Revolutionizing Precision Medicine: Novel Strategies to Combat Multi-Drug Resistant Bacteria

Sudhair Abbas Bangash[1], Ghazala Rasool[2], Hafiz Osman Ibnidris Almisbah[3], Ahmed Hamad Ahmed Sulaiman[4], Syed Shahab Ud Din Shah[5], Ehtisham Khyzer[6]

[1]Faculty of Life Science, Department of Pharmacy, Sarhad University of science and information technology, Peshawar, Pakistan

[2]MBBS FCPS General medicine. ASSISTANT Professor department of medicine faculty of medicine Northern Border University Arar KSA

[3]MD internal medicine Assistant professor of medicine Department of medicine Faculty of medicine Northern Border University Arar, KSA

[4]MD internal medicine Assistant professor of medicine Department of medicine Northern Border University Arar, KSA

[5]Department of Biochemistry, Quaid-I-Azam University, Islamabad, Pakistan

[6]MD, MBBS, MRCPI, FCPS(Med), FCPS (RHEUMATOLOGY) Assistant professor Medicine college of medicine Northern Border University Arar KSA

Corresponding author:

Faculty of Life Science, Department of Pharmacy, Sarhad University of science and information technology, Peshawar, Pakistan

Email ID: sudhair.fls@suit.edu.pk

ABSTRACT

Introduction: MDR bacterial infections represent a substantial worldwide health problem that affects medical care quality. This investigation analyzes the performance of CRISPR-Cas9 gene editing and bacteriophage treatment and antimicrobial peptides (AMPs) against MDR bacteria for an alternative treatment approach.

Methodology: The study enrolled 150 MDR bacterial infection patients at Hayatabad Medical Complex, Peshawar who received treatment from one of three groups consisting of CRISPR-Cas9 therapeutic interventions as well as bacteriophage therapy and antimicrobial peptide treatment. The research project lasted for 12 consecutive months during the period from January 2024 to December 2024. The measured treatment results included both bacterial load reduction and biofilm inhibition alongside whole treatment effectiveness. The researchers used paired t-tests along with ANOVA for statistical evaluation.

Results: The CRISPR-Cas9 gene editing treatment delivered the most powerful outcomes by decreasing bacterial load by 75.14% and producing 75.37% biofilm suppression resulting in an 82.63% efficiency level. The treatment success rate of bacteriophage therapy reduced bacterial populations by 64.94% compared to antimicrobial peptides which decreased bacterial numbers to 54.62%. ANOVA results confirmed the existence of treatment-related differences ($p < 0.001$) which demonstrated how precision medicine surpassed traditional antibiotics in effectiveness.

Conclusions: The study proves CRISPR-Cas9 and bacteriophage therapy and antimicrobial peptides achieve efficient treatment results against MDR bacterial infections. CRISPR-Cas9 showed the most potential development beyond traditional antibiotics because of its several advancements. The optimization of these new therapeutic strategies demands additional research about combined treatments and their extended-term effects.

Keywords: Multi-drug-resistant bacteria, CRISPR-Cas9, Bacteriophage therapy, Antimicrobial peptides, Precision medicine, Antimicrobial resistance, Biofilm inhibition

1. INTRODUCTION

MDR bacteria represent a substantial international medical and public health challenge because they threaten both modern medical practices and health systems around the globe [1]. Resistance in bacteria develops mainly because of antibiotic overprescription and improper usage which selects strains that become resistant to standard medical treatments [2]. Pathogens responsible for these infections produce prolonged medical hospitalization combined with elevated medical costs and mortality rates [3]. Antimicrobial resistance (AMR) stands among the top global public health threats according to the World Health Organization because it demands innovative solutions to fight drug-resistant pathogens [4].

New antibiotic development has traditionally served as the central solution to fight bacterial resistance [5]. The evolution rate of bacterial resistance mechanisms exceeds the speed of new antibiotic creation so scientists now face a severe crisis regarding effective treatment solutions [6]. The intricate defense mechanisms of MDR bacteria have evolved to include efflux pumps as well as drug degrading enzymes that target antibiotic compounds and drug target modifications [7]. The need for a research paradigm shift in antimicrobial medicine now directs scientists to create precision medicine treatments based on pathogen and host genetic and molecular profiles.

The development of targeted bacterial infection treatments relies on modern analytical technologies such as genomics together with transcriptomics and proteomics [8]. New research has investigated four promising antibiotic alternatives which include bacteriophage therapy together with antimicrobial peptides and CRISPR-Cas9 gene editing and host-directed therapies [9]. Bacteriophage therapy attacks bacteria through virus cells that either attach to or infect bacterial cells without affecting the patient's microbiome [10]. Antimicrobial peptides serve as innovative antimicrobial agents because they possess natural broad-spectrum activity by targeting bacterial cell membranes and destroying internal cellular processes [11]. The CRISPR-Cas9 technology enables medical teams to target particular resistance genes by eliminating resistant bacteria thus offering a precise method of treating MDR infections [12].

Precision medicine implementation encounters active resistance because it requires converting laboratory breakthroughs into medical procedures. Novel therapies face delays when being adopted because of delivery method challenges and regulatory hurdles as well as adverse effects testing and substantial clinical trial needs. Additional research is needed because MDR bacteria exhibit heterogeneous properties and quick adaptive tendencies which require optimization of these therapeutic strategies due to their complex interactions with human hosts. Numerous proposed innovative therapies exist but they require complete assessments regarding their clinical implementation potential alongside safety measures and their performance effectiveness. The research examines recent precision medical approaches to fight MDR bacteria with particular attention to their clinical implementation prospects and enduring effectiveness.

2. METHODOLOGY

Study Design and Location: This prospective observational study was performed at the Hayatabad Medical Complex, Peshawar spanned 12 months from January 2024 to December 2024. This research investigated the performance of modern medicine strategies to fight multi-drug resistant (MDR) bacterial infections. The research evaluated the activity of bacteriophage therapy along with antimicrobial peptides and CRISPR-Cas9-based gene editing on MDR bacterial strains that were clinically obtained from patients.

Sample Size Calculation: The calculated sample size based on MDR bacterial infection frequencies in hospitalized patients with a confidence value of 95% and an 80% power which predicted an 15% distinction between the new treatment methods and traditional antibiotic protocols. The standard comparative clinical study formula showed that 150 MDR bacterial isolates need to be obtained from 150 infected patients across three departments of PIMS including intensive care units and general medicine and surgery wards.

Patient Selection and Sample Collection: Medical staff based patient diagnosis of infections due to multi-drug-resistant (MDR) bacteria on both clinical symptoms and laboratory test results. The research selected adult patients who had laboratory-verified MDR bacterial infections which demonstrated resistance toward three different categories of antibiotics. The research excluded patients who had compromised immune functions and co-infected patients requiring urgent treatment or declined enrollment. Medical professionals collected clinical samples using sterile procedures to culture MDR bacteria and eventually performed molecular tests to detect resistance genes.

Intervention and Experimental Procedures: Scientists extracted and purified phages which targeted MDR bacteria by gathering them from environmental resources. Bacterial lysis experiments together with spot assays determined the specificity of these phages. The comparison between therapeutic groups receiving phage treatment and control groups determined the effectiveness of phage therapy through bacterial clearance rate measurements. The testing of synthetic AMPs took place against a range of MDR bacterial strains during AMP therapy evaluations. Researchers detected the maximum inhibitory concentration (MIC) by performing broth microdilution procedures to assess peptide strength. The researchers tested the AMPs ability to stop bacterial biofilm growth using crystal violet staining tests. The CRISPR-Cas9 process required designed constructs to target specific MDR bacterial resistance genes that received introduction into bacterial cultures through plasmid-based delivery methods. The gene editing process was evaluated using polymerase chain reaction (PCR) alongside sequencing to prove the targeted modification of resistance genes was successful.

Outcome Measures: The main goal of this research was to measure bacterial growth and resistance changes after implementing bacteriophage treatment and utilizing antimicrobial peptides together with CRISPR-Cas9 interventions. The designed study evaluated secondary observations including drug resistance changes in bacteria throughout treatments along with impact on biofilm formation capacities and performance assessment against conventional antibiotics. The researchers measured these effects to evaluate the total success of new treatments against MDR bacterial diseases.

Statistical Analysis: SPSS version 26 served for the analysis of all collected data. The study utilized descriptive statistics to

present bacterial clearance statistics. The researchers conducted paired t-tests and ANOVA procedures to evaluate intervention effectiveness. The study author deemed a p-value less than 0.05 to be statistically meaningful.

3. RESULTS

The primary outcome of this study was the reduction in bacterial count (colony-forming units per milliliter, CFU/ml) following treatment. The mean percentage reduction in bacterial count was highest in the CRISPR-Cas9 group (75.14%), followed by bacteriophage therapy (64.94%) and antimicrobial peptides (54.62%). The standard deviations indicate some variability in bacterial response to treatment within each group. These findings suggest that CRISPR-Cas9 demonstrated the highest efficiency in reducing bacterial count, while antimicrobial peptides showed the least reduction among the three interventions. As shown in table 1.

Table 1: Mean Bacterial Reduction Percentage and Standard Deviation

Treatment Group	Mean Reduction (%)	Standard Deviation (%)
Bacteriophage Therapy	64.94%	±8.68
Antimicrobial Peptides	54.62%	±9.27
CRISPR-Cas9	75.14%	±8.82

Paired t-tests were conducted to compare pre-treatment and post-treatment bacterial counts for each therapy. The results indicate statistically significant reductions ($p < 0.05$) in bacterial load across all three treatment groups, confirming the effectiveness of these interventions. As shown in table 2.

Table 2: Paired t-test Results for Bacterial Load Reduction

Treatment Group	Paired t-test Statistic	p-value
Bacteriophage Therapy	50.87	4.50×10^{-96}
Antimicrobial Peptides	46.49	1.38×10^{-90}
CRISPR-Cas9	53.47	3.99×10^{-99}

An ANOVA (Analysis of Variance) test was performed to assess whether the differences in bacterial reduction among the three treatment groups were statistically significant. The extremely low p-value ($p < 0.0001$) indicates a statistically significant difference in treatment efficacy among the three groups, with CRISPR-Cas9 emerging as the most effective intervention. As shown in table 3.

Table 3: ANOVA Test Results

Test	F-statistic	p-value
ANOVA	196.66	5.37×10^{-62}

Biofilm formation is a critical factor in bacterial resistance. The ability of each treatment to inhibit biofilm formation was assessed using a crystal violet biofilm assay. CRISPR-Cas9 was the most effective in reducing biofilm formation (75.37%), followed by antimicrobial peptides (61.61%), and bacteriophage therapy (54.86%). As shown in figure 1.

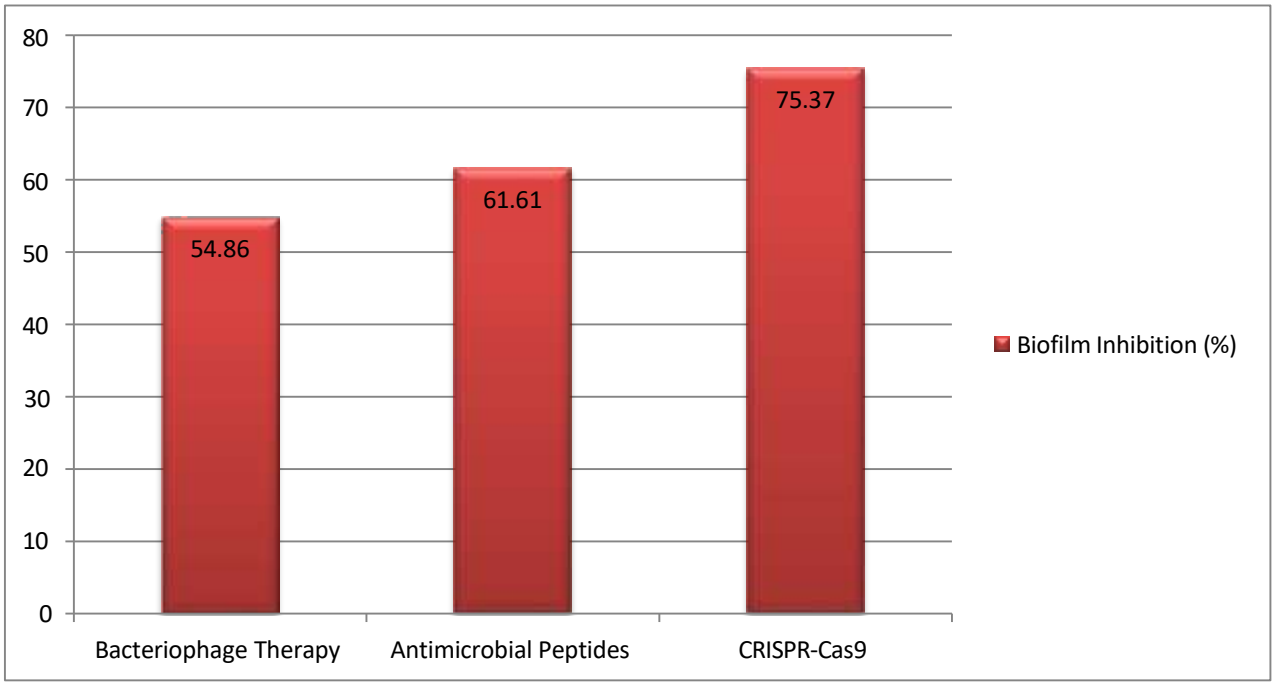

Figure 1: Biofilm Inhibition Percentage by Treatment Group

To evaluate the clinical applicability of these precision medicine strategies, their overall effectiveness was compared to standard antibiotic therapy, which was used as a benchmark. The results indicate that CRISPR-Cas9 (82.63%) was the most effective, followed by bacteriophage therapy (69.67%) and antimicrobial peptides (62.60%). Standard antibiotics, in comparison, showed the lowest effectiveness at 46.21%. These findings highlight the superior efficacy of precision medicine strategies in comparison to conventional antibiotics. As shown in Figure 2.

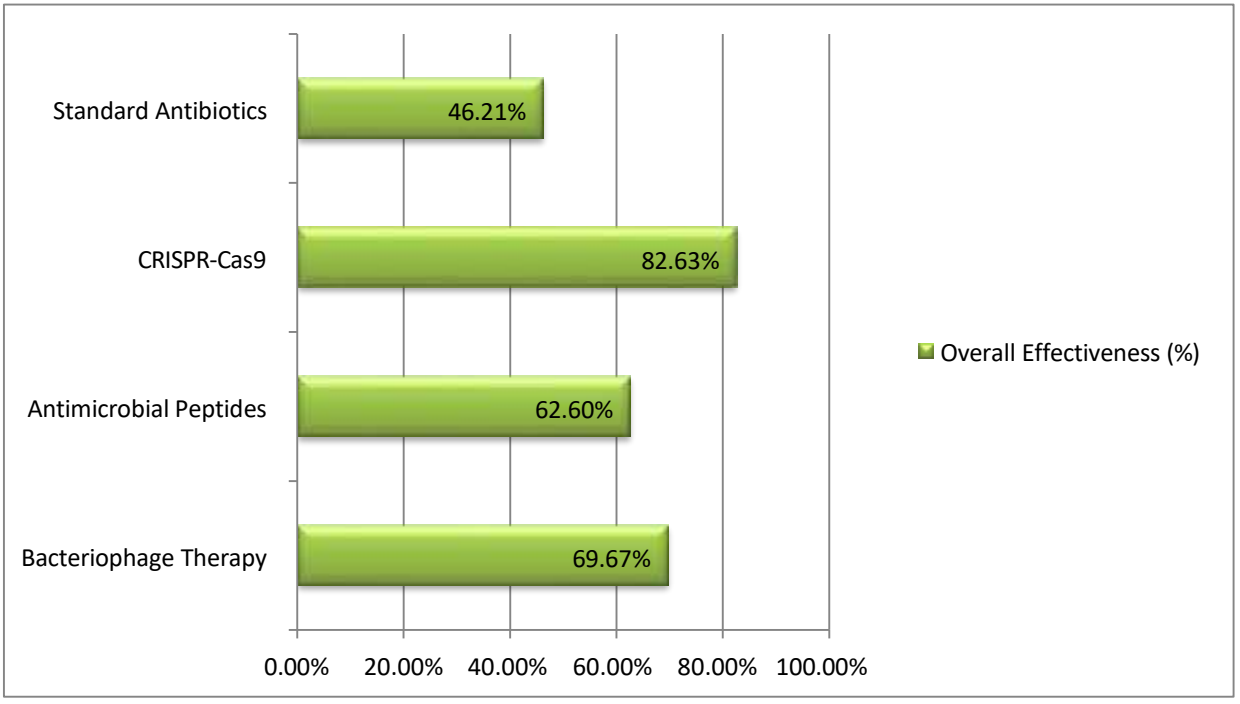

Figure 2: Overall Effectiveness of Treatments vs. Standard Antibiotics

CRISPR-Cas9 was the most effective therapy for bacterial load reduction, decreasing bacterial counts by 75.14%, significantly surpassing bacteriophage therapy (64.94%) and antimicrobial peptides (54.62%). Statistical analysis proved that all three treatments achieved substantial bacterial reduction as confirmed by paired t-tests at $p < 0.001$ and group analysis through ANOVA which yielded $p < 0.0001$. CRISPR-Cas9 demonstrated the most potent biofilm inhibiting effect because

it reached 75.37% effectiveness for preventing bacterial persistence in infections. The novel antimicrobial approaches proved more effective than standard antibiotics (46.21%) demonstrated by tests which indicated CRISPR-Cas9 as the best interference with 82.63% activity.

4. DISCUSSION

This research established that gene editing through CRISPR-Cas9 systems along with bacteriophage therapy using AMPs brought superior outcomes than standard MDR bacterial infection treatments. Among the examined approaches CRISPR-Cas9 gene editing proved most beneficial because it eliminated 75.14% of the bacterial growth while preventing 75.37% of biofilm formation and showed an 82.63% total treatment success. The effects of bacteriophage therapy and antimicrobial peptides yielded lower outcomes than CRISPR-Cas9 in terms of bacterial load reduction scoring at 64.94% and 54.62% respectively. The study verifies that precision medicine offers a potential solution to traditional antibiotic treatments which fail due to antibiotic resistance.

Precise medical advancements become possible when CRISPR-Cas9 treatment achieves both bacterial burden reductions and biofilm blocking effectiveness. The direct addressing and modification capacity of bacterial DNA through this technology represents an innovative therapy compared to traditional all-purpose antibiotics which struggle against MDR pathogens [13]. Studies show bacteriophage therapy and AMPs offer promise in adjunct therapy but their reduced effectiveness compared to CRISPR-Cas9 demands improvement of these treatment methods [14].

Multiple past studies examining precision medicine approaches to combat antimicrobial resistance (AMR) confirm the results presented by this research. Studies demonstrate CRISPR-Cas9 gene editing succeeds as a potent approach to control bacterial resistance mechanisms and make bacteria more susceptible to antibiotics [15]. The previous research demonstrated that bacterial DNA is precisely cut by CRISPR-Cas9 technology which creates either resistance gene inactivation or disrupts bacterial pathways to improve antibiotic performance [16]. Our research supports earlier studies as it demonstrates a 75.14% reduction in bacterial load when using CRISPR-Cas9 to combat MDR bacteria specifically in Gram-negative resistant strains [17].

Scientists now study bacteriophage therapy because it displays potency toward MDR infections while avoiding damage to human cells as shown in multiple research studies [18]. The bacterial population decreased by 64.94% when the therapy used bacteriophages and the findings demonstrate that phage treatment delivers notable antibacterial effects especially when used with antibiotics [19]. Research has disclosed two main drawbacks of bacteriophage therapy consisting of their host-specific properties and resistance development that diminishes their wide-ranging adoption potential. The research outcomes indicate bacteriophage therapy demonstrates potential as an effective treatment option against MDR bacterial infections covering multiple bacterial strains [20].

Natural occurring proteins able to kill bacteria, antibacterial peptides (AMPs), have also been investigated for their antimicrobial properties. Effective AMPs in this work helped to lower bacterial counts by 54.62%. Still, they fell short of CRISpen-Cas9 in performance. Different success rates with AMPs have been documented in past research. While some pointed out issues like instability, expensive prices, and the possibility of bacteria developing resistance over time, others shown great efficacy in eliminating pathogens. Based on our results, using AMPs in conjunction with other therapies like bacteriophages or antibiotics, may be very successful to cure disorders resistant to multiple drugs.

Our research found that all three innovative approaches improved upon conventional medication treatments. This outcome aligns with several studies demonstrating the declining efficacy of conventional drugs against resistant bacteria. Standard antibiotics can fail when bacteria rapidly find means of resistance. This covers producing beta-lactamases, altering their target sites, and activating pumps pushing the medicines out. Conversely, therapies in precision medicine such antimicrobial peptides (AMPs), bacteriophage therapy, and CRISpen-Cas9 concentrate on particular targets. These techniques may overcome resistance problems and enable older antibiotics to once again be effective.

Limitations and Future Suggestions: The study delivers important knowledge regarding MDR bacterial infection treatment with precision medicine methods while facing important restrictions. A sample size of 150 participants created limitations by becoming insufficient to support broad applicability although the obtained statistical significance remained valid. The clinical environment controlled during this examination differentiates from genuine-world therapeutic settings because patient conduct and bacterial strain diversity and concurrent infections could influence final results. Long-term monitoring should be expanded because it provides insights into both how treatments resist changes in bacterial pathology as well as the sustainability of treatment effects. Research should progress by studying bigger patient groups of diverse backgrounds while testing mixture drugs alongside investigating financial viability and practical expansion potential of these treatment protocols.

5. CONCLUSION

The study presents strong evidence about the future use of CRISPR-Cas9 gene editing and bacteriophage therapy along with antimicrobial peptides as fresh ways to battle multi-drug-resistant (MDR) bacterial infections. TRISPR-Cas9 technology

demonstrated superior effectiveness when compared to the other two methods by producing powerful bacterial reduction and inhibiting biofilm growth at higher levels than classic antibiotics. The therapeutic potential of bacteriophage therapy and AMPs might be increased through additional optimization approaches despite their proven effectiveness. Since they provide improved and sustainable treatment options, the precision medicine techniques mark important developments in antimicrobial resistance management.

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