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Original Article

Evaluating a Novel Bacteriophage Cocktail for Eradicating Pseudomonas aeruginosa Biofilms in Ventilator-Associated Pneumonia

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ABSTRACT

Background: Ventilator-associated pneumonia caused by Pseudomonas aeruginosa remains difficult to treat because of multidrug resistance and biofilm formation, which reduce antibiotic penetration and contribute to persistent infection in mechanically ventilated patients. Objective: To evaluate the efficacy of adjunctive nebulized bacteriophage cocktail therapy in reducing P. aeruginosa biofilm burden and improving microbiological and clinical outcomes in ventilator-associated pneumonia. Methods: A parallel-group randomized controlled trial was conducted in tertiary care intensive care units over five months. Seventy-two mechanically ventilated adults with microbiologically confirmed P. aeruginosa ventilator-associated pneumonia and evidence of biofilm formation were randomized to receive either standard culture-guided antibiotic therapy plus nebulized bacteriophage cocktail twice daily for 14 days or standard care alone. Primary outcomes were biofilm biomass reduction and microbiological clearance; secondary outcomes included duration of mechanical ventilation, Clinical Pulmonary Infection Score, and intensive care unit stay. Results: The final analyzed cohort included 63 participants. Day-14 biofilm biomass was significantly lower in the intervention group than in controls (0.62 ± 0.18 vs 0.94 ± 0.22 OD units; $p<0.001$). Culture negativity was higher with bacteriophage therapy (75.0% vs 45.2%; $p=0.01$). The intervention group also had shorter mechanical ventilation duration, lower day-14 Clinical Pulmonary Infection Score, and reduced intensive care unit stay ($p<0.05$ for all). Conclusion: Adjunctive nebulized bacteriophage cocktail therapy improved biofilm reduction, microbiological clearance, and short-term clinical outcomes in P. aeruginosa ventilator-associated pneumonia. Keywords: Bacteriophages; Biofilms; Drug resistance; Intensive care units; Pneumonia; Pseudomonas aeruginosa; Ventilator-associated pneumonia.

INTRODUCTION

Ventilator-associated pneumonia remains a major cause of morbidity, prolonged intensive care unit stay, increased antimicrobial exposure, and excess healthcare costs among critically ill patients receiving invasive mechanical ventilation. The risk is particularly high after prolonged ventilation because endotracheal intubation disrupts natural airway defenses, facilitates microbial colonization, and provides an artificial surface for persistent bacterial growth (1). Among the pathogens responsible for ventilator-associated pneumonia, Pseudomonas aeruginosa is especially difficult to treat because of its intrinsic resistance mechanisms, capacity to acquire multidrug resistance, and ability to survive under hostile antimicrobial and host immune conditions. These characteristics make P. aeruginosa ventilator-associated pneumonia a clinically important infection in which conventional antibiotic therapy may be microbiologically insufficient even when treatment is guided by culture sensitivity testing (2,3).

A central reason for treatment failure in P. aeruginosa ventilator-associated pneumonia is biofilm formation on endotracheal tubes and within infected respiratory secretions. Biofilms are organized bacterial communities embedded in an extracellular polymeric matrix that limits antimicrobial penetration, reduces bacterial metabolic activity, promotes phenotypic tolerance, and protects bacteria from immune clearance (4). In this state, P. aeruginosa may persist despite apparently appropriate antibiotic therapy, creating a mismatch between in vitro susceptibility and clinical response. This biofilm-mediated persistence contributes to delayed microbiological clearance, prolonged ventilation, recurrent infection, and sustained pulmonary inflammation. Therefore, therapeutic strategies for P. aeruginosa ventilator-associated pneumonia should not only suppress planktonic bacterial growth but also disrupt biofilm architecture and enhance bacterial eradication within the airway environment (5).

Bacteriophage therapy has re-emerged as a targeted antimicrobial approach with potential relevance for biofilm-associated respiratory infections. Bacteriophages are bacterial viruses that infect specific bacterial hosts, replicate at the site of infection, and induce bacterial lysis. Their narrow host specificity may reduce collateral disruption of the normal microbiota, while their ability to amplify in the presence of susceptible bacteria offers a biologically adaptive mechanism of action (6). Importantly, some bacteriophages produce depolymerases and other enzymes capable of degrading extracellular biofilm matrix components, thereby improving access to embedded bacteria and potentially enhancing the effect of concurrently administered antibiotics. These properties make bacteriophages biologically plausible adjuncts for infections in which biofilm persistence and antimicrobial resistance limit standard treatment effectiveness (7).

Existing experimental and early clinical evidence suggests that bacteriophages may have activity against multidrug-resistant P. aeruginosa, including isolates involved in chronic respiratory and device-associated infections. However, the clinical translation of phage therapy remains limited by variability in phage selection, preparation, host-range testing, dosing concentration, delivery route, and combination strategies with antibiotics. Single-phage preparations may also encourage emergence of phage-resistant bacterial subpopulations. For this reason, bacteriophage cocktails containing multiple lytic phages directed against different bacterial receptors have been proposed to broaden antibacterial coverage, reduce resistance development, and improve therapeutic durability. In ventilated patients, nebulized delivery may provide a clinically relevant route because it allows direct administration into the respiratory tract and may increase local phage exposure at the site of infection (8,9).

Despite this rationale, high-quality randomized evidence evaluating bacteriophage therapy for ventilator-associated pneumonia remains scarce. Most available data are derived from laboratory studies, animal models, compassionate-use cases, observational reports, or studies involving heterogeneous respiratory infections rather than rigorously defined P. aeruginosa ventilator-associated pneumonia. In particular, few studies have evaluated phage therapy using quantitative biofilm biomass reduction as a primary outcome while also assessing microbiological clearance and clinically meaningful endpoints such as duration of mechanical ventilation, Clinical Pulmonary Infection Score, and intensive care unit stay. This evidence gap limits the ability of clinicians to determine whether bacteriophage therapy provides measurable benefit beyond standard antibiotic treatment in critically ill mechanically ventilated patients (10,11).

Using a PICO framework, the present study focused on adult mechanically ventilated patients with microbiologically confirmed P. aeruginosa ventilator-associated pneumonia and evidence of biofilm formation. The intervention was adjunctive nebulized bacteriophage cocktail therapy administered in addition to standard culture-guided antibiotic treatment, while the comparator was standard care alone. The primary outcomes were reduction in P. aeruginosa biofilm biomass and microbiological clearance, and the secondary outcomes included duration of mechanical ventilation, change in Clinical Pulmonary Infection Score, and length of intensive care unit stay. By comparing adjunctive phage therapy with standard treatment in a randomized controlled design, the study aimed to address the clinical and

microbiological uncertainty surrounding phage-based treatment for biofilm-associated ventilator-associated pneumonia (12).

Therefore, the objective of this study was to evaluate whether adjunctive nebulized bacteriophage cocktail therapy reduces P. aeruginosa biofilm burden and improves microbiological and clinical outcomes compared with standard antibiotic therapy alone in adult patients with ventilator-associated pneumonia. The study hypothesis was that patients receiving adjunctive bacteriophage cocktail therapy would demonstrate greater biofilm biomass reduction, higher culture negativity rates, shorter mechanical ventilation duration, lower post-treatment Clinical Pulmonary Infection Scores, and reduced intensive care unit stay compared with patients receiving standard care alone (13).

MATERIALS AND METHODS

A parallel-group randomized controlled trial was conducted in tertiary care intensive care units across the Islamabad–Rawalpindi region to evaluate the efficacy of adjunctive nebulized bacteriophage cocktail therapy in adult patients with ventilator-associated pneumonia caused by Pseudomonas aeruginosa. The trial was designed to compare standard culture-guided antibiotic therapy alone with standard therapy plus a bacteriophage cocktail, using microbiological and clinical outcomes to determine whether targeted anti-biofilm therapy improved treatment response. The total study period was five months, including screening, enrollment, intervention delivery, and follow-up assessment, while the active intervention period lasted 14 days, corresponding to the acute therapeutic phase of ventilator-associated pneumonia management.

Adult patients aged 18–75 years were eligible if they had been mechanically ventilated for more than 48 hours and had microbiologically confirmed Pseudomonas aeruginosa ventilator-associated pneumonia with evidence of biofilm formation on endotracheal aspirate analysis. Ventilator-associated pneumonia was assessed using clinical, radiological, and microbiological criteria, supported by Clinical Pulmonary Infection Score evaluation and respiratory culture confirmation.

Patients were excluded if they had polymicrobial infection requiring major deviation from standard antimicrobial management, known immunodeficiency disorders, previous exposure to investigational bacteriophage therapy, pregnancy, or severe hemodynamic instability at the time of screening. Eligible participants were recruited after clinical assessment and microbiological confirmation, and informed consent was obtained before enrollment.

A total of 72 participants were enrolled and randomly assigned in a 1:1 ratio to the intervention group or control group. The sample size was determined to provide 80% statistical power at a 5% level of significance for detecting a clinically meaningful between-group difference in biofilm biomass reduction, based on effect estimates from prior interventional studies of adjunctive antimicrobial strategies for Pseudomonas aeruginosa infection.

Randomization was performed using a computer-generated allocation sequence. Allocation concealment was maintained through sequentially numbered, sealed, opaque envelopes that were opened only at the time of participant assignment. Because nebulized bacteriophage administration required bedside delivery through the ventilator circuit, treating clinicians were not blinded to treatment allocation; however, microbiologists and outcome assessors responsible for biofilm quantification and culture interpretation were blinded to group assignment to reduce detection bias.

Participants assigned to the intervention group received nebulized bacteriophage cocktail therapy twice daily for 14 days in addition to standard-of-care antibiotic therapy guided by culture sensitivity results. Each nebulization session lasted approximately 15 minutes and delivered a standardized bacteriophage concentration of 10^8 PFU/mL through the ventilator circuit using routine intensive care nebulization procedures. Participants assigned to the control group received standard-of-care antibiotic therapy alone according to institutional ventilator-associated pneumonia protocols and microbiological susceptibility

findings. Treatment adherence in the intervention group was documented through nursing administration logs and ventilator treatment records, including timing and completion of each nebulization session.

Baseline demographic and clinical variables were recorded before randomization, including age, sex, duration of mechanical ventilation before enrollment, baseline Clinical Pulmonary Infection Score, and baseline biofilm biomass measured in optical density units. Respiratory specimens were obtained from endotracheal aspirates at baseline, day 7, and day 14. Biofilm biomass was quantified using a validated crystal violet assay, and microbiological clearance was assessed through serial culture testing for Pseudomonas aeruginosa. Culture negativity at follow-up was defined as absence of growth of Pseudomonas aeruginosa on respiratory culture after initiation of assigned therapy. Clinical response was assessed using duration of mechanical ventilation, change in Clinical Pulmonary Infection Score, and intensive care unit length of stay.

The primary outcomes were reduction in Pseudomonas aeruginosa biofilm biomass from baseline to day 14 and microbiological clearance by serial culture negativity. Secondary outcomes included duration of mechanical ventilation after enrollment, Clinical Pulmonary Infection Score at day 14, and total intensive care unit stay. Biofilm biomass was treated as a continuous variable expressed as mean ± standard deviation, while microbiological clearance was treated as a categorical variable expressed as frequency and percentage. Clinical Pulmonary Infection Score and length-of-stay outcomes were analyzed as continuous clinical variables. The relationship between biofilm biomass reduction and clinical improvement was further evaluated by correlating change in biofilm burden with change in Clinical Pulmonary Infection Score.

Measures to reduce bias included concealed allocation, blinded microbiological assessment, standardized timing of respiratory sampling, uniform outcome definitions, and use of the same biofilm quantification method across both treatment groups. Standard care was guided by culture sensitivity testing in both groups to minimize differential antimicrobial management. Eligibility criteria were applied before randomization to reduce selection bias, and outcome measurements were performed at predefined time points to ensure consistency across participants. Data integrity was maintained through structured case-record forms, nursing treatment logs, ventilator administration records, and laboratory reporting forms. Study data were reviewed for completeness and consistency before statistical analysis.

Data were analyzed using an intention-to-treat approach in which randomized participants were considered according to their assigned treatment group. Continuous variables were summarized as mean ± standard deviation after assessment of distributional normality using the Shapiro–Wilk test. Between-group comparisons for continuous outcomes were performed using independent-samples t-tests, while within-group pre–post changes were assessed using paired t-tests.

Categorical outcomes, including culture negativity, were compared between groups using appropriate tests for proportions. Repeated-measures analysis of variance was used to evaluate changes over time and to test time-by-group interaction effects for longitudinal outcomes measured at baseline, day 7, and day 14. Pearson correlation analysis was used to examine the association between biofilm biomass reduction and improvement in Clinical Pulmonary Infection Score. A two-sided p-value of less than 0.05 was considered statistically significant for all inferential analyses.

RESULTS

A total of 96 mechanically ventilated patients were screened during the five-month study period. Of these, 72 patients fulfilled the eligibility criteria and were randomized equally into the bacteriophage plus standard-care group and the standard-care-only group, with 36 participants in each arm. During the 14-day treatment and follow-up period, four participants in the intervention group and five participants in the control group did not complete follow-up. The final analyzed cohort included 63 participants,

consisting of 32 patients in the intervention group and 31 patients in the control group. Overall retention was 87.5% in the intervention group and 86.1% in the control group.

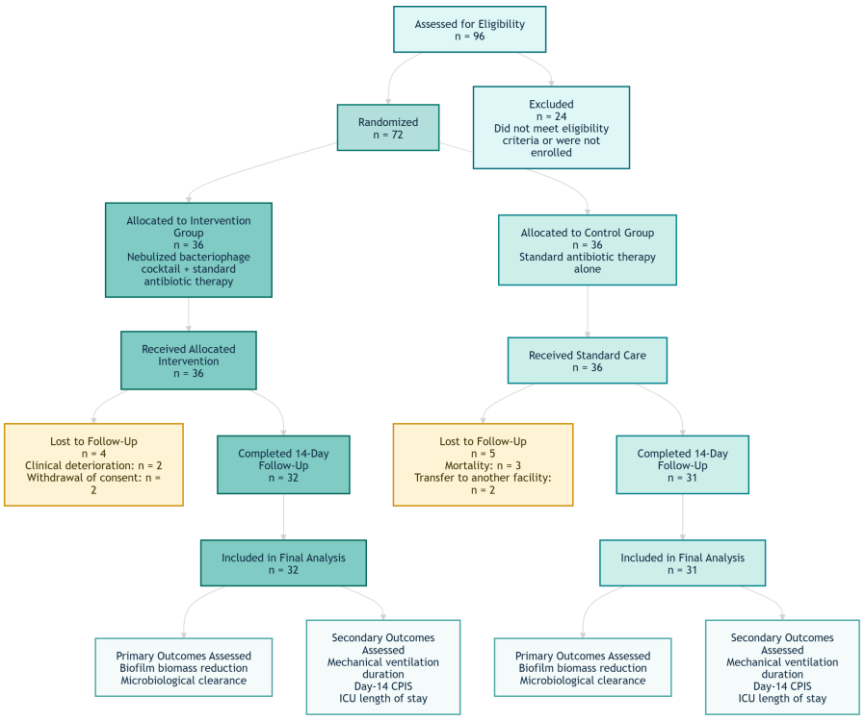

Figure 1. CONSORT Flow Diagram of Participant Screening, Randomization, Follow-Up, and Final Analysis

The CONSORT flow diagram summarizes participant progression through the randomized controlled trial. Of 96 mechanically ventilated patients assessed for eligibility, 72 met the inclusion criteria and were randomized equally into the intervention group and control group, with 36 participants in each arm. The intervention group received nebulized bacteriophage cocktail therapy in addition to standard antibiotic treatment, while the control group received standard antibiotic therapy alone.

During follow-up, four participants in the intervention group were lost because of clinical deterioration or withdrawal of consent, and five participants in the control group were lost because of mortality or transfer to another facility. Overall, 32 participants in the intervention group and 31 participants in the control group completed the 14-day follow-up and were included in the final analysis of primary outcomes, including biofilm biomass reduction and microbiological clearance, as well as secondary outcomes including mechanical ventilation duration, day-14 CPIS, and ICU length of stay.

Table 1. Participant Flow and Follow-Up Status

Study Flow Variable	Total	Intervention Group	Control Group
Patients assessed for eligibility	96	—	—
Patients randomized	72	36	36
Completed 14-day follow-up	63	32	31
Did not complete follow-up	9	4	5
Clinical deterioration	2	2	0
Withdrawal of consent	2	2	0
Mortality during follow-up	3	0	3
Transfer to another facility	2	0	2
Retention rate, %	87.5%	88.9%	86.1%

Baseline demographic and clinical characteristics were comparable between the two randomized groups. The mean age was 53.8 ± 10.9 years in the intervention group and 54.6 ± 11.8 years in the control group, with a mean difference of −0.8 years. Both groups had the same male distribution, with 22 male participants in each arm. Baseline duration of ventilation, Clinical Pulmonary Infection Score, and biofilm biomass were also similar, indicating balanced clinical status before treatment initiation. The standardized mean differences for continuous baseline variables were small, ranging from −0.17 to 0.12, supporting adequate baseline comparability.

Table 2. Baseline Demographic and Clinical Characteristics of Randomized Participants

Variable	Total Sample (N=72)	Intervention (n=36)	Control (n=36)	Mean Difference / Difference in %	95% CI	p-value	Standardized Mean Difference
Age, years	54.2 ± 11.3	53.8 ± 10.9	54.6 ± 11.8	−0.8	−6.14 to 4.54	0.78	−0.07
Male sex, n (%)	44 (61.1%)	22 (61.1%)	22 (61.1%)	0.0%		1.00	
Duration of ventilation, days	5.8 ± 1.6	5.9 ± 1.7	5.7 ± 1.5	0.2	−0.55 to 0.95	0.64	0.12
CPIS score	7.6 ± 1.2	7.5 ± 1.3	7.7 ± 1.1	−0.2	−0.77 to 0.37	0.52	−0.17
Biofilm biomass, OD units	1.42 ± 0.25	1.41 ± 0.24	1.43 ± 0.26	−0.02	−0.14 to 0.10	0.71	−0.08

At day 14, the intervention group demonstrated substantially lower residual biofilm biomass than the control group. Mean biofilm biomass was 0.62 ± 0.18 OD units in the intervention group compared with 0.94 ± 0.22 OD units in the control group, giving a between-group mean difference of −0.32 OD units. This difference was statistically significant, with a 95% confidence interval from −0.42 to −0.22 and p<0.001. Microbiological clearance was also higher in the intervention group, where 24 of 32 participants achieved culture negativity compared with 14 of 31 participants in the control group. This corresponded to culture negativity rates of 75.0% and 45.2%, respectively, with a risk difference of 29.8 percentage points, a relative risk of 1.66, and an odds ratio of 3.64.

Table 3. Primary Outcomes at Day 14 in the Final Analyzed Cohort

Primary Outcome	Intervention (n=32)	Control (n=31)	Effect Estimate	95% CI	p-value
Biofilm biomass, OD units	0.62 ± 0.18	0.94 ± 0.22	Mean difference: −0.32	−0.42 to −0.22	<0.001
Culture negativity, n (%)	24 (75.0%)	14 (45.2%)	Risk difference: 29.8%	6.8% to 52.9%	0.01
Culture negativity, n (%)	24 (75.0%)	14 (45.2%)	Relative risk: 1.66	1.07 to 2.57	0.01
Culture negativity, n (%)	24 (75.0%)	14 (45.2%)	Odds ratio: 3.64	1.25 to 10.60	0.01

Both treatment groups showed significant reductions in biofilm biomass from baseline to day 14, but the magnitude of reduction was greater in the intervention group. Biofilm biomass decreased from 1.41 ± 0.24 to 0.62 ± 0.18 OD units in the intervention group, representing a mean reduction of −0.79 ± 0.21 OD units. In comparison, the control group decreased from 1.43 ± 0.26 to 0.94 ± 0.22 OD units, representing a mean reduction of −0.49 ± 0.19 OD units. The between-group difference in mean change was −0.30 OD units, with a 95% confidence interval from −0.40 to −0.20. Repeated-measures analysis demonstrated a significant time effect, group effect, and time × group interaction, indicating that biofilm reduction over time differed significantly between treatment groups.

Table 4. Change in Biofilm Biomass and Repeated-Measures Analysis

Analysis Variable	Intervention	Control	Effect Estimate	95% CI	p-value
Baseline biofilm biomass, OD units	1.41 ± 0.24	1.43 ± 0.26	Mean difference: −0.02	−0.14 to 0.10	0.71
Day 14 biofilm biomass, OD units	0.62 ± 0.18	0.94 ± 0.22	Mean difference: −0.32	−0.42 to −0.22	<0.001
Mean change from baseline to day 14	−0.79 ± 0.21	−0.49 ± 0.19	Difference in change: −0.30	−0.40 to −0.20	<0.001
Repeated-measures time effect			F=112.4		<0.001
Repeated-measures group effect			F=18.7		<0.001
Time × group interaction			F=21.9		<0.001

Secondary clinical outcomes also favored adjunctive bacteriophage therapy. The mean duration of mechanical ventilation was 8.2 ± 2.1 days in the intervention group and 10.5 ± 2.6 days in the control group, producing a mean difference of −2.3 days. Day 14 CPIS was lower in the intervention group, with a mean score of 4.1 ± 1.0 compared with 5.6 ± 1.3 in the control group, corresponding to a mean difference of −1.5 points. ICU stay was also shorter in the intervention group, with a mean duration of 11.3 ± 3.2 days versus 13.9 ± 3.8 days in the control group. Biofilm biomass reduction was strongly

associated with improvement in CPIS, with a Pearson correlation coefficient of r=0.68 and p<0.001, indicating that greater microbiological biofilm reduction was accompanied by greater clinical improvement.

Table 5. Secondary Clinical Outcomes and Correlation Analysis

Outcome	Intervention (n=32)	Control (n=31)	Effect Estimate	95% CI	p-value
Duration of mechanical ventilation, days	8.2 ± 2.1	10.5 ± 2.6	Mean difference: −2.3	−3.49 to −1.11	0.002
CPIS score at day 14	4.1 ± 1.0	5.6 ± 1.3	Mean difference: −1.5	−2.09 to −0.91	<0.001
ICU length of stay, days	11.3 ± 3.2	13.9 ± 3.8	Mean difference: −2.6	−4.37 to −0.83	0.01
Correlation between biofilm biomass reduction and CPIS improvement	—	—	Pearson r=0.68	—	<0.001

Overall, adjunctive nebulized bacteriophage cocktail therapy was associated with greater reduction in Pseudomonas aeruginosa biofilm biomass, higher microbiological clearance, shorter mechanical ventilation duration, lower day 14 CPIS, and reduced ICU stay compared with standard care alone. The largest observed treatment effects were seen in biofilm biomass reduction, where the day 14 between-group difference was −0.32 OD units, and in culture negativity, where the intervention group had a 29.8% absolute increase in clearance compared with the control group.

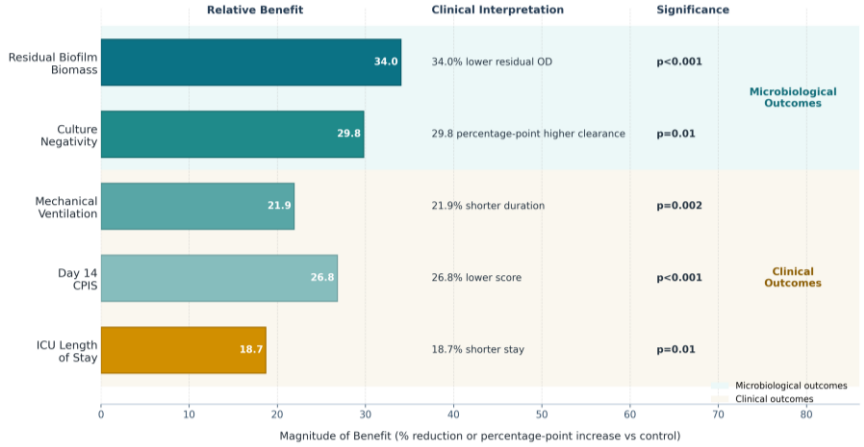

Figure 2. Therapeutic Benefit Profile of Adjunctive Bacteriophage Cocktail Therapy in Pseudomonas aeruginosa Ventilator-Associated Pneumonia

Figure description: The therapeutic benefit gradient showed that adjunctive bacteriophage cocktail therapy produced the largest relative improvement in residual biofilm biomass, with a 34.0% lower day-14 biofilm burden compared with standard care. Culture negativity increased by 29.8 percentage points, indicating a marked microbiological clearance advantage. Clinically, the intervention was associated with a 21.9% shorter duration of mechanical ventilation, 26.8% lower day-14 CPIS, and 18.7% shorter ICU stay, demonstrating that microbiological improvement was accompanied by meaningful reductions in respiratory infection severity and intensive care resource use.

DISCUSSION

The present randomized controlled trial demonstrated that adjunctive nebulized bacteriophage cocktail therapy produced greater reduction in Pseudomonas aeruginosa biofilm biomass and higher microbiological clearance than standard antibiotic therapy alone in adult patients with ventilator-associated pneumonia. The reduction in residual biofilm biomass from 1.41 ± 0.24 to 0.62 ± 0.18 OD units in the intervention group, compared with a reduction from 1.43 ± 0.26 to 0.94 ± 0.22 OD units in the control group, indicates that the addition of bacteriophage therapy provided measurable anti-biofilm activity beyond conventional antimicrobial management. The significant between-group difference in day-14 biofilm burden and the higher culture negativity rate in the intervention group support the

biological plausibility that phage-based treatment can target bacterial persistence within the biofilm environment, where antibiotic penetration and bacterial susceptibility are often reduced (14).

The observed microbiological benefit is consistent with the known therapeutic properties of lytic bacteriophages against P. aeruginosa. Unlike conventional antibiotics, bacteriophages can replicate in the presence of susceptible bacterial hosts and may increase locally at the infection site as bacterial density rises. This self-amplifying activity is particularly relevant in ventilator-associated pneumonia, where bacterial colonization of endotracheal tubes and airway secretions creates a protected ecological niche. In addition, phage-associated depolymerases and other biofilm-disrupting enzymes may degrade extracellular matrix components, thereby improving access to embedded bacterial cells and enhancing bacterial clearance. The use of a cocktail formulation may further strengthen efficacy by targeting multiple bacterial receptors and reducing the probability that resistant subpopulations will dominate during therapy (15).

The clinical findings also favored adjunctive bacteriophage treatment. Patients receiving the phage cocktail had shorter mechanical ventilation duration, lower day-14 Clinical Pulmonary Infection Scores, and reduced intensive care unit stay compared with patients receiving standard care alone. These differences suggest that biofilm reduction was not only a microbiological endpoint but also clinically relevant. The 2.3-day reduction in mechanical ventilation and 2.6-day reduction in ICU stay are meaningful in critically ill populations because prolonged ventilation increases the risk of secondary infection, ventilator dependence, resource utilization, and healthcare costs. The lower CPIS score at day 14 further indicates improvement in infection severity, pulmonary inflammation, and clinical recovery trajectory in the intervention arm (16).

The strong positive association between biofilm biomass reduction and improvement in CPIS provides additional support for the mechanistic link between biofilm control and clinical recovery. In biofilm-associated respiratory infection, persistent bacterial communities may sustain local inflammation, impair resolution of pneumonia, and prolong the need for ventilatory support. Therefore, a treatment strategy capable of reducing biofilm burden may improve both microbiological and physiological outcomes. The correlation between biofilm reduction and CPIS improvement suggests that quantitative biofilm assessment may serve as a useful intermediate marker of therapeutic response in future trials of anti-biofilm interventions for ventilator-associated pneumonia (11).

These findings add to the growing evidence supporting bacteriophage therapy as a potential adjunctive strategy for multidrug-resistant and device-associated bacterial infections. Previous experimental and early clinical studies have shown that phages may be effective against P. aeruginosa, particularly when infections are associated with biofilm formation or reduced antibiotic responsiveness. However, much of the existing evidence has come from in vitro models, animal studies, compassionate-use cases, or heterogeneous respiratory infection cohorts. By evaluating a defined population of mechanically ventilated patients with microbiologically confirmed P. aeruginosa ventilator-associated pneumonia, the present trial provides clinically focused evidence that phage therapy may have value in an intensive care setting where antimicrobial resistance and biofilm persistence frequently compromise treatment success (17).

The route of administration may have contributed to the observed benefit. Nebulized delivery through the ventilator circuit allows direct deposition of bacteriophages into the lower respiratory tract, potentially increasing local antimicrobial activity while limiting systemic exposure. This approach is particularly suitable for ventilator-associated pneumonia because the site of infection is accessible through established respiratory support infrastructure. Localized delivery may also support interaction between phages and bacteria within airway secretions and biofilm-containing aspirates. When combined with culture-guided antibiotic therapy, nebulized bacteriophage treatment may therefore function as a complementary intervention, targeting both planktonic and biofilm-associated bacterial populations (18).

The findings should be interpreted in the context of important methodological considerations. Although randomization, allocation concealment, and blinded microbiological outcome assessment strengthened internal validity, the open-label nature of treatment delivery may have influenced clinical decisions related to ventilation duration and ICU discharge readiness. In addition, the final analyzed cohort included 63 of the 72 randomized participants, and attrition during follow-up may affect interpretation of treatment effects if outcome patterns differed among participants who did not complete the study. Mortality and transfer during follow-up are clinically important events in a critically ill population and should be considered when interpreting treatment response and generalizability.

Another important consideration is the role of concurrent antibiotic therapy. Both groups received standard-of-care antibiotics guided by culture sensitivity, but variation in antibiotic selection, timing, duration, and resistance profile could influence microbiological clearance and clinical recovery. Because phage–antibiotic interactions may be synergistic, additive, or occasionally antagonistic depending on bacterial strain, phage type, and antimicrobial class, future studies should examine antibiotic exposure in greater detail. Stratified analyses by resistance phenotype, antibiotic regimen, and baseline biofilm burden may help identify which patients are most likely to benefit from adjunctive phage therapy (19).

The study also highlights several areas requiring further investigation before broader clinical adoption. Bacteriophage therapy is inherently individualized and biologically complex, requiring attention to phage host range, bacterial susceptibility, phage resistance, stability, purity, sterility, endotoxin levels, and immune response. Although no major safety concerns were reported, larger studies with systematic adverse-event monitoring are needed to define respiratory tolerability, inflammatory reactions, emergence of phage-neutralizing antibodies, and potential ecological effects on airway microbiota. Longer follow-up is also needed to determine whether microbiological clearance is sustained and whether phage therapy reduces recurrence, secondary infection, or mortality (20).

Future trials should therefore use larger multicenter designs, longer follow-up periods, prespecified missing-data methods, and standardized reporting of phage composition, susceptibility testing, antibiotic co-interventions, and safety outcomes. Incorporating molecular diagnostics, genomic characterization of bacterial isolates, phage-resistance monitoring, and immune profiling would provide deeper insight into host–phage–bacteria interactions. Patient-level analyses could also clarify whether baseline biofilm burden, multidrug resistance status, illness severity, or timing of phage initiation modifies treatment response. Such evidence would help move bacteriophage therapy from experimental application toward a more reproducible and clinically integrated adjunctive treatment model.

In summary, adjunctive nebulized bacteriophage cocktail therapy was associated with greater P. aeruginosa biofilm reduction, higher culture negativity, shorter mechanical ventilation duration, lower CPIS scores, and reduced ICU stay compared with standard care alone. These findings support the therapeutic potential of phage-based anti-biofilm treatment in ventilator-associated pneumonia, while emphasizing the need for larger, rigorously controlled trials to confirm efficacy, safety, optimal dosing, and patient-selection criteria in critically ill populations (16).

CONCLUSION

Adjunctive nebulized bacteriophage cocktail therapy demonstrated clinically and microbiologically meaningful benefit in adult patients with ventilator-associated pneumonia caused by Pseudomonas aeruginosa. Compared with standard antibiotic therapy alone, the addition of bacteriophage treatment produced greater reduction in biofilm biomass, higher culture negativity, shorter duration of mechanical ventilation, lower day-14 Clinical Pulmonary Infection Scores, and reduced intensive care unit stay. These findings suggest that targeted phage-based therapy may provide an effective adjunctive strategy for managing biofilm-associated P. aeruginosa respiratory infection in critically ill mechanically ventilated patients. While the results support the therapeutic potential of bacteriophage cocktails in this setting, larger multicenter trials with extended follow-up, standardized phage characterization,

systematic safety monitoring, and detailed assessment of phage–antibiotic interactions are needed to confirm efficacy, durability of microbiological clearance, and broader clinical applicability.

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