One possible explanation for this variability is the time of sample collection. *Pseudomonas aeruginosa* is one of the main pathogens associated with many serious infections, including UTIs, lung infections, and pneumonia [30]. There is an increased number of drug resistance *P. aeruginosa* clinical isolates. Among recent studies conducted in Iraq, one conducted in Basra Province examined samples collected from different hospitals, of which over 70% were resistant to cephalosporins and ciprofloxacin, and 68% were resistant to carbapenem [4]. Another study reported that 24% of tested *P. aeruginosa* isolates were resistant to carbapenem [5]. Given the declining effectiveness of existing antimicrobial therapies, interest is growing in identifying effective alternatives, which include bacteriophages.

Bacteriophage Pa25IQ showed a narrow host range, inhibiting the growth of only 6 of the 25 *P. aeruginosa* clinical isolates tested. Similarly, one study reported the isolation of two bacteriophages with a narrow host range against *P. aeruginosa* strain PAO1 [31]. These narrow host ranges may reflect highly specific tail-spike proteins, high heterogeneity in surface receptors among the tested *P. aeruginosa* clinical isolates, or intracellular defense mechanisms that confer resistance [32,33]. A narrow host range is desirable, as bacteriophages target only specific bacterial species, leaving the rest of the host microbiome intact [34]. However, a narrow host range may limit the therapeutic efficacy of bacteriophages. Possible solutions to overcome this limitation include the use of bacteriophage cocktails or polyvalent bacteriophages [35], the isolation of a broader range of bacteriophages from the environment [36], genetically engineering bacteriophages to recognize more hosts [37], or combining bacteriophages with antibiotics [38,39].

Genome sequencing of both the bacteriophage and the host could be a promising approach to more accurately predict bacteriophage host range [40,41]. Therefore, our study examined the genomes of the bacteriophage Pa25IQ and host *P. aeruginosa* strain using WGS. The host genome size indicates a large, complex genome, similar to those of other *P. aeruginosa* strains [17].

For successful bacteriophage therapy, the dissemination patterns of prophages in environmental and clinical bacterial isolates need to be molecularly determined, as some carry antibiotic resistance genes [42]. In our study, genome analysis revealed five prophage regions in the host *P. aeruginosa* genome. Bacteriophage infection of bacteria results in one of two outcomes: lysis or lysogeny. Lysogeny is a temperate bacteriophage infection, in which the bacteriophage genome is incorporated into the host genome (prophage) and replicates via host cell division [43]. The genes carried and expressed by a prophage may increase host fitness, a process known as lysogenic conversion. The ability of prophages to encode these genes may facilitate bacteriophage resistance via different mechanisms [44]. For instance, various prophages encode superinfection exclusion proteins that prevent additional bacteriophage infection by modifying the bacterial cell envelope [45]. However, further experimental studies are required to confirm these predictions and the presence of these genes in the genome.

Bacteria use the CRISPR-Cas system as an adaptive immune system to protect against bacteriophage infections [46]. This system records short sequences of the invading bacteriophage's genome and adds them to the host cell's CRISPR array, enabling it to locate and destroy bacteriophage DNA during subsequent infections. CRISPR-Cas specifically targets bacteriophage DNA, allowing bacteria to control bacteriophage replication and survive in environments where bacteriophages are present [47].

The genome of bacteriophage Pa25IQ is approximately 94,292 bp in length and has an average GC content of 55.14%. Functional annotation identified proteins involved in bacteriophage replication, including DNA helicase and terminase, as well as holin, which is involved in lysis, and structural proteins, such as the head, tail, and fiber proteins. The presence of one tRNA emphasizes a strong dependence on the host's translation machinery. Bacteriophages tend to minimize tRNA sets by eliminating nonessential and redundant genes. This strategy of genome economy balances space (capsid) and survival [13]. Further studies are required to specify the exact life cycle type for the isolated phage.

Comparative whole-genome sequence analysis using BLASTn revealed that bacteriophage Pa25IQ exhibited 99% genomic similarity with *Samunavirus* SM1 (NCBI GenBank ID: NC_041877.1), a member of the Caudoviricetes class. The genome of bacteriophage SM1 was 93,191 bp long and had a GC content of 55.24%, while the genome of bacteriophage Pa25IQ was slightly longer at 94,292 bp, but had a similar GC content of 55.14%, indicating adaptation to the same bacterial host. The genome of bacteriophage SM1 contained 129 CDSs, compared to 158 predicted ORFs in the genome of bacteriophage Pa25IQ (≥300 bp). The additional ORFs in bacteriophage Pa25IQ may represent accessory or hypothetical genes acquired through recombination or horizontal gene transfer. Despite similarities with bacteriophage SM1, bacteriophage Pa25IQ contained several more predicted ORFs. It also exhibited a relatively narrow host range, infecting only 24% of the tested *P. aeruginosa* clinical isolates, suggesting that it has adapted to local niches. Considering that bacteriophage Pa25IQ was isolated from hospital wastewater, this should also be considered a potential local niche-defining factor, as wastewater may actively shape bacteriophage–host coevolution and refine bacteriophages targeting MDR bacterial strains of clinical concern.

Despite the valuable information on bacteriophage and host genomes addressed, our study had several limitations that should be acknowledged. Firstly, WGS was not performed for all six sensitive *P. aeruginosa* clinical isolates to assess the similarity of their CRISPR-Cas systems to those of bacteriophage Pa25IQ. Secondly, since only one bacteriophage was isolated, no comparisons across bacteriophages were possible. Nevertheless, our findings lay the foundation for future research. The exact functions of the hypothetical genes identified in the bacteriophage Pa25IQ genome remain unknown and require further investigation. The phage genome resulted from a draft assembly composed of nine contigs generated from short-read sequencing; therefore, genome termini and genomic topology could not be definitively resolved. Further studies using long-read sequencing and dedicated termini prediction tools (e.g., PhageTerm) are recommended to obtain a complete phage genome sequence and exactly determine genome architecture. In addition, the anti-biofilm activity of bacteriophage Pa25IQ should be assessed, as *P. aeruginosa* is a well-established biofilm producer. Moreover, the *in vivo* safety of bacteriophage Pa25IQ should be assessed in animal models to improve its future clinical application in humans. Furthermore, bacteriophage superinfection immunity needs to be studied. Finally, testing the effects of various environmental factors on the survival and infectivity of bacteriophage Pa25IQ is needed.

5. CONCLUSION

Since bacteriophage therapy is re-emerging as an attractive alternative for treating pathogenic *P. aeruginosa*, determining both host and bacteriophage genomes is essential for developing effective bacteriophage-based treatments. Our study represents the first in Iraq to comprehensively characterize both the host *P. aeruginosa* strain and its specific bacteriophage. It successfully isolated a bacteriophage, Pa25IQ, from hospital wastewater that could infect pathogenic *P. aeruginosa*. Bacteriophage Pa25IQ appeared stable across different temperatures and pHs and had an optimal MOI of 1. Genome sequencing revealed that bacteriophage Pa25IQ belongs to the Caudoviricetes class and is a close relative of *Samunavirus* SM1 (99% similarity), and contains no virulence or antibiotic resistance genes. Thus, deep genomic analysis contributes to our understanding of *Pseudomonas* bacteriophage diversity and evolutionary history, and supports the development of biocontrol strategies against *P. aeruginosa* infections.

6. ABBREVIATIONS

A, Alkalinity; BHI: brain heart infusion; BOD, Biochemical oxygen demand; Cas, CRISPR-associated genes; *CDSs*, protein-coding sequences; CRISPR, clustered regularly-interspaced palindromic repeats; DNA, deoxyribonucleic acid; EC, Electrical Conductivity; ETT, endotracheal tube; FESEM, Field Emission Scanning Electron Microscope; Kb, kilo base; MDR, Multidrug resistant; MOI, Multiplicity of infection; O&G, Oil and grease; OD, oxygen demand; ORFs, open reading frame; *P. aeruginosa, Pseudomonas aeruginosa*; PBS, phosphate buffer saline; Pfu, plaque forming unit; Rpm, round per minute; TDS, Total dissolved solids; TH, Total Hardness; tRNA, transfer RNA; Turb, Turbidity; UTI, urinary tract infections.

7. ANTIBIOTICS

AK, Amikacin; FEP, Cefepime; CAZ, Ceftazidime; CIP, Ciprofloxacin; GM, Gentamicin; IMI, Imipenem; LVX, Levofloxacin; MEM, Meropenem; TZP, Piperacillin/tazobactam; TOB, Tobramycin.

8. ACKNOWLEDGMENTS

The authors would like to thank the Mustansiriyah University-Baghdad for their support in the present work.

9. AUTHOR'S CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be authors as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

10. FUNDING

There is no funding to report.

11. CONFLICT OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

12. ETHICAL APPROVAL

The study protocol was approved by the Ethical Approval Board of the Middle Technical University, Iraq (Approval No. MEC 140).

13. DATA AVAILABILITY STATEMENT

The genomic sequencing data of the isolated phage is available in the NCBI under the BioSample accession SAMN51761234, and the

corresponding raw sequence reads can be accessed via SRA Run accession SRR35559211.

14. PUBLISHER'S NOTE

All claims expressed in this article are solely those of the authors and do not necessarily represent those of the publisher, the editors, and the reviewers. This journal remains neutral with regard to jurisdictional claims in published institutional affiliations.

15. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that they have not used artificial intelligence (AI) tools for writing and editing of the manuscript, and no images were manipulated using AI.

REFERENCES

1. Koujalagi T, Ruhal R. Mitigating Health Risks Through Environmental Tracking of *Pseudomonas aeruginosa*. Curr Microbiol 2025;82(1):57; doi: https://doi.org/10.1007/s00284-024-04036-6
2. Diggle SP, Whiteley M. Microbe Profile: *Pseudomonas aeruginosa*: opportunistic pathogen and lab rat. Microbiology 2020;166(1):30–3; doi: https://doi.org/10.1099/mic.0.000860
3. Hemmati J, Nazari M, Abolhasani FS, Ahmadi A, Asghari B. *In vitro* investigation of relationship between quorum-sensing system genes, biofilm forming ability, and drug resistance in clinical isolates of *Pseudomonas aeruginosa*. BMC Microbiol 2024;24(1):99; doi: https://doi.org/10.1186/s12866-024-03249-w
4. Alkhulaifi ZM, Mohammed KA. Prevalence and molecular analysis of antibiotic resistance of *Pseudomonas aeruginosa* isolated from clinical and environmental specimens in Basra, Iraq. Iran J Microbiol 2023;15(1):45–54; doi: https://doi.org/10.18502/ijm.v15i1.11917
5. Jawad SS, Al-Azawi IH. Aminoglycoside Susceptibility among Extensive Drug Resistant *Pseudomonas aeruginosa* from Hospitalized Patients in Al-Diwaniyah, Iraq. Med J Babylon 2024;21(3):590–8; doi: https://doi.org/10.4103/MJBL.MJBL_694_23
6. Jameel ZH, Alwash MS, Abdulla AA. Molecular Detection, Antibiotic Resistance, and Biofilm Formation of Clinical *Pseudomonas aeruginosa* Isolates from Patients in Al-Hillah, Babylon Province, Iraq. Med J Babylon 2024;21(2):292–7; doi: https://doi.org/10.4103/MJBL.MJBL_388_23
7. Keen EC. A century of phage research: bacteriophages and the shaping of modern biology. BioEssays News Rev Mol Cell Dev Biol 2015;37(1):6; doi: https://doi.org/10.1002/bies.201400152
8. Naureen Z, Dautaj A, Anpilogov K, Camilleri G, Dhuli K, Tanzi B, *et al.* Bacteriophages presence in nature and their role in the natural selection of bacterial populations. Acta Bio Medica Atenei Parm 2020;91(Suppl 13):2020024; doi: https://doi.org/10.23750/abm.v91i13-S.10819
9. Abdelghafar A, El-Ganiny A, Shaker G, Askoura M. Isolation of a bacteriophage targeting *Pseudomonas aeruginosa* and exhibits a promising *in vivo* efficacy. AMB Express 2023;13(1):79; doi: https://doi.org/10.1186/s13568-023-01582-3
10. Damar Celik D, Karaynir A, Salih Dogan H, Bozdogan B, Ozbek Celik B. Characterization and genomic analysis of PA-56 Pseudomonas phage from Istanbul, Turkey: antibacterial and antibiofilm efficacy alone and with antibiotics. Heliyon 2024;10(17): e36243; doi: https://doi.org/10.1016/j.heliyon.2024.e36243
11. Lipps WC, Braun-Howland EB, Baxter TE. Standard methods for the examination of water and wastewater. American Public Health Association (APHA), Washington, D.C, 2023; doi: https://doi.org/10.2105/SMWW.2882.004
12. Abdulhussein AA, Abdulsattar BO. Isolation and characterization of two novel phages as a possible therapeutic alternative against multi-drug resistant *E. coli*. Gene Rep 2022;28:101644; doi: https://doi.org/10.1016/j.genrep.2022.101644
13. Alotaibi AN, Alqahtani FH, ALfayez NM, Alnassar HM, Alomair AH, Aleyiydi MS, *et al.* Isolation and genomic characterization of selective lytic *Pseudomonas* phage Amjad_SA from a desert urban pond in Riyadh. Front Microbiol 2026;17:1750744; doi: https://doi.org/10.3389/fmicb.2026.1750744
14. Liu Y, Huang Y, Zhu D, Zhang L, Zhang J, Tong Y, *et al.* Characterization and complete genomic analysis of a novel bacteriophage BUCT775 for acinetobacter baumannii and its elimination efficiency in the environment. Int J Mol Sci 2025;26(15):7279; doi: https://doi.org/10.3390/ijms26157279
15. Lyu S, Xiong F, Qi T, Shen W, Guo Q, Han M, *et al.* Isolation and characterization of a novel temperate bacteriophage infecting *Aeromonas hydrophila* isolated from a *Macrobrachium rosenbergii* larvae pond. Virus Res 2024;339:199279; doi: https://doi.org/10.1016/j.virusres.2023.199279
16. Young J, Shariyate MJ, Razavi AH, Nazarian A, Rodriguez EK. Bacteriophage dosing and its effect on bacterial growth suppression in a *Staphylococcus epidermidis* model: an *in vitro* study. PHAGE Ther Appl Res 2024;5(4):223–9; doi: https://doi.org/10.1089/phage.2024.0001
17. De Sousa T, Hébraud M, Dapkevicius MLNE, Maltez L, Pereira JE, Capita R, *et al.* Genomic and metabolic characteristics of the pathogenicity in *Pseudomonas aeruginosa*. Int J Mol Sci 2021;22(23):12892; doi: https://doi.org/10.3390/ijms222312892
18. Pires DP, Vilas Boas D, Sillankorva S, Azeredo J. Phage therapy: a step forward in the treatment of *Pseudomonas aeruginosa* infections. J Virol 2015;89(15):7449–56; doi: https://doi.org/10.1128/jvi.00385-15
19. Podlacha M, Grabowski Ł, Kosznik-Kawśnicka K, Zdrojewska K, Stasiłojć M, Węgrzyn G, *et al.* Interactions of bacteriophages with animal and human organisms—safety issues in the light of phage therapy. Int J Mol Sci 2021;22(16):8937; doi: https://doi.org/10.3390/ijms22168937
20. Aghaee BL, Mirzaei MK, Alikhani MY, Mojtahedi A. Sewage and sewage-contaminated environments are the most prominent sources to isolate phages against *Pseudomonas aeruginosa*. BMC Microbiol 2021;21:132; doi: https://doi.org/10.1186/s12866-021-02197-z
21. Kwiatek M, Mizak L, Parasion S, Gryko R, Olender A, Niemcewicz M. Characterization of five newly isolated bacteriophages active against *Pseudomonas aeruginosa* clinical strains. Folia Microbiol (Praha) 2015;60(1):7–14; doi: https://doi.org/10.1007/s12223-014-0333-3
22. Latz S, Krüttgen A, Häfner H, Buhl E, Ritter K, Horz HP. Differential effect of newly isolated phages belonging to PB1-like, phiKZ-like and LUZ24-like viruses against multi-drug resistant *Pseudomonas aeruginosa* under varying growth conditions. Viruses 2017;9(11):315; doi: https://doi.org/10.3390/v9110315
23. Nordstrom HR, Evans DR, Finney AG, Westbrook KJ, Zamora PF, Hofstaedter CE, *et al.* Genomic characterization of lytic bacteriophages targeting genetically diverse *Pseudomonas aeruginosa* clinical isolates. Iscience 2022;25(6):104372; doi:https://doi.org/10.1016/j.isci.2022.104372
24. Yuan Y, Qu K, Tan D, Li X, Wang L, Cong C, *et al.* Isolation and characterization of a bacteriophage and its potential to disrupt multi-drug resistant *Pseudomonas aeruginosa* biofilms. Microb Pathog 2019;128:329–6; doi: https://doi.org/10.1016/j.micpath.2019.01.032
25. Jiang L, Tan J, Hao Y, Wang Q, Yan X, Wang D, *et al.* Isolation and characterization of a novel myophage Abp9 against pandrug resistant *Acinetobacter baumannii*. Front Microbiol 2020;11:506068; doi: https://doi.org/10.3389/fmicb.2020.506068
26. Scarascia G, Yap SA, Kaksonen AH, Hong PY. Bacteriophage infectivity against Pseudomonas aeruginosa in saline conditions. Front Microbiol 2018;9:875; doi: https://doi.org/10.3389/fmicb.2018.00875
27. Zemb O, Manefield M, Thomas F, Jacquet S. Phage adsorption to bacteria in the light of the electrostatics: a case study using *E.*

Coli, T2 and flow cytometry. J Virol Methods 2013;189(2):283–9; doi: http://doi.org/10.1016/j.jviromet.2013.02.007
28. Ali S, Assafi M. Prevalence and antibiogram of *Pseudomonas aeruginosa* and *Staphylococcus aureus* clinical isolates from burns and wounds in Duhok City, Iraq. J Infect Dev Ctries 2024;18(01):82–92; doi: https://doi.org/10.3855/jidc.18193
29. Al-Saffar MF, Jarallah EM. Isolation and characterization of *Pseudomonas aeruginosa* from Babylon province. Biochem Cell Arch 2019;19(1):203–209; doi: https://doi.org/10.13140/RG.2.2.22943.20644
30. Litwin A, Rojek S, Gozdzik W, Duszynska W. *Pseudomonas aeruginosa* device associated–healthcare associated infections and its multidrug resistance at intensive care unit of University Hospital: polish, 8.5-year, prospective, single-centre study. BMC Infect Dis 2021;21(1):180; doi: https://doi.org/10.1186/s12879-021-05883-5
31. Yuanyuan N, Xiaobo Y, Shang W, Yutong Y, Hongrui Z, Chenyu L, *et al.* Isolation and characterization of two homolog phages infecting *Pseudomonas aeruginosa*. Front Microbiol 2022;13:946251; doi: https://doi.org/10.3389/fmicb.2022.946251
32. Sui B, Li X, Wang L, Xu Y, Hou Y, Khan MSI, *et al.* Intra-strain and inter-strain heterogeneity shape phage-host interactions and phenotypic adaptation in *Pseudomonas aeruginosa*. Appl Environ Microbiol 2026;92(4):e00050; doi: https://doi.org/10.1128/aem.00050-26
33. Pourcel C, Midoux C, Vergnaud G, Latino L. The basis for natural multiresistance to phage in *Pseudomonas aeruginosa*. Antibiotics 2020;9(6):339; doi: https://doi.org/10.3390/antibiotics9060339
34. Alipour-Khezri E, Skurnik M, Zarrini G. *Pseudomonas aeruginosa* bacteriophages and their clinical applications. Viruses 2024;16(7):1051; doi: https://doi.org/10.3390/v16071051
35. Yang Y, Shen W, Zhong Q, Chen Q, He X, Baker JL, et al. Development of a bacteriophage cocktail to constrain the emergence of phage-resistant *Pseudomonas aeruginosa*. Front Microbiol 2020;11:327; doi: https://doi.org/10.3389/fmicb.2020.00327
36. Knezevic P, Kostanjsek R, Obreht D, Petrovic O. Isolation of *Pseudomonas aeruginosa* specific phages with broad activity spectra. Curr Microbiol 2009;59(2):173–80; doi: https://doi.org/10.1007/s00284-009-9417-8
37. Jia HJ, Jia PP, Yin S, Bu LK, Yang G, Pei DS. Engineering bacteriophages for enhanced host range and efficacy: insights from bacteriophage-bacteria interactions. Front Microbiol 2023;14:1172635; doi: https://doi.org/10.3389/fmicb.2023.1172635
38. Chaudhry WN, Concepción-Acevedo J, Park T, Andleeb S, Bull JJ, Levin BR. Synergy and order effects of antibiotics and phages in killing *Pseudomonas aeruginosa* biofilms. PLoS One 2017;12(1):e0168615; doi: https://doi.org/10.1371/journal.pone.0168615
39. Lin Y, Chang RYK, Britton WJ, Morales S, Kutter E, Chan HK. Synergy of nebulized phage PEV20 and ciprofloxacin combination against *Pseudomonas aeruginosa*. Int J Pharm 2018;551(1-2):158–65; doi: https://doi.org/10.1016/j.ijpharm.2018.09.024
40. Edwards RA, McNair K, Faust K, Raes J, Dutilh BE. Computational approaches to predict bacteriophage–host relationships. FEMS Microbiol Rev 2016;40(2):258–72; doi: https://doi.org/10.1093/femsre/fuv048
41. Villarroel J, Kleinheinz K, Jurtz V, Zschach H, Lund O, Nielsen M, *et al.* HostPhinder: a phage host prediction tool. Viruses 2016;8(5):116; doi: https://doi.org/10.3390/v8050116
42. Xavier KVM, Silva AMDA, Luz ACDO, Da Silva FSC, De Melo BST, Pitta JLDLP, *et al.* Diversity and Role of Prophages in *Pseudomonas aeruginosa*: resistance Genes and Bacterial Interactions. Genes 2025;16(6):656; doi: https://doi.org/10.3390/genes16060656
43. Zhang M, Zhang T, Yu M, Chen YL, Jin M. The life cycle transitions of temperate phages: regulating factors and potential ecological implications. Viruses 2022;14(9):1904; doi: https://doi.org/10.3390/v14091904
44. Bondy-Denomy J, Qian J, Westra ER, Buckling A, Guttman DS, Davidson AR, *et al.* Prophages mediate defense against phage infection through diverse mechanisms. ISME J 2016;10(12):2854–66; doi: https://doi.org/10.1038/ismej.2016.79
45. Newton GJ, Daniels C, Burrows LL, Kropinski AM, Clarke AJ, Lam JS. Three-component-mediated serotype conversion in Pseudomonas aeruginosa by bacteriophage D3. Mol Microbiol 2001;39(5):1237–47; doi: https://doi.org/10.1111/j.1365-2958.2001.02311.x.
46. Gholizadeh P, Aghazadeh M, Asgharzadeh M, Kafil HS. Suppressing the CRISPR/Cas adaptive immune system in bacterial infections. Eur J Clin Microbiol Infect Dis 2017;36(11):2043–51; doi: https://doi.org/10.1007/s10096-017-3036-2
47. Watson BNJ, Steens JA, Staals RHJ, Westra ER, Van Houte S. Coevolution between bacterial CRISPR-Cas systems and their bacteriophages. Cell Host Microbe 2021;29(5):715–25; doi: https://doi.org/10.1016/j.chom.2021.03.018

How to cite this article:
Abdulsattar BO, Abdulhussein AA, Samanje JN, Ali MR, Abdulsattar JO. Isolation and genomic characterization of drug-resistant *Pseudomonas aeruginosa* and its phage from Iraqi hospital wastewater. J Appl Biol Biotech 2026;14(6):107-118. DOI: 10.7324/jabb.2026.315277