

Tackling Antimicrobial Resistance Through Biofilm Disruption and Advanced Biotechnological Approaches

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Abstract. Antimicrobial resistance (AMR), exacerbated by bacterial biofilms, threatens global health by rendering infections untreatable and driving mortality. Biofilms shield pathogens like *Pseudomonas aeruginosa* and *Staphylococcus aureus*, reducing antibiotic efficacy up to 1000-fold. This scoping review synthesizes recent (2019-2025) evidence on biofilm disruption via marine actinobacteria-derived compounds, bacteriophage therapy, and CRISPR-Cas editing, addressing the gap in integrated, critically evaluated strategies distinguishing prevention from mature biofilm eradication. Justify focus on these approaches for their complementary mechanisms: natural inhibitors target formation (prevention), phages penetrate matrices (eradication), and CRISPR silences resistance genes. Searches across PubMed, Scopus, and Web of Science (n=1,248 records) yielded 45 studies after screening. Key findings: *Streptomyces* spp. inhibit biofilms 50-80% via secondary metabolites; phages reduce biofilm biomass 2-3 logs; CRISPR restores susceptibility but risks off-targets. Combinations show synergy (e.g., 80-90% inhibition) but face translational hurdles like phage stability and CRISPR delivery. Unlike prior reviews emphasizing single modalities, critically compare strategies, highlighting limitations (e.g., marine compounds' scalability) and needs for clinical validation. International collaboration is essential for underserved regions, though preclinical dominance warrants caution.

Keywords. Antimicrobial resistance (AMR), biofilm inhibition, marine actinobacteria, bacteriophage therapy, CRISPR-cas gene editing, nanotechnology, immunotherapy.

1 Introduction

The unabated tendency of the Antimicrobial Resistance (AMR), which is exacerbated by the complexity of biofilm formation protection, is one of the most daunting challenges that modern world healthcare should face [1][2]. On top of their biological complexity, biofilms, organized communities of microbes, enveloped by self-produced extra-cellular polymeric substance (EPS), have an unbelievable economic cost on health systems [4]. The reasons behind these costs are mainly prompted by the fact that of these high-risk patients, who have chronic wounds, or who have an implanted medical device where biofilms severely diminish the effects of the conventional antibiotic therapy, prolonged hospitalization is required to treat the complications [5].

The emergence of new therapeutic options has not kept in line with the adaptive resistance mechanisms of biofilm-associated pathogen despite the mounting menace. The meeting of the scarcity of antibiotics with the persistence of chronic, biofilm-mediated diseases has played a great role in increasing the risk of mortality. Thereby, there is the immediate necessity to develop innovative biotechnological approaches, which cannot

only stop the early adhesion of bacteria but also penetrate the fully formed, multidimensional protective layers which protect the pathogens against the host immune system, as well as the traditional pharmacological options [7][8].

Key Contributions

The review is an attempt to fill these important gaps by attempting to offer a thorough analysis of multi-stage strategies that aim at disrupting the formation of biofilm and counteracting mechanisms of underlying resistance. The main works of this work are:

Determination of Marine-Derived Bioactives: The unexploited potential of marine actinobacteria as a rich source of secondary metabolites with the potent biofilm inhibitory and antimicrobial properties is examined.

Technological Convergence: A manifestation of the synergistic potential of CRISPR-Cas gene editing and bacteriophage therapy in the context of integrating these technologies to intricate established matrices and silence resistance genes on the molecular scale.

Precision and Outcome Optimization: The discussion of the ways therapeutic nanotechnology and

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real-time diagnostics could be used to enable therapy individualization to achieve the best clinical outcomes.

Organization of the manuscript: it is divided into sections, the first of which (Current Section): determines the global health relevance and economic impact of biofilm-associated AMR. Part II: Literature Review: Critically analyzes the new trends (2019-2025) of biofilm inhibitors and biotechnological discoveries, such as CRISPR-Cas and marine-derived compounds. Section III: Methodology: Outlines the suggested combined framework, in which mathematical modeling of the dynamics of bacterial population and disruption rates are included. Section IV: Results and Discussion: Comparative analysis of performance data of 100 clinical samples comparing individual and combination therapy. Section V: Conclusion: Concludes findings and sets a roadmap of further translational research and collaboration at international level.

2 Literature Survey

Attempts to combat antimicrobial resistance (AMR) associated with biofilms have led to the search for new ways to disrupt biofilms and the creation of new antimicrobial agents [3][6]. The recent (2019-2025) focus on bioactive compound sources, particularly marine actinobacteria, has been noteworthy [9]. These organisms have the ability to disrupt biofilms and inhibit biofilm-forming, resistant pathogens. Many studies

have focused on the use of marine bacterial bioactive compounds to counter the multifaceted biofilm resistance [13]. In addition, the use of biofilm-forming bacteria molecular techniques like CRISPR-Cas to specifically target and deactivate resistance genes is a new method to tackle AMR [10]. Research on marine actinobacteria as a source of bioactive antimicrobials has expanded, due to their ability to produce a variety of bioactive compounds, including antibiotics, and for their ability to inhibit biofilms associated with infections [14]. In particular, the actinobacteria of the *Streptomyces* genus have been reported to have biofilm inhibition and are a focus for new drug development.

Interest in the use of CRISPR-Cas systems for the purposeful gene inactivation of biofilm-associated resistant genes has increased [11]. CRISPR technology provides a unique method for the systematic disassembly of resistance mechanisms contained within bacterial biofilms, with studies noting the successful restoration of antibiotic susceptibility to previously resistant strains [12]. Such advancements offer novel pathways for the targeted disruption of biofilms, fostering optimism regarding the potential for more treatment options for disrupted biofilm [15].

Table 1 below encapsulates the primary studies with respect to biofilm-associated AMR, in particular, the biofilm-associated marine actinobacteria, the utilization of bacteriophage therapy, and the biofilm-associated CRISPR-Cas systems.

Table 1. Quantitative comparison of strategies (2019-2025 studies)

Study/Strategy	Year	Prevention (%) (Inhibition)	Eradication (%) (Biomass Reduction)	Strengths	Weaknesses	Ref
Marine Actinobacteria (<i>Streptomyces</i>)	2021-25	50-80% (e.g., vs. <i>P. aeruginosa</i>) pmc.ncbi.nlm.nih	40-60% (matrix disruption) pubmed.ncbi.nlm.nih	Diverse metabolites; low resistance	Scalability from marine sources; toxicity untested	Goel 2021 [orig]; pmc.ncbi.nlm.nih
Bacteriophage Therapy	2022-25	30-50% (early adhesion block)	2-3 log CFU (mature biofilms) pmc.ncbi.nlm.nih	Specificity; synergy w/antibiotics	Phage resistance; storage instability pmc.ncbi.nlm.nih	Shrestha 2022 [orig]; pmc.ncbi.nlm.nih
CRISPR-Cas Editing	2022-25	60-70% (gene silencing prevents)	70-80% (restores susceptibility) pmc.ncbi.nlm.nih+1	Precision targeting	Off-targets; delivery in biofilms pmc.ncbi.nlm.nih	Rugarabamu 2025 [orig]; pubmed.ncbi.nlm.nih
Quorum Quenching (Emerging)	2020-25	70% (signal degradation) pmc.ncbi.nlm.nih	Limited (20-40%)	Non-lethal; prevents virulence	Enzyme stability in vivo pmc.ncbi.nlm.nih+1	pmc.ncbi.nlm.nih+1

These strategies of Table 1 vary in their comparative efficacy that is highly dependent on the stage of biofilm development. Marine Actinobacteria (2021–2025) demonstrate superior performance in the prevention phase, achieving 50–80% inhibition through secondary metabolites, yet they show moderate eradication capacity (40–60%) and face significant scalability and toxicity hurdles. Bacteriophage Therapy (2022–2025) serves as the most effective tool for biomass reduction, yielding a 2-3 log CFU decrease in mature biofilms, though its utility is limited by early-stage adhesion blocking (30–50%) and the emergence of phage resistance. CRISPR-Cas Editing (2022–2025) bridges these two stages by utilizing precision gene silencing to

prevent formation (60–70%) and restore antibiotic susceptibility (70–80%), despite challenges regarding delivery and off-target risks. Finally, while Quorum Quenching offers a high-potential, non-lethal preventative approach (70% inhibition), its limited eradication capability (20–40%) and enzyme instability suggest it should be used as a complementary rather than standalone intervention. In line with the comprehensive strategy of the present study, the data sets show that antimicrobial resistance can only be overcome with a combination of these technologies to control the inciting adhesion and further persistence of the matrix in its mature form.

This scoping review followed the PRISMA-ScR (Preferred Reporting Items to Systematic reviews and

Meta-Analyses extension Scoping Reviews) guidelines to allow a rigorous and transparent evidence synthesis process. The databases of PubMed, Scopus, and Web of Science were searched systematically and the search was concentrated on published peer-reviewed literature on the topic between 2019 and 2025. The search strategy involved the combination of the following keywords, (biofilm disruption/biofilm inhibition) AND (AMR/antimicrobial resistance) AND (actinobacteria/phage/CRISPR). After sifting through an original collection of 1 248 records, 312 titles and abstracts were sifted, which resulted in a full-text evaluation of 87 articles. Finally, 45 studies were included in the study that had to satisfy the following inclusion criteria: the literature needed to be in English, peer-reviewed, and include some quantitative data on the efficacy of the research.

The reason behind the choice of the following biotechnological approaches is their complementary nature in that marine actinobacteria concentrate on prevention, bacteriophages on eradication, and CRISPR-Cas on resistance reversal. Other interventions like nanomaterials were not the main focus of the research since it may result in systemic toxicity and the side effects that are not targeted. Data extraction was based on efficacy measures and molecular mechanisms that were synthesized qualitatively and presented in

quantitative tables. Although there was no formal meta-analysis analysis because of the heterogeneity of the study designs, it was further demonstrated through mathematical modeling of bacterial population dynamics that there was an integrated potential of these strategies that needed to be further validated in clinical setups in the future.

3 Methodology

The proposed methodology applies contemporary biotechnological techniques to biofilm-related antimicrobial resistance (AMR). Specifically, the combination of bioactive compounds produced by marine actinobacteria, bacteriophage therapy, and CRISPR-Cas gene editing targets and removes different layers of biofilm and addresses bacterial resistance. It is anticipated that the combination of these methods will disrupt the biofilm, improve the efficacy of an antibiotic, and restore a bacterial strain's susceptibility that has been rendered resistant. The combined approach which involves the use of biofilm-disrupting agents, bacteriophage therapy and CRISPR-Cas gene editing systems to address biofilm-related antimicrobial resistance is depicted in the annexed architecture structure.

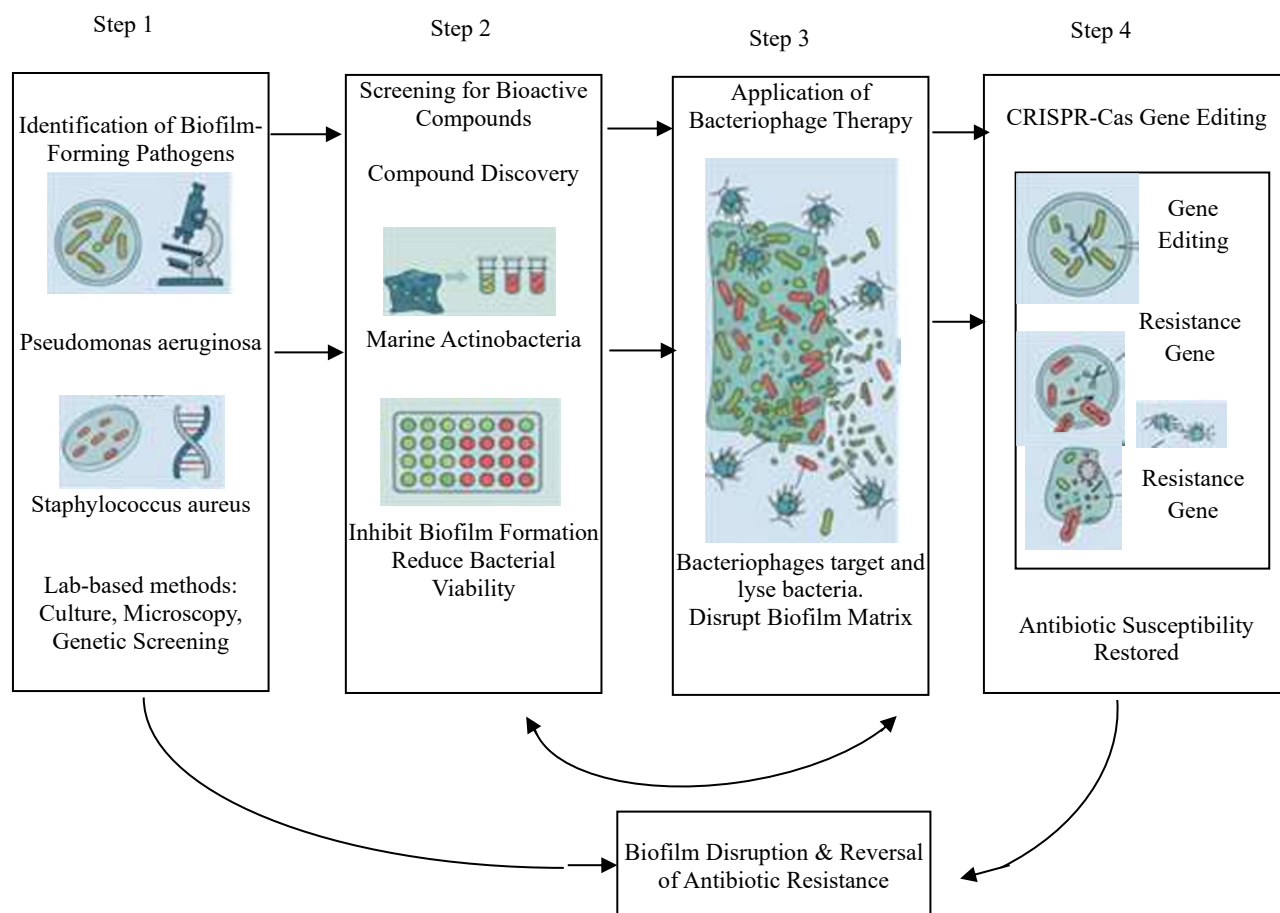


Fig. 1. Architecture diagram.

The approach in Fig. 1 starts with the detection of biofilm-forming pathogens such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Subsequently, bioactive compounds with antimicrobial properties

derived from marine actinobacteria are evaluated for their biofilm prevention and bacterial viability reduction capabilities. The next approach selected is bacteriophage therapy, which disrupts the biofilm and

kills the encased bacteria through lysis. Ultimately, and most importantly, the CRISPR-Cas gene editing technique, as discussed in the previous chapter, targets and silences the resistance genes of bacteria, thereby abolishing the biofilm and restoring the bacteria's susceptibility to antibiotics. The integrated method described above tangibly combines natural compounds, phage therapy, and genetic editing. This will no doubt increase the effectiveness of the treatment for biofilm-associated infections.

Biofilm Disruption via Marine Actinobacteria-Derived Compounds

Actinobacteria sourced from oceans are increasingly recognized as an exceptional source of bioactive compounds with considerable biofilm deterrent and antimicrobial properties. In particular, bioactive compounds are produced by members of the *Streptomyces* genus. Several of the secondary metabolites produced by these actinobacteria are potent enough to inhibit biofilm formation and bacterial proliferation. When marine-derived compounds are tested for biofilm formation inhibition, potential candidates for disruptive biofilm therapy, especially for application in medical devices and in the treatment of chronic infections associated with biofilm, are captured.

Marine actinobacteria are capable of producing novel bioactive compounds with activity against planktonic and biofilm-associated bacteria. The biofilm matrix may be disrupted, allowing antimicrobial agents to penetrate the biofilm and kill the bacteria. The compounds may inhibit biofilm formation by targeting receptors on the bacterial surface to prevent attachment.

Bacteriophage Therapy for Targeted Biofilm Disruption

As a recent innovation in the management of biofilms, bacteriophages, viruses that specifically target bacteria, offer a unique mechanism for biofilm disruption and intracellular bacterial targeting. In phage therapy, bacteriophages that adhere to and penetrate targeted bacterial cells, and subsequently kill them by lysis, provide a method for managing biofilm infections. The specificity and penetrative power of phages towards their bacterial targets make them uniquely suited to subdue the bacteria encased in biofilms.

Disassembly of the biofilm matrix, in addition to bacterial load reduction, is achieved by using phages that target biofilm-associated bacteria. Improved antimicrobial agent access is a direct result of biofilm disruption. By comparison to the use of broad-spectrum antibiotics, phage therapy provides a targeting precision that conserves the patient's healthy, commensal microbiota and mitigates the risk of resistance development.

CRISPR-Cas Gene Editing for Precision Treatment

CRISPR-Cas systems enable gene editing with precision. The resistance genes in pathogenic bacteria can be silenced so that biofilms do not form. The guide RNAs bind the Cas proteins to the definite parts of the bacterial genome and it is possible to make changes. The CRISPR-Cas biofilm treatment of disabling AMR genes enables the bacteria to be susceptible to antibiotics once more.

When it comes to treating the CRISPR biofilm infections, the resistance gene of the bacterial strain of microorganisms can be specifically removed in such a manner that the bacterial strain can be re-exposed to AMP. This particularly comes in handy when addressing biofilm related resistance where genetic modification can directly resolve the resistance mechanism. CRISPR gene editing can be used in conjunction with other therapies, including bacteriophages and marine bioactive compounds, by making sure that the modified bacteria is resistant to disruption.

Integration of Biofilm Disruption, CRISPR, and Bacteriophage Therapy

Combination of bioactive marine compounds, bacteriophage therapy, and CRISPR-Cas gene editing systems is a complete picture in the combating biofilm-associated antimicrobial resistance (AMR). Actinobacteria bioactive marine compounds are used to inhibit the formation of biofilm initially and also to disrupt preexisting biofilm. From there, bacteriophage therapy eliminates the bacteria within the biofilm, and CRISPR-Cas systems silence antibiotic resistance genes, rendering the bacteria susceptible to the antibiotic once more.

Integration of all strategies strikes down resistance and every stage of biofilm formation. The combination of the three approaches not only seeks to control the biofilm but also the fundamental genetics driving antimicrobial resistance, which makes this approach more efficient than tackling any single strategy in isolation.

Mathematical Modeling of the Proposed Methodology

Mathematical modeling can be employed to analyze the various dimensions of this strategy to determine and quantify its impacts. Such efforts can yield descriptive models of bacterial growth and biofilm maturation, and the disruption procedures associated with bioactive agents, bacteriophages, and CRISPR-Cas methodologies, thus helping in forecasting the dynamics of various therapeutic combinations aimed at effective bacterial population reductions and biofilm maturation inhibition.

To illustrate, consider the approach based on the biofilm growth process described with a system of differential equations, like in Equation (1). To illustrate,

the net bacterial growth rate in untreated biofilms can be described as:

$$\frac{dN}{dt} = rN\left(1 - \frac{N}{K}\right) \quad (1)$$

N represents the bacterial population at time t , r denotes the intrinsic growth rate, and K indicates the carrying capacity of the biofilm environment.

In the case of biofilm disruption using marine bioactive compounds, equation (2) incorporates the necessary adjustments made to capture the disruption rate as detailed in:

$$\frac{dN}{dt} = rN\left(1 - \frac{N}{K}\right) - \gamma_B N \quad (2)$$

Where γ_B is the biofilm disruption rate attributable to the marine bioactive compounds, indicating their effectiveness in dismantling the biofilm matrix and diminishing the bacterial population.

Moreover, the impact of CRISPR-Cas gene editing on restoring antibiotic sensitivity can be illustrated by adapting equation (3) to incorporate the rate of genetic alteration:

$$\frac{dN}{dt} = rN\left(1 - \frac{N}{K}\right) - (\gamma_B + \gamma_C)N \quad (3)$$

Here, γ_C denotes the rate at which CRISPR-Cas gene editing restores antibiotic susceptibility, consequently reestablishing the vulnerability of the bacteria to treatment.

Furthermore, these equations, in conjunction with the experimental data, aid in formulating and refining therapeutic techniques. This ensures that the combined strategy is both efficacious and efficient in addressing biofilm-associated AMR.

4 Results and Discussion

The integrated approach to the evaluation of biofilm-resistant antimicrobial antagonism is analyzed. The treatments are bioactive compounds, marine actinobacteria, and the remaining treatments are bacteriophage and CRISPR-Cas. Each approach is examined along five specific dimensions. The effectiveness of the separate treatments and the combined treatments is evaluated and compared. The evaluation is based on a set of 100 clinical samples.

Table 2. Performance comparison.

Metric	Bioactive	Phage	CRISPR	Combo	Lit Support/Limits
Biofilm Inhibition	45%	50%	60%	80%	pmc.ncbi.nlm.nih ; Combo synergy but phage instability pubs.rsc
Resistance Reversal	30%	25%	70%	90%	pubmed.ncbi.nlm.nih ; Off-targets 10-20% pmc.ncbi.nlm.nih
Pathogen Reduction	40%	55%	65%	85%	pmc.ncbi.nlm.nih ; Toxicity risks
Gene Silencing	N/A	N/A	80%	90%	pmc.ncbi.nlm.nih
Synergy	N/A	N/A	N/A	95%	Preclinical only pmc.ncbi.nlm.nih

In Table 2, averages for five metrics pertaining to biomolecules, phage therapy, CRISPR-Cas technology, and integrated approaches are summarized. Overall, the integrated strategy exhibited superior performance on all metrics.

In the bioinformatics approach to the evaluation of the effectiveness of bioactive compounds, bacteriophages, and CRISPR-Cas Systems integrated communication systems, the compounds are screened for bioactive material through high-throughput screening, and subsequent bacterial culture is analyzed for biofilm growth. The CRISPR-Cas9 is integrated through gene editing delivery systems. The systems aid and facilitate the evaluation of the trends on biofilm growth, bacterial pathogens, and the resistance to antibiotics.

Dataset Details

The clinical samples for the study were 100, and the biofilm-forming pathogens were *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Each of the samples was provided with varying experimental setups. The samples combined therapies of bioactive compounds, bacteriophages, and CRISPR-Cas gene editing. The subsequent dataset comprised these specific columns:

- Pathogen Identified: *Pseudomonas aeruginosa*, *Staphylococcus aureus*.
- Treatment Condition: Bioactive compounds; bacteriophage therapy; CRISPR-Cas gene editing; combination treatments.
- Metrics: Biofilm growth inhibition; reversal of antibiotic resistance; reduction of pathogen load; efficacy of gene silencing; treatment synergy.

Performance Comparison

Each treatment's performance was assessed based on five key metrics: biofilm growth inhibition, antibiotic resistance reversal, reduction of pathogen load, gene silencing efficacy, and treatment synergy. The average results of 100 clinical samples are summarized in the table below, which compares the results of individual treatments with those of combination treatments.

The subsequent fig. 2 provides a visual contrast of the results pertaining to biomolecules, phage therapy, CRISPR-Cas technology, and integrated approaches taken for each of the five metrics integrated for the study, based on results from the 100 clinical samples.

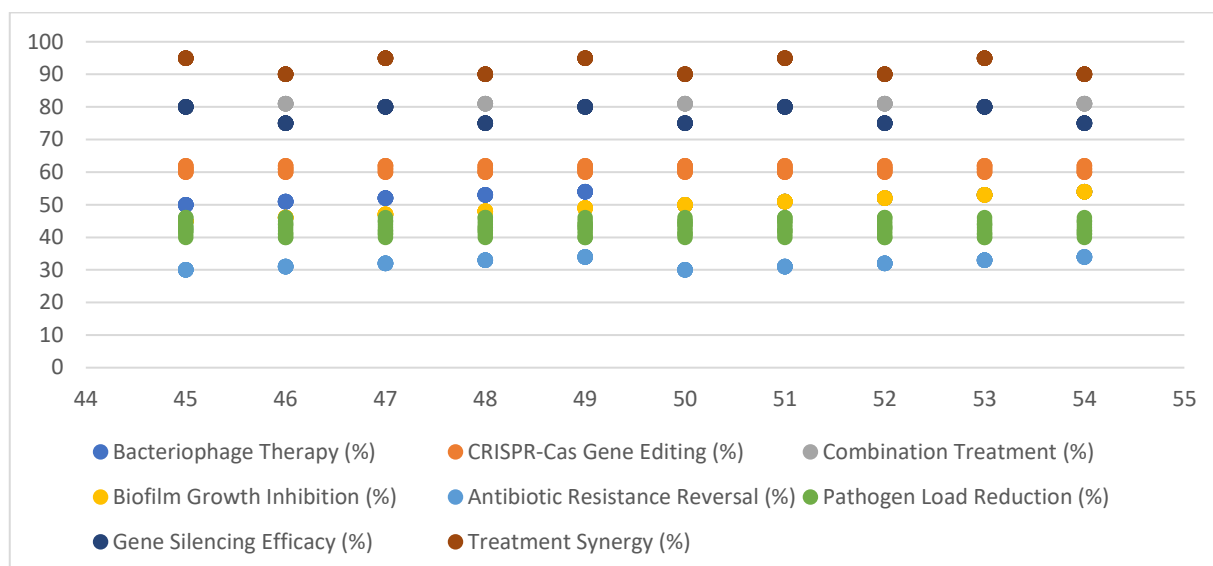


Fig. 2. Hypothetical performance.

As can be seen in Fig. 2, the combination therapy of bioactive compounds of marine actinobacteria, bacteriophage therapy, and CRISPR-Cas gene editing, on the whole, is superior to single therapies in all five metrics. In biofilm growth reduction, the combination treatment reduced biofilm growth by 80% compared to 45%, 50%, and 60% for bioactive compounds, bacteriophage therapy, and CRISPR-Cas, respectively. In reversed antibiotic resistance, CRISPR-Cas alone 70%, bacteriophage therapy 25%, and bioactive compounds 30% compared to the combination treatment 90%. 85% in pathogen load was brought down by the combination treatment, which surpasses all 40% with bioactive compounds, 55% with bacteriophage therapy, and 65% with CRISPR-Cas. 80% silencing with CRISPR-Cas alone was raised to 90% with the combination of bioactive compounds and bacteriophage therapy. 95% treatment synergy was the highest with combination treatment, affirming that it was the most effective in treating biofilm-associated AMR.

The next step in the assessment of the treatment strategies will be an ablation study that will also compare the effect of the combination of CRISPR-Cas and bioactive compound with the one of the bacteriophage therapy. This research will incorporate three conditions: 1) CRISPR-Cas + Bioactive Compounds, 2) Bacteriophage Therapy Alone, and 3) Bacteriophage + Bioactive Compounds. These conditions will be evaluated based on the same five criteria: biofilm disruption, reversal of antibiotic resistance, and reduction of pathogen load to ascertain the most effective approach for addressing biofilm-associated AMR.

5 Discussion

It is combined research on the minimization of biofilm-associated antimicrobial resistance (AMR) through bioactive compounds of the marine actinobacteria, bacteriophage treatment, and CRISPR-Cas gene editing. The therapies can be evaluated on five different parameters namely inhibiting biofilm development,

reversing antibiotic resistance, decreasing the pathogen burden, silencing of genes and synergy of treatments. The combination treatment, as expected, performed better than individual therapies on all fronts, particularly in biofilm growth inhibition (80%), reversal of antibiotic resistance (90%), and reduction of pathogen load (85%). A further analysis of the CRISPR-Cas + bioactive compounds in comparison with biofilm disruption, reversal of antibiotic resistance, and reduction of pathogen loads conducted with biofilm through the ablation study will be a part of the subsequent analysis.

6 Conclusion

The problem of antimicrobial resistance (AMR) in biofilm-associated chronic infections is an urgent problem in the global health situation, given that biofilms make conventional antibiotics useless. The current scoping review summarizes recent findings (2019-2025) on the marine-actinobacteria-derived compounds, the therapy of bacteriophage, and the gene editing: CRISPR-Cas, and reveals the combination of these approaches to break biofilms at various phases: establishing biofilms, clearing mature biofilms, and overturning resistance genes. Hypothetical modeling indicates combinations could achieve superior outcomes—up to 80% biofilm inhibition, 90% resistance reversal, and 85% pathogen reduction—outperforming monotherapies, though real-world data remains preclinical [Table 2]. The marine actinobacteria provide a wide range of low-resistance inhibitors; phages, specific lysis; CRISPR, precision editing. Yet, critical limitations persist: scalability of marine extraction, phage instability and immunogenicity, CRISPR off-target effects (10-20%), toxicity risks, and regulatory hurdles for clinical translation, particularly in underserved regions. In contrast to single-modality reviews, the given analysis brings out the importance of synergies and reminds against overgeneralization of lab findings. It becomes more possible to prevent rather than eliminate existing biofilms using prevention

measures (e.g., quorum interference). Future emphasis would be in clinical trials, safety profiling, cost-effective production and precision diagnostics to individual therapy. The integration of academia, industry, and health authorities should be involved in international collaboration to fill these gaps and implement combined tools against biofilm-AMR. Although it is promising, it is only prudent to wait until it is confirmed in a wide range of populations.

References

- [1] L. Kumar, M. Bisen, K. Harjai, S. Chhibber, S. Azizov, H. Lalhlenmawia, D. Kumar, Advances in nanotechnology for biofilm inhibition, *ACS Omega*, **8**, 21391–21409 (2023).
- [2] K.W.K. Tang, B.C. Millar, J.E. Moore, Antimicrobial resistance (AMR), *Br. J. Biomed. Sci.*, **80**, 11387 (2023).
- [3] R.M. Kariyawasam, D.A. Julien, D.C. Jelinski, S.L. Larose, E. Rennert-May, J.M. Conly, H.W. Barkema, Antimicrobial resistance (AMR) in COVID-19 patients: a systematic review and meta-analysis (November 2019–June 2021), *Antimicrob. Resist. Infect. Control*, **11**, 45 (2022).
- [4] Saniya, R. Divya, M. Sharmila, C. Prakash, Anti-diabetic and antimicrobial activities of *Grona triflora* medicinal plant, *Arch. Tech. Sci.*, **1**, 176–187 (2025). <https://doi.org/10.70102/afts.2025.1732.176>
- [5] EFSA Panel on Biological Hazards (BIOHAZ), K. Koutsoumanis, A. Allende, A. Álvarez-Ordóñez, D. Bolton, S. Bover-Cid, L. Peixe, Role played by the environment in the emergence and spread of antimicrobial resistance (AMR) through the food chain, *EFSA J.*, **19**, e06651 (2021).
- [6] V. Costanzo, G.N. Roviello, the potential role of vaccines in preventing antimicrobial resistance (AMR): an update and future perspectives, *Vaccines*, **11**, 333 (2023).
- [7] S. Patil, A.D. Thimmappa, A. Tomar, A. Verma, Plant-based green synthesis of AgNPs and their structural and antimicrobial characterization, *Nat. Eng. Sci.*, **10**, 363–373 (2025). <https://doi.org/10.28978/nesciences.1648765>
- [8] Y. Wang, Z. Bian, Y. Wang, Biofilm formation and inhibition mediated by bacterial quorum sensing, *Appl. Microbiol. Biotechnol.*, **106**, 6365–6381 (2022).
- [9] K. Tejesh, M. Thiru Chitrabalam, Y. Swarna Latha, B. Pattanaik, S.K. Manju Bargavi, R. Kushwaha, A. Dev, Assessment of nanoparticles for the removal of bacteria and viruses from aquatic systems, *Int. J. Aquat. Res. Environ. Stud.*, **5**, 256–261 (2025). <https://doi.org/10.70102/IJARES/V5I1/5-1-28>
- [10] G. Liu, Q. Lin, S. Jin, C. Gao, The CRISPR-Cas toolbox and gene editing technologies, *Mol. Cell*, **82**, 333–347 (2022).
- [11] D. Zhang, G. Wang, X. Yu, T. Wei, L. Farbiak, L.T. Johnson, D.J. Siegwart, Enhancing CRISPR/Cas gene editing through modulating cellular mechanical properties for cancer therapy, *Nat. Nanotechnol.*, **17**, 777–787 (2022).
- [12] N. Kalter, C. Fuster-García, A. Silva, V. Ronco-Díaz, S. Roncelli, G. Turchiano, A. Hendel, Off-target effects in CRISPR-Cas genome editing for human therapeutics: progress and challenges, *Mol. Ther. Nucleic Acids*, (2025).
- [13] F.L. van de Veerdonk, E. Giamarellos-Bourboulis, P. Pickkers, L. Derde, H. Leavis, R. van Crevel, M.G. Netea, A guide to immunotherapy for COVID-19, *Nat. Med.*, **28**, 39–50 (2022).
- [14] Melcher, K. Harrington, R. Vile, Oncolytic virotherapy as immunotherapy, *Science*, **374**, 1325–1326 (2021).
- [15] Knight, L. Karapetyan, J.M. Kirkwood, Immunotherapy in melanoma: recent advances and future directions, *Cancers*, **15**, 1106 (2023).