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ORIGINAL ARTICLE

Isolation and characterization of bacteriophages against antibiotic-resistance *Pseudomonas aeruginosa*

Parisa Abedi Elkhichi[1], Sara Bahonar[2], Hanieh Shirazi[3], Aghil Bahramian[1], Maryam Kazemi Aghdam[3], Mona Ghazi[2*]

[1]Infectious Diseases Research Center, AJA University of Medical Sciences, Tehran, Iran
[2]Department of Microbiology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[3]Pediatric Pathology Research Center, Research Institute for Children's Health, Shahid Beheshti University of Medical Sciences, Tehran, Iran

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ABSTRACT

Background and Objectives: As an important opportunistic pathogen, *Pseudomonas aeruginosa* is associated with severe infections and increasing antimicrobial resistance. The growing prevalence of multidrug-resistant (MDR) strains has renewed interest in bacteriophages as alternative antibacterial agents. The present study sought to isolate and characterize lytic bacteriophages from hospital wastewater active against antibiotic-resistant clinical *P. aeruginosa* isolates.

Materials and Methods: Fifty clinical isolates of *P. aeruginosa* were obtained and tested for antimicrobial susceptibility. Bacteriophages were isolated from hospital wastewater by enrichment and spot assay screening, followed by plaque purification and titration using the double-layer agar method. The host range of purified phages was evaluated against the clinical isolates. Adsorption assays and one-step growth experiments were performed to determine adsorption efficiency, latent period, and burst size. Transmission electron microscopy (TEM) was used to examine phage morphology.

Results: A considerable proportion of the clinical isolates showed multidrug-resistant phenotypes, and a considerable number were also classified as extensively drug-resistant. Four lytic bacteriophages, designated HSP1-HSP4, were successfully isolated and purified. All produced clear plaques and reached high titers. Host range analysis showed variable lytic spectra, with HSP1 exhibiting the broadest activity and showing lytic activity against 70% of the tested isolates, followed by HSP2 (58%), HSP3 (48%), and HSP4 (40%). Adsorption analysis indicated that over 80% of phage particles attached to their host cells within 10 minutes, while HSP1 exhibited the highest adsorption efficiency at 93.6%. A short latent period of approximately 20 minutes was observed for all four phages, and burst sizes ranged from 56 to 65 PFU/cell. Transmission electron microscopy revealed isometric heads and contractile tails, morphologically consistent with tailed phages of the class *Caudoviricetes*.

Conclusion: Hospital wastewater can serve as a practical source for the isolation of lytic bacteriophages active against antibiotic-resistant *P. aeruginosa.* The in vitro properties of the isolated phages support their further evaluation as candidate antibacterial agents against resistant *P. aeruginosa*. However, genomic characterization, phage-antibiotic interaction analyses, and in vivo investigations are required before their broader applicability can be determined.

Keywords: Bacteriophages; *Pseudomonas aeruginosa*; Antibiotic resistance

*Corresponding author: Mona Ghazi, Ph.D, Department of Microbiology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Tel: +98-21-23872556 Fax: +98-21-22439952 Email: mghazi@sbmu.ac.ir

INTRODUCTION

Pseudomonas aeruginosa, a Gram-negative opportunistic pathogen, is frequently implicated in nosocomial infections, particularly in cystic fibrosis patients and immunocompromised individuals. Its remarkable capacity for antibiotic resistance complicates eradication efforts and has accelerated interest in alternative therapeutic approaches (1). *P. aeruginosa* is frequently associated with device-related infections, including those involving catheters and ventilators, and is likewise a leading agent in urinary tract infections, wound infections, pneumonia, and infections at surgical sites (2, 3). Infections caused by *P. aeruginosa* are often associated with poor clinical outcomes, and reported mortality rates range from 13% to nearly 50%, influenced by both the site of infection and the patient's underlying condition (4-6).

The most pressing issue in managing *P. aeruginosa* infections is the rise of antimicrobial resistance, which has resulted in major therapeutic challenges. Widespread and inappropriate antibiotic use has contributed to the development and dissemination of both intrinsic and acquired resistance mechanisms, thereby reducing the effectiveness of currently available antimicrobial agents and posing a serious threat to public health worldwide (7, 8). As a result, the growing prevalence of antibiotic-resistant bacteria has made it imperative to explore antibacterial alternatives beyond conventional strategies.

Bacteriophages—viruses that specifically infect bacterial cells—have garnered considerable research interest as a potential alternative to conventional antibiotics (9). Lytic phages are of particular interest because they can specifically infect and destroy bacterial cells while causing minimal disruption to the normal microbiota (10). In addition, their ability to co-evolve with bacterial hosts may improve their activity against resistant strains and help overcome existing bacterial resistance mechanisms (11).

Hospital wastewater contains diverse microbial populations and represents a valuable source for the isolation of bacteriophages active against clinically important bacterial pathogens such as *P. aeruginosa* (12). However, the diversity and in vitro lytic properties of hospital wastewater-derived phages active against clinical *P. aeruginosa* isolates remain insufficiently characterized.

Given this background, the current investigation sought to isolate and characterize lytic bacteriophages from hospital wastewater samples that are active against clinical *P. aeruginosa* isolates and to evaluate their host range, adsorption characteristics, replication parameters, and morphology.

MATERIALS AND METHODS

Bacterial isolation and characterization. This descriptive cross-sectional study comprised 50 clinical *P. aeruginosa* isolates obtained from respiratory specimens at Mofid Hospital, Tehran, Iran, between 2022 and 2024. The isolates were cultured on selective cetrimide agar (Merck, Germany) and incubated at 37°C for 24 h. Presumptive *Pseudomonas* colonies were subjected to Gram staining, evaluation of colony morphology, and oxidase testing. Species-level identification of *P. aeruginosa* was subsequently confirmed based on pigment production, growth at 42°C, and standard biochemical tests, in accordance with established reference methods (13).

Ethics statement. The ethical approval of the current study was obtained from the Ethics Committee of Shahid Beheshti University of Medical Sciences (IR.SBU.MSP.REC.1402.59).

Antibiotic susceptibility test. Antimicrobial susceptibility testing of the confirmed isolates was performed as per Clinical and Laboratory Standards Institute (CLSI) recommendations (14). Susceptibility to gentamicin (10 µg), amikacin (30 µg), ceftazidime (30 µg), cefepime (30 µg), imipenem (10 µg), meropenem (10 µg), ciprofloxacin (5 µg), piperacillin-tazobactam (30+6 µg), and aztreonam (30 µg) was assessed by the disk diffusion method using antibiotic disks supplied by MAST Group (Merseyside, UK). Colistin susceptibility was assessed by broth microdilution, with *P. aeruginosa* ATCC 27853 serving as the quality control strain in each assay series. The confirmed clinical isolates were subsequently used as host strains for bacteriophage isolation.For the purpose of this study, resistance phenotypes were categorized based on the antimicrobial classes included in the test panel. Isolates resistant to at least one agent in three or more antimicrobial classes were classified as multidrug-resistant (MDR), while those susceptible to no more than two classes were designated as extensively drug-resistant (XDR). Isolates resistant to all antimicrobial agents included in the study panel were considered possible pan-drug-resistant (possible PDR).

Bacteriophage isolation. Untreated wastewater samples were obtained from the wastewater outlet of Mofid Children's Hospital (Tehran, Iran) to isolate lytic bacteriophages effective against *P. aeruginosa*. Sterile containers were used for sample collection, and the specimens were transported to the laboratory under aseptic conditions. After transport, the samples were stored overnight at 4°C to allow suspended particles and sediment to settle (15). The samples were then centrifuged at 4000 × g for 10 minutes to remove cell debris and suspended particles. The supernatant was filtered using a 0.45 µm syringe filter (Jet Biofil, Alicante, Spain) to obtain a clear solution. For enrichment, 10 mL of the filtered wastewater was mixed with 10 mL of Luria-Bertani (LB) medium (Merck, Germany) and 2 mL of an exponential phase culture of the host *P. aeruginosa*. The mixture was incubated at 37°C for 18–24 hours with gentle agitation (120 rpm), after which the culture was centrifuged at 10,000 × g for 10 minutes and the supernatant filtered through a 0.45 µm syringe filter to obtain a bacteria-free phage lysate.

Spot test assay. To detect the presence of lytic bacteriophages, the filtrate was subjected to a spot test assay. Briefly, 100 µL of an overnight culture of *P. aeruginosa* was mixed with 4 mL of molten soft LB agar (0.7% w/v; Merck, Germany) and overlaid onto solidified LB agar plates (1.5% w/v). Following solidification of the overlay, 10 µL of purified bacteriophage suspension with an approximate titer of 10^8 plaque-forming units per milliliter (PFU/mL) was spotted onto the surface of the bacterial lawn. After being allowed to dry at room temperature, the plates were incubated at 37°C for 18–24 hours. The formation of clear lysis zones at the site of inoculation was considered indicative of lytic bacteriophage activity (9).

Plaque assay and bacteriophage titration. Bacteriophage quantification was performed by means of the double-layer agar plaque assay. Serial tenfold dilutions of the bacteriophage lysates were prepared in SM buffer. For each dilution, 100 µL of the diluted phage suspension was mixed with 100 µL of an exponential-phase *P. aeruginosa* culture and added to 4 mL of molten soft LB agar (0.7% w/v agar supplemented with 1 mM $CaCl_2$, maintained at 45°C). The mixture was immediately poured onto LB agar plates (1.5% w/v) and allowed to solidify. Plates were incubated overnight at 37°C, after which individual plaques were counted. Bacteriophage titers were calculated and expressed as plaque-forming units per milliliter (PFU/mL) (16).

Purification and concentration of bacteriophages. Following initial isolation, bacteriophage-containing lysates were purified by the plaque-picking method to ensure monoclonality. Individual plaques showing clear zones of lysis were excised from double-layer agar plates using a sterile Pasteur pipette or micropipette tip and suspended in 1 mL of SM buffer (50 mM Tris-HCl [pH 7.5], 100 mM NaCl, 10 mM $MgSO_4$, and 0.01% gelatin). The suspension was briefly vortexed and incubated at 4°C for 4–6 h to allow bacteriophage particles to diffuse into the buffer. The recovered bacteriophage suspension was then filtered through a 0.45 µm syringe filter to remove residual bacterial cells. This plaque-picking and replating process was repeated at least three times to ensure phage homogeneity and eliminate contaminating phage types (17). Purified bacteriophage lysates were subjected to concentration by polyethylene glycol (PEG) precipitation as described earlier (32).

Host range testing. Spot test assay was employed to determine the host range of the isolated phage using a panel of clinical *P. aeruginosa* isolates. Briefly, each bacterial strain was cultured overnight in LB broth at 37°C and standardized to an optical density at 600 nm (OD_{600}) of 0.1–0.2 using a UV–Vis spectrophotometer (BioPhotometer, Eppendorf, Germany). Subsequently, 100 µL of the standardized bacterial suspension was mixed with 4 mL of molten soft LB agar (0.7% w/v agar) and overlaid onto LB agar plates (1.5% agar). After the top agar layer had solidified, 10 µL of the purified phage suspension (approximately 10^8 PFU/mL) was spotted onto the surface of each bacterial lawn. After being dried at room temperature, the plates were incubated at 37°C for 18-24 hours. The presence of clear zones at the inoculation sites was interpreted as susceptibility of the tested isolates to the phage. The host range was determined based on the number of bacterial isolates showing visible lysis (17).

Bacteriophage adsorption assay. Bacteriophage adsorption assay was conducted to determine the rate of phage attachment to host cells. Each phage was tested against its corresponding host strain used for isolation. Briefly, an exponential-phase culture of *P.*

aeruginosa ($OD_{600} \approx 0.2$) was mixed with a phage suspension at a multiplicity of infection (MOI) of 0.1, resulting in an initial phage concentration of approximately 10^7 PFU/mL, and incubated at 37°C. At regular time intervals (every 2 min for up to 20 min), 100 µL aliquots were collected and immediately centrifuged at 10,000 × g for 5 min at 4°C to pellet the bacterial cells. The supernatant containing unadsorbed bacteriophages was titrated using the double-layer agar method. The adsorption rate was determined by comparing the difference between the initial titer and the unadsorbed titer in the supernatant.

Transmission electron microscopy (TEM). Transmission electron microscopy (TEM) was employed to examine the morphology of the purified phages (18). A 10 µL drop of high-titer phage suspension (~10^8–10^9 PFU/mL) was applied onto carbon-coated copper grids (300-mesh) and allowed to adsorb for approximately 4 minutes at room temperature. Filter paper was used to gently wick away excess liquid. Negative staining was performed using 2% (w/v) phosphotungstic acid (PTA, pH 7.0). for 30–60 seconds, air-dried at room temperature, and examined under a transmission electron microscope (Zeiss LEO 906 TEM, Carl Zeiss, Germany) operated at an accelerating voltage of 80 kV. Imaging was performed at the Electron Microscopy Facility, Tabriz, Iran.

One-step growth and burst size. The latent period and burst size of the isolated bacteriophages were determined through one-step growth experiment. Each phage was evaluated using its corresponding host strain. An exponential-phase bacterial culture was infected at a low multiplicity of infection (MOI) of 0.01, resulting in an initial phage concentration of approximately 10^6 PFU/mL, and incubated for 10 min at 37°C to allow phage adsorption. The mixture was then centrifuged at 10,000 × g for 5 min to remove unadsorbed particles, and the resulting pellet was resuspended in fresh pre-warmed LB broth. The infected bacterial culture was incubated at 37°C on a shaking incubator, with samples harvested at 5-minute time points over a total incubation period of 90 minutes. Each aliquot was promptly diluted and plated using the double-layer agar technique to determine phage titers at each time point. Using the PFU counts recorded at each time point, the latent period and burst size were derived from the one-step growth curve.

Statistical analysis. Adsorption and one-step growth experiments were carried out in triplicate. Data were plotted using GraphPad Prism version 9 (GraphPad Software, USA). Because the study was primarily descriptive in nature, no inferential statistical analyses were performed.

RESULTS

Antibiotic susceptibility test results. Antimicrobial susceptibility testing demonstrated a high prevalence of resistance among the clinical *P. aeruginosa* isolates. Most strains were resistant to at least three antimicrobial classes, with particularly high resistance observed against β-lactams and fluoroquinolones. Detailed resistance profiles are presented in Table 1. Resistance to imipenem and meropenem was highly prevalent among the isolates, highlighting the need for alternative antibacterial strategies. Based on the antimicrobial categories included in the study panel, 32 of 50 isolates (64%) were classified as multidrug-resistant (MDR), 11 (22%) as extensively drug-resistant (XDR), and 7 (14%) as possible pan-drug-resistant (possible PDR). For the purposes of this study, possible PDR was assigned to isolates resistant to all tested antimicrobial categories. Nonetheless, definitive PDR status would necessitate susceptibility testing against all clinically relevant antipseudomonal antimicrobial classes.

Bacteriophage isolation and titer determination. Four lytic bacteriophages were successfully isolated from untreated hospital wastewater and designated HSP1, HSP2, HSP3, and HSP4 (Hospital Sewage Phages 1-4). Although the four phages exhibited similar virion morphology, they were considered distinct phage isolates based on reproducible differences in plaque morphology, host range, adsorption efficiency, and one-step growth characteristics following repeated plaque purification. All four phages produced clear plaques on double-layer agar, with titers ranging from 10^7 to 10^9 PFU/mL after enrichment and purification. Representative plaques were circular and measured approximately 1-3 mm in diameter.

Bacteriophage morphology. Transmission electron microscopy (Fig. 1) showed that all four isolated bacteriophages (HSP1-HSP4) exhibited a characteristic head-tail morphology, consisting of isometric heads

Table 1. Antibiotic susceptibility test results for 50 clinical strains. Percentage Susceptibility (S), Intermediate (I) and Resistance (R) were determined as per CLSI guidelines.

Antibiotic	Antibiotic class	Breakpoints (CLSI)[a]	Susceptible % (n)	Intermediate % (n)	Resistant % (n)
Cefepime	Cephems	S≥25, R≤18	40% (20)	2% (1)	58% (29)
Ceftazidime	Cephems	S≥21, R≤17	32% (16)	2% (1)	66% (33)
Ciprofloxacin	Quinolones	S≥25, R≤21	48% (24)	4% (2)	48% (24)
Imipenem	Carbapenems	S≥23, R≤19	48% (24)	2% (1)	50% (25)
Meropenem	Carbapenems	S≥23, R≤19	52% (26)	2% (1)	46% (23)
Amikacin	Aminoglycosides	S≥17, R≤14	52% (26)	2% (1)	46% (23)
Piperacillin–Tazobactam	β-lactam/inhibitor	S≥21, R≤17	54% (27)	6% (3)	40% (20)
Aztreonam	Monobactams	S≥27, R≤21	60% (30)	4% (2)	36% (18)
Gentamicin	Aminoglycosides	S≥15, R≤12	56% (28)	4% (2)	40% (20)
Colistin	Polymyxins	S≤2[b]	_	72% (36)	28% (14)

a: Breakpoints are based on zone diameter (mm) as per CLSI guidelines.

b: Colistin breakpoints are based on Minimum Inhibitory Concentration (MIC, µg/mL).

c: According to CLSI M100 guidelines, *P. aeruginosa* isolates with MIC ≤2 µg/mL are categorized as "Intermediate" rather than "Susceptible."

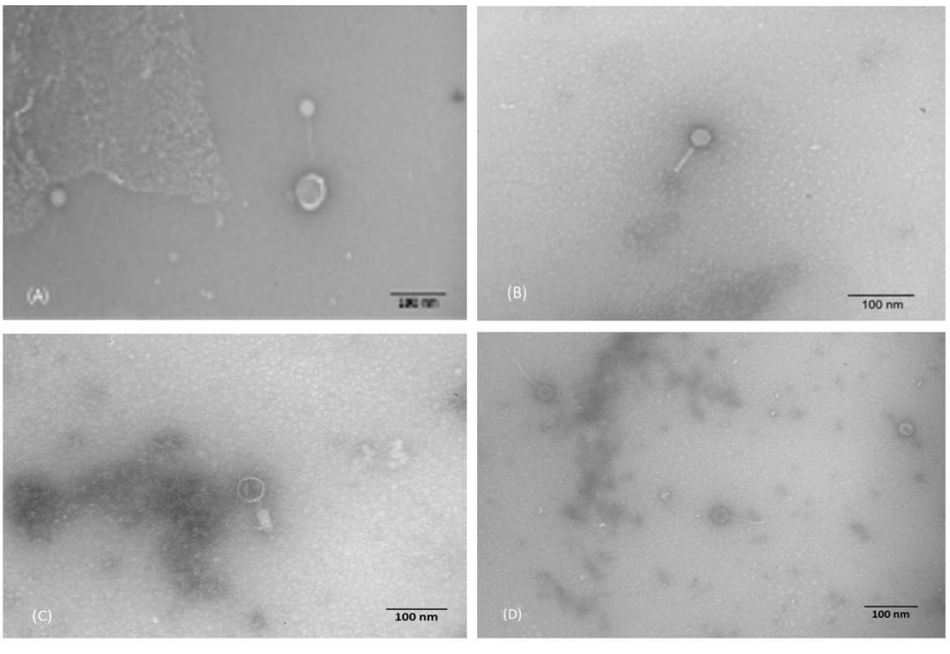

Fig. 1. Transmission electron micrographs of the isolated bacteriophages. (A) HSP1, (B) HSP2, (C) HSP3, and (D) HSP4, showing icosahedral heads and contractile tails. Scale bar = 100 nm.

and contractile tails. These features were consistent with tailed bacteriophages belonging to the class *Caudoviricetes* (Table 2). The phage heads showed noticeable size variation, with diameters ranging from approximately 70 to 90 nm based on the TEM scale bars. Tail appendages were visible in some particles in Fig. 1A and 1B, and a baseplate-like structure was observed in Fig. 1C.

Host range analysis. The host range of the four isolated bacteriophages (HSP1, HSP2, HSP3, and HSP4) was evaluated by spot assay against 50 clinical *P.*

Table 2. Electron microscopic characteristics of the bacteriophages

Bacteriophages	Head shape	Head diameter (nm)	Tail length (nm)	Tail type	Class
HSP1	Icosahedral	88 ± 5	110 ± 5	Contractile	Caudoviricetes
HSP2	Icosahedral	85 ± 6	105 ± 8	Contractile	Caudoviricetes
HSP3	Icosahedral	70 ± 5	95 ± 6	Contractile	Caudoviricetes
HSP4	Icosahedral	82 ± 5	115 ± 7	Contractile	Caudoviricetes

aeruginosa isolates. The analysis revealed distinct lytic activity profiles among the phages. HSP1 showed the broadest activity, producing visible lysis zones on 35 of 50 isolates (70%). HSP2 showed lytic activity against 29 isolates (58%), while HSP3 produced lysis zones on 24 isolates (48%). HSP4 exhibited a comparatively narrower host range but still showed lytic activity against 20 isolates (40%).

Bacteriophage adsorption efficiency. All four bacteriophages exhibited rapid adsorption kinetics. Over 80% of phages adsorbed to host cells within the first 10 minutes. For most phages, the adsorption curve approached a plateau by 15 min, with only minimal additional reduction in the free phage titer thereafter. Among the four phages, HSP1 showed the highest adsorption efficiency (93.6%), followed by HSP4 (90.7%), HSP2 (89.2%), and HSP3 (87.5%) (Fig. 2).

One-step growth curve. One-step growth curve assays were conducted for HSP1, HSP2, HSP3, and HSP4 to evaluate their replication kinetics and burst size. Each phage was tested at a low MOI of 0.01 using its corresponding host strain, and free phage titers were measured at 5-min intervals over a 90-min period. All four bacteriophages displayed comparable replication kinetics, characterized by an initial latent period of approximately 20 min, followed by a marked increase in phage titer. Burst sizes showed modest variation among the phages, with HSP4 exhibiting the highest burst size (~65 PFU/cell), followed by HSP1 (~61 PFU/cell), HSP3 (~60 PFU/cell), and HSP2 (~56 PFU/cell) (Fig. 3).

DISCUSSION

The emergence of antibiotic-resistant bacterial pathogens has become a major public health crisis (19). Multidrug-resistant *P. aeruginosa* infections remain a major therapeutic challenge, yet phage therapy has emerged as a promising alternative, with accumulating clinical and experimental support (20). One promising approach is the use of bacteriophages as natural antibacterial agents (21). Bacteriophages are viruses that exclusively infect bacteria and do not directly infect human cells (22). Sewage and wastewater are recognized as suitable sources for bacterio-

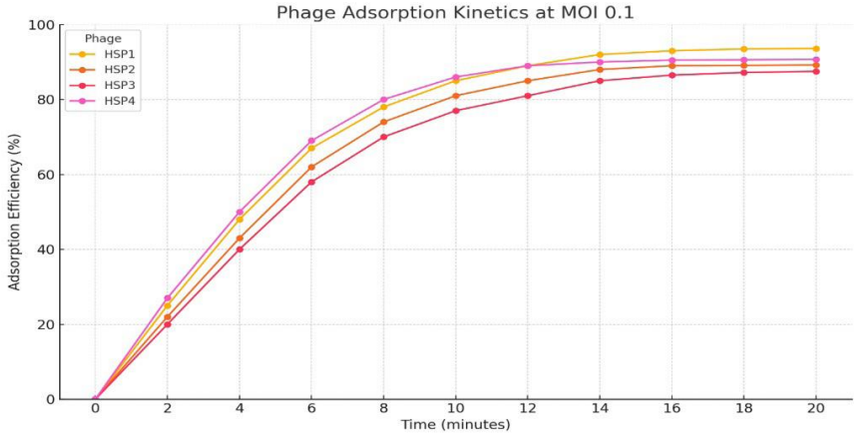

Fig. 2. Adsorption kinetics of bacteriophages HSP1, HSP2, HSP3, and HSP4 at MOI 0.1. More than 80% of bacteriophage particles were adsorbed within the first 10 minutes, and the adsorption curves reached a plateau by 15 minutes.

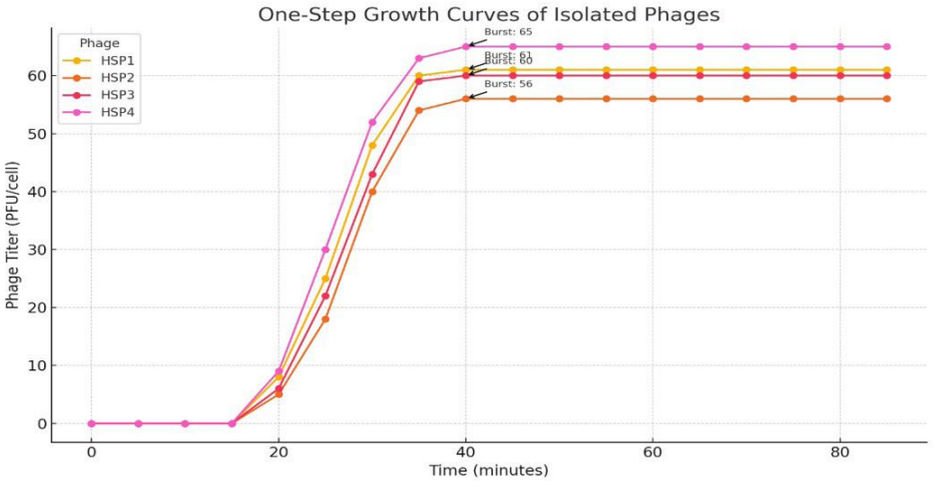

Fig. 3. One-step growth curves of bacteriophages HSP1–HSP4 against *P. aeruginosa*. The curve shows an initial incubation period of approximately 20 minutes, followed by a rapid increase in phage titers (PFU). Arrows indicate the time points used for burst size determination and the corresponding burst sizes (PFU/cell).

phage isolation, and in this study, hospital wastewater was used to isolate bacteriophages active against resistant clinical *P. aeruginosa* isolates (12, 23). This ecological context may have contributed to the isolation of phages showing substantial lytic activity against resistant *P. aeruginosa* isolates in the present study. Our findings revealed a high prevalence of MDR *P. aeruginosa* among the clinical isolates tested, with a substantial subset meeting the criteria for XDR or possible PDR. These resistance profiles underscore the growing clinical challenge associated with *P. aeruginosa* infections and highlight pressing need for novel therapeutic approaches, particularly phage therapy (20, 21). Accordingly, the present study aimed to isolate lytic bacteriophages active against resistant *P. aeruginosa* strains. Four distinct lytic phages (HSP1-HSP4) were isolated from hospital wastewater. Each produced clear plaques and reached high titers following purification. Among the four phages, HSP1 showed the broadest activity, producing visible lysis zones on 70% of the clinical isolates tested, followed by HSP2 (58%), HSP3 (48%), and HSP4 (40%). This diversity in host range may support the future development of phage cocktails to improve coverage against heterogeneous *P. aeruginosa* populations. Our findings corroborate those of Shokri et al. and Barazandeh et al., which also demonstrated variability in host range among *P. aeruginosa* bacteriophages active against clinical isolates (24, 25). The relatively broader lytic activity observed among the isolated bacteriophages may reflect adaptation to the hospital wastewater environment, where bacteriophages are continuously exposed to diverse and highly resistant *P. aeruginosa* populations (26). However, this interpretation remains speculative and would require further genomic and receptor-level investigation.Bacteriophage adsorption kinetics and one-step growth curves further indicated favorable in vitro properties of these bacteriophages. Transmission electron microscopy revealed that all four isolates (HSP1-HSP4) possessed isometric heads and contractile tails, features consistent with tailed bacteriophages of the class *Caudoviricetes* (27). However, further classification at the family or genus level would require whole-genome sequencing and was therefore beyond the scope of the present study. Although this morphology is commonly observed among lytic tailed bacteriophages, morphology alone is insufficient to determine genome type or detailed taxonomic placement. In another study, Barazandeh et al. characterized a novel lytic bacteriophage active against XDR *P. aeruginosa* isolates and reported that, despite its relatively narrow host range, the phage was effective against 47 out of 70 clinical isolates (25). That study also demonstrated favorable in vitro properties, including rapid adsorption, a short latent period, and a high burst size, all of which support the therapeutic relevance of bacteriophages targeting resistant *P. aeruginosa* strains (25). However, the bacteriophages described in our study (HSP1-HSP4) showed broader diversity in lytic activity across the tested clinical isolates. Together with their

favorable adsorption and replication characteristics, these findings support the need for further evaluation of the isolated bacteriophages as potential antibacterial agents against resistant *P. aeruginosa*. In another study, Shokri et al. reported a bacteriophage cocktail consisting of three bacteriophages from the families Cystoviridae, Leviviridae, and Inoviridae that were also active against *P. aeruginosa* (24). These findings collectively highlight the diversity among *P. aeruginosa* bacteriophages and emphasize the need for broader studies to expand existing knowledge in this area.

All isolates exhibited rapid adsorption (>80% within 10 min), with HSP1 showing the highest efficiency (93.6%). The phages also displayed a short latent period (~20 min) and high burst sizes (56-65 PFU/cell), indicating efficient lytic replication under in vitro conditions, consistent with therapeutic *Pseudomonas* bacteriophages described by Marashi et al. (16). Compared with bacteriophages reported in other studies, including those described by Wintachai et al., our bacteriophages demonstrated favorable in vitro characteristics in terms of adsorption rate and lytic activity profile, although direct comparison between studies should be interpreted cautiously because of differences in host strains, bacterial species, assay conditions, and experimental design (29, 30). Bacteriophages with these characteristics can propagate rapidly under in vitro conditions, and such features are generally regarded as favorable for lytic bacteriophages, although their relevance in vivo remains to be determined (30). Although spot assays were used for host range assessment and are indicative of lytic activity, they may not fully predict productive infection or in vivo efficacy because of factors such as biofilm penetration and host immune interactions (17, 31).

Our study successfully isolated clinically relevant bacteriophages by using hospital wastewater as a reservoir, where there is a higher likelihood of finding bacteriophages adapted to antibiotic-resistant strains. However, several limitations should be acknowledged. The host range was assessed only in vitro, without biofilm or synergy testing. Furthermore, we did not perform full genomic characterization or screening for toxin-related, lysogeny-associated, or other undesirable genes, all of which are important for clinical safety assessment. Despite these limitations, the present findings indicate that hospital wastewater can serve as a useful source for isolating lytic bacteriophages active against resistant clinical *P. aeruginosa* isolates and provide a basis for further investigation of their antibacterial potential.

CONCLUSION

In conclusion, the present study showed that hospital wastewater is a practical source for the isolation of lytic bacteriophages active against antibiotic-resistant clinical isolates of *P. aeruginosa*. The recovered phages exhibited favorable in vitro characteristics, including clear lytic activity, relatively broad host range in some isolates, rapid adsorption, short latent periods, and moderate-to-high burst sizes. These results highlight the potential of these phages as promising candidates for further evaluation against drug-resistant *P. aeruginosa*. Nevertheless, comprehensive genomic characterization, assessment in biofilm-associated and phage–antibiotic combination models, and in vivo studies remain necessary to better define their safety, biological suitability, and possible future applicability.

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