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Original Article

Phage Mediated Degradation of *Pseudomonas aeruginosa* Exopolysaccharides

Sujana Prabell[1], Sarika Suresh[2], Ramya Premanath[2], Juliet Roshini Mohan Raj[1*]

Departments of [1]Infectious Diseases and Microbial Genomics and [2]Bio and Nano Technology, Nitte University Centre for Science Education and Research, NITTE (Deemed to be University), Mangaluru, Karnataka, India

ABSTRACT

Objectives: *Pseudomonas aeruginosa* is a highly adaptive, nosocomial, and multi-drug-resistant opportunistic bacterium known for the production of extracellular polymeric substances (EPS). The presence of EPS hinders the penetration of antibiotics and other antimicrobial agents and thus poses a significant challenge in healthcare. The interaction between phages and the EPS of *P. aeruginosa* in terms of carbohydrate hydrolysis and structural changes were studied to assess the potential of phages to overcome the barrier of the EPS.

Material and Methods: Twenty-six *P. aeruginosa* isolates, including 18 multidrug-resistant clinical isolates and 8 drug-sensitive environmental isolates, were tested for sensitivity to a panel of 18 non-redundant phage lysates. EPS was extracted from each bacterial culture, and the total carbohydrate content was assayed before and after phage treatment using the phenol-sulfuric acid method. The effect of candidate phage activity on the structure of the EPS was further analysed using Fourier transform infrared spectroscopy (FTIR), comparing EPS that were treated with phages for different time intervals with untreated controls. The combinatorial effect of phages that degraded EPS but could not lyse the host cell, with cell wall and cell membrane targeting antibiotics, was determined.

Results: Of the 468 phage-host combinations tested, 380 (80%) exhibited host cell lysis, indicating phage sensitivity. However, only 30% of these phage-sensitive instances showed phage-mediated hydrolysis of the extracted EPS. However, it was interesting to note that phage-mediated EPS hydrolysis was observed in 40% of instances where the cultures were resistant to lysis due to phage infection. Phages that do not lyse the bacteria but degrade the EPS are effective in facilitating cell envelope-targeting antibiotics to lyse the bacterial pathogen.

Conclusion: This study highlights the effectiveness of bacteriophage in overcoming the hurdle of the exopolysaccharides in *Pseudomonas aeruginosa* in antimicrobial therapy.

Keywords: Bacteriophage, Combinatorial effect, Extracellular polymeric substance, Multidrug resistance, *Pseudomonas aeruginosa*

INTRODUCTION

Extracellular polymeric substances (EPS) are high-molecular-weight organic polymers secreted by microorganisms into their environment, playing a crucial role in biofilm formation and maintenance. *Pseudomonas aeruginosa*, a multidrug-resistant pathogen, is well-known for its ability to form biofilms, which significantly contributes to its tolerance and resistance to antibiotics and antimicrobial therapies. This organism is an opportunistic pathogen, frequently implicated in nosocomial infections, particularly in immunocompromised patients.[1] The EPS matrix is essential for the structural and functional integrity of biofilms, governing key physicochemical properties such as adhesion, mechanical stability, and protection from external threats.[2] The composition of the *P. aeruginosa* biofilm matrix can vary depending on the strain, biofilm age, and environmental conditions, including pH, oxygen levels, and nutrient availability.[3] Notably, the presence of the *pslA* gene in 90% of multidrug-resistant (MDR) *P. aeruginosa* isolates is strongly associated with enhanced biofilm formation and increased antibiotic resistance.[4]

Given the rising challenge of antibiotic resistance in *P. aeruginosa*, alternative therapeutic strategies are urgently required. Bacteriophage (phage) therapy has emerged as a promising approach. Phages, viruses that specifically infect bacteria, have been successfully used in various industries, including food production and wastewater treatment, to target and eradicate bacterial biofilms.[5-7] Phage therapy has

***Corresponding author:** Dr. Juliet Roshini Mohan Raj, Department of Infectious Diseases and Microbial Genomics, Nitte University Centre for Science Education and Research, NITTE (Deemed to be University), Mangaluru, Karnataka, India. julietm@nitte.edu.in

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demonstrated efficacy in lysing MDR *P. aeruginosa* strains and disrupting biofilms.[8] In this study, we investigated the depolymerisation of the EPS matrix in both *P. aeruginosa* PAO1 and drug-resistant strains by previously characterised lytic bacteriophages with known biofilm-degrading capabilities.

MATERIAL AND METHODS

Bacterial cultures

Pseudomonas aeruginosa PAO1 strain, along with multidrug-resistant clinical isolates from sputum (n=10) and wound (n=8) samples,[9] and drug-sensitive environmental isolates (n=8),[9] were obtained from glycerol stocks (10%) stored at -80°C at Nitte University Centre for Science Education and Research, Mangaluru, India. The cultures were revived in Asparagine broth (HiMedia, India) and subsequently maintained on Luria-Bertani (LB) agar for routine use. All bacterial cultures were incubated at 37°C.

Pseudomonas phages

A total of 18 *Pseudomonas* bacteriophages, previously designated Pa1 to Pa189, along with 4 newly characterised phages (Pa19 to Pa22), were propagated using *P. aeruginosa* PAO1 as the host strain. The bacteriophage lysates were concentrated by ultracentrifugation at 26,300 g for 4 hours (Optima XPN-100 Ultracentrifuge, Beckman Coulter), following filtration through a 0.22 µm membrane. The resulting phage pellet was suspended in SM buffer, and the phage titre was determined using the routine test dilution method. For phage susceptibility testing, *P. aeruginosa* PAO1 and clinical isolates were lawned on LB agar plates. Each phage (Pa1–Pa18) (5 µL) was spotted on the bacterial lawns, which were incubated at 37°C for 6 hours to observe for lysis.

Extraction of exopolysaccharide

EPS was extracted from *P. aeruginosa* cultures as per the method described by Chug *et al*.[10] Briefly, bacterial cultures were grown in 50 mL LB broth for 96 hours at 37°C. Cells were harvested by centrifugation at 10,621 g for 30 minutes (Eppendorf® Centrifuge 5804R). The EPS in the supernatant was precipitated using 95% ethanol in a 1:3 v/v ratio at 4°C overnight. The EPS was pelleted by centrifugation at 15,295 g for 20 minutes, resuspended in 30 mL Milli-Q water, and passed through a 0.45 µm membrane filter to remove residual cells. The extracted EPS was stored at 4°C till further analyses.

Colorimetric estimation of EPS depolymerization

The phenol-sulfuric acid method was used to quantify the carbohydrate concentration in the extracted EPS before and after phage treatment. Briefly, 500 µL of extracted EPS was mixed with an equal volume of bacteriophage lysate (~5 *10^8 PFU/mL) and incubated for 1 hour and 18-hour time points. At each time point, 40 µL of 80% phenol prepared from 99.5% phenol (LOBAChemie,05168) and 2.5 mL of 98% extra pure sulfuric acid (LOBAChemie,00289) were added, and the mixture was incubated at room temperature for 20 minutes in the dark. The absorbance was measured at 490 nm using a photoelectric colorimeter (SYSTRONICS Type: 113). A standard curve was generated using a sodium alginate concentration gradient (HiMedia MB114) to extrapolate EPS concentrations.[11] Control reactions included EPS alone, phage alone, and SM buffer.

Statistical analysis: Analysis of variance (ANOVA) was employed to determine if time affected phage-mediated EPS degradation. A significance level of $p \leq 0.05$ was considered statistically significant.

Fourier transform infrared spectroscopy of the EPS on phage treatment

The structural changes in EPS after phage treatment were analysed using FTIR spectroscopy. EPS samples treated with bacteriophages were treated with $ZnCl_2$ (HiMedia MB046) in a 1:50 ratio to remove the phages[9,12]). The samples were centrifuged at 4300 g (Eppendorf Minispin®/Minispin Plus® centrifuge), and the supernatant was collected in a fresh 1.5 mL tube. The FTIR spectra were obtained using an ALPHA II FTIR spectrometer (Bruker Optics GmbH & Co) at the Nitte Gulabi Shetty Memorial Institute of Pharmaceutical Sciences, Mangaluru, India. Controls of phage suspensions and phage suspensions treated with $ZnCl_2$ in the absence of EPS were also analysed via FTIR to monitor for any noise /peaks due to either $ZnCl_2$ or the phages.

Combinatorial effect of antibiotics and phages

The minimal inhibitory concentration (MIC) for the combinational therapy of phage with antibiotics was assessed in bacterial isolates that were resistant to lysis by the bacteriophages but whose EPS was degraded by the specific phage. The antibiotics utilised included Cefotaxime (Himedia MB134-1G, India), Colistin (Himedia PCT1142-1G, India), and Penicillin G (Himedia TC187-1MU, India). MIC was determined in a 96-well plate as per CLSI guidelines using antibiotic concentrations ranging from 64 mg/L to 0.125 mg/L with bacteriophages at a concentration of 10^9 PFU/mL. Appropriate controls, including antibiotic-only controls, media-only controls, phage only and culture controls, were included to ensure the reliability of the results. 96-well plates were incubated at 37°C for 18 hours. The absorbance at 620 nm was used to record culture turbidity. Wells with the least

concentration of antibiotic showing > 90% reduction in absorbance compared to cell control was considered the MIC_{80}.

RESULTS

Host range of the phages

The host range, reflecting the susceptibility or resistance of clinical *P. aeruginosa* isolates to the tested bacteriophages, has been summarised in Table 1. Notably, isolate ADS-34 was resistant to lysis by all phages tested, while isolates ADS-03 and ADS-13 exhibited resistance to more than 80% of the phages.

EPS degradation by phages

Phages demonstrated the ability to lyse *P. aeruginosa* biofilms, reducing biofilm formation by >50% in *P. aeruginosa* PAO1 and other isolates, as previously confirmed by the crystal violet biofilm assay.[9] In this study, phages were able to degrade between 20% to 75% of the carbohydrate content in extracted EPS, as shown in Figure 1. A statistically significant reduction in EPS carbohydrate content was observed after phage treatment compared to controls ($p_{1\ hour}$ = 2.28481E-18 and $p_{18\ hours}$ =5.32412 E-16). However, the difference in EPS degradation between 1 and 18 hours of phage treatment was not statistically significant (= 0.035603327).

The composition of EPS can vary depending on factors such as strain type and environmental conditions. While phages were able to lyse 93% of isolates from wound specimens, EPS degradation was only observed in 56% of these cases. In sputum-derived isolates, although lysis sensitivity was lower (74%), phage-mediated EPS degradation occurred in

Table 1: Host range of phages on the isolates used

Isolates/phages		Bacteriophages																	
		Pa1	Pa3	Pa5	Pa6	Pa7	Pa9	Pa10	Pa11	Pa12	Pa13	Pa14	Pa15	Pa16	Pa17	Pa18	Pa19	Pa20	Pa22
Sputum isolates	ADS 03	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
	ADS 04	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	ADS 13	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
	ADS 14	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	ADS 26	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	ADS 28	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	ADS 34	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	ADS 46	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	ADS 47	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	ADS 49	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Environmental isolates	AMO1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	FTOA1	+	+	+	-	+	-	-	+	+	+	-	+	+	+	-	+	+	+
	FLO1	+	-	+	-	-	-	-	+	-	-	+	+	+	+	+	+	+	+
	JMO2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	FO1	+	+	+	+	-	+	-	+	-	+	-	+	+	+	+	+	+	+
	FTOA	+	+	+	+	-	+	-	+	-	+	-	+	+	+	+	+	+	+
	NOA	-	+	+	-	-	-	-	+	-	+	-	+	+	+	+	+	+	+
	FO2	+	+	+	-	+	-	+	+	+	+	+	-	+	+	-	+	+	+
Wound isolates	ADWS-11	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
	ADWS-44	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	ADWS-36	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	ADWS-25	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-
	ADWS-62	+	+	+	+	-	+	-	-	-	+	+	+	+	+	-	+	+	-
	ADW-24	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	ADW-37	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	ADW-22	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

ADS: Asparagine diabetic sputum isolate; Pa: phage, +: Isolate shows lysis on exposure to the phage, -: Isolate resistant to phage infection.

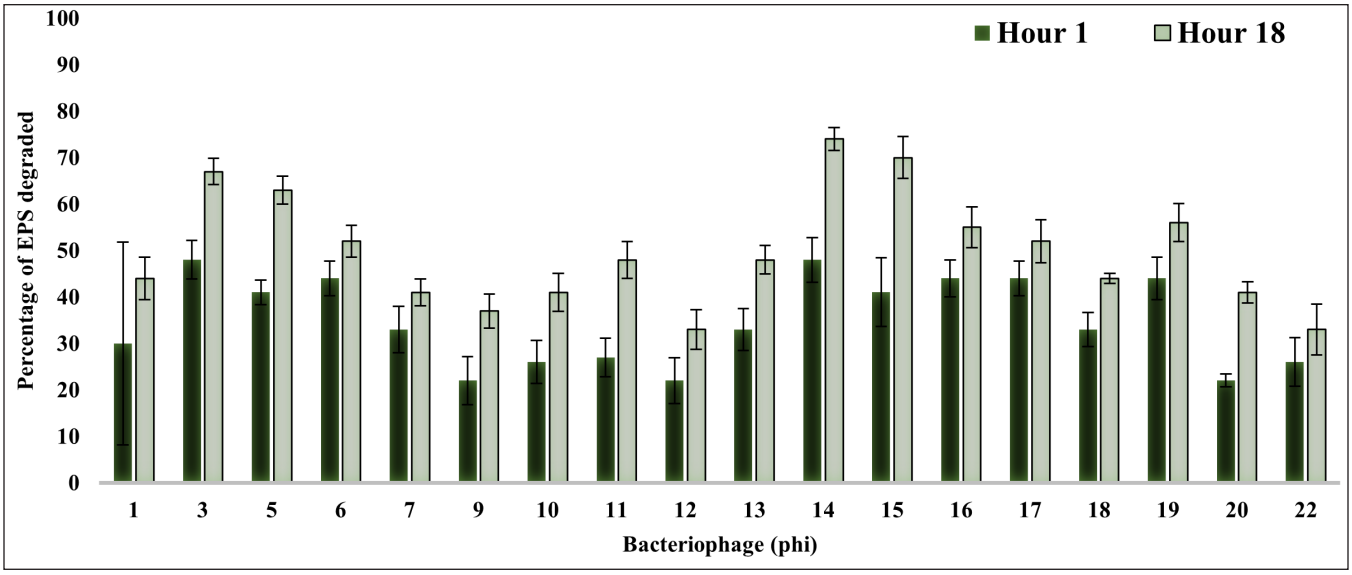

Figure 1: The average ability of the 18 bacteriophages to degrade the exopolysaccharides of *Pseudomonas aeruginosa*. EPS: Extracellular polymeric substances.

50% of phage-resistant isolates. Environmental isolates were generally less susceptible to both phage infection and EPS degradation [Figure 2].

Phage Pa1 degraded the EPS of 37.5% of environmental isolates and 62.5% of wound isolates after 1 hour but failed to degrade the EPS of sputum isolates. After 18 hours, Pa1 exhibited EPS degradation in 62.5% of environmental, 62.5% of wound, and 10% of sputum isolates. Phage Pa3 degraded the EPS of 37.5% of environmental, 50% of wound, and 50% of sputum isolates after 1 hour, with this increasing to 50%, 62.5%, and 80%, respectively, after 18 hours. However, phages Pa14 to Pa20 were less effective, degrading less than 40% of the EPS content. For clinical isolates from wounds and sputum, ~50% EPS degradation occurred among those isolates resistant to phage lysis.

Fourier transform infrared spectroscopy (FTIR/IR spectroscopy)

FTIR spectroscopy confirmed the structural digestion of specific molecules within the EPS [Figure 3 and Supplementary Tables S1 to S4]. The untreated EPS of *P. aeruginosa* PAO1 [Figure 3a] exhibited characteristic absorption peaks at 1260.84 cm^{-1} (CO-C-CO stretching), 1747.38 cm^{-1} (C=O stretching), 2359.42 cm^{-1} (CO2), and 2850.87 cm^{-1} (C-H stretching). After treatment with phage Pa1, these peaks disappeared, indicating phage-induced structural alterations in the EPS.

Phage Pa10 treatment at 1 hour resulted in the disappearance of the 1167.08 cm^{-1} (C-O stretching) peak, further indicating phage-mediated structural changes. Additionally, new peaks at 3673.88 cm^{-1}, 3648.71 cm^{-1}, 3629.21 cm^{-1}, 3587.33 cm^{-1}, and 3566.70 cm^{-1} emerged, indicating the presence of free alcohols, which suggests EPS digestion [Figure 3b].

FTIR spectra of the clinical isolate ADS-28 showed the disappearance of the 2357.50 cm^{-1} (O=C=O) peak and the appearance of a 3735.39 cm^{-1} peak, suggesting a distinct alteration in the EPS molecular structure due to phage treatment [Figure 3c]. In contrast, the EPS of isolate ADS-46, which did not exhibit any significant carbohydrate reduction after phage treatment, showed no significant changes in the FTIR spectra [Figure 3d], consistent with the phenol-sulfuric acid estimation results.

Combinatorial effect of antibiotics and phages

The MIC_{80} values for antibiotics alone and in combination with bacteriophages were determined for bacterial isolates resistant to bacteriophage-mediated lysis but whose EPS was effectively degraded by the phage. Of 12 such combinations tested, only 4 phage-antibiotic combinations showed a synergistic effect [Table 2]. MIC_{80} for isolate FLO1 decreased from 64 mg/L to 32 mg/L for Penicillin in the presence of phage Pa10, and for ADWS-62, a significant reduction was observed with the MIC_{80} decreasing from 64 mg/L to < 0.125 mg/L in the presence of phage Pa10. The isolate ADS-34, which was MDR and showed complete resistance to phage lysis, displayed a reduction of MIC for colistin from 8 mg/L to 4 mg/L with a combinatorial effect with phage Pa14. ADWS-11 showed a significant reduction in MIC_{80} from 64 mg/L with cefotaxime alone to 2 mg/L when cefotaxime was used in combination with phage Pa22.

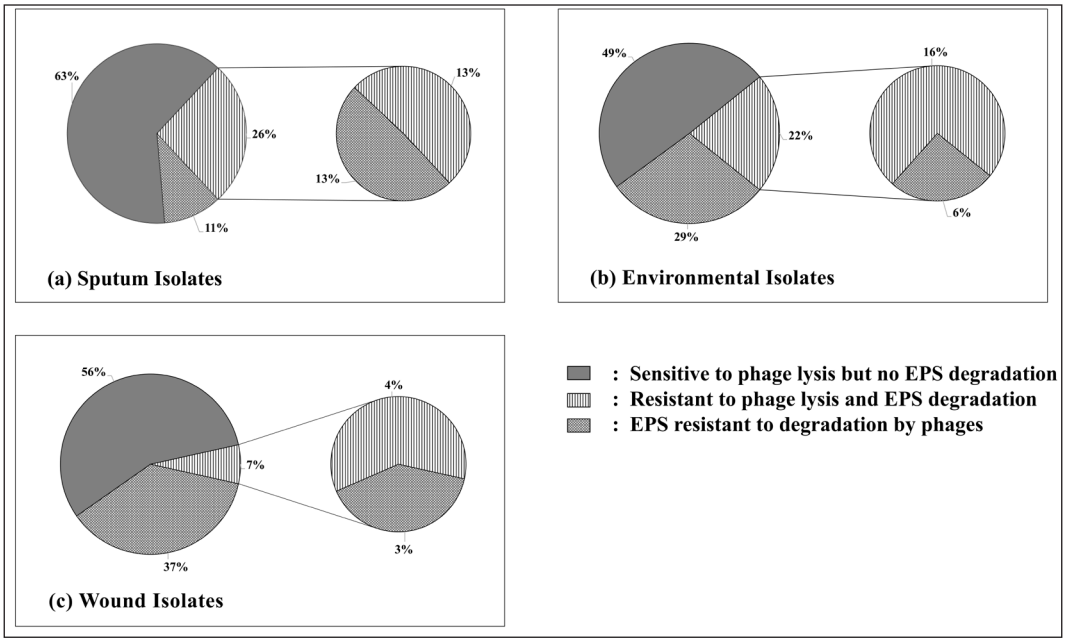

Figure 2: Sensitivity of isolates to phage-mediated EPS degradation. (a) Sputum isolates: 74% phage-host combinations (133/180) showed lysis, but of these, only 11% (19/180) showed EPS degradation. Of the 26% phage-host combinations (49/180) that did not show lysis, EPS degradation was observed in 23/180 combinations. (b) Environmental isolates: 78% phage-host combinations (113/ 144) showed lysis, but 29% (42 /144) showed EPS degradation. Of the 22% phage-host combinations (31/144) that resisted phage infections, 8 /144 showed EPS degradation. (c) Wound Isolates: 93% combinations (134/144) were positive for lysis, of which 53/144, i.e., (37%), showed EPS degradation. Four of ten combinations that did not show lysis were positive for EPS degradation. Phage -host combinations were calculated as the number of phages multiplied by the number of isolates. The bigger pie chart represents phage sensitivity, wherein the area in complete grey represents cell lysis due to phage activity, but the phages did not degrade the EPS of these isolates. The stripped area indicates isolates that were resistant to phage infection, as indicated by the absence of lysis. The dotted areas represent isolates that lyses on phage infection and whose EPS was degraded by the phages. The smaller pie chart represents only the proportion of isolates that were resistant to phage infection. In this small pie chart, the striped area represents resistance to phage infection and degradation of EPS. The dotted areas represent isolates whose EPS was degraded by the phages, even though cell lysis was not observed in the plaque assay.

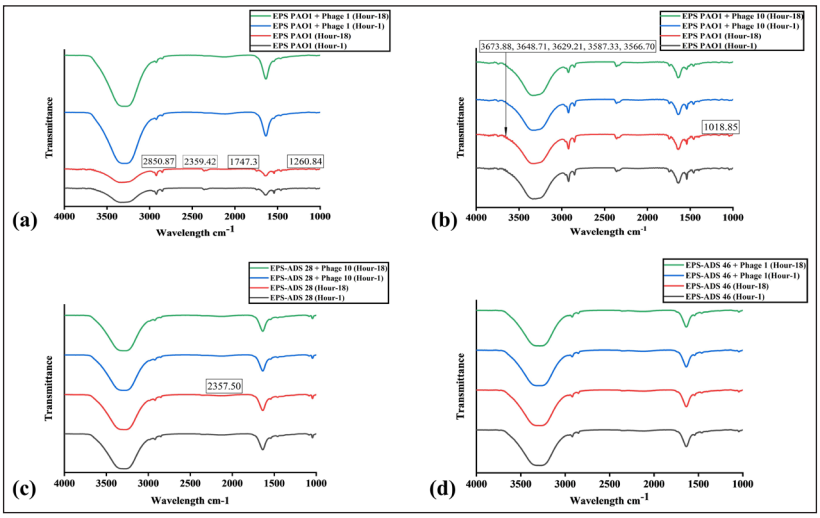

Figure 3: Fourier-transform infrared (FTIR) spectra of *P. aeruginosa* PAO1 EPS: (a) With phage Pa1 treatment at hour one and hour eighteen, specific peak disappearance at 2850.87 cm^{-1}, 2359.42 cm^{-1}, 1747.38 cm^{-1}, and 1260.84 cm^{-1} were observed. (b) With phage Pa10 treatment, peak disappearance at 1167.08 cm^{-1} and the appearance of new peaks in the range 3674 to 3566 cm^{-1} were observed. (c) FTIR spectra of *P. aeruginosa* clinical isolate ADS-28 EPS with phage Pa1 treatment at hours one and eighteen showed peak disappearance at 2357.50 cm^{-1}. (d) FTIR spectra of *P. aeruginosa* clinical isolate ADS-46 EPS with and without phage treatment showed no significant difference as phage Pa1 was incapable of degrading the exopolysaccharide of this particular strain. EPS: Extracellular polymeric substances, PAO1: Pseudomonas aeruginosa strain.

Table 2: Minimal inhibitory concentration (mic) in combination with phages

Antibiotic	Isolate	Bacteriophage degrading EPS but not capable of host lysis	MIC_{80} for antibiotics only (mg/L)	MIC_{80} breakpoint for antibiotics with bacteriophage (combinational therapy) (mg/L)
Penicillin	FLO1	Pa10	> 64	32
	ADWS-62	Pa10	> 64	< 0.125
Colistin	ADS-03	Pa14	2	8
	ADS-13	Pa14	2	4
	ADWS-11	Pa22	8	16
	ADS-34	Pa14	8	4
	FLO1	Pa10	4	4
Cefotaxime	ADS-03	Pa14	4	4
	ADS-13	Pa14	4	8
	ADS-34	Pa14	2	4
	FLO1	Pa10	1	8
	ADWS-11	Pa22	> 64	2
Penicillin	PAO1	Pa22	< 0.125	< 0.125
Colistin		Pa12	< 0.125	< 0.125
Cefotaxime		Pa15	0.25	0.25

FLO1, ADWS, ADS, PAO1: Pseudomonas aeruginosa strain.

DISCUSSION

Extracellular polymeric compounds, including polysaccharides, proteins, lipids, and nucleic acids, are produced by bacteria in biofilms and contribute to their pathogenicity and resistance.[13-15] *P. aeruginosa* is known to colonise the lungs of cystic fibrosis patients, diabetic wounds, and urinary catheters, forming biofilms that hinder antimicrobial diffusion and lead to chronic infections.[16,17]

Although phages can lyse bacterial cells, the extracellular matrix can protect the host in response to antimicrobial agents, especially antibiotics.[18,19] For instance, *P. aeruginosa* shows increased biofilm formation in response to sub-inhibitory concentrations of aminoglycoside antibiotics like tobramycin.[20,21] Additionally, the alginate matrix in the biofilm protects *P. aeruginosa* from leukocyte destruction mediated by IFN-γ.[22] Several studies confirm that microbial EPS hinders sulfamethizole and sulfonamide antibiotics diffusion, hence alleviating the inhibitory effect.[23] Further, the EPS reduces the negative effects of certain antibiotics like fluoroquinolones and β-lactams that generate reactive oxygen species.[24] Phage cocktails have been shown to eradicate infections in *in vivo* studies.[25,26] Alemayehu *et al*.,[27] using a phage cocktail, demonstrated complete elimination of *P. aeruginosa* biofilm in a murine model. While some strains can resist phage lysis due to the presence of EPS, phage-mediated degradation of EPS can weaken the biofilm matrix, making the host more susceptible to antimicrobial agents.[28,29] Thus, for successful therapeutic application, phages must navigate the EPS to find suitable adhesion sites, which is a challenge due to their lack of intrinsic motility.[30] Combinational therapy remains an ongoing area of investigation.[31] This study evaluated the ability of bacteriophages to degrade the EPS produced in *P. aeruginosa* recovered from clinical and environmental isolates. FTIR spectroscopy confirmed bond breakages, indicating the digestion of exopolysaccharide molecules by the bacteriophages.

Bacteriophages were able to degrade EPS of the bacterial strain ADS-34, which was resistant to all tested antibiotics,[9] and phage-resistant isolates ADS-03 and ADS-13 (resistant to 80% of the phages). While the sensitivity of sputum isolates to phage lysis was 93%, the EPS degradation was observed in only 56% of the isolates. A substantial reduction in EPS content following phage treatment suggests that phage therapy could effectively diminish the biofilm matrix. However, the lack of a significant difference in the degradation between 1 and 18 hours of treatment implies that the initial phase of phage interaction is critical for EPS degradation. The following criteria must be accomplished for a phage to successfully degrade the biofilm and lyse the bacteria: Host susceptibility to the phage and the ability of the phage polysaccharide depolymerase to degrade the EPS. A substantial degree of biofilm degradation can occur even if only one of these criteria is met, and phage-free depolymerase enzyme activity alone results in rapid biofilm degradation.[32]

Phage Pa1 and Pa3 displayed different efficiencies in degrading EPS from various sources over time. Phage Pa1 showed a marked increase in degradation efficacy from 1 hour to 18 hours for environmental and wound isolates but was notably less effective against EPS from sputum isolates. Phage Pa3, on the other hand, demonstrated a consistent and increasing degradation capacity across all isolate types over time. This indicates that while some phages may act quickly, others may require longer exposure to achieve significant EPS degradation, and their effectiveness can vary based on the EPS composition of bacterial isolate and the EPS degradation mechanism that the phage possesses[33] or the amount of exopolysaccharide in the biofilm[34] or that the phages response to biofilm is strain-dependent.[35] Phage-derived depolymerases show significant anti-biofilm and bactericidal effects.[29] Studying phage enzymatic properties is crucial, as novel phage proteins could provide targeted strategies against biofilm degradation.[36]

FT-IR spectroscopy has been used to study structural attributes like macromolecular conformation and molecular interaction.[37,38,39] In this study, FTIR was used to study any structural alteration of the EPS upon phage treatment. The disappearance of characteristic bonds in the EPS of PAO1 after treatment with phage Pa1 and the appearance of peaks in Pa10-treated EPS indicates effective enzymatic activity and structural degradation. Distinct spectral changes observed in clinical isolate ADS-28 suggest that phage treatment can significantly alter EPS composition, supporting the potential for phage therapy to disrupt biofilms. Conversely, the lack of significant spectral changes in ADS-46 after phage treatment, though the host was susceptible to all phages tested, indicated that some EPS compositions resist phage-mediated degradation.

Biofilms present a major obstacle in treating infections as they impede antibiotic penetration and provide a protective barrier for bacteria. The EPS, which represents the "house of the biofilm cells," is thus a primary barrier that needs to be overcome for effective antimicrobial action.[40] Antibiotic combinatorial therapies and non-antibiotic treatments are being actively researched as promising strategies to address the growing threat of antibiotic-resistant infections. Phage-antibiotic combinations in *Staphylococcus aureus* indicate an enhanced susceptibility of biofilms to antibiotics after subsequent phage treatment.[41,42] *Burkholderia* species pretreated with sublethal concentrations of antibiotics show higher susceptibility to phage.[43] A combination of phages with antibiotics targeting bacterial protein synthesis reduces the lytic activity of bacteriophages and thus does not function with synergy.[44] Hence, in this study, antibiotics targeting the cell envelop were tested for phage-antibiotic synergy. However, combinatorial treatment can show mixed outcomes, including synergy, no effect, and antagonism were observed in concordance with other reports.[45,46] The drastic decrease in the MIC_{80} of penicillin toward ADWS-62 and that of cefotaxime to ADWS-11 when used in combination with phages that could not lyse these isolates is a promising beacon in the application of phages in therapy. These findings emphasize the need for tailored phage-antibiotic combinations to overcome biofilm-associated resistance and improve therapeutic outcomes against MDR *P. aeruginosa* infections.

CONCLUSION

Certain bacteriophages demonstrated significant EPS degradation, effectively weakening biofilm structures and enhancing bacterial susceptibility to antimicrobial agents. FTIR analysis provided structural evidence of EPS digestion by specific phages, corroborating the colorimetric results and confirming phage-induced alterations in EPS composition.

The ability of phages to degrade EPS can play a more critical role in their therapeutic potential when used in combination with antibiotics compared to their lytic ability alone. A deeper understanding of the composition differences in the EPS and mechanisms underlying phage-mediated EPS degradation and bacterial lysis could facilitate the development of novel antimicrobial strategies. Further research in this area could enable the use of bacteriophages in targeted, combinatorial, and personalised therapies against biofilm-forming and antibiotic-resistant bacteria.

Ethical approval: The research/study approved by the Central Ethics Committee Review Board at NITTE (Deemed to be University), number NU/CEC/2021/159, dated 7th September 2021.
Declaration of patient consent: Patient's consent not required as patients identity is not disclosed or compromised.
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Conflicts of interest: There are no conflicts of interest.
Use of artificial intelligence (AI)-assisted technology for manuscript preparation: The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

REFERENCES

1. Jack AA, Khan S, Powell LC, Pritchard MF, Beck K, Sadh H, *et al*. Alginate oligosaccharide-induced modification of the lasI-lasR and rhlI-rhlR quorum-sensing systems in Pseudomonas aeruginosa. Antimicrob Agents Chemother 2018;62:e02318-17.
2. Zahedani SS, Tahmasebi H, Jahantigh M. Coexistence of virulence factors and efflux pump genes in clinical isolates of *Pseudomonas aeruginosa*: Analysis of biofilm-forming strains from Iran. Int J Microbiol 2021;2021:5557361.

3. Powell LC, Pritchard MF, Ferguson EL, Powell KA, Patel SU, Rye PD, *et al*. Targeted disruption of the extracellular polymeric network of *Pseudomonas aeruginosa* biofilms by alginate oligosaccharides. NPJ Biofilms Microbiomes 2018;4:13.
4. Abdulhaq N, Nawaz Z, Zahoor MA, Siddique AB. Association of biofilm formation with multi drug resistance in clinical isolates of *Pseudomonas aeruginosa*. EXCLI J 2020;19:201-8.
5. Laxmi M, Bhat SG. Biofilms in food industry: Mitigation using bacteriophage. In: Advances in Biotechnology for Food Industry; 2018. p. 393-342.
6. Zhang Y, Hu Z. Combined treatment of Pseudomonas aeruginosa biofilms with bacteriophages and chlorine. Biotechnol Bioeng 2013;110:286-95.
7. Goldman G, Starosvetsky J, Armon R. Inhibition of biofilm formation on UF membrane by use of specific bacteriophages. J Memb Sci 2009;342:145-52.
8. Chaudhary N, Narayan C, Mohan B, Taneja N. Characterization and in vitro activity of a lytic phage RDN37 isolated from community sewage water active against MDR uropathogenic *E.coli*. Indian J Med Microbiol 2021;39:343-8.
9. Suresh S, Saldanha J, Bhaskar Shetty A, Premanath R, Akhila DS, Mohan Raj JR. Comparison of antibiofilm activity of *Pseudomonas aeruginosa* phages on isolates from wounds of diabetic and non-diabetic patients. Microorganisms 2023;11:2230.
10. Chug R, Mathur S, Kothari SL, Harish N, Gour VS. Maximizing EPS production from *Pseudomonas aeruginosa* and its application in CR and Ni sequestration. Biochem Biophys Rep 2021;26:100972.
11. Orgad O, Oren Y, Walker SL, Herzberg M. The role of alginate in *Pseudomonas aeruginosa* EPS adherence, viscoelastic properties and cell attachment. Biofouling 2011;27:787-98.
12. Czajkowski R, Ozymko Z, Lojkowska E. Application of zinc chloride precipitation method for rapid isolation and concentration of infectious Pectobacterium spp. and Dickeya spp. lytic bacteriophages from surface water and plant and soil extracts. Folia Microbiol (Praha) 2016;61:29-33.
13. Houari A, Picard J, Habarou H, Galas L, Vaudry H, Heim V, *et al*. Rheology of biofilms formed at the surface of NF membranes in a drinking water production unit. Biofouling 2008;24:235-40.
14. Toyofuku M, Roschitzki B, Riedel K, Eberl L. Identification of proteins associated with the *Pseudomonas aeruginosa* biofilm extracellular matrix. J Proteome Res 2012;11:4906-15.
15. Murugan K, Selvanayaki K, Al-Sohaibani S. Urinary catheter indwelling clinical pathogen biofilm formation, exopolysaccharide characterization and their growth influencing parameters. Saudi J Biol Sci 2016;23:150-9.
16. Valderrey AD, Pozuelo MJ, Jiménez PA, Maciá MD, Oliver A, Rotger R. Chronic colonization by *Pseudomonas aeruginosa* of patients with obstructive lung diseases: cystic fibrosis, bronchiectasis, and chronic obstructive pulmonary disease. Diagn Microbiol Infect Dis 2010;68:20-7.
17. Bouhrour N, Nibbering PH, Bendali F. Medical device-associated biofilm infections and multidrug-resistant pathogens. Pathogens 2024;13:393.
18. Al-Mathkhury HJ, Ali AS, Ghafil JA. Antagonistic effect of bacteriocin against urinary catheter associated *Pseudomonas aeruginosa* biofilm. N Am J Med Sci 2011;3:367-70.
19. Vestby LK, Grønseth T, Simm R, Nesse LL. Bacterial biofilm and its role in the pathogenesis of disease. Antibiotics (Basel) 2020;9:59.
20. Hoffman LR, D'Argenio DA, MacCoss MJ, Zhang Z, Jones RA, *et al*. Aminoglycoside antibiotics induce bacterial biofilm formation. Nature 2005;436:1171-5.
21. Bagge N, Schuster M, Hentzer M, Ciofu O, Givskov M, Greenberg EP, *et al*. *Pseudomonas aeruginosa* biofilms exposed to imipenem exhibit changes in global gene expression and beta-lactamase and alginate production. Antimicrob Agents Chemother 2004;48:1175-87.
22. Leid JG, Willson CJ, Shirtliff ME, Hassett DJ, Parsek MR, Jeffers AK. The exopolysaccharide alginate protects *Pseudomonas aeruginosa* biofilm bacteria from IFN-gamma-mediated macrophage killing. J Immunol 2005;175:7512-8.
23. Wang L, Li Y, Wang L, Zhang H, Zhu M, Zhang P, *et al*. Extracellular polymeric substances affect the responses of multi-species biofilms in the presence of sulfamethizole. Environ Pollut 2018;235:283-92.
24. Jiang B, Zeng Q, Hou Y, Li H, Shi S, Chen Z, *et al*. The responses of activated sludge to membrane cleaning reagent H_2O_2 and protection of extracellular polymeric substances. Environ Res 2022;203:111817.
25. Basu S, Agarwal M, Kumar Bhartiya S, Nath G, Kumar Shukla V. An in vivo wound model utilizing bacteriophage therapy of Pseudomonas aeruginosa biofilms. Ostomy Wound Manage 2015;61:16-23.
26. Forti F, Roach DR, Cafora M, Pasini ME, Horner DS, Fiscarelli EV, *et al*. Design of a broad-range bacteriophage cocktail that reduces *Pseudomonas aeruginosa* biofilms and treats acute infections in two animal models. Antimicrob Agents Chemother 2018;62:e02573-17.
27. Alemayehu D, Casey PG, McAuliffe O, Guinane CM, Martin JG, Shanahan F, *et al*. Bacteriophages φMR299-2 and φNH-4 can eliminate *Pseudomonas aeruginosa* in the murine lung and on cystic fibrosis lung airway cells. mBio 2012;3:e00029-12.
28. Glonti T, Chanishvili N, Taylor PW. Bacteriophage-derived enzyme that depolymerizes the alginic acid capsule associated with cystic fibrosis isolates of *Pseudomonas aeruginosa*. J Appl Microbiol 2010;108:695-702.
29. Kim S, Rahman M, Seol SY, Yoon SS, Kim J. *Pseudomonas aeruginosa* bacteriophage PA1Ø requires type IV pili for infection and shows broad bactericidal and biofilm removal activities. Appl Environ Microbiol 2012;78:6380-5.
30. Hanlon GW, Denyer SP, Olliff CJ, Ibrahim LJ. Reduction in exopolysaccharide viscosity as an aid to bacteriophage penetration through *Pseudomonas aeruginosa* biofilms. Appl Environ Microbiol 2001;67:2746-53.
31. Chung J, Eisha S, Park S, Morris AJ, Martin I. How three self-secreted biofilm exopolysaccharides of *Pseudomonas aeruginosa*, psl, pel, and alginate, can each be exploited for antibiotic adjuvant effects in cystic fibrosis lung infection. Int J Mol Sci 2023;24:8709.
32. Sutherland IW, Hughes KA, Skillman LC, Tait K. The interaction of phage and biofilms. FEMS Microbiol Lett 2004;232:1-6.
33. Mi L, Liu Y, Wang C, He T, Gao S, Xing S, *et al*. Identification of a lytic *Pseudomonas aeruginosa* phage depolymerase and its anti-biofilm effect and bactericidal contribution to serum. Virus Genes 2019;55:394-405.

34. Phee A, Bondy-Denomy J, Kishen A, Basrani B, Azarpazhooh A, Maxwell K. Efficacy of bacteriophage treatment on *Pseudomonas aeruginosa* biofilms. J Endod 2013;39:364-9.
35. Holguín AV, Rangel G, Clavijo V, Prada C, Mantilla M, Gomez MC, *et al*. Phage ΦPan70, a putative temperate phage, controls *Pseudomonas aeruginosa* in planktonic, biofilm and burn mouse model assays. Viruses 2015;7:4602-23.
36. Pires DP, Oliveira H, Melo LD, Sillankorva S, Azeredo J. Bacteriophage-encoded depolymerases: their diversity and biotechnological applications. Appl Microbiol Biotechnol 2016;100:2141-51.
37. Mohite BV, Koli SH, Rajput JD, Patil VS, Agarwal T, Patil SV. Production and characterization of multifacet exopolysaccharide from an agricultural isolate, *Bacillus subtilis*. Biotechnol Appl Biochem 2019;66:1010-23.
38. Mostaço-Guidolin LBüttner, Murakami LS, Nomizo A, Bachmann L. Fourier transform infrared spectroscopy of skin cancer cells and tissues. Applied Spectroscopy Reviews 2009;44:438-55.
39. Chen CC, Nargotra P, Kuo CH, Liu YC. High-molecular-weight exopolysaccharides production from *Tuber bronchii* cultivated by submerged fermentation. Int J Mol Sci 2023;24:4875.
40. Flemming HC, Neu TR, Wozniak DJ. The EPS matrix: The "house of biofilm cells". J Bacteriol 2007;189:7945-7.
41. Dickey J, Perrot V. Adjunct phage treatment enhances the effectiveness of low antibiotic dose against Staphylococcus aureus biofilms in vitro. PLoS One 2019; 14:e0209390.
42. Loganathan A, Bozdogan B, Manohar P, Nachimuthu R. Phage-antibiotic combinations in various treatment modalities to manage MRSA infections. Front Pharmacol 2024;15:1356179.
43. Kamal F, Dennis JJ. *Burkholderia cepacia* complex Phage-Antibiotic Synergy (PAS): an-tibiotics stimulate lytic phage activity. Appl Environ Microbiol 2015;81:1132-8.
44. Vashisth M, Yashveer S, Anand T, Virmani N, Bera BC, Vaid RK. Antibiotics targeting bacterial protein synthesis reduce the lytic activity of bacteriophages. Virus Res 2022;321:198909.
45. Manohar P, Loh B, Nachimuthu R, Leptihn S. Phage-antibiotic combinations to control *Pseudomonas aeruginosa Candida* two-species biofilms. Sci Rep 2024;14:9354.
46. Akturk E, Oliveira H, Santos SB, Costa S, Kuyumcu S, Melo LDR, *et al*. Synergistic action of phage and antibiotics: Parameters to enhance the killing efficacy against mono and dual-species biofilms. Antibiotics (Basel) 2019;8:103.

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