Journal of Carcinogenesis
Covering Genesis to Therapeutics

Journal of Carcinogenesis, ISSN: 1477-3163
2025; Vol 24: Issue 9s, https://carcinogenesis.com/

Bacteriophage Therapy Against Biofilm-Forming MDR Pseudomonas aeruginosa: Insights into Efficacy and Mechanisms

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ABSTRACT

Bacteria possess an outer layer known as a biofilm, which contributes to their resistance. Genetic mutations in pathogenic microbiota posed significant challenges to human health. The treatment of harmful bacteria has become increasingly difficult, leading pharmaceutical companies to withdraw from developing drugs targeting bacterial resistance. Consequently, there are limited alternative approaches available for treating bacterial infections, with phage therapy emerging as one of the more accessible options in certain countries, such as Russia, Georgia, and Poland, as well as in some states within the United States. Phages act as a bactericidal agent, targeting certain bacteria. There are various types of phages, each with distinct properties that influence the microbial environment. Beneficial phages have important roles in Phage therapy and are the focus of our study, which specifically examines the interactions between biofilm-forming bacteria and Pseudomonas bacteriophages. The study primarily focuses on the predominant multidrug-resistant strain, Pseudomonas aeruginosa, and its interactions with both strong and weak bacteriophage activities, which play a significant role in healthcare systems. This research may benefit populations by offering an alternative treatment strategy for diseases caused by biofilm – producing Pseudomonas aeruginosa.

KEYWORDS: *Biofilm, Bacteriophages, Phage Therapy, Multi Drug Resistance (MDR), Bacteriophage – biofilm interaction*

How to Cite: Kandhan Pooncholai, Suresh Dhanaraj, Rajyoganandh S.Vijayaraman, Ashok Kumar K, Somanathan Thirunavukkarasu., (2025) Bacteriophage Therapy Against Biofilm-Forming MDR Pseudomonas aeruginosa: Insights into Efficacy and Mechanisms, *Journal of Carcinogenesis*, *Vol.24, No.9s*, 19-27.

1. INTRODUCTION

Pseudomonas aeruginosa is a Gram -negative bacterium characterized by the presence of a key signaling molecule, cyclic di-GMP which is typically found at low levels within the organism.[1] This bacterium is motile; however, an increase in cyclic di-GMP levels occurs when the bacteria are stimulated by various surfaces, such as rock ,plastic or host tissue, this stimulation leads to a rapid transformation of cyclic di-GMP which subsequently triggers the production of pili the outer layer of *Pseudomonas aeruginosa* maintains an active connection under favorable conditions leading to the formation of a pili matrix that remains intact, concurrently, the connections associated with flagella are disrupted, resulting in the cessation of motility in *Pseudomonas aeruginosa* [2]. During this period of halted movement the biofilm levels decrease in response to specific treatment, within the biofilm matrix of *Pseudomonas aeruginosa*, nucleic acid, amino acid, carbohydrates and various ions play crucial roles.

The biofilm can disrupt the cell cycle by lowering cyclic di-GMP levels, which is projected to have physiological

implications when comparing planktonic and biofilm cells, specific characterization reveals that disruption affects the integrity of cells, including macrophages and *Caenorhabditis elegans,* in relation of increased virulence.[3,4,5] Additionally, planktonic cells exhibit heightened sensitivity to irons stress , Bacteriophages have been identified as potential agents for targeting and eliminating biofilm associated bacteria [6].

Biofilms formed by *Pseudomonas aeruginosa* contribute to chronic opportunistic infections, posing challenges to healthcare systems, particularly among immunocompromised individuals and the elderly [7,8]. Traditional antibiotics often fail against these infections as biofilms shield bacteria from external factors, including the host's immune system and antibiotics. *Pseudomonas aeruginosa* causes hospital – acquired infections and is a model organism for studying antibiotic resistant bacteria [9]. Research must deepen the understanding of the molecular mechanisms facilitating the transition from planktonic growth to a biofilm phenotype, and the role of quorum sensing in treatment resistance. This knowledge will improve the clinical management of chronic infections and aid in developing novel therapeutic agents [10].

Researchers have been investigating the genetic factors contributing to the antibiotic resistance of *Pseudomonas aeruginosa* [11, 12, 13], particularly against drugs such as tobramycin. One significant genetic locus identified is ndvB, which encodes periplasmic glucans that may interact with antibiotics, leading to their sequestration within the periplasmic space [14, 15, 16]. These findings suggest a genetic basis for antibiotic resistance, beyond the biofilm's role as a diffusion barrier. Bacteriophages with exceptional capabilities can effectively eliminate drug resistant biofilm bacteria [20, 21]. Bacteriophages are viruses that replicate exclusively within bacterial cells [14], commonly found in sewage, soil, and animal sources [22, 23, 24]. Their isolation is increasingly used in pharmaceutical applications due to their potential in addressing antibiotic resistance [15]. Pharmaceutical companies are revisiting phage research to develop new treatment strategies for resistant strains. Phages offer an alternative to antibiotics in managing hard-to-treat infections [25, 26], although phenotypic diversity in both phages and bacteria can influence treatment outcomes [18, 19]. Understanding host –phage interactions is vital, as they are influenced by multiple physical and biological factors. This study aims to characterize lytic bacteriophages from various environments, using biofilm forming, and multi-drug resistant *Pseudomonas aeruginosa* isolated from clinical sources as the host bacteria [16].

2. MATERIAL AND METHODS

Place of the Study

The current research was carried out at the ICF Hospital Chennai, Tamil Nadu, India and the Department of Microbiology at Vels Institute of Science Technology and Advanced Studies in Chennai, Tamil Nadu, India.

MDR strains isolated from Hospital Environment

The ICF hospital has compiled a diverse collection of clinical samples (bio medical waste). Biofilm-producing multidrug-resistant Pseudomonas bacteria were isolated from non-lactose fermenting colonies on MacConkey plates [17]. The Kirby-Bauer disc diffusion method and the Vitek method were employed for antibiotic susceptibility testing (AST). The antibiotic resistance patterns examined included Penicillin, Aminoglycosides, Fluoroquinolones, Cephalosporins, and the Carbapenem group. The Vitek method confirmed that all isolated bacteria exhibited drug resistance.

Selected Host – Microorganism

The study utilized ATCC 27835 strains of drug-sensitive *Pseudomonas aeruginosa*, as well as clinical isolates of *Escherichia coli*, *Klebsiella*, *Proteus* species, *Staphylococcus, Enterococcus, Acinetobacter baumannii*, and non-Mycobacterium tuberculosis species as hosts for the isolation of bacteriophages

Identification of Bio film formed bacteria by Tube test method

A 100 µL aliquot of log-phase multidrug-resistant (MDR) *Pseudomonas aeruginosa* culture ($1x10^6$ CFU/mL) was inoculated into 5mL of Brain Heart Infusion (BHI) broth and incubated overnight at 37°C. [27, 28].

Congo Red Assay used for biofilm conformation

The strains were streaked on modified Congo Red Agar (CRA), and streak inoculation was tested. A 10µL sample of bacterial suspension (10^6 CFU/ mL) was inoculated and incubated at 37°C under microaerophilic conditions for 48 hrs. Three strains were tested per plate, and results were assessed by two observers [29]. CRA experiments were repeated at least twice. *Pseudomonas aeruginosa* ATCC 27835 (Strain A) served as the negative control, while strains B and C were used as positives. A tube test was also conducted to observe ring formation by biofilm producing multidrug resistant *Pseudomonas aeruginosa,* confirming biofilm screening on CRA, indicated by black colored colonies (Fig. 1).

Elisa- Optical Density method used for Biofilm production

Model for biofilm production in *MDR Pseudomonas aeruginosa* procedure overview: Sample collection and bacterial

isolation: clinical sample were collected from biomedical waste associated with *P. aeruginosa* at ICF Hospital Tamil Nadu, India. The bacteria were isolated through culture on MacConkey agar plate [30, 31]. Biofilm formation: the isolated strains were incubated in 96 well plates to promote biofilm formation crystal violet is utilized to stain the biofilm color visualized. Elisa setup; the wells of the plate are coated with an antigen that subsequently the culture samples were added to the well. After incubation a secondary conjugated introduced along with a significant presence of biofilm. The Interpretation of result, highly optical Density value (>0.5). This result signifies strong biofilm production, which is indication of increased virulence and antibiotic resistance, commonly seen in multidrug -resistance *P. aeruginosa* strain. In our study samples data: samples type optical density value (450nm) Negative control 0.05, MDR *P. aeruginosa* biofilm positive 1.2. MDR *P. aeruginosa* control biofilm positive; 1.3. An optical density value greater than 0.5 indicates significant biofilm production, which is associated with high resistant level and complications in managing chronic infection, particularly in MDR *Pseudomonas aeruginosa* strains.

Sample collection and processing for Bacteriophage

Hospital sewage water samples containing various human excretions were collected from three locations: 30 samples from Chengalpattu, 10 from Rameshwaram, and 10 from Pattinampakkam, Tamil Nadu, India, totaling 50 samples. Samples were drawn from three strata: top, mid –layer (15 cm), and bottom (45 cm), using a 30cm sterile disposable pipette, pipette bulb, and conical flask [32, 33]. Samples were processed following the method of Jothi Kumar et al., with minor modifications. The samples were homogenized for 3hrs, then centrifuged at 3000 rpm for 20 min. The supernatant was centrifuged at 5000 rpm for 20 min using a cooling centrifuge. The final supernatant was filtered through a 0.45 µm Millipore syringe filter. The filtrate was then evaluated for lytic activity, revealing both strong and weak interactions against biofilm forming MDR *Pseudomonas aeruginosa.*

***Pseudomonas* Bacteriophage isolation:**

Direct spot test for Bacteriophage

A volume of $1x10^6$ 50 microliters of bacteriophage suspension was applied to a streak plate containing Nutrient agar, utilizing 10 micro liter $2x10^4$ Six different strains of biofilm-forming multidrug-resistant *Pseudomonas aeruginosa***.**

Double layer agar method used for *Pseudomonas* Bacteriophage

Samples exhibiting distinct plaque morphology were chosen for the preparation of phage lysates. A method outlined by Adam et al. involved the preparation of a sterile vial containing 500 microliters of filtrate, which was subsequently combined with 500 microliters of a bacterial culture that had been incubated for four hours [34]. To facilitate the adsorption of phage onto the bacterial surface, a solution of magnesium chloride (2.4 g/L) was introduced. The mixture was then gently agitated for 20 minutes. Then, 2.5mL of molten soft agar (45°C) was added to the tube and poured onto a nutrient agar plate. After solidification, the plate was incubated at 37°C for 48hrs, with plaque formation observed at 6, 12, 24, and 48hrs intervals.

Characterization of *Pseudomonas Phages*

Host-range Analysis

Newly isolated phages were evaluated for their efficacy against pathogenic bacteria, including ATCC *Pseudomonas*, biofilm-forming *Pseudomonas aeruginosa*, *Escherichia coli, Klebsiella, Proteus, Acinetobacter baumannii, Staphylococcus, and Enterococci*, as well as non-tuberculous mycobacteria [35].

Plaque Morphology for pseudomonas phage

The morphology of the plaques was documented based on their size, with margins and outlines categorized as small (≤ 2mm), (2 mm), and large (≥ 2mm), clearly distinguishing between well- defined and diffused plaque types, as shown in Fig.2 [36].

Bacteriophage Specific condition Temperature and pH

The phage was tested at temperatures between 40 – 70°C for 1, 2 and 3 min, and the suitable temperature was obtained at 40°C and viability before and after heat exposure was assessed using the Double Agar Layer Method (DAL). Similarly, phage stability in broth was evaluated across pH levels from 3 to 11, and the suitable pH obtained at 7.5 [36].

Titration of phage lysate.

A 100 µL phage sample was mixed with 200 µL of host bacteria (1×10^6 cfu/ml with 2.4 g/L $MgCl_2$, followed by 2.5mL of molten soft agar at 45°C. Serial dilutions of the phage lysate were performed, and plaques were formed using the DAL method. Phage titer was calculated as PFU/mL = Number of plaques x Dilution Factor. Isolated phages showed specific activity and stability, with both strong and weak responses against biofilm forming *Pseudomonas aeruginosa* [37].

Characterization of bacteriophage by TEM

To prepare the grid, 5.0 µl of phage were added, followed by the addition of 2.0 µl of 2.0% Uranyl Acetate after a 5-minute interval. The sample was then allowed to dry for subsequent examination using 150 kv Transmission electron microscopy. [41] Fig. (3).

UV confocal microscopy

A slide for UV confocal microscopy was prepared using Acridine Orange as a strain to study the interaction between bacteria – Strong bacteriophages &Weak bacteriophage. MDR *Pseudomonas aeruginosa* (10^6 cfu/mL) was treated with 20µL of logarithmic phase culture and 20 µL of specific bacteriophage (MOI). The mixture was incubated for 20 -30 min at room temperature. Then 10µL of Acridine Orange was added and incubated for 10 – 15 min to stain the bacterial cell wall and internal structures. To preserve the integrity of the bacterial structure and the bacteriophage, 20 microliters of methanol was applied for 10 to 15 minutes. After fixation, the sample was washed with PBS to eliminate any excess dye. A small amount of the stained bacteria-bacteriophage mixture was then placed onto a clean glass slide, and a cover slip was gently positioned. The sample was examined using a a wavelength of 500-550 nm under UV confocal microscopy at IIT Madras, Chennai, Tamil Nadu, India, using a wavelength of 500-550 nm. The fluorescence emitted by the Acridine Orange dye can be visualized under UV light, allowing for the observation of bacterial cells infected with the bacteriophage and the interaction between the two. The bacteriophage may appear as small particles within the bacteria during the infection stage. This advanced microscopy technique provides valuable insights into bacterial-phage interactions through UV fluorescence imaging [42].

3. RESULTS AND DISCUSSION

Biofilm forming as the result of MDR *Pseudomonas aeruginosa*

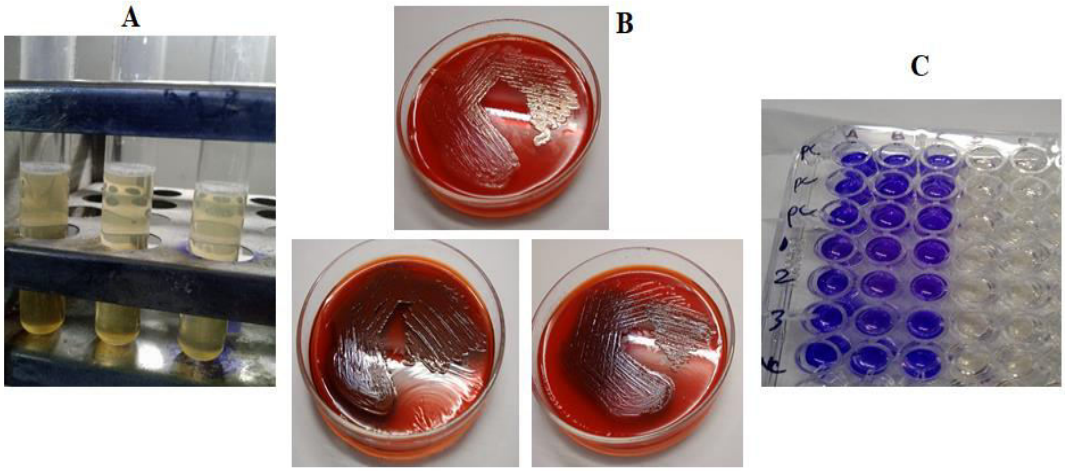


Fig. (1). A. Tube test; B. Congo Red Assay; C. ELISA O.D value

A.Tube test -Ring formation for the biofilm Postive
B.Congo Assay-Block colour colony formed for the Bioflim Positve
C.ELISA O.D value Test- The thest value is highly for the Biofilm Postive.

Recovery of Lytic phages status

Phage recovery was notably strong, though limited in sewage from hospitalized patients. Lytic activity exceeded 75% using the turbidity reduction method, compared to 55% in both the streak plate and DAL methods. A 100% correlation was observed between the streak plate and DAL methods.

Table (1). To calculate the percentage for each turbidity reduction method and streak plate method based on the total samples (50)

S.No.	Sample source	State of sampling	Total samples	Rapid screening	
				Turbidity reduction method	Streak plate method
1	Strong phages	Top	15	6(12%)	7(14%)
		middle	15	7(14%)	8(16%)
		bottom	20	7(14%)	10(20%)
		Total	**50**	20(40%)	25(50%)

2	**Weak phages**	Top	15	3(06%)	0(0)%
		Middle	15	2(04%)	3(6%)
		Bottom	20	3(6%)	2(4%)
		Total	**50**	8(16%)	5(10%)

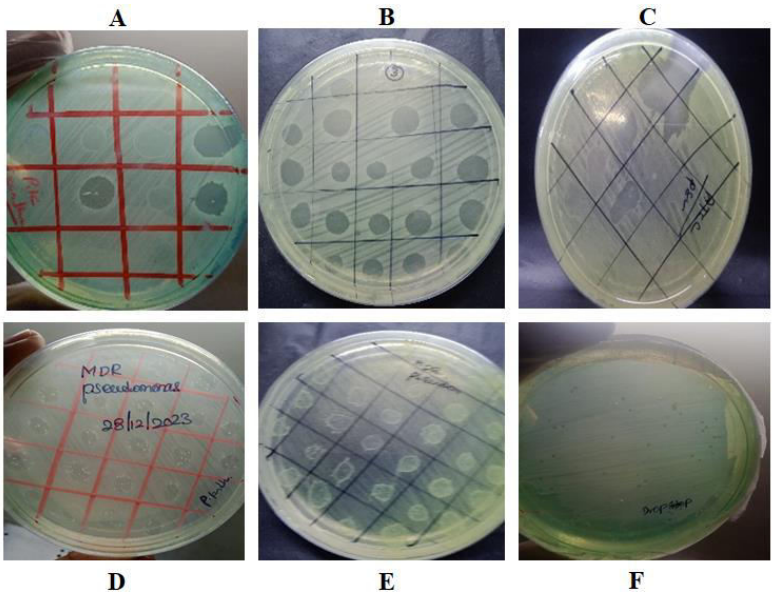


Fig. (2). Direct spot test; Strong phage A.B.C and Weak phage D.E.F

Table (2). Plaque Morphology of Phage Lysates.

Phage Types	**Cfu/mL MDR *Pseudomonas***	**Pfu/mL *Pseudomonas* phage**	**Number of plaques**	**Plaques Morphology**
Strong Phage				
BIOPA1	1x10^6	2x10^4	>50	Small sized pin head clear
BIOPA2	1x10^6	2x10^4	45	Small size diffused
BIOPA3	1x10^6	2x10^4	25	Large sized clear
Weak phage				
BIO PAA	1x10^6	2x10^4	50	Small lysed unclear
BIO PAB	1x10^6	2x10^4	30	Large sized un diffused
BIO PAK	1x10^6	2x10^4	10	Elevated types un haloed

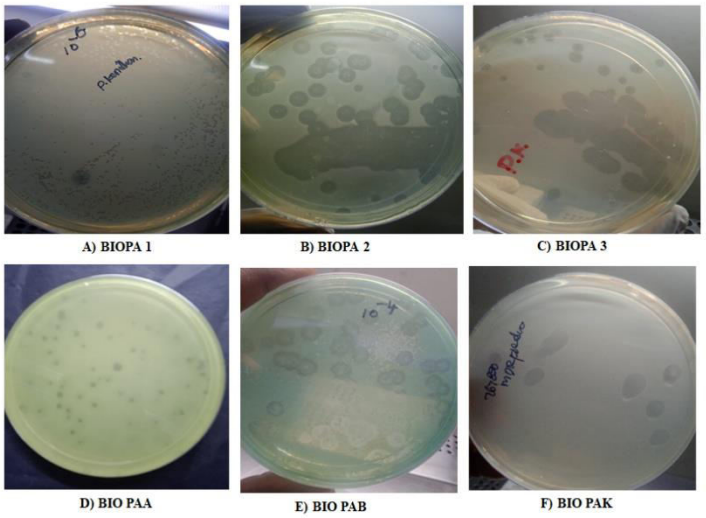
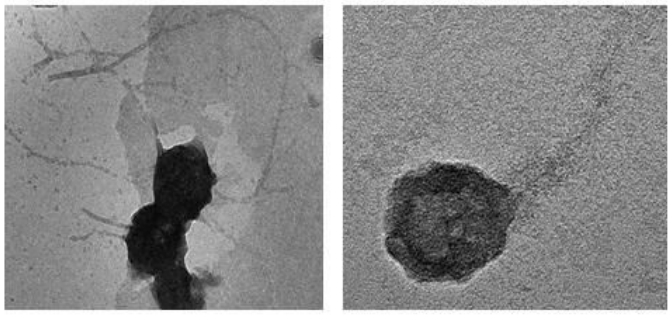


Fig. (3). Lytic activity of Strong and weak phages

Strong phages (BIOPA1, BIOPA2, and BIOPA3) show higher plaque counts with clearer and more defined plaque morphologies. Weak phages (BIO PAA, BIO PAB, BIO PAK) have lower plaque counts and more unclear plaque morphologies, suggesting less efficient bacterial lysis.

Calculation:

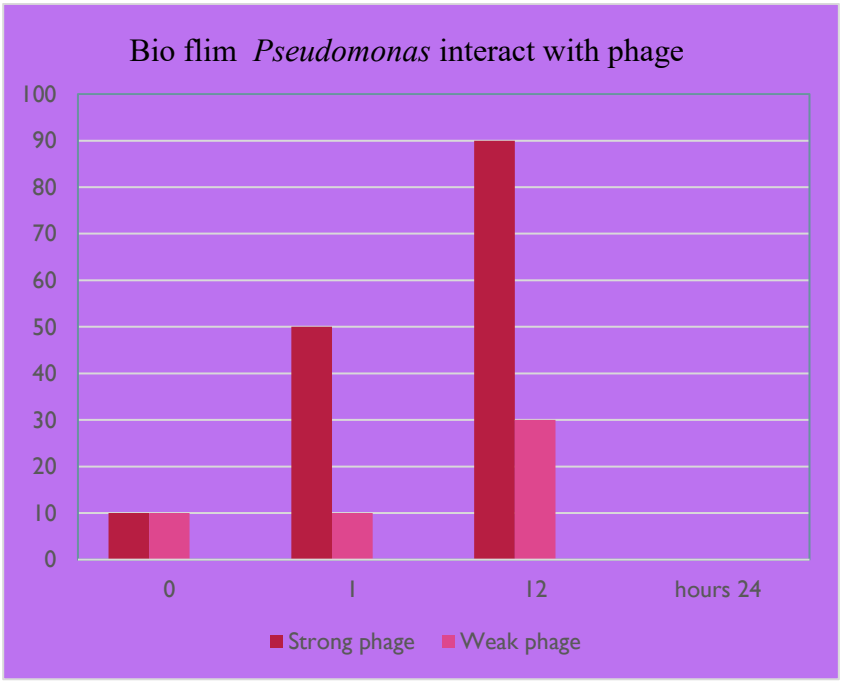
Plaque were calculated by the phage forming unit /ml



Filamentous Phage

Fig. 4. Transmission Electron Microscopy (TEM) analysis and observed filamentous phage.

Chart: (1) Bio film Pseudomonas interact with bacteriophage phage in 24hours of incubation.



A strong bacteriophage interaction (90% at 12 hours) indicates a high level of effectiveness in bacterial infection and lysis, with nearly complete bacteriophage activity. In contrast, a weak bacteriophage interaction (30% at 12 hours) shows limited effectiveness, suggesting slower or less efficient bacteriophage activity in infecting the bacteria. This difference in interaction strength highlights the importance of time and bacteriophage potency in determining the success of bacterial control.

Confocal UV Microscopic Examination
Fluresence Dye as a Agriden Orange

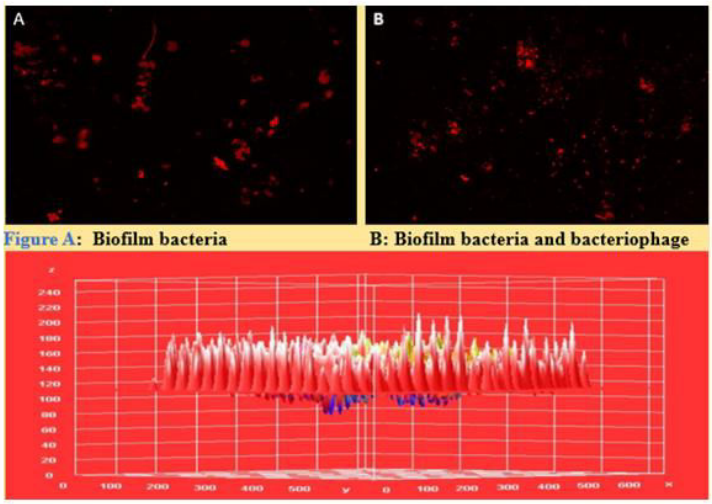


Fig.4. C: Confocal UV Microscopic examination graphical interaction of biofilm bacteria - Specific Strong and weak bacteriophage reaction

These represent the 3Dspatial distribution of bacteria and bacteriophage interaction across the sample. The fluorescence data :80% pin color likely indicates the strongest binding or interaction between bacteria and bacteriophage 30% yellow partial binging weak interaction 70% green indicates a strong bacterial response ,interaction with the bacteriophage 60% white high concentration of bacteria and bacteriophage interacting strongly 20% green and yellow weaker binding interaction in some areas likely less active sites. The majority of the interaction is concentrated in specific region 80% pink color with varying intensities indicating different interaction stages. The green and yellow pattern show bacteriophage -bacteria binding strength and spatial distribution.

Pseudomonas aeruginosa, a Gram- negative bacterium found widely in the environment, causes acute and chronic infections in clinical settings [38]. Its treatment is increasingly difficult due to rising antibiotic resistance, especially with the emergence of multidrug resistant (MDR) strains. A major factor in this resistance is its ability to form biofilms, which shield the bacteria from antibiotics and the host immune response, significantly reducing the effectiveness of standard therapies.

Given these challenges, alternative therapeutic approaches are being explored, with bacteriophage therapy emerging as a promising solution [39]. Bacteriophages, viruses that specifically infect bacteria, have gained renewed interest due to their ability to target and kill antibiotic-resistant bacteria. Countries like Russia, Poland, Georgia, and the United States have made significant progress in the clinical application of bacteriophage therapy, demonstrating its potential as an alternative treatment for resistant bacterial infections.

In our study, we focused on MDR *Pseudomonas aeruginosa* strains capable of biofilm formation, which were tested for their interaction with bacteriophages displaying lytic activity. The bacteriophages were isolated and characterized based on their lytic properties, which were further confirmed using transmission electron microscopy (TEM) [40, 41]. TEM provided visual confirmation of the bacteriophages interacting with bacterial cells, showing clear signs of bacterial cell lysis.

Furthermore, advanced confocal microscopy was utilized to examine the interaction between biofilm-producing MDR *Pseudomonas aeruginosa* and the bacteriophages. Confocal microscopic Using a fluorescence dye, Acridine Orange, we observed and compared the interaction of both strong and weak bacteriophages with the biofilm. The results indicated that the strong bacteriophage completely lysed the MDR *P. aeruginosa* biofilm, whereas the weak bacteriophage exhibited minimal lytic activity, demonstrating a weaker interaction with the biofilm [42].

However, the weak interaction observed with certain bacteriophages suggests the need for further research to understand the factors influencing bacteriophage efficacy, including phage-host interactions and the optimization of phage therapy protocols for better clinical outcomes. While bacteriophage therapy presents a promising alternative for treating *MDR Pseudomonas aeruginosa* infections, especially in biofilm-associated cases, additional studies are required to explore the full potential of phage therapy. Future research should focus on identifying and characterizing bacteriophages with higher lytic activity against *P. aeruginosa*, improving phage delivery systems, and understanding the molecular mechanisms

underlying phage resistance and biofilm disruption.

4. CONCLUSION

Bacteriophage therapy holds strong promise as an alternative treatment for multidrug resistant *Pseudomonas aeruginosa,* especially in biofilm associated infections. Strong bacteriophages demonstrated promising efficacy in lysing biofilm and bacterial cells, while weak phages showed minimal activity. Further research is needed to optimize phage therapy, including identifying more potent bacteriophages, improving delivery systems, and understanding the molecular mechanisms of phage resistance and biofilm disruption. This could pave the way for more effective treatments against resistant bacterial infections in clinical settings.

Outcomes of the Study
This study investigates the interaction between biofilm forming *Pseudomonas aeruginosa* and *Pseudomonas* specific bacteriophages. The study focuses on identifying highly potent phages, and the impact of phage efficacy on biofilm disruption, proposing potential candidates for phage therapy against multidrug resistant (MDR) strains, and exploring alternative strategies for managing biofilm associated infections.

Rationale of the Study
Antibiotic resistant, biofilm bacteria such as *Pseudomonas aeruginosa* represent a major public health threat. As the development of new antibiotics remains limited, bacteriophage therapy offers a promising alternative approach. This study investigates the potential of bacteriophages as therapeutic agents for treating where conventional antibiotics fail.

Implications for Research Outcomes
This study contributes to the development of targeted phage therapies for multidrug resistant (MDR) infections and proposes effective strategies for biofilm management. It advances the understating of bacterial resistance mechanisms against phages and may inform policy frameworks to support the broader clinical application of phage therapy. It also highlights phage therapy's potential to reduce antibiotic resistance infections and improve public health outcomes.

Acknowledgments
The authors gratefully acknowledge the support provided by ICF Hospital, Chennai, Tamil Nadu, India, and the Department of Microbiology, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Chennai, Tamil Nadu, India, where the current research was carried out. The facilities, resources and guidance provided by both institutions were invalusble to the successful completion of this research.

Financial support and sponsorship
Nil

Conflicts of interest
There are no conflicts of interest.

REFERENCES

[1] Rybtke MT, Yang L, Thauland TJ, Wang Y, Molin S, Tolker-Nielsen T. Cyclic-di-GMP signaling in *Pseudomonas aeruginosa* biofilm formation and pathogenesis. *Environ Microbiol*. 2015;17(2):110–22.
[2] Friedman L, Kolter R. Genes involved in matrix formation in *Pseudomonas aeruginosa* biofilms. *Environ Microbiol*. 2004;6(10):1145–56.
[3] Huang MC, Zhou ZY, Wu QL, Guo Y. Effects of cyclic diguanylate on biofilm formation by *Pseudomonas aeruginosa*. *FEMS Microbiol Lett*. 2015;362(9):fnv073.
[4] Pamp SJ, Sternberg C, Tolker-Nielsen T. Characterization of *Pseudomonas aeruginosa* biofilm development and disassembly using a single-cell resolution system. *Nat Commun*. 2009;4:1436–44.
[5] Stewart PS, Franklin MJ. Physiology of bacterial biofilms. *Annu Rev Microbiol*. 2008;62:173–93.
[6] Sauer K, Camper AK, Ehrlich GD, Costerton JW, Davies DG. Cyclic di-GMP and its role in biofilm formation and virulence in *Pseudomonas aeruginosa*. *J Bacteriol*. 2002;184(10):2716–26.
[7] Gordeeva AL, Slesarev AA, Zhuravlev VY. Bacteriophages as potential agents for targeting *Pseudomonas aeruginosa* biofilm infections. *Bacteriophage*. 2019;9(1):1–13.
[8] Gates SN, Dahotre SN, Kearns DB. Cyclic di-GMP signaling and biofilm formation in *Pseudomonas aeruginosa*. *J Clin Invest*. 2015;125(2):459–69.
[9] Costerton JW, Lewandowski Z, Caldwell DE, Korber DR, Lappin-Scott HM. Bacterial biofilms in nature and disease. *Annu Rev Microbiol*. 1999;53:711–46.

[10] O'Toole GA, Kaplan HB, Kolter R. *Pseudomonas aeruginosa* biofilm formation in the presence of antibiotics. *J Antimicrob Chemother*. 2003;52(5):771–7.
[11] Davies D. Understanding biofilm resistance to antibacterial agents. *Nat Rev Drug Discov*. 2003;2(2):114–22.
[12] Leid JG, Willson CJ, Shirtliff ME, Hassett DJ, Parsek MR, Jeffers AK. Bacterial biofilm formation in chronic wounds. *Am J Surg*. 2005;190 (6):620–5.
[13] Choi KH, Kumar A, Schweizer HP. The *Pseudomonas aeruginosa* ndvB gene, involved in periplasmic glucan biosynthesis, contributes to biofilm formation and resistance to antibiotic treatment. *J Bacteriol*. 2011;193 (16):4173–82.
[14] Fong JN, Yildiz FH. Biofilm formation under flow conditions. *Curr Opin Microbiol*. 2015;26:69–77.
[15] Sullivan JT, Whiteley M, Phage therapy against antibiotic-resistant bacteria. *J Clin Microbiol*. 2018;56(9):e00875–18.
[16] Gorski A, Międzybrodzki R, Borysowski J, Weber-Dąbrowska B, Lobocka M, Fortuna W, et al. Bacteriophage therapy in the treatment of bacterial infections: 60 years later. *J Clin Microbiol*. 2015;53(2):431–7.
[17] Khan SA, Asif A, Tahir H, Altaf R. Bacteriophage therapy against *Pseudomonas aeruginosa* biofilms and antibiotic-resistant strains: current status and future perspectives. *Microorganisms*. 2018;6(2):70.
[18] Hyman P, Abedon ST. Bacteriophage treatment of bacterial infections: a review. *Bacteriophage*. 2010;1(2):1–10.
[19] Costerton JW, Lewandowski Z, Caldwell DE, Korber DR, Lappin-Scott HM. Bacterial biofilms in nature and disease. *Annu Rev Microbiol*. 1999;53:711–46.
[20] Brogden KA. Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? *Nat Rev Microbiol*. 2005;3(3):238–50.
[21] O'Toole GA, Kaplan HB, Kolter R. *Pseudomonas aeruginosa* biofilm formation in the presence of antibiotics. *J Antimicrob Chemother*. 2003;52(5):771–7.
[22] Hassan MS, Jassim SA. Bacteriophage therapy for multidrug-resistant *Pseudomonas aeruginosa* infections: a review. *Antimicrob Agents Chemother*. 2013;57(9):4534–8.
[23] Chhibber S, Harjai K. Bacteriophages and their role in biofilm eradication: a review. *BioControl*. 2013;58(3):299–313.
[24] Fong JN, Yildiz FH. Biofilm formation under flow conditions. *Curr Opin Microbiol*. 2015;26:69–77.
[25] Schooley RT, Biswas B, Gill JJ, Hernandez-Morales A, Lancaster J, Lessor L, et al. Phage therapy of *Pseudomonas aeruginosa* infections in cystic fibrosis patients. *J Clin Invest*. 2017;127(8):2858–69.
[26] Hyman P, Abedon ST. Bacteriophage treatment of bacterial infections: a review. *Bacteriophage*. 2010;1(2):1–10.
[27] Wright A, Hawkins CH, Änggård EE, Harper DR. Bacteriophage therapy for *Pseudomonas aeruginosa* infections in cystic fibrosis patients. *J Clin Microbiol*. 2009;47(10):3375–81.
[28] Kutter E, De Vos D, Gvasalia G, Alavidze Z, Gogokhia L, Kuhl S, et al. Phage therapy: a renewed approach to fight bacterial infections. *Antimicrob Agents Chemother*. 2015;59(9):5761–70.
[29] Adams MH. *Bacteriophages*. New York: Interscience Publishers; 1959.
[30] Gorski A, Międzybrodzki R, Weber-Dąbrowska B, Fortuna W, Letkiewicz S, Rogóz P, et al. Bacteriophage therapy in the treatment of bacterial infections: 60 years later. *J Clin Microbiol*. 2015;53(2):431–7.
[31] Liu M, Li Y, Chen Y, Wan J. Evaluation of the biofilm-forming capacity of *Pseudomonas aeruginosa* strains using a multi-method approach. *FEMS Microbiol Lett*. 2017;364(11):fnx129.
[32] Feng X, Zhu H, Zhang X, Wang X, Lin H. Characterization of bacteriophages isolated from wastewater and their use in treating multidrug-resistant *Pseudomonas aeruginosa* biofilms. *Front Microbiol*. 2019;10:1981.
[33] Hancock RE, Speert DP. *Pseudomonas aeruginosa*: a key pathogen in cystic fibrosis and hospital-acquired infections. *Lancet*. 2000;356(9235):1427–30.
[34] Davies J, Davies D. Origins and evolution of antibiotic resistance. *Microbiol Mol Biol Rev*. 2010;74(3):417–33.
[35] Hall-Stoodley L, Stoodley P. Evolving concepts in biofilm infections. *Cell Microbiol*. 2009;11(7):1034–43.
[36] O'Toole GA, Kolter R. Flagellar and twitching motility are necessary for *Pseudomonas aeruginosa* biofilm development. *Mol Microbiol*. 1998;30(2):295–304.
[37] Abedon ST, Kuhl SJ. *Bacteriophage applications for antibiotic-resistant bacterial infections*. New York: Springer; 2012.
[38] Pirnay JP, Blasdel BG, Bretaudeau L, Buckling A, Chanishvili N, Clark JR, et al. The potential of bacteriophages in the treatment of multidrug-resistant *Pseudomonas aeruginosa* infections. *J Antimicrob Chemother*. 2011;66(7):1457–60.
[39] Alavidze Z, et al. Bacteriophage therapy in the treatment of multidrug-resistant bacterial infections. *J Clin Microbiol*. 2017;55(1):124–34. doi:10.1128/JCM.02657-16.
[40] Adams MH. *Bacteriophages*. New York: Interscience Publishers; 1959. ISBN: 9780471787353.
[41] Goepfert PA, et al. Transmission electron microscopy of bacteriophages infecting *Pseudomonas aeruginosa*. *J Bacteriol*. 1965;90(1):146–54. doi:10.1128/JB.90.1.146-154.1965.
[42] Wimpenny JW, et al. Confocal laser scanning microscopy of biofilms. *J Microbiol Methods*. 2000;39(2):87–104. doi:10.1016/S0167-7012(00)00261-2.