INTERNATIONAL JOURNAL OF ADVANCED RESEARCH
ISSN: 3106-7980: 3106-7972
Volume 3, Issue 2, 2026

ISOLATION AND PHYSICOCHEMICAL CHARACTERIZATION OF LYTIC BACTERIOPHAGES AGAINST MULTI-DRUG-RESISTANT *PSEUDOMONAS AERUGINOSA* FROM HOSPITAL SEWAGE SAMPLE

Fasiha[1], Sabeen Sabri[2], Arfat Yousaf Shaheen[3], Furqan malik[4], Muhammad Hussnain Afzal[5], Muhammad Afzal[6], Muhammad Saleem Khan[*7]

[1,2,3,4,5]*Department of Microbiology and Molecular Genetics, Faculty of Life Sciences, University of Okara, Okara, 56130 Pakistan*
[6, *7]*Department of Zoology, Faculty of Life Sciences, University of Okara, Okara, 56130 Pakistan*

[*7]samiikhan@uo.edu.pk

DOI: https://doi.org/10.5281/zenodo.21413409

Keywords
P. aeruginosa, multi-drug resistant, antimicrobial resistance, Nosocomial infections, Lytic bacteriophages, Host range, Latent period, Burst size, MOI

Article History
Received: 28 April 2026
Accepted: 15 June 2026
Published: 30 June 2026

Corresponding Author: *
Muhammad Saleem Khan

Abstract
The rapid emergence of antibiotics resistance has become one of the most pressing global health challenges with Pseudomonas aeruginosa recognized as leading cause of health care associated infections and multi-drug resistant bacterial infections. Bacteriophages have gained attention as a potential alternative to conventional antibiotics. This study aimed to isolate and characterize lytic bacteriophages against MDR P. aeruginosa from hospital sewage samples (n=25), were collected from Okara and Sahiwal region, Pakistan. Two isolated bacteriophages designated as P. a Ø and P. a Ø1 were selected for characterization. The phages exhibited titer of 3×10^{12} PFU/mL and 2×10^{10} PFU/mL, respectively, and produced a clear plaque measuring 1.2 - 1.5 mm in diameter. Host range analysis demonstrated that both the phages were highly specific to MDR P. aeruginosa. Latent period of isolated phages P. a Ø and P. a Ø1 were approximately 25 minutes and the burst size of 154 PFU/infected cell and 118 virus particles released per infected cell. Physicochemical characterization showed that both the phages remain stable over a pH range of 3-11 and temperature range of 70°C while reduced viability or complete inactivation at 80°C. No detectable activity was found against the ethanol exposure (63%) and acetone (90%). A decreased stability was observed against normal saline and chloroform (70%). In addition, both the bacteriophages retained viability during storage at 4°C and -20°C for time up to three months, although the gradual decline in the titer was observed. The findings of this study provide foundation for in vitro and in vivo investigations to evaluate the therapeutically potential of these bacteriophages against multi-drug resistant P. aeruginosa.

Introduction:

Pseudomonas aeruginosa is a Gram-negative, rod shaped, facultative aerobic opportunistic human pathogen (Khan et al., 2023). *P. aeruginosa* is widely distributed in nature and can thrive in variety of environmental niches. It can be isolated from numerous living host, including humans, animals and plants. In humans, it may exist as a part of normal skin microbiota, without causing disease under healthy conditions (Barrow, 2001). It is one

of the leading causes of healthcare associated infections, and responsible for 11% of nosocomial infection worldwide, leading to the significant morbidity particularly in immunocompromised patients. As a highly virulent pathogen, it is responsible for wide range of infections including pneumonia, bacteremia, urinary tract infections (UTIs) and wound infections (Khan et al., 2023; Kumari et al., 2009). *P. aeruginosa* can cause life threatening infections in a patients with cancer, severe burns, AIDS, cystic fibrosis or those undergoing surgery, with persistent lung colonization and recurrent chronic pulmonary infections being particularly common in individuals with cystic fibrosis (Gale et al., 2015; Krylov, 2014). During chronic infections it forms biofilms that enhances resistance to antibiotics, host immune defense and mechanical clearance (Thi et al., 2020).

Although, *P. aeruginosa* is associated with the health care associated infections, community acquired infections are increasingly reported in developing countries, including Pakistan. Due to its remarkable capacity to acquire resistance to multiple antimicrobial agents, *P. aeruginosa* is classified among the ESKAPE pathogen (Sharma et al., 2021). The rapid emergence and dissemination of antimicrobial resistance (AMR) in this organism poses a serious global health challenge, with the estimate indicating that mortality associated with the Multi-Drug resistant (MDR) and Pan-Drug resistant (PDR) infections could eventually exceeds that of major diseases such as cancer (Chan et al., 2023). The world health organization (WHO) designate *P. aeruginosa* as high priority multi drug resistant bacterial pathogens requiring urgent research and development of new therapeutic strategies (Sati et al., 2025).

Among the emerging alternative to conventional antibiotics, bacteriophages has gained considerable attention because of their ability to specifically infect and lyse bacterial cells. First discovered by the Frederick Twort in the early 20th century through the observation of "Glassy Transformation" in bacterial colonies (Żaczek et al., 2020). Phages are ubiquitous in nature and considered as a cost-effective approach for treating bacterial infections. Commonly referred to as bacteria controller, they are most abundant biological entities on Earth, with an estimated population of about 10^{31} to 10^{32} particles (Marashi et al., 2022). Lytic bacteriophages exert their antibacterial activity by hijacking the bacterial genetic machinery, ultimately causing bacterial cell lysis (Dennehy & Abedon, 2021). However, the phage therapy became less common after the antibiotics were widely introduced, but the emergence of antibiotic resistant bacteria due to overuse and misuse of antibiotics has renewed interest in phage therapy. Unlike, broad-spectrum antibiotics, bacteriophages have high host specificity, selectivity eliminating pathogenic bacteria while preserving the beneficial microbiota (Gordillo Altamirano & Barr, 2019). Recent advancement has been made in the isolation and characterization of lytic bacteriophages targeting multi drug resistant *P. aeruginosa.* Therapeutic bacteriophages exhibited potential antibacterial activity against the clinical isolates have been frequently recovered from hospital sewage, waste water and other environmental sources (Shi et al., 2024). *P. aeruginosa* specific bacteriophages have demonstrated considerable potential as bio control agents by effectively disrupting biofilms and serving as therapeutic agent against several bacterial infections (Chegini et al., 2020), while the geographical variation in the phage diversity underscores the need for continued isolation and characterization of locally circulating bacteriophages (Majdani & Hatefirad, 2022). Hospital sewage serves as an ideal source for bacteriophage isolation because it receive waste from large human population, resulting in high diversity of bacterial hosts and their corresponding bacteriophages. Therefore, the present study aimed to isolate and physicochemically characterize lytic bacteriophages against the multi-drug resistant *P. aeruginosa* from hospital sewage samples collected in District Sahiwal and Okara.

Materials and Methods:

Revival of Bacterial Isolates:

Clinical isolates of *Pseudomonas aeruginosa* were obtained from Microbiology and Molecular Genetics lab, University of Okara. These isolates were preserved as glycerol stock at -20°C and revived by inoculation in Luria Bertani broth medium followed by incubation at 37°C. Fresh culture were then streak on Nutrient Agar and plates were then incubate at 37°C to obtain well isolated colonies for further analysis.

Gram Staining and Biochemical Confirmation:

The purity and identity of revived isolates were also confirmed by Gram Staining according to standard protocol described by Hans Christian Gram. Prepared slides were examined under the 100x oil immersion objective of light microscope (Tripathi & Sapra, 2024). These isolates were confirmed using oxidase and catalase biochemical tests following established procedure (Hemraj et al., 2013).

Antibiotic Susceptibility Testing:

Antibiotic susceptibility profile of *P. aeruginosa* isolates was determined using the Kirby Bauer Disc fusion method following Clinical and Laboratory Standard Institute (CLSI) (Hudzicki, 2009). A total of seventeen antibiotic discs of different concentrations were tested to determine the resistant and susceptibility patterns of isolates (Jamal et al., 2017).

Collection and Processing of Sewage Sample:

A total of twenty-five (n=25) sewage samples were collected from District Headquarter Hospital, Okara, District Headquarter South City, Okara and District Headquarter Sahiwal for the isolation of bacteriophages active against P. *aeruginosa*. The collected sewage samples were centrifuged at 4000 rpm for 25 minutes to remove particulate debris. The supernatant was filtered through 0.45µm syringe filter and filtrate were stored at 4°C until further processing (Nyachieo et al., 2021).

Enrichment and Isolation of Bacteriophages:

Enrichment of Bacteriophages:

For the enrichment of bacteriophages, 15ml of Luria Bertani (LB) Broth, 15 mL of sewage filtrate, 0.5mL of logarithmic phase newly grown *P. aeruginosa*, and 30 µl of $MgCl_2$ were mixed in a sterile test tube. The mixture was incubated overnight in shaking incubator at 37°C. Following incubation, the culture was centrifuged and the supernatant was filtered through 0.22µm syringe filter to obtain enriched phase lysate (Yin et al., 2019).

Screening of Bacteriophages:

The presence of lytic bacteriophages was determined using double agar overlay spot assay. Briefly, 200µl of log phase *P. aeruginosa* culture was mixed with 3-5ml of molten soft agar and overlaid onto nutrient agar plates. After solidification, 10µl of enriched sewage filtrate was spotted on three separate locations on bacterial lawn. Plates were incubated at 37°C for 24hrs and examined the zone of lysis indicative of bacteriophage activity (Yilmaz et al., 2025).

Plaque Assay:

Positive samples identified during screening were subjected to plaque assay using the double agar overlay technique. Serial ten-fold dilutions of the phage lysate were prepared, and 1 mL of each dilution was mixed with the host bacterium before adding approximately 5 mL of molten soft agar. The mixture was poured over nutrient agar plates, allowed to solidify, and incubated at 37°C for 24 hours. Plaque morphology and plaque-forming units (PFU) were recorded (Yang et al., 2025).

Purification of Bacteriophages:

Individual well-isolated plaques were carefully picked and propagated in fresh host cultures. The resulting lysates were filtered through sterile 0.22-µm syringe filters, serially diluted, and subjected to repeated plaque assays using the double agar overlay method. This purification process was repeated five successive times to obtain purified lytic bacteriophage isolates against multidrug-resistant *P. aeruginosa* (Yilmaz et al., 2025).

Biological Characterization of Bacteriophages:

Host Range Determination of *P. Aeruginosa* Phages:

The host range of the purified bacteriophages was evaluated using the spot assay method. Briefly, 100 µL of logarithmic-phase cultures of *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, and *Acinetobacter spp*. were individually mixed with 5 mL of molten soft agar and overlaid onto nutrient agar plates. After solidification, 10 µL of purified phage lysate was spotted onto three different locations on each bacterial lawn. Plates were incubated at 37°C for 24 hours, and lytic activity was recorded based on the appearance of clear inhibition zones (Amankwah et al., 2022).

One Step Growth Curve Analysis:

One-step growth kinetics of the purified bacteriophages were determined to estimate the latent period and burst size. A logarithmic-phase culture of *P. aeruginosa* was mixed with purified phage lysate and $MgCl_2$ to facilitate phage adsorption. Following adsorption, the mixture was centrifuged at 4,000 rpm for 20 minutes, and the pellet was resuspended in fresh Luria Bertani (LB) broth before incubation in a shaking incubator. Samples were collected at 10-minute intervals over a period of 60 minutes, and phage titers were determined using the double agar overlay method. The latent period and burst size were calculated from the resulting one-step growth curve (Sharma et al., 2021).

Physiochemical Characterization of Bacteriophages

Temperature Stability

The thermal stability of purified bacteriophages was evaluated by incubating phage suspensions in LB broth at 25, 37, 45, 60, 70, and 80°C for one hour using a water bath. Residual phage activity was subsequently determined by the double agar overlay plaque assay (Cao et al., 2015)

PH stability:

The stability of purified bacteriophages under different pH conditions was assessed by exposing phage suspensions to pH values ranging from 2 to 12. Following incubation, phage viability was determined using the double agar overlay plaque assay (Oliveira et al., 2020).

Organic Solvents Tolerance:

The tolerance of purified bacteriophages to organic solvents was evaluated by exposing phage suspensions to 70% chloroform, 63% ethanol, 90% acetone, and normal saline. Phage survival was assessed using the plaque assay based on the double agar overlay technique (Majdani & Hatefirad, 2022).

Cold Temperature Storage Tolerance:

To evaluate storage stability, purified bacteriophages were stored at 4°C and −20°C. Phage viability was assessed after one, two, and three months using the plaque assay to determine the effect of prolonged cold storage on phage activity (Aghaee et al., 2021).

Results:

Isolation and Biochemical Confirmation of *Pseudomonas aeruginosa*:

Pseudomonas aeruginosa produced, opaque flat circular colonies, with characteristic greenish pigmentation (Pyocanin) on nutrient agar, accompanied by the distinctive fruity, grape like odor. Gram staining revealed short pink rod-shaped rods under the 100x oil immersion objectives confirming the Gram-negative nature of the isolates. Biochemical characterization further confirmed the identity of *P. aeruginosa* with the positive catalase activity indicating the rapid formation of bubbles within 45 seconds and positive oxidase reaction demonstrated by the development of purple colour within 10 to 15 seconds.

Antibiotics Susceptibility Testing:

The antibiotic susceptibility profile of *P. aeruginosa* was determined against 17 antibiotics using Kirby Bauer disc fusion method showed complete resistance characterized by the absence of zone of inhibition (0 mm) was recorded against erythromycin, penicillin, piperacillin, Septran and tetracycline as shown in Table 1.

Table 1: Antibiotic Susceptibility profile of *P. aeruginosa*

Antibiotic Class	Antibiotics	Zone (Diameter)	Interpretation
Aminoglycosides	Amikacin	19 mm	Susceptible
	Tobramycin	19 mm	Susceptible
Macrolides	Azithromycin	20 mm	Susceptible
	Erythromycin	0	Resistant
β-Lactam Penicillin	Penicillin	0	Resistant
	Piperacillin	0	Resistant
β-Lactam Penicillin/ β-Lactamase Inhibitor combination	Augmentin (Amoxicillin + Clavulanic Acid)	20 mm	Intermediate
Cephalosporin	Ceftriaxone	21 mm	Intermediate
	Cefepime	22 mm	Susceptible
Fluroquinolones	Ciprofloxacin	28 mm	Susceptible
	Levofloxacin	24 mm	Intermediate
Polymixins	Colistin	13 mm	Intermediate
	Polymixins B	14 mm	Intermediate
Tetracyclines	Tetracycline	0 mm	Resistant
Sulfonamides	Septran	0 mm	Resistant
Phenicols	Chloramphenicol	10 mm	Intermediate
Glycopeptides	Vancomycin	21 mm	Intermediate

Isolation of Bacteriophages:

A total of 25 sewage samples were processed for bacteriophage isolation. Following centrifugation and enrichment, the clarified filtrate was collected in sterile falcon tubes and stored at 4°C until further analysis. Screening by double agar overlay spot assay identify lytic bacteriophages in 8 out of 25 sewage samples. Of the positive samples, 4 (n=4/8) samples were obtained from main DHQ, hospital Okara, 2 (n=2/9) were from DHQ hospital South City, Okara and 2 (n=2/8) samples were from DHQ hospital, Sahiwal as shown in Table 2. The presence of bacteriophage was confirmed by clear lytic zone (Spot) on bacterial lawn after 24hrs of incubation.

Table 2: Screening of hospital sewage samples for bacteriophages against *P. aeruginosa* by Spot Assay

Sample Area	Sewage Samples (n)	Positive (n)	Percentage (%)
Main DHQ Hospital, Okara	8	4	50%
DHQ Hospital South City, Okara	9	2	22%
DHQ Hospital, Sahiwal	8	2	25%
Total	25	8	32%

Plaque Assay of Phage Lysate:

The eight bacteriophage positive samples identified by spot assay were further evaluated using double agar overlay plaque assay. Of these 5 produced distinct plaques, confirming the presence of lytic bacteriophages. Based on the plaque morphology and phage titer, two bacteriophages were selected for subsequent characterization as shown in Table 3. Phages produces clear plaques with the well-defined margins at 10^{-10} dilution using the double agar overlay method.

Table 3: Characteristics of Lytic bacteriophage isolates *P. aeruginosa* through Plaque Assay

Phage Isolate	Source Hospital	Diameter (mm)	Phage titer PFU/ml
P. a Ø	Main DHQ Hospital, Okara	1.5 mm	$3x10^{12}$
P. a Ø1	DHQ Hospital South City, Okara	1.2 mm	$2x10^{10}$

Host Range Determination of *P. aeruginosa* Phage:

The host ranges of purified bacteriophages *P. a* Ø and *P. a* Ø1were evaluated using spot assay. Both phages produced distinct lytic zone against MDR *P. aeruginosa* confirming the lytic activity towards the target host. In contrast, no lytic activity was observed against *E. coli*, *S. aureus* and *A. baumannii* indicating that both bacteriophages were exhibited the narrow host range as shown in Table 4.

Table 4: Host range determination of P. a Ø1 and P. a Ø1 against different bacterial species using Spot Assay

Bacterial Host	*P. a* Ø1	*P. a* Ø1	Interpretation
P. aeruginosa (MDR)	+	+	Lytic Activity
E. coli	-	-	No Lytic Activity
S. aureus	-	-	No Lytic Activity
A. baumannii	-	-	No Lytic Activity

(+) Presence of clear lytic zone (Positive Spot Test), (-) Absence of lytic zone (Negative Spot Test)

One Step Growth Kinetics of Selected Bacteriophages:

The One step growth curve of the selected bacteriophages was determined using double agar overlay method at the Multiplicity of Infection (MOI) of 0.1. Both *P. a* Ø and *P. a* Ø1 exhibited the latent period of approximately 25 minutes. Following the latent phase, a marked increase in phage titer was observed corresponding to the release of mature phage particle. The estimated burst size was 154 plaque forming unit (PFU) per infected bacterial cells for *P. a* Ø and 118 PFU per infected bacterial cells for *P. a* Ø1indicating efficient replication of both bacteriophages against MDR *P. aeruginosa* as shown in Fig 1.

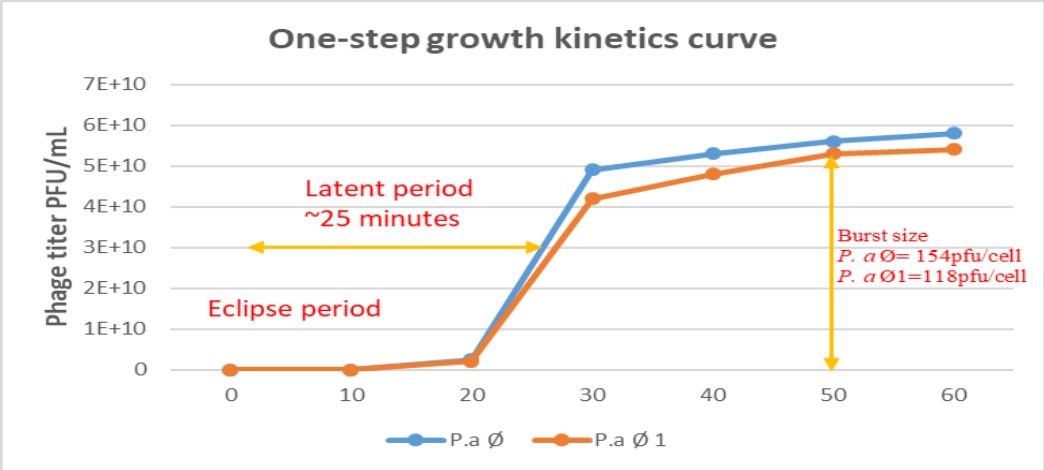

Figure 1: One Growth Curve of Bacteriophages *P. a* Ø and *P. a* Ø1against MDR *P. aeruginosa* illustrating efficient phage replication and bacterial lysis

Physiochemical Characterization of *P. aeruginosa* phages:

Thermal Stability of *P. aeruginosa* Phages:

The thermal stability of bacteriophages *P. a* Ø and *P. a* Ø1 was evaluated over the range of temperatures. *P. a* Ø remained stable up to 70°C with reduction in viability was observed at 80°C. Similarly, *P. a* Ø1 remains stable up to 70°C but was completely inactivated at 80°C as shown in Figure 2.

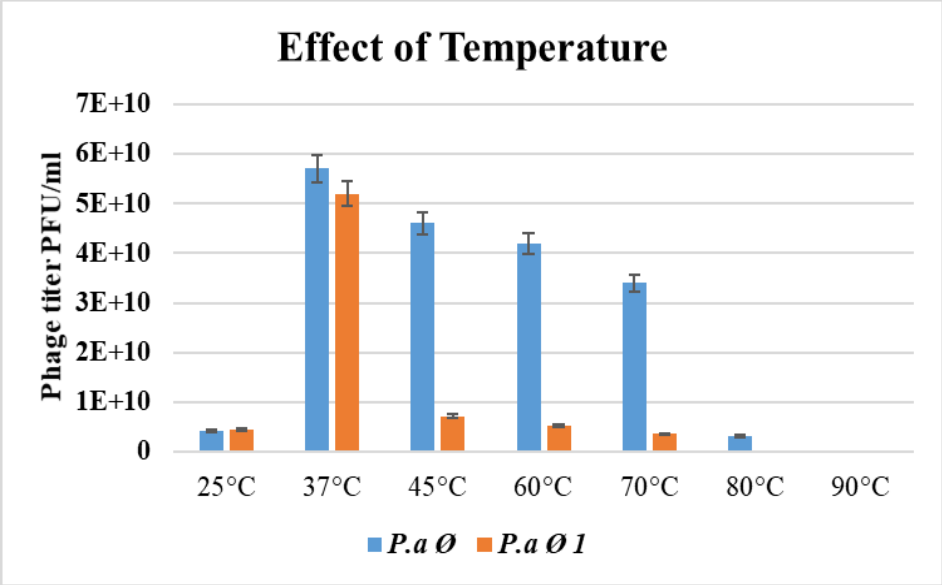

Figure 2: Thermal Stability of Bacteriophages *P. a* Ø and *P. a* Ø1 following 1hr incubation at pH 7 across temperature ranges from 25°C to 80°C.

pH stability *P. aeruginosa* phages:

Physiochemical characterization revealed that bacteriophage *P. a* Ø remain stable over a pH ranges from 3-11, whereas *P. a* Ø1 maintained stability between pH 3 and 10 as shown in figure 3.

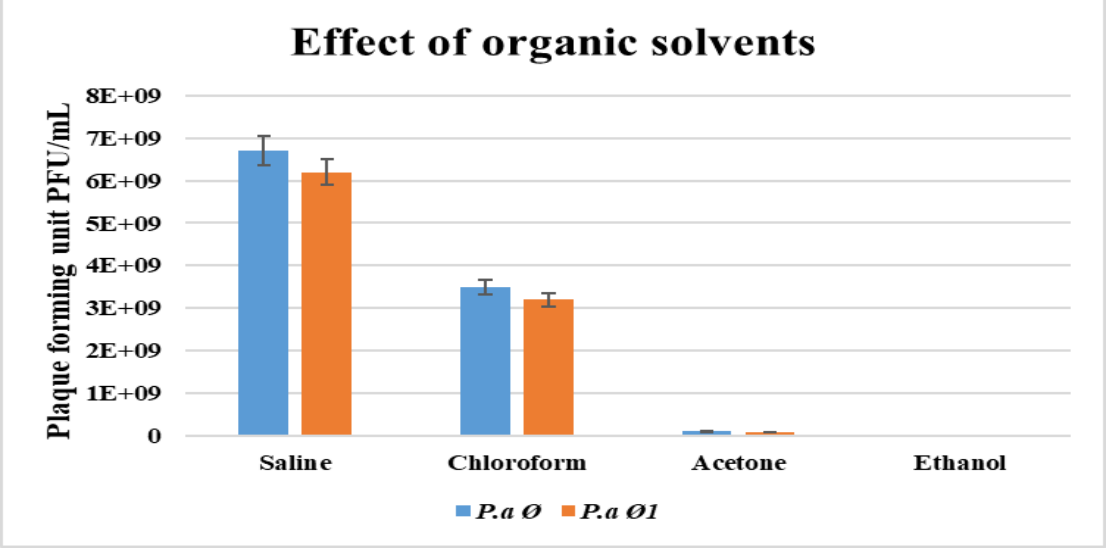

Figure 3: pH stability of Bacteriophages *P. a* Ø and *P. a* Ø1 following 1hr incubation at 37°C over a pH range of 2-12.

Stability of *P. aeruginosa* phages against organic solvents

The stability of both bacteriophages in organic solvent were determined by double agar overlay method. Both phages stablility was decresed against chloroform and normal saline, whereas an extreme decraseds in titer was observed following exposure to acetone. No phage viability was detected after treatment with ethanol as shown in figure 4.

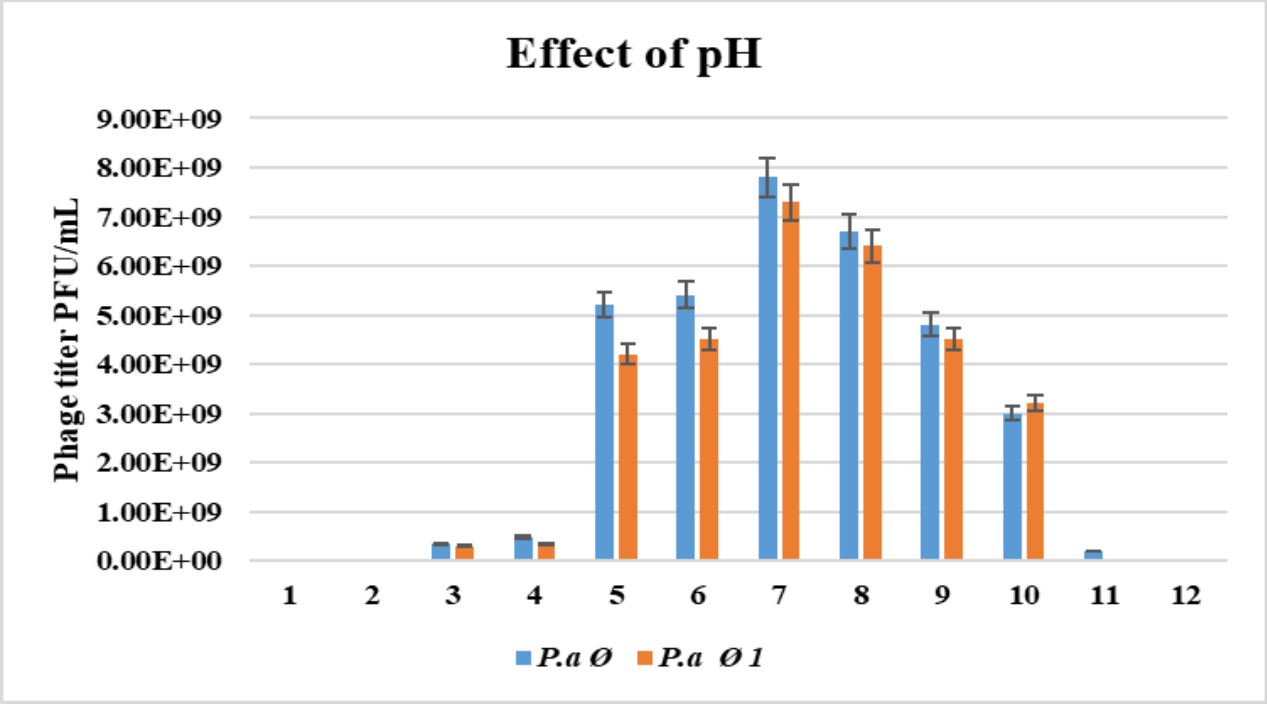

Figure 4: Effect of Organic Solvents on the viability of Bacteriophages

Cold storage temperature stability of *P. aeruginosa* phages

The stability of *P. a* Ø and *P. a* Ø1 was evaluated during the storage at 4˚C and -20˚C. Both phages remain viable during these storage temperature, however a gradual decline in phage titer was observed over a three month of storage period as shown in figure 5.

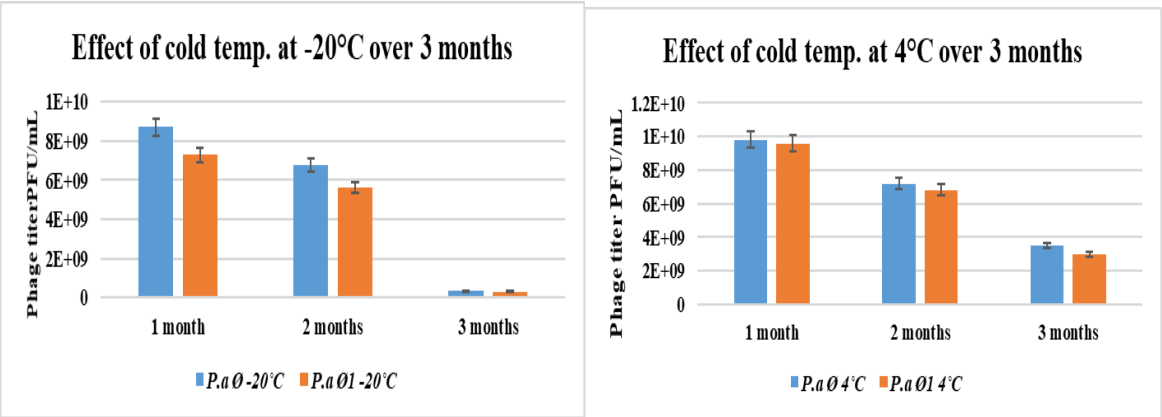

Figure 5: Stability of Bacteriophages *P. a* Øand *P. a* Ø1 at cold temperatures

Discussion:

Hospital sewage is considered an important reservoir of bacteriophages due to its high diversity of bacterial hosts making it a valuable place for the isolation of phages targeting MDR pathogens. Accordingly, hospital sewage samples were selected in present study for the isolation of lytic bacteriophages against MDR *P. aeruginosa*. This approach is consistent with the previous investigations that successfully isolated the therapeutic bacteriophages from the hospital sewage and wastewater (Jia et al., 2020; Li et al., 2025; Sharma et al., 2021). Similarly, Aghaee et al., 2021 and Menon et al., 2021demonsterated that environmental samples, including hospital sewage, river water and soil serve as a rich reservoir for *P. aeruginosa* specific bacteriophages (Aghaee et al., 2021; Menon et al., 2021). The isolated bacteriophages designated as *P. a* Ø and *P. a* Ø1produced clear circular plaque with well-defined margins using double agar overlay methods indicating their lytic nature. Comparable plaque morphology was reported by Menon et al., 2021 who observed round plaques with clear centers and slightly turbid edges (Menon et al., 2021). Likewise, Amgarten et al 2017 describe the bacteriophages ZC01 as producing clear plaques measuring approximately 2.0–2.5 mm in diameter with distinct boundaries, supporting the plaque characteristics observed in the present study (Amgarten et al., 2017). Host range analysis revealed that both *P. a* Ø and *P. a* Ø1 were specific to MDR *Pseudomonas aeruginosa*, producing lytic activity only against the target bacterium, while no lysis was observed against *E. coli*, *S. aureus*, or *A. baumannii*. Similar findings were reported by Ni et al. (2021), who found that their isolated phage lysed 29 of 35 *P. aeruginosa* strains but showed no lytic activity against other bacterial species. This host specific nature is an important feature of therapeutic bacteriophages, as it allows selective elimination of the target pathogen while preserving the normal beneficial microbiota (Ni et al., 2021). One-step growth analysis demonstrated that both bacteriophages exhibited efficient replication at a multiplicity of infection (MOI) of 0.1, with a latent period of approximately 25 minutes. The burst sizes of *P. a* Ø and *P. a* Ø1 were estimated to be 154 PFU and 118 PFU per infected bacterial cell, respectively. Similar growth characteristics have been reported previously, although variations exist among bacteriophages. Ni et al. 2021 reported a latent period of 20 minutes and a burst size of 51 PFU per infected cell, whereas Li et al. (2025) observed a shorter latent period of 15 minutes with a substantially larger burst size of 3,370 virus particles per infected cell (Ni et al., 2021). Similarly, Cui et al. (2023) reported that bacteriophage ASP23 exhibited a latent period of 10 minutes and a burst size of 140 PFU per infected cell. These differences are likely attributable to variations in phage genotype, bacterial host, and experimental conditions (Cui et al., 2023). Physicochemical characterization further demonstrated that both bacteriophages remained stable over a broad pH range, maintaining viability between pH 3 and 11. Thermal stability analysis showed that both phages remained stable up to 70°C, with a marked reduction or complete loss of viability at 80°C. These findings indicate relatively high environmental stability and compare favorably with previous reports. Akremi et al. 2022 reported bacteriophages that remained stable only between 4°C and 50°C, whereas Cong et al. 2024 found that bacteriophage PpY1 lost viability at 70°C, with no plaque formation observed beyond this temperature. The broader thermal tolerance observed in the present study may enhance the potential applicability of these bacteriophages under diverse environmental and storage conditions (Akremi et al., 2022; Cong et al., 2024). Overall, the successful isolation and biological characterization of bacteriophages *P. a* Ø and *P. a* Ø1 highlight the therapeutic potential of locally isolated lytic phages against MDR P. aeruginosa. Considering the remarkable diversity of bacteriophages across different ecological niches, continued isolation and characterization of region-specific phages are essential for expanding phage libraries and facilitating the development of safe and effective phage-based therapies against multidrug-resistant bacterial infections.

The findings of the present study also have important implications for the emerging field of nanotechnology-based antimicrobial therapeutics. Recent advancement in the nanotechnology demonstrates that have good antimicrobial activity such as cobalt iodide nanoparticle (Umar et al., 2025), copper carbonate nanoparticle (Aslam et al., 2025) and silver carbonate nanoparticles (Khan et al., 2024). These nanoparticles can also be used in the drug delivery and nano-senser in the future.

Conclusion:

The present study successfully isolated and characterized lytic bacteriophages from the hospital sewage that showed potent lytic activity against clinically isolated MDR *P. aeruginosa*. The selected bacteriophages demonstrated the effective lytic activity, broad physiochemical stability, and high host specificity underscoring their potential as environmentally sustainable alternatives to conventional antibiotics. These findings indicate that locally isolated bacteriophages represent promising candidates for the treatment of MDR *P. aeruginosa* infections. Further research and clinical studies are required to overcome existing challenges and facilitate the wider application of phage therapy in post antibiotic era.

REFERENCE:

Aghaee, B. L., Khan Mirzaei, M., Alikhani, M. Y., Mojtahedi, A., & Maurice, C. F. (2021). Improving the inhibitory effect of phages against Pseudomonas aeruginosa isolated from a burn patient using a combination of phages and antibiotics. *Viruses*, *13*(2), 334.

Akremi, I., Merabishvili, M., Jlidi, M., Haj Brahim, A., Ben Ali, M., Karoui, A., Lavigne, R., Wagemans, J., Pirnay, J.-P., & Ben Ali, M. (2022). Isolation and characterization of lytic Pseudomonas aeruginosa bacteriophages isolated from sewage samples from Tunisia. *Viruses*, *14*(11), 2339.

Amankwah, S., Adisu, M., Gorems, K., Abdella, K., & Kassa, T. (2022). Assessment of phage-mediated inhibition and removal of multidrug-resistant Pseudomonas aeruginosa biofilm on medical implants. *Infection and Drug Resistance*, 2797-2811.

Amgarten, D., Martins, L. F., Lombardi, K. C., Antunes, L. P., de Souza, A. P. S., Nicastro, G. G., Kitajima, E. W., Quaggio, R. B., Upton, C., & Setubal, J. C. (2017). Three novel Pseudomonas phages isolated from composting provide insights into the evolution and diversity of tailed phages. *BMC genomics*, *18*(1), 346.

Aslam, M. W., Sabri, S., Umar, A., Khan, M. S., Abbas, M. Y., Khan, M. U., & Wajid, M. (2025). Exploring the antibiotic potential of copper carbonate nanoparticles, wound healing, and glucose-lowering effects in diabetic albino mice. *Biochemical and Biophysical Research Communications*, *754*, 151527.

Barrow, P. A. (2001). The use of bacteriophages for treatment and prevention of bacterial disease in animals and animal models of human infection. *Journal of Chemical Technology & Biotechnology*, *76*(7), 677-682.

Cao, Z., Zhang, J., Niu, Y. D., Cui, N., Ma, Y., Cao, F., Jin, L., Li, Z., & Xu, Y. (2015). Isolation and characterization of a "phiKMV-like" bacteriophage and its therapeutic effect on mink hemorrhagic pneumonia. *PLoS One*, *10*(1), e0116571.

Chan, B., Stanley, G., Kortright, K., Modak, M., Ott, I., Sun, Y., Würstle, S., Grun, C., Kazmierczak, B., & Rajagopalan, G. (2023). Personalized inhaled bacteriophage therapy decreases multidrug-resistant Pseudomonas aeruginosa. *MedRxiv*, 2023.2001. 2023.22283996.

Chegini, Z., Khoshbayan, A., Taati Moghadam, M., Farahani, I., Jazireian, P., & Shariati, A. (2020). Bacteriophage therapy against Pseudomonas aeruginosa biofilms: a review. *Annals of clinical microbiology and antimicrobials*, *19*(1), 45.

Cong, X., Zhao, S., Zhang, Q., Liu, S., Zhang, Y., & Yan, F. (2024). Isolation, characterization, and genome engineering of a lytic pseudomonas aeruginosa phage. *Microorganisms*, *12*(11), 2346.

Cui, J., Shi, X., Wang, X., Sun, H., Yan, Y., Zhao, F., Zhang, C., Liu, W., Zou, L., & Han, L. (2023). Characterization of a lytic Pseudomonas aeruginosa phage vB_PaeP_ASP23 and functional analysis of its lysin LysASP and holin HolASP. *Frontiers in microbiology*, *14*, 1093668.

Dennehy, J. J., & Abedon, S. T. (2021). Phage infection and lysis. In *Bacteriophages: biology, technology, therapy* (pp. 341-383). Springer.

Gale, M. J., Maritato, M. S., Chen, Y.-L., Abdulateef, S. S., & Ruiz, J. E. (2015). Pseudomonas aeruginosa causing inflammatory mass of the nasopharynx in an immunocompromised HIV infected patient: A mimic of malignancy. *IDCases*, *2*(2), 40-43.

Gordillo Altamirano, F. L., & Barr, J. J. (2019). Phage therapy in the postantibiotic era. *Clinical microbiology reviews*, *32*(2), 10.1128/cmr. 00066-00018.

Hemraj, V., Diksha, S., & Avneet, G. (2013). A review on commonly used biochemical test for bacteria. *Innovare Journal of Life Science*, *1*(1), 1-7.

Hudzicki, J. (2009). Kirby-Bauer disk diffusion susceptibility test protocol. *American society for microbiology*, *15*(1), 1-23.

Jamal, M., Andleeb, S., Jalil, F., Imran, M., Nawaz, M. A., Hussain, T., Ali, M., & Das, C. R. (2017). Isolation and characterization of a bacteriophage and its utilization against multi-drug resistant Pseudomonas aeruginosa-2995. *Life sciences*, *190*, 21-28.

Jia, K., Yang, N., Zhang, X., Cai, R., Zhang, Y., Tian, J., Raza, S. H. A., Kang, Y., Qian, A., & Li, Y. (2020). Genomic, morphological and functional characterization of virulent bacteriophage IME-JL8 targeting Citrobacter freundii. *Frontiers in microbiology*, *11*, 585261.

Khan, M. F., Khattak, A. A., Saleem, A., Rizwan, M., Asif, M., & Alvi, I. A. (2023). Efficient Reduction of Pseudomonas aeruginosa Biofilms Using the Myoviridae Lytic Bacteriophage vBPaeM MLG. *Lahore Garrison University Journal of Life Sciences*, *7*(03), 262-279.

Khan, T., Umar, A., Subhan, Z., Khan, M. S., Ali, H. Z., Ullah, H., Sabri, S., Wajid, M., Iqbal, R., & Bhat, M. A. (2024). Therapeutic potential of novel silver carbonate nanostructures in wound healing and antibacterial activity against pseudomonas chengduensis and Staphylococcus aureus. *Pharmaceuticals*, *17*(11), 1471.

Krylov, V. N. (2014). Bacteriophages of Pseudomonas aeruginosa: long-term prospects for use in phage therapy. *Advances in virus research*, *88*, 227-278.

Kumari, S., Harjai, K., & Chhibber, S. (2009). Characterization of Pseudomonas aeruginosa PAO specific bacteriophages isolated from sewage samples. *Am J Biomed Sci*, *1*(2), 91-102.

Li, A., Chen, C., Li, Y., Wang, Y., Li, X., Zhu, Q., Zhang, Y., Tian, S., & Xia, Q. (2025). Characterisation of a new virulent phage isolated from Hainan Island with potential against multidrug-resistant Pseudomonas aeruginosa infections. *Research in Microbiology*, *176*(1-2), 104250.

Majdani, R., & Hatefirad, R. (2022). Isolation and characterization of two lytic bacteriophages against Pseudomonas aeruginosa and evaluation of their antibacterial effects on clinical isolates of Staphylococcus aureus and Escherichia coli in the northwest of Iran.

Marashi, S. M. A., Nikkhahi, F., Hamedi, D., & Shahbazi, G. (2022). Isolation, characterization and in vitro evaluation of specific bacteriophages targeting extensive drug resistance strains of Pseudomonas aeruginosa isolated from septic burn wounds. *Infection & Chemotherapy*, *54*(1), 153.

Menon, N. D., Kumar, M. S., Satheesh Babu, T., Bose, S., Vijayakumar, G., Baswe, M., Chatterjee, M., D'Silva, J. R., Shetty, K., & Haripriyan, J. (2021). A Novel N4-Like Bacteriophage isolated from a wastewater source in South India with activity against several multidrug-resistant clinical Pseudomonas aeruginosa isolates. *Msphere*, *6*(1), 10.1128/msphere. 01215-01220.

Ni, P., Wang, L., Deng, B., Jiu, S., Ma, C., Zhang, C., Almeida, A., Wang, D., Xu, W., & Wang, S. (2021). Characterization of a lytic bacteriophage against Pseudomonas syringae pv. actinidiae and its endolysin. *Viruses*, *13*(4), 631.

Nyachieo, A., Alafi, S., Mutai, I. J., Ngolobe, B., Nabunje, R., & Nakavuma, J. L. (2021). Isolation and Characterization of Novel Lytic Phages to Combat Multidrug-Resistant E. coli and Salmonella spp. *Journal of Microbiology and Infectious Diseases*, *11*(04), 183-190.

Oliveira, V. C., Bim, F. L., Monteiro, R. M., Macedo, A. P., Santos, E. S., Silva-Lovato, C. H., Paranhos, H. F., Melo, L. D., Santos, S. B., & Watanabe, E. (2020). Identification and characterization of new bacteriophages to control multidrug-resistant Pseudomonas aeruginosa biofilm on endotracheal tubes. *Frontiers in microbiology*, *11*, 580779.

Sati, H., Carrara, E., Savoldi, A., Hansen, P., Garlasco, J., Campagnaro, E., Boccia, S., Castillo-Polo, J. A., Magrini, E., & Garcia-Vello, P. (2025). The WHO Bacterial Priority Pathogens List 2024: a prioritisation study to guide research, development, and public health strategies against antimicrobial resistance. *The Lancet infectious diseases*, *25*(9), 1033-1043.

Sharma, S., Datta, S., Chatterjee, S., Dutta, M., Samanta, J., Vairale, M. G., Gupta, R., Veer, V., & Dwivedi, S. K. (2021). Isolation and characterization of a lytic bacteriophage against Pseudomonas aeruginosa. *Scientific Reports*, *11*(1), 19393.

Shi, Z., Hong, X., Li, Z., Zhang, M., Zhou, J., Zhao, Z., Qiu, S., & Liu, G. (2024). Characterization of the novel broad-spectrum lytic phage Phage_Pae01 and its antibiofilm efficacy against Pseudomonas aeruginosa. *Frontiers in microbiology*, *15*, 1386830.

Thi, M. T. T., Wibowo, D., & Rehm, B. H. (2020). Pseudomonas aeruginosa biofilms. *International journal of molecular sciences*, *21*(22), 8671.

Tripathi, N., & Sapra, A. (2024). Gram Staining.[Updated 2023 Aug 14]. *StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing*.

Umar, A., Khan, M. S., Hassan, W., Ullah, H., Sabri, S., Ali, H. Z., Khan, M. U., Nabi, M., Wajid, M., & Alzahrani, A. Y. A. (2025). Pharmaceutical potential of cobalt iodide nanoparticles in enhancing wound healing and modulating endocrine, immune, metabolic and antibacterial functions. *Biochemical and Biophysical Research Communications*, 152320.

Yang, X., Hussain, W., Chen, Y., Xu, P., Yang, X., Wang, H., Zhang, X., Fu, Q., & Wang, S. (2025). A new type of Pseudomonas aeruginosa phage with potential as a natural food additive for eradicating biofilms and combating multidrug-resistant strains. *Food Control*, *168*, 110888.

Yilmaz, N., Yildirim, Z., Sahin, T. S., & Karaoğlan, M. (2025). Isolation and characterization of lytic bacteriophages specific to foodborne pathogenic Pseudomonas aeruginosa and use as a biopreservative in chicken meat. *Food and Bioprocess Technology*, *18*(3), 2545-2560.

Yin, Y., Ni, P. e., Deng, B., Wang, S., Xu, W., & Wang, D. (2019). Isolation and characterisation of phages against Pseudomonas syringae pv. actinidiae. *Acta Agriculturae Scandinavica, Section B–Soil & Plant Science*, *69*(3), 199-208.

Żaczek, M., Weber-Dąbrowska, B., Międzybrodzki, R., Łusiak-Szelachowska, M., & Górski, A. (2020). Phage therapy in Poland–a centennial journey to the first ethically approved treatment facility in Europe. *Frontiers in microbiology*, *11*, 1056.