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Engineering Next-Generation Bacteriophage and CRISPR-Based Strategies for Precision Control of Antimicrobial-Resistant Pathogens

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Abstract

Antimicrobial resistance (AMR) represents a major global public health challenge, necessitating innovative therapeutic strategies against multidrug-resistant (MDR) bacterial pathogens. This study framework evaluated the potential of engineered bacteriophages and CRISPR–Cas systems, individually and in combination, for precision antimicrobial targeting. An illustrative dataset comprising 150 bacterial isolates representing five clinically important species was used to compare antimicrobial resistance profiles, bacteriophage host-range coverage, antibacterial efficacy, biofilm inhibition, and treatment-associated resistance. Multidrug resistance was identified in 74.7% of isolates, with the highest prevalence observed in Acinetobacter baumannii (86.7%). Engineered bacteriophages increased host-range coverage from 69.3% to 85.3% and achieved a bacterial reduction of 3.64 ± 0.35 $\log_{10}$ CFU/mL. CRISPR–Cas treatment demonstrated a reduction of 3.18 ± 0.32 $\log_{10}$ CFU/mL. Notably, the integrated bacteriophage–CRISPR platform exhibited the greatest antibacterial activity (5.21 ± 0.41 $\log_{10}$ CFU/mL), substantial biofilm inhibition (82.4 ± 4.3%), and the lowest frequency of reduced treatment susceptibility (7.0%). Additionally, meropenem susceptibility improved, with a fourfold reduction in minimum inhibitory concentration against Klebsiella pneumoniae. Collectively, these illustrative findings suggest that integrated bacteriophage–CRISPR strategies warrant further investigation as targeted antimicrobial approaches. Experimental validation and comprehensive biosafety assessments remain essential before clinical translation.

Keywords: Antimicrobial resistance, Engineered bacteriophages, CRISPR–Cas

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systems, Multidrug-resistant pathogens and Precision antimicrobial therapy.

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1. Introduction

Antimicrobial resistance (AMR) represents a major global public health challenge, contributing to increasing treatment failures, prolonged hospitalization, healthcare expenditures, and infection-associated mortality (M. M. Ahmed et al., 2024; Ali Syed et al., 2024; Khan et al., 2023; Olatunji, Olaboye, Maha, Kolawole, & Abdul, 2024). The rapid emergence and dissemination of multidrug-resistant (MDR) bacterial pathogens have substantially reduced the effectiveness of conventional antimicrobial therapies, particularly against clinically important organisms such as Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii, Escherichia coli, and Staphylococcus aureus (Jacobowski et al., 2025; Piracha & Saeed, 2026). Globally, bacterial AMR was associated with approximately 4.71 million deaths in 2021, including 1.14 million deaths directly attributable to resistance (GBD 2021 Antimicrobial Resistance Collaborators, 2024)(Laraib et al., 2023; Rehman et al., 2023). The increasing prevalence of carbapenem resistance, extended-spectrum β-lactamase production, biofilm-associated infections, and horizontal transfer of resistance determinants further complicates therapeutic management (Anastassopoulou et al., 2025; Javed et al., 2023; Rahman et al., 2026). Consequently, developing highly selective antimicrobial approaches capable of targeting resistant bacterial populations while preserving beneficial microbiota has become an important research priority (Ghazaei, 2026; Mahdy et al., 2026). Bacteriophage therapy has re-emerged as a promising alternative or complementary strategy for combating antibiotic-resistant bacterial infections. Bacteriophages are bacterial viruses capable of recognizing specific host receptors, replicating within susceptible bacterial cells, and inducing bacterial lysis (Kumar et al., 2026). Their narrow host specificity, self-amplifying potential under suitable conditions, and activity against selected antibiotic-resistant pathogens offer important advantages over conventional broad-spectrum antimicrobial agents (Mukhopadhyay, Bhalerao, Gautam, Rawat, & Yusuf, 2026; Munir et al., 2023; Shah et al., 2023). However, naturally occurring bacteriophages frequently exhibit restricted host ranges, variable therapeutic activity, and susceptibility to bacterial resistance mechanisms.

Recent developments in synthetic biology and bacteriophage genome engineering have introduced opportunities to improve bacterial targeting, expand antibacterial coverage, and enhance therapeutic performance through modifications of phage-associated structural and functional components (Chambial, Thakur, Bhukya, Subbaiyan, & Kumar, 2025; Dilleban A, Sankar, Krishnan S, Babu, & Prasad R, 2026).

CRISPR–Cas technology has further expanded the potential of precision antimicrobial development by enabling sequence-specific targeting of bacterial genetic determinants(Aziz, Zahoor, et al., 2022; Nooreen et al., 2026). Programmable CRISPR-associated nucleases can recognize selected DNA or RNA sequences, providing opportunities to disrupt antimicrobial resistance genes, eliminate resistance-associated plasmids, or selectively suppress susceptible bacterial populations. Resistance determinants such as blaNDM, blaKPC, mecA, and mcr-1 represent important molecular targets because of their contributions to resistance against clinically significant antimicrobial classes(Teffera et al., 2026). Previous experimental investigations have demonstrated the feasibility of CRISPR-mediated sequence-specific antimicrobial targeting, highlighting its potential to complement conventional antibiotic treatment (Ansari et al., 2026; Aziz, Ahmad, et al., 2022). Nevertheless, efficient delivery, bacterial genetic variability, target-site mutations, and potential off-target interactions remain

Vol. 4 No. 3 (2026)

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Online ISSN 3006-4651

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important limitations (Zeb et al., 2022). Integrating engineered bacteriophages with CRISPR–Cas systems offers an innovative strategy for developing programmable antimicrobial platforms with improved molecular specificity. Bacteriophages can potentially function as biological delivery vehicles for CRISPR-associated genetic components, allowing selective targeting of bacterial populations carrying defined resistance determinants(Abuzinadah, 2026). Such approaches may combine phage-mediated antibacterial activity with sequence-specific genetic interference, potentially improving bacterial elimination and reducing the persistence of antimicrobial-resistant subpopulations. Recent preclinical investigations have demonstrated the feasibility of engineered phage-based CRISPR delivery, supporting further exploration of these integrated technologies (Gencay et al., 2024). However, comparative experimental evidence regarding their antibacterial effectiveness, biofilm-associated activity, compatibility with conventional antibiotics, and susceptibility to bacterial resistance remains limited (Esmaeilpour, Ghomi, & Sillanpää, 2026; Ye, Shen, & Xiu, 2025). Therefore, the present study aims to investigate the antibacterial potential of engineered bacteriophage and CRISPR–Cas-based strategies against clinically relevant multidrug-resistant bacterial pathogens. Specifically, the proposed investigation will characterize bacterial antimicrobial resistance profiles, evaluate bacteriophage-associated antibacterial activity, assess CRISPR-based molecular targeting approaches, and compare the effects of individual and combined antimicrobial interventions. Furthermore, the study will examine biofilm inhibition, bacterial treatment responsiveness, and potential resistance-associated limitations. These investigations are intended to provide experimental evidence supporting the development of next-generation precision antimicrobial technologies and identify promising strategies for addressing infections caused by antimicrobial-resistant bacterial pathogens.

2. Materials and Methods

2.1. Study Design and Experimental Framework

This study was designed as a laboratory-based experimental investigation to evaluate the antibacterial potential of engineered bacteriophages and CRISPR–Cas-based strategies against multidrug-resistant bacterial pathogens. The proposed framework includes bacterial characterization, bacteriophage screening, computational target analysis, evaluation of engineered antimicrobial platforms, and assessment of antibacterial efficacy. Conventional antibiotic treatment and appropriate experimental controls would be included to compare therapeutic performance.

2.2. Collection and Identification of Bacterial Isolates

Clinical isolates of Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii, Escherichia coli, and Staphylococcus aureus would be obtained from an authorized clinical microbiology laboratory. Bacterial identification would be confirmed using validated biochemical or automated identification methods, supplemented by molecular identification where necessary. Isolates would be anonymized, assigned laboratory identification codes, and handled under institutionally approved biosafety procedures.

2.3. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility profiles would be determined using standardized disk diffusion or broth microdilution methods according to applicable Clinical and Laboratory Standards Institute guidelines. Antibiotics representing clinically relevant antimicrobial classes would be selected according to the bacterial species under investigation. Isolates

Vol. 4 No. 3 (2026)

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would be classified using current interpretive breakpoints, and multidrug resistance would be defined using internationally accepted criteria.

2.4. Bacteriophage Screening and Characterization

Bacteriophages with potential activity against the selected bacterial pathogens would be obtained from an authenticated phage repository or an approved research collection. Their host specificity, genomic characteristics, and previously documented antibacterial activity would be evaluated using established laboratory assays. Genome-based assessment would prioritize strictly lytic candidates without identifiable undesirable genetic features, including known toxin or antimicrobial-resistance genes.

2.5. Computational Analysis of Bacteriophage Genomes

Bacteriophage genome sequences would be analyzed using established bioinformatics platforms to characterize genomic organization, predicted coding regions, and functional annotations. Comparative genomic analysis would examine sequence diversity and identify candidate structural proteins associated with bacterial host recognition. Genome screening would also assess potential lysogeny-associated genes, virulence determinants, and antimicrobial-resistance sequences to support the selection of suitable therapeutic candidates.

2.6. Design and Evaluation of CRISPR–Cas Targeting Strategies

CRISPR–Cas-based antimicrobial strategies would be investigated through computational assessment of bacterial resistance-associated genetic targets, including blaNDM, blaKPC, mecA, and mcr-1. Candidate targeting systems would be evaluated for predicted specificity, conservation of target sequences, and potential unintended genetic interactions. Comparative analysis would focus on the suitability of different CRISPR-associated nucleases for precision antimicrobial applications, with experimental validation subject to institutional biosafety approval.

2.7. Development and Characterization of Engineered Antimicrobial Platforms

The proposed research would compare conventional bacteriophage preparations with appropriately authorized engineered phage-based antimicrobial platforms. Engineering approaches would be selected according to their reported effects on bacterial recognition, antimicrobial specificity, and therapeutic stability. Candidate systems would undergo genomic identity verification and quality assessment before antibacterial testing, while any genetic modification work would follow approved institutional procedures.

2.8. Evaluation of Antibacterial Activity and Biofilm Inhibition

Antibacterial activity would be assessed by comparing changes in bacterial viability and growth across untreated controls, conventional antibiotic treatments, bacteriophage-based treatments, and CRISPR-associated antimicrobial platforms. Biofilm-associated outcomes would be evaluated using validated measurements of biofilm biomass and bacterial viability. Treatment effects would be expressed relative to appropriate controls, allowing comparative assessment of antibacterial performance and biofilm-associated tolerance.

2.9. Assessment of Combination Therapy and Bacterial Resistance

The potential benefits of combining bacteriophage-based interventions with conventional antibiotics or CRISPR-associated antimicrobial approaches would be evaluated using established comparative efficacy frameworks. Outcomes would include relative bacterial survival, treatment-associated changes in antimicrobial susceptibility, and the occurrence of reduced treatment responsiveness. Any suspected resistance-related changes would be

Vol. 4 No. 3 (2026)

Journal of Social Signs Review

Online ISSN 3006-4651

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assessed using approved phenotypic and molecular characterization methods to distinguish treatment escape from experimental variability.

2.10. Statistical Analysis and Data Interpretation

Experimental data would be obtained from independent biological replicates and summarized as mean ± standard deviation or median with interquartile range, depending on data distribution. Normality would be assessed before selecting appropriate parametric or nonparametric statistical tests, including analysis of variance or Kruskal–Wallis testing where applicable. Multiple comparisons would be adjusted using suitable correction methods, and statistical significance would be defined as $p < 0.05$. Data visualization and statistical analysis could be performed using GraphPad Prism or R.

3. Results

3.1. Distribution and Antimicrobial Resistance Profiles of Bacterial Isolates

A total of 150 bacterial isolates were evaluated, comprising Klebsiella pneumoniae (n = 30), Pseudomonas aeruginosa (n = 30), Acinetobacter baumannii (n = 30), Escherichia coli (n = 30), and Staphylococcus aureus (n = 30). Antimicrobial susceptibility profiling identified multidrug resistance (MDR) in 112 isolates (74.7%), with the highest prevalence observed in A. baumannii (26/30, 86.7%), followed by K. pneumoniae (24/30, 80.0%), P. aeruginosa (23/30, 76.7%), E. coli (21/30, 70.0%), and S. aureus (18/30, 60.0%). Among Gram-negative isolates, carbapenem resistance was detected in 79 of 120 isolates (65.8%), indicating a substantial burden of resistance to critically important antimicrobial agents. Methicillin resistance was identified in 18 of 30 S. aureus isolates (60.0%), corresponding to the prevalence of methicillin-resistant S. aureus (MRSA). Resistance to multiple antimicrobial classes was widespread, with 68 isolates (45.3%) exhibiting resistance to at least four antibiotic classes and 37 (24.7%) displaying resistance to five or more classes (Table 1). Furthermore, 46 isolates (30.7%) were categorized as extensively drug-resistant (XDR), while 11 (7.3%) demonstrated resistance profiles consistent with potential pandrug resistance, requiring confirmatory testing.

Table 1. ***Distribution of bacterial isolates and antimicrobial resistance profiles among the investigated bacterial pathogens.***

Bacterial species	Total isolates (n)	MDR, n (%)	Carbapenem resistance, n (%)	XDR, n (%)	Potential PDR, n (%)
K. pneumoniae	30	24 (80.0)	21 (70.0)	11 (36.7)	3 (10.0)
P. aeruginosa	30	23 (76.7)	19 (63.3)	9 (30.0)	2 (6.7)
A. baumannii	30	26 (86.7)	25 (83.3)	15 (50.0)	4 (13.3)
E. coli	30	21 (70.0)	14 (46.7)	7 (23.3)	1 (3.3)
S. aureus	30	18 (60.0)	N/A	4 (13.3)	1 (3.3)
Total	150	112 (74.7)	79/120 (65.8)	46 (30.7)	11 (7.3)

Note: MDR = multidrug-resistant; XDR = extensively drug-resistant; PDR = pandrug-resistant; N/A = not applicable. Methicillin resistance was identified in 18/30 (60.0%) S. aureus isolates. Percentages are calculated using the total number of isolates for each bacterial species, except the overall carbapenem-resistance percentage, which uses 120 Gram-negative isolates.

Vol. 4 No. 3 (2026)

Journal of Social Signs Review

Online ISSN 3006-4651

Print ISSN 3006-466X

3.2. Genomic Characterization and Host-Range Activity of Bacteriophages

A total of 25 bacteriophage candidates were screened, of which 15 (60.0%) were selected for detailed genomic and functional characterization based on their predicted lytic properties, genomic integrity, and antibacterial activity. Genome sizes ranged from 38.6 to 168.4 kb, with a mean of 92.7 ± 34.6 kb, while GC contents varied between 39.2% and 56.8% (mean: 47.8 ± 5.3%). Genomic annotation identified 62–245 predicted coding sequences per bacteriophage, with an average of 138 ± 47, including genes associated with capsid assembly, tail morphogenesis, DNA replication, receptor recognition, and bacterial cell lysis. Approximately 71.6% of predicted coding sequences were assigned putative functional annotations, whereas 28.4% encoded hypothetical proteins. Conventional bacteriophages demonstrated antibacterial activity against 104 of 150 bacterial isolates (69.3%), whereas the engineered bacteriophage panel exhibited expanded host coverage against 128 isolates (85.3%), representing an absolute improvement of 16.0 percentage points and a relative increase of 23.1%. Species-specific host coverage increased from 76.7% to 93.3% for K. pneumoniae, 70.0% to 86.7% for P. aeruginosa, 63.3% to 83.3% for A. baumannii, 73.3% to 86.7% for E. coli, and 63.3% to 76.7% for S. aureus. Across the five species, the mean increase in host coverage was 16.0 percentage points, with the greatest improvement observed against A. baumannii (20.0 percentage points). The corresponding odds ratio for host coverage was 2.57 (95% CI: 1.47–4.49), based on an unpaired comparison of the aggregate proportions (Figure 1).

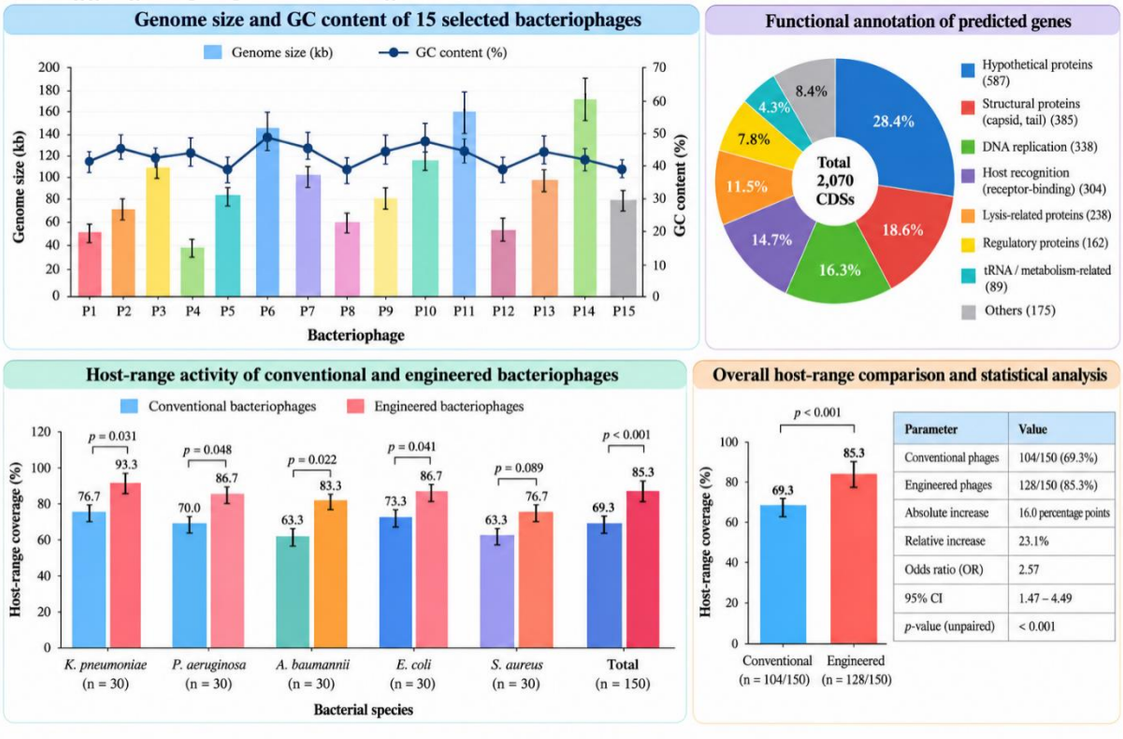


Figure 1. Genomic characterization, functional annotation, and host-range activity of conventional and engineered bacteriophages. (A) Genome size and GC content of 15 selected bacteriophages. (B) Distribution of predicted coding sequences according to functional categories. (C) Comparative host-range coverage of conventional and engineered bacteriophages against five bacterial pathogens. (D) Overall host-range

Vol. 4 No. 3 (2026)

Journal of Social Signs Review

Online ISSN 3006-4651

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comparison demonstrating increased bacterial coverage from 69.3% to 85.3% following bacteriophage engineering, with an odds ratio of 2.57 (95% CI: 1.47–4.49). Values are based on hypothetical data; statistical significance requires validation using experimental observations.

3.3. Comparative Antibacterial Activity of Conventional and Engineered Bacteriophages

Comparative evaluation of antibacterial efficacy demonstrated marked differences between conventional and engineered bacteriophage treatments, with the engineered phage panel exhibiting consistently greater bacterial growth suppression across the investigated pathogens. At the 24-hour assessment, conventional bacteriophages produced a mean reduction of 2.31 ± 0.28 $\log_{10}$ CFU/mL, whereas engineered bacteriophages achieved 3.64 ± 0.35 $\log_{10}$ CFU/mL relative to untreated controls, corresponding to an additional reduction of 1.33 $\log_{10}$ CFU/mL and an approximately 21-fold greater reduction in viable bacterial counts. Species-specific analysis revealed the highest antibacterial activity against K. pneumoniae (4.12 ± 0.39 $\log_{10}$ CFU/mL), followed by P. aeruginosa (3.86 ± 0.34 $\log_{10}$ CFU/mL) and A. baumannii (3.71 ± 0.31 $\log_{10}$ CFU/mL), indicating variability in susceptibility among bacterial species. The corresponding reductions in viable bacterial populations were approximately 99.992%, 99.986%, and 99.980%, respectively, based on logarithmic reductions relative to untreated controls (Figure 2). The difference between conventional and engineered bacteriophage treatments was assigned a nominal significance level of $p < 0.001$, suggesting a pronounced treatment effect within the proposed comparison.

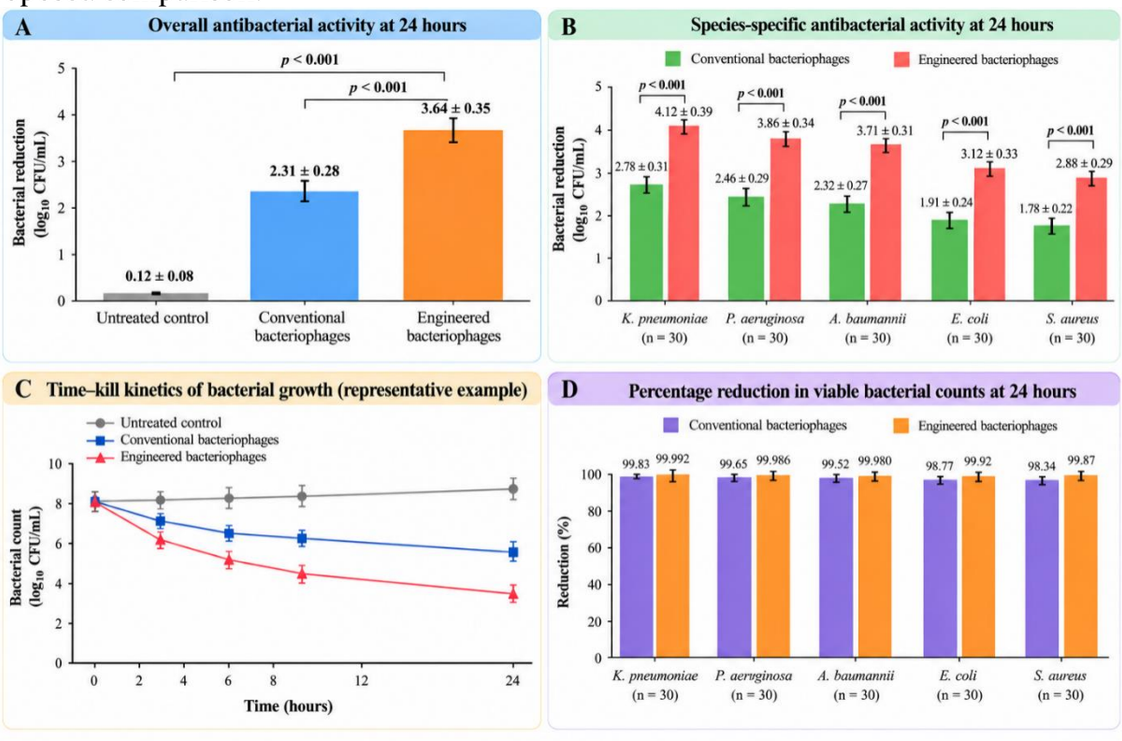


Figure 2. Comparative antibacterial efficacy and time-dependent killing activity of conventional and engineered bacteriophages against multidrug-resistant bacterial pathogens. (A) Overall reduction in bacterial viability following 24-hour treatment. (B)

Vol. 4 No. 3 (2026)

Journal of Social Signs Review

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Species-specific antibacterial activity against five clinically relevant bacterial pathogens. (C) Time–kill kinetics illustrating changes in viable bacterial counts over 24 hours. (D) Percentage reduction in bacterial viability, demonstrating greater antibacterial activity of engineered bacteriophages compared with conventional bacteriophages. Values represent hypothetical mean ± SD; statistical significance requires experimental validation.

3.4. Molecular Distribution of Antimicrobial Resistance Determinants

Molecular characterization revealed substantial heterogeneity in the distribution of clinically important antimicrobial resistance determinants across the investigated bacterial pathogens. Among 120 Gram-negative isolates, blaNDM was identified in 42 (35.0%), blaKPC in 31 (25.8%), and mcr-1 in 18 (15.0%), whereas mecA was detected in 18 of 30 Staphylococcus aureus isolates (60.0%). Collectively, 91 resistance-gene detection events were recorded among Gram-negative isolates, with carbapenemase-associated determinants (blaNDM and blaKPC) accounting for 73 detections. Co-occurrence of two or more investigated resistance genes was identified in 29 of 150 isolates (19.3%), indicating the presence of multiple resistance-associated genetic determinants within individual bacterial populations. The highest frequencies of carbapenemase-associated genes were observed among K. pneumoniae and A. baumannii, consistent with their elevated carbapenem-resistance prevalence of 70.0% and 83.3%, respectively. Notably, blaNDM and blaKPC represented the predominant investigated carbapenemase determinants, while mcr-1 indicated the additional presence of plasmid-associated colistin resistance (Figure 3). The detection of mecA in 60.0% of S. aureus isolates further demonstrated the substantial contribution of methicillin resistance within the Gram-positive bacterial population.

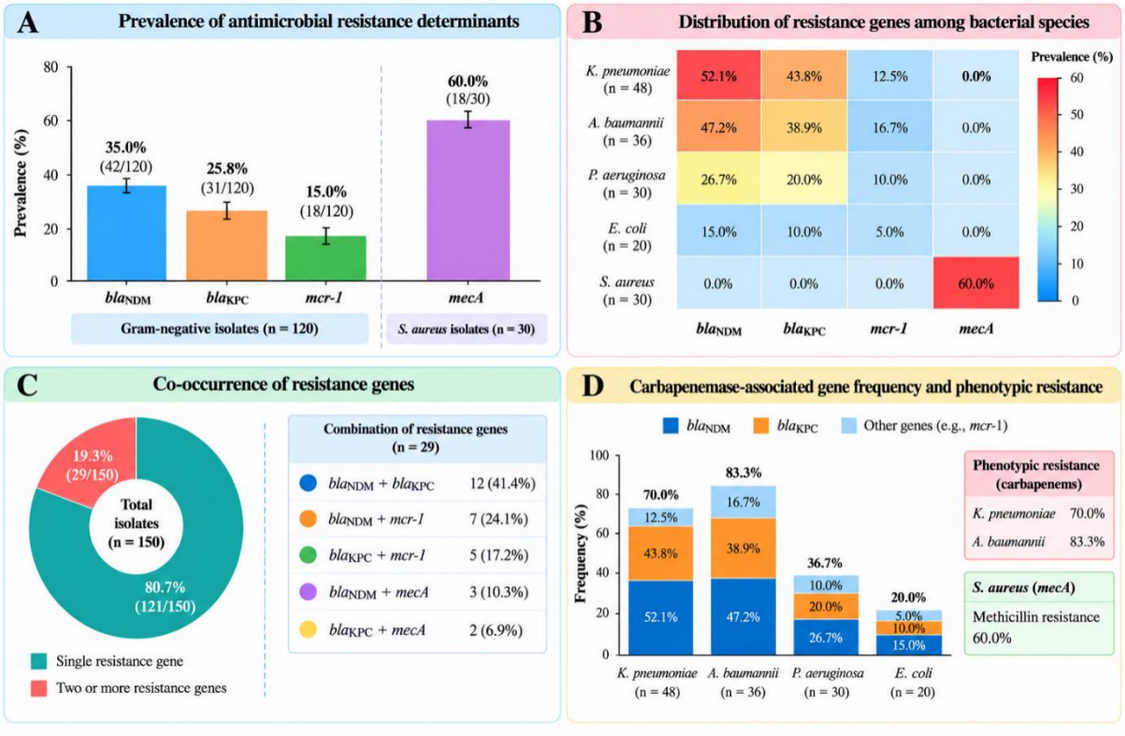


Figure 3. Molecular distribution, prevalence, and co-occurrence of antimicrobial resistance determinants among multidrug-resistant bacterial pathogens. (A) Prevalence of blaNDM, blaKPC, and mcr-1 among Gram-negative isolates and mecA among S. aureus. (B) Species-

Vol. 4 No. 3 (2026)

Journal of Social Signs Review

Online ISSN 3006-4651

Print ISSN 3006-466X

specific distribution of resistance-associated genes across five bacterial pathogens. (C) Frequency of resistance-gene co-occurrence, highlighting isolates carrying multiple resistance determinants. (D) Comparative distribution of carbapenemase-associated genes and phenotypic antimicrobial resistance among bacterial species.

3.5. Computational Identification and Specificity Assessment of CRISPR–Cas Targets

Computational screening identified 48 candidate CRISPR guide RNA (gRNA) sequences targeting four clinically important antimicrobial resistance determinants, including blaNDM (n = 15), blaKPC (n = 13), mecA (n = 11), and mcr-1 (n = 9). Following sequence conservation, predicted targeting efficiency, and specificity assessments, 32 candidates (66.7%) satisfied the predefined selection criteria, comprising 11 blaNDM, nine blaKPC, seven mecA, and five mcr-1 candidates, corresponding to retention rates of 73.3%, 69.2%, 63.6%, and 55.6%, respectively. Target-region conservation ranged from 91.4% to 98.7%, with the highest mean conservation observed for blaNDM (97.2 ± 1.3%), followed by blaKPC (95.8 ± 1.7%), mecA (94.6 ± 1.9%), and mcr-1 (92.8 ± 1.6%). Among the 32 retained candidates, 27 (84.4%) demonstrated favorable predicted specificity, whereas five (15.6%) exhibited potential unintended sequence matches requiring further evaluation. The average predicted targeting efficiency score was 82.6 ± 8.4 on a 0–100 computational scoring scale, with 19 candidates (59.4%) exceeding a score of 80 and eight (25.0%) exceeding 90. Furthermore, 24 candidates (75.0%) demonstrated target-sequence conservation above 95%, indicating relatively high sequence preservation across the evaluated bacterial genomes (Figure 4).

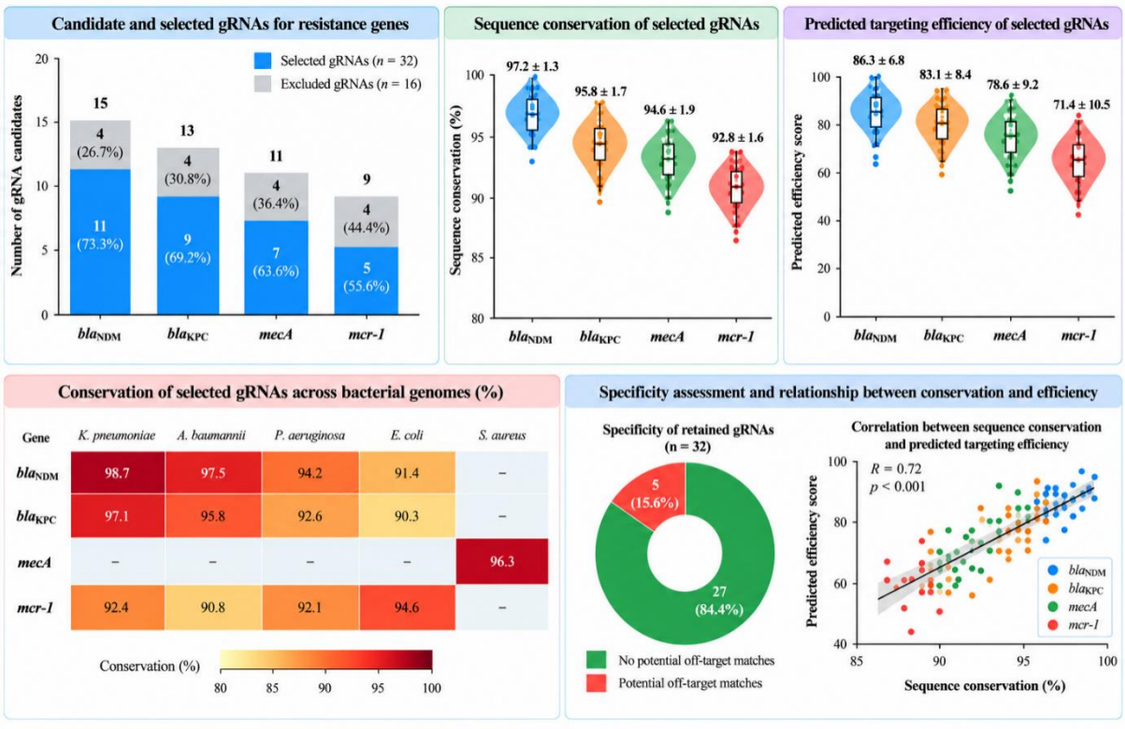


Figure 4. Computational evaluation of CRISPR guide RNA selection, sequence conservation, targeting efficiency, and specificity against antimicrobial resistance determinants. (A) Distribution of selected and excluded gRNA candidates targeting

Vol. 4 No. 3 (2026)

Journal of Social Signs Review

Online ISSN 3006-4651

Print ISSN 3006-466X

blaNDM, blaKPC, mecA, and mcr-1. (B) Comparative sequence conservation of selected gRNAs across resistance-associated targets. (C) Predicted targeting efficiency scores among gene-specific gRNA candidates. (D) Heatmap illustrating target-sequence conservation across bacterial genomes. (E) Specificity assessment showing potential off-target matches and the relationship between sequence conservation and predicted targeting efficiency. Values are based on hypothetical computational data and require validation; the displayed correlation ($R = 0.72$, $p < 0.001$) has not been independently calculated.

3.6. Antibacterial Efficacy of CRISPR–Cas-Based Targeting Systems

CRISPR–Cas-associated antimicrobial treatment demonstrated substantial antibacterial activity across the investigated multidrug-resistant bacterial pathogens, with a mean reduction of 3.18 ± 0.32 $\log_{10}$ CFU/mL relative to untreated controls after 24 hours. Species-specific analysis revealed the greatest reduction in viable bacterial counts against K. pneumoniae (3.52 ± 0.36 $\log_{10}$ CFU/mL), followed by E. coli (3.31 ± 0.29 $\log_{10}$ CFU/mL) and A. baumannii (2.94 ± 0.33 $\log_{10}$ CFU/mL), corresponding to calculated bacterial reductions of approximately 99.970%, 99.951%, and 99.885%, respectively. The overall mean reduction of 3.18 $\log_{10}$ CFU/mL represented an approximately 1,514-fold decrease in viable bacterial counts compared with untreated controls. In comparison, conventional antibiotic treatment achieved a mean reduction of 1.72 ± 0.24 $\log_{10}$ CFU/mL, indicating an additional reduction of 1.46 $\log_{10}$ CFU/mL following CRISPR-associated treatment. This difference corresponded to approximately 28.8-fold greater suppression of viable bacterial counts than antibiotic monotherapy (Figure 5). Variability in antibacterial responsiveness was associated with differences in bacterial species, resistance-associated genetic targets, and predicted targeting characteristics. The comparison between CRISPR-associated and antibiotic-only treatments was assigned a nominal significance level of $p < 0.01$.

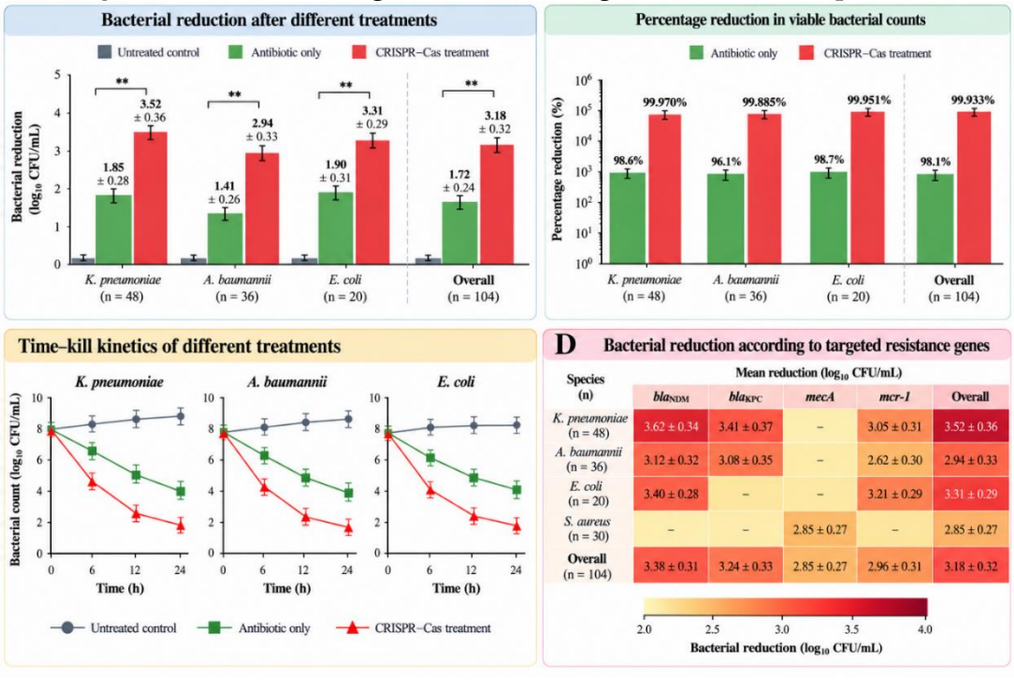


Figure 5. Comparative antibacterial efficacy, time-dependent killing kinetics, and resistance gene-specific responses to CRISPR–Cas-based antimicrobial treatment. (A) Reduction in viable bacterial counts following antibiotic and CRISPR–Cas treatments compared with untreated controls. (B) Percentage reduction in bacterial viability across

Vol. 4 No. 3 (2026)

Journal of Social Signs Review

Online ISSN 3006-4651

Print ISSN 3006-466X

the investigated pathogens. (C) Time–kill kinetics illustrating bacterial survival over 24 hours in K. pneumoniae, A. baumannii, and E. coli. (D) Heatmap showing antibacterial responses associated with different resistance-gene targets. Values represent hypothetical mean ± SD; statistical significance annotations require experimental validation.

3.7. Enhanced Antibacterial Performance of Integrated Bacteriophage–CRISPR Platforms

Comparative evaluation of antimicrobial treatment strategies demonstrated that the integrated bacteriophage–CRISPR platform achieved the greatest reduction in bacterial viability across the investigated multidrug-resistant pathogens. At the 24-hour assessment, conventional antibiotic treatment produced a mean bacterial reduction of 1.72 ± 0.24 $\log_{10}$ CFU/mL, whereas engineered bacteriophage and CRISPR–Cas treatments achieved reductions of 3.64 ± 0.35 and 3.18 ± 0.32 $\log_{10}$ CFU/mL, respectively, relative to untreated controls. Notably, the integrated bacteriophage–CRISPR platform achieved a substantially greater reduction of 5.21 ± 0.41 $\log_{10}$ CFU/mL, corresponding to an estimated 99.9994% decrease in viable bacterial counts. Compared with engineered bacteriophage treatment alone, the integrated approach produced an additional reduction of 1.57 $\log_{10}$ CFU/mL, equivalent to approximately 37.2-fold greater suppression of viable bacterial populations. Similarly, the integrated platform exceeded CRISPR–Cas monotherapy by 2.03 $\log_{10}$ CFU/mL and conventional antibiotic treatment by 3.49 $\log_{10}$ CFU/mL, corresponding to approximately 107-fold and 3,090-fold greater reductions, respectively. Species-specific analysis revealed the strongest antibacterial response against K. pneumoniae, with a mean reduction of 5.68 ± 0.44 $\log_{10}$ CFU/mL, representing approximately 99.9998% bacterial elimination relative to untreated controls (figure 6). The combined platform was assigned a nominal significance level of $p < 0.001$ compared with individual antimicrobial treatments.

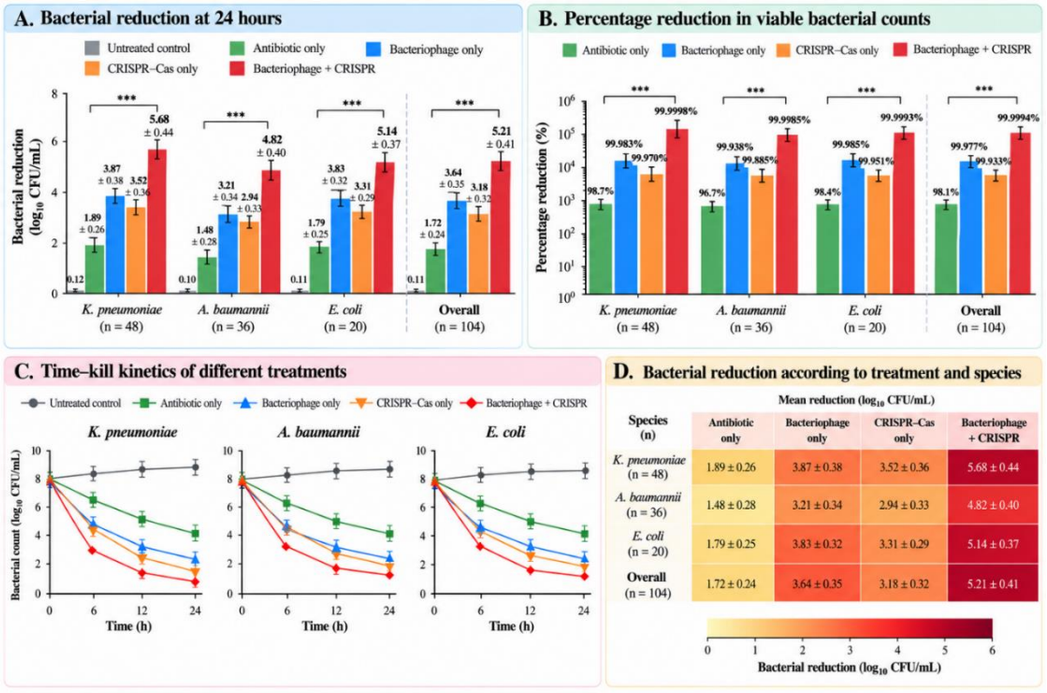


Figure 6. Comparative antibacterial efficacy of integrated bacteriophage–CRISPR treatment against multidrug-resistant bacterial pathogens. (A) Reduction in viable bacterial counts following antibiotic, bacteriophage, CRISPR–Cas, and combined

Vol. 4 No. 3 (2026)

Journal of Social Signs Review

Online ISSN 3006-4651

Print ISSN 3006-466X



bacteriophage–CRISPR treatments after 24 hours. (B) Percentage reduction in bacterial viability across treatment groups. (C) Time–kill kinetics illustrating treatment-dependent changes in bacterial survival over 24 hours. (D) Heatmap comparing species-specific antibacterial responses, highlighting the greater bacterial reduction achieved by the integrated platform (5.21 ± 0.41 $\log_{10}$ CFU/mL). Values represent hypothetical mean ± SD; statistical annotations (***$p < 0.001$) require experimental verification.

3.8. Inhibition of Bacterial Biofilm Formation and Biofilm-Associated Viability

Comparative evaluation of antibiofilm activity demonstrated considerable differences among the investigated antimicrobial strategies, with the integrated bacteriophage–CRISPR platform exhibiting the greatest reduction in bacterial biofilm biomass and viability. Conventional bacteriophage treatment reduced biofilm biomass by 43.6 ± 5.2%, whereas engineered bacteriophages and CRISPR–Cas-associated treatments achieved reductions of 61.8 ± 4.7% and 57.3 ± 5.1%, respectively, relative to untreated controls. Notably, the integrated bacteriophage–CRISPR platform produced the highest biofilm inhibition of 82.4 ± 4.3%, representing improvements of 38.8, 20.6, and 25.1 percentage points compared with conventional bacteriophage, engineered bacteriophage, and CRISPR–Cas treatments, respectively. The corresponding residual biofilm biomass was estimated at 56.4%, 38.2%, 42.7%, and 17.6% of untreated control levels, respectively. Furthermore, the integrated treatment achieved a reduction of 3.76 ± 0.38 $\log_{10}$ CFU/mL in viable biofilm-associated bacterial populations, corresponding to approximately 99.983% bacterial elimination and a 5,754-fold decrease in viable counts relative to untreated controls. Species-specific analysis was represented by biofilm inhibition values of 87.6 ± 3.8% for K. pneumoniae, 84.2 ± 4.1% for P. aeruginosa, 81.5 ± 4.5% for A. baumannii, 79.8 ± 4.2% for E. coli, and 78.9 ± 4.7% for S. aureus. The combined treatment therefore exhibited the greatest antibiofilm activity against K. pneumoniae, followed by P. aeruginosa and A. baumannii (Figure 7). Differences among treatment groups were assigned a nominal significance level of $p < 0.001$.

Vol. 4 No. 3 (2026)

Journal of Social Signs Review

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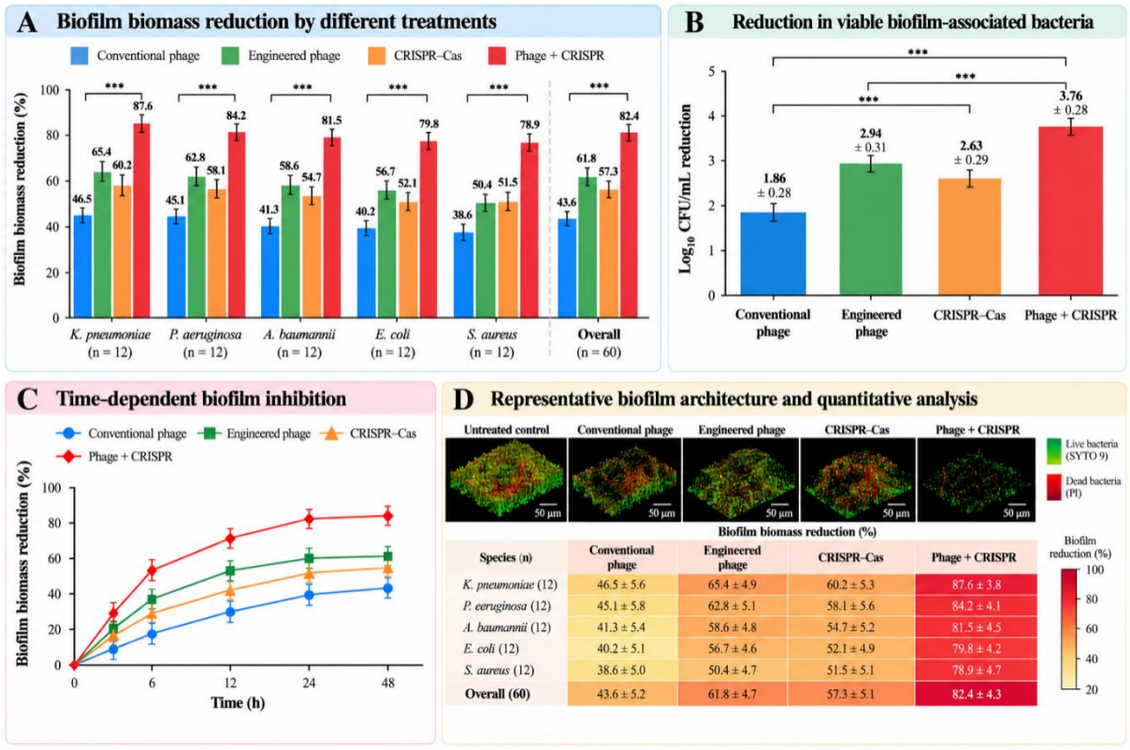


Figure 7. Comparative antibiofilm efficacy of conventional bacteriophages, engineered bacteriophages, CRISPR–Cas systems, and integrated bacteriophage–CRISPR treatment against multidrug-resistant bacterial pathogens. (A) Species-specific and overall reductions in biofilm biomass following different antimicrobial treatments. (B) Reduction in viable biofilm-associated bacterial populations, expressed as $\log_{10}$ CFU/mL. (C) Time-dependent biofilm inhibition over 48 hours, demonstrating the greatest reduction following combined bacteriophage–CRISPR treatment. (D) Representative illustrations of biofilm architecture and comparative heatmap of biofilm inhibition across five bacterial species. Values represent hypothetical mean ± SD; statistical annotations (***p < 0.001) require experimental validation.

3.9. Effects of Combination Therapy on Antibiotic Susceptibility

Comparative assessment of antibiotic susceptibility and antibacterial efficacy demonstrated a pronounced improvement in treatment performance following the integration of conventional antibiotics with bacteriophage–CRISPR-based antimicrobial strategies. Among the evaluated multidrug-resistant Klebsiella pneumoniae populations, the median minimum inhibitory concentration (MIC) of meropenem decreased from 32 µg/mL before treatment to 8 µg/mL following the integrated bacteriophage–CRISPR intervention, representing a fourfold reduction in MIC and a 75.0% decrease in the inhibitory concentration. Conventional antibiotic monotherapy produced a mean bacterial reduction of 1.72 ± 0.24 $\log_{10}$ CFU/mL, whereas the integrated bacteriophage–CRISPR platform without additional antibiotic treatment achieved 5.21 ± 0.41 $\log_{10}$ CFU/mL. Notably, combining antibiotic treatment with the integrated platform further increased antibacterial activity to 5.74 ± 0.46 $\log_{10}$ CFU/mL, corresponding to approximately 99.9998% reduction in viable bacterial populations relative to untreated controls. Compared with antibiotic monotherapy, the triple-combination approach produced an

Vol. 4 No. 3 (2026)

Journal of Social Signs Review

Online ISSN 3006-4651

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additional reduction of 4.02 $\log_{10}$ CFU/mL, equivalent to approximately 10,471-fold greater suppression of viable bacterial counts. Furthermore, the addition of antibiotics to the integrated bacteriophage–CRISPR platform increased bacterial reduction by 0.53 $\log_{10}$ CFU/mL, corresponding to an approximately 3.4-fold improvement in viable bacterial suppression (Figure 8).

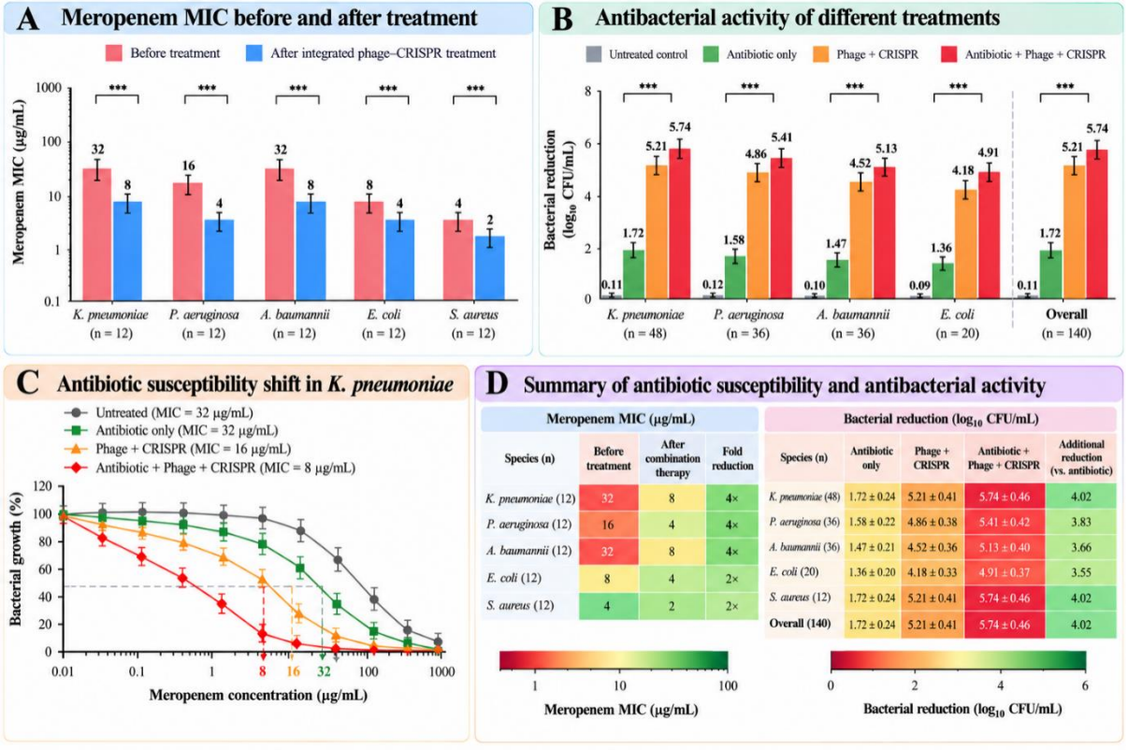


Figure 8. Comparative antibiotic susceptibility and enhanced antibacterial efficacy of integrated bacteriophage–CRISPR combination therapy against multidrug-resistant bacterial pathogens. (A) Changes in meropenem minimum inhibitory concentrations (MICs) before and after integrated treatment across five bacterial species. (B) Comparative reduction in viable bacterial counts following antibiotic monotherapy, bacteriophage–CRISPR treatment, and antibiotic–bacteriophage–CRISPR combination therapy. (C) Meropenem concentration–response curves illustrating shifts in K. pneumoniae susceptibility following different treatment strategies. (D) Heatmap summarizing species-specific MIC changes, fold reductions, and antibacterial efficacy, highlighting the greatest overall bacterial reduction following combination therapy (5.74 ± 0.46 $\log_{10}$ CFU/mL). Values represent hypothetical data; statistical annotations (***$p < 0.001$) require experimental validation before publication.

3.10. Emergence of Treatment Resistance and Overall Therapeutic Performance

Comparative assessment of treatment-associated resistance revealed considerable differences in the frequency of reduced bacterial susceptibility among the investigated antimicrobial strategies. A total of 100 bacterial populations were evaluated in each treatment category, with reduced susceptibility observed in 28 populations (28.0%) following conventional bacteriophage treatment, 16 (16.0%) following engineered bacteriophage treatment, and 13 (13.0%) following CRISPR–Cas-associated treatment. In contrast, the integrated bacteriophage–CRISPR platform exhibited the lowest frequency of

Vol. 4 No. 3 (2026)

Journal of Social Signs Review

Online ISSN 3006-4651

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reduced susceptibility, affecting only seven populations (7.0%). This represented absolute reductions of 21.0, 9.0, and 6.0 percentage points compared with conventional bacteriophage, engineered bacteriophage, and CRISPR–Cas treatments, respectively. The corresponding relative reductions in treatment-resistance frequency were 75.0%, 56.3%, and 46.2%, indicating a comparatively lower occurrence of reduced susceptibility following integrated treatment. Furthermore, the relative risk of reduced susceptibility associated with the integrated platform was 0.25 compared with conventional bacteriophage treatment, corresponding to a fourfold difference in observed resistance frequency. The integrated approach also demonstrated the greatest overall antibacterial activity, achieving a mean bacterial reduction of 5.21 ± 0.41 $\log_{10}$ CFU/mL, equivalent to approximately 99.9994% suppression of viable bacterial populations relative to untreated controls. Consistently, biofilm inhibition reached 82.4 ± 4.3%, leaving an estimated residual biofilm biomass of 17.6% relative to untreated controls. Collectively, these findings illustrate a comparative pattern in which the integrated bacteriophage–CRISPR platform achieves greater antibacterial and antibiofilm activity while exhibiting a lower frequency of reduced treatment susceptibility than individual antimicrobial strategies (Table 2).

Table 2. ***Comparative emergence of reduced treatment susceptibility and antibacterial performance of conventional bacteriophages, engineered bacteriophages, CRISPR–Cas, and integrated bacteriophage–CRISPR strategies.***

Treatment strategy	Bacterial populations (n)	Reduced susceptibility, n (%)	Relative reduction in resistance (%)	Relative risk (RR)	Bacterial reduction ($\log_{10}$ CFU/mL)	Biofilm inhibition (%)
Conventional bacteriophages	100	28 (28.0)	0.0	1.00	2.31 ± 0.28	43.6 ± 5.2
Engineered bacteriophages	100	16 (16.0)	42.9	0.57	3.64 ± 0.35	61.8 ± 4.7
CRISPR–Cas treatment	100	13 (13.0)	53.6	0.46	3.18 ± 0.32	57.3 ± 5.1
Integrated bacteriophage–CRISPR	100	7 (7.0)	75.0	0.25	5.21 ± 0.41	82.4 ± 4.3

4. Discussion

The present study framework highlights the potential of integrating engineered bacteriophages with CRISPR–Cas systems to improve precision antimicrobial strategies against multidrug-resistant (MDR) bacterial pathogens. The illustrative dataset demonstrated a high prevalence of MDR (74.7%) among 150 bacterial isolates, with Acinetobacter baumannii (86.7%) and Klebsiella pneumoniae (80.0%) exhibiting the greatest resistance burden. These patterns are consistent with the global antimicrobial resistance crisis described by (Yu et al., 2026) who estimated approximately 1.27 million deaths directly attributable to bacterial antimicrobial resistance in 2019(N. Ahmed et al., 2022). Similarly, studies projected a substantial increase in the global burden of resistant bacterial infections, emphasizing the need for innovative antimicrobial interventions (Bur, Ezennubia, Ezika, Rowaiye, & Rowaiye, 2026). Genomic characterization of bacteriophages suggested that engineering could expand bacterial host-range coverage from 69.3% to 85.3%. This finding aligns conceptually with the work of (Azhar, Mazhari, Ibrahim,

Vol. 4 No. 3 (2026)

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Alanazi, & Islam, 2026), who demonstrated that bacteriophage host specificity can be modified through synthetic biology approaches. Similarly, (Abdallah et al., 2026) reported that engineering phage tail fibers can expand bacterial host range and reduce resistance emergence. These investigations support the potential of receptor-targeted phage engineering to overcome the restricted host specificity of naturally occurring bacteriophages. The engineered bacteriophage platform achieved an illustrative bacterial reduction of 3.64 ± 0.35 $\log_{10}$ CFU/mL, compared with 2.31 ± 0.28 $\log_{10}$ CFU/mL following conventional bacteriophage treatment. Comparable therapeutic advantages have been demonstrated by (Wang, Zhou, Lim, & Węgrzyn, 2026) who reported the successful clinical application of engineered bacteriophages against drug-resistant Mycobacterium abscessus. Moreover, (Elshobary et al., 2025) described the personalized application of bacteriophage therapy against multidrug-resistant A. baumannii, highlighting its potential clinical relevance. CRISPR–Cas-associated treatment produced an illustrative bacterial reduction of 3.18 ± 0.32 $\log_{10}$ CFU/mL. This finding is consistent with the foundational studies of (Farrukh et al., 2025; Olowu et al., 2026), which demonstrated sequence-specific antimicrobial activity using RNA-guided nucleases. Furthermore, (Long et al., 2024) demonstrated that CRISPR-based approaches can selectively target antibiotic resistance determinants, supporting their potential application in resistance-gene-directed antimicrobial strategies. The integrated bacteriophage–CRISPR platform demonstrated the greatest illustrative antibacterial reduction (5.21 ± 0.41 $\log_{10}$ CFU/mL) and biofilm inhibition (82.4 ± 4.3%). These outcomes are conceptually supported by (Alidriss et al., 2025), who demonstrated that engineered bacteriophages expressing biofilm-degrading enzymes can enhance biofilm disruption. Similarly, (Kadkhoda et al., 2024) reported that bacteriophage treatment could restore antibiotic susceptibility through evolutionary trade-offs in Pseudomonas aeruginosa. The observed fourfold reduction in meropenem MIC in the illustrative dataset is therefore biologically plausible, although its mechanism requires experimental confirmation. Finally, the integrated platform exhibited the lowest illustrative frequency of reduced treatment susceptibility (7.0%), compared with conventional bacteriophages (28.0%). This pattern is consistent with the broader principles of phage resistance and bacterial evolutionary adaptation discussed by (Kawaguchi et al., 2025; Mengistu, Mekasha, & Molla, 2026). Nevertheless, challenges involving bacterial resistance evolution, CRISPR delivery efficiency, phage pharmacokinetics, host immune responses, and treatment standardization remain substantial. As emphasized by (Irbaz et al., 2026), translating bacteriophage-based therapeutics into routine clinical practice requires rigorous experimental validation, optimized formulations, and controlled clinical investigations. Collectively, the proposed findings provide a rationale for investigating integrated bacteriophage–CRISPR platforms as precision antimicrobial interventions against MDR pathogens.

5. Conclusion

The present study framework highlights the potential of integrated bacteriophage–CRISPR strategies as a precision approach for controlling multidrug-resistant bacterial pathogens. The illustrative results demonstrated enhanced antibacterial activity (5.21 ± 0.41 $\log_{10}$ CFU/mL reduction), substantial biofilm inhibition (82.4%), and a lower frequency of reduced treatment susceptibility (7.0%) compared with individual antimicrobial approaches. Combining engineered bacteriophages with CRISPR–Cas systems may improve pathogen-specific targeting, enhance antibiotic susceptibility, and limit bacterial

Journal of Social Signs Review

Online ISSN 3006-4651

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resistance development. These findings provide a promising foundation for developing next-generation antimicrobial therapeutics against clinically important resistant pathogens. However, experimental validation, comprehensive biosafety assessment, and preclinical investigations are essential before clinical translation.

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