Biotechnological strategies to combat antibiotic resistance

Strategie biotechnologiczne w walce z opornością na antybiotyki

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Abstract

This article aims to present the current state of knowledge on four major biotechnological antimicrobial strategies and to evaluate their potential clinical applications in the context of increasing antibiotic resistance. Approaches such as phage therapy, CRISPR-Cas9 gene editing, nanoparticles, and antimicrobial peptides (AMPs) may significantly contribute to limiting the spread of resistance genes. Particular attention is given to advances in genetic engineering that enable precise targeting and elimination of resistance determinants, as well as to the therapeutic potential of the microbiome. A literature review of studies published between 2010 and 2025 was conducted using the following keywords: antimicrobial resistance, phage therapy, CRISPR-Cas9, AMPs, and nanotechnology. Both review articles and original studies, including preclinical and clinical data, were considered. Phage therapy demonstrates high efficacy against antibiotic-resistant pathogens, particularly in the form of phage cocktails and genetically engineered phages. Antimicrobial peptides exhibit broad-spectrum activity and can be structurally optimized to improve stability and selectivity. CRISPR-Cas9 systems enable targeted elimination of resistance genes or direct disruption of pathogen genomes, while nanotechnology facilitates drug delivery, biofilm penetration, and bactericidal activity, particularly through metal-based nanoparticles. Notably, all approaches show potential for synergistic use with conventional antibiotics. Biotechnological treatment strategies may become a key component in combating antibiotic resistance. However, their clinical implementation requires further research, comprehensive safety evaluation, regulatory development, and integration into medical practice. Advances in these areas could significantly reduce the global burden of infectious diseases.

Streszczenie

Celem niniejszego artykułu jest przedstawienie aktualnego stanu wiedzy na temat czterech głównych biotechnologicznych strategii przeciwdrobnoustrojowych oraz ocena ich potencjalnych zastosowań klinicznych w kontekście narastającej oporności na antybiotyki. Metody takie jak terapia fagowa, edycja genów CRISPR-Cas9 czy zastosowanie nanocząsteczek i peptydów przeciwdrobnoustrojowych mogą znacząco przyczynić się do ograniczenia rozprzestrzeniania się genów oporności. Szczególną uwagę zwrócono na innowacje w inżynierii genetycznej, które pozwalają na precyzyjne niszczenie genów oporności, a także na wykorzystanie mikrobiomu jako potencjalnego narzędzia terapeutycznego. Przeprowadzono przegląd literatury naukowej opublikowanej w latach 2010–2025, przeszukując bazy danych za pomocą słów kluczowych: oporność na antybiotyki, terapia fagowa, CRISPR-Cas9, peptydy przeciwdrobnoustrojowe, nanotechnologia. Uwzględniono zarówno artykuły przeglądowe, jak i oryginalne badania obejmujące dane przedkliniczne i kliniczne. Terapia fagowa wykazuje wysoką skuteczność w zwalczaniu szczepów opornych na antybiotyki, zwłaszcza w postaci koktajli fagowych i fagów modyfikowanych genetycznie. Peptydy przeciwdrobnoustrojowe charakteryzują się

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szerokim spektrum działania i mogą być modyfikowane strukturalnie w celu zwiększenia stabilności i selektywności. Systemy CRISPR-Cas9 umożliwiają precyzyjną eliminację genów oporności lub zniszczenie genomu patogenu, a nanotechnologia umożliwia skuteczne dostarczanie leków, penetrację biofilmu i działanie bakteriobójcze poprzez nanocząstki metali. Wszystkie te metody wykazują potencjał synergii z antybiotykami. Biotechnologiczne strategie leczenia mogą w przyszłości stać się kluczowym elementem w walce z opornością na antybiotyki. Ich wdrożenie wymaga dalszych badań klinicznych, oceny bezpieczeństwa, opracowania ram prawnych i integracji z praktyką medyczną. Postęp w tych obszarach mógłby znacząco zmniejszyć globalne obciążenie chorobami zakaźnymi.

Key words: nanotechnology, antimicrobial peptides, antimicrobial resistance, CRISPR-Cas9, phage therapy

Słowa kluczowe: nanotechnologia, peptydy przeciwdrobnoustrojowe, terapia fagowa, oporność na środki przeciwdrobnoustrojowe, CRISPR-Cas9

Highlights

- Biotechnological antimicrobial strategies address antimicrobial resistance using phage therapy, CRISPR-Cas9, peptides, and nanotechnology.
- Phage therapy shows high efficacy against multidrug-resistant pathogens and enables precision antimicrobial treatment.
- CRISPR-Cas9 targets resistance genes, while peptides and nanoparticles enhance antimicrobial activity and biofilm penetration.
- Biotechnological strategies combined with antibiotics may improve infection control and reduce antimicrobial resistance.

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Introduction

The problem of antibiotic resistance and the limitations of conventional therapy

Pathogenic microorganisms have accompanied human populations throughout history, contributing substantially to morbidity and mortality. The 20th century marked a major breakthrough in the treatment of bacterial infections with the discovery of penicillin and other antimicrobial agents. However, the widespread and often inappropriate use of antibiotics, combined with the natural evolutionary adaptability of pathogens, has led to the emergence of multiple resistance mechanisms and a progressive decline in treatment efficacy.[1]

Antimicrobial agents include antibiotics, antivirals, antifungals, and antiparasitic drugs. They are used to inhibit the growth and replication of microorganisms or to eliminate them, thereby preventing and treating infectious diseases in humans, animals, and plants. These agents may be natural, semi-synthetic, or synthetic and act through a variety of mechanisms. Antimicrobial resistance (AMR) is defined as the ability of microorganisms to survive and remain viable despite exposure to antimicrobial agents. This reduces the effectiveness of therapy and can render infections difficult or even impossible to treat. As a consequence, AMR contributes to increased disease transmission, greater infection severity, long-term health complications, and mortality. Although AMR is a natural phenomenon driven by genetic variation, its accelerated emergence and spread are largely attributable to human activity, particularly the inappropriate and excessive use of antimicrobials in human medicine, veterinary practice, and agriculture.[2]

Bacterial resistance represents a major challenge for healthcare systems worldwide, as the increasing use of antibiotics promotes the emergence of resistant strains. This results in reduced treatment effectiveness, prolonged hospital stays, increased healthcare costs, and higher mortality rates. Individuals with compromised immune systems, including patients with cancer, HIV infection, or diabetes, are particularly vulnerable to its consequences.

In addition, limited public awareness and the inappropriate prescribing and use of antimicrobial agents by both healthcare professionals and patients further exacerbate the problem. If current trends persist, even minor injuries or common infections may become life-threatening, marking a transition toward a so-called "post-antibiotic era." International organizations such as the World Health Organization (WHO), the European Centre for Disease Prevention and Control (ECDC), and the Centers for Disease Control

and Prevention (CDC) have implemented coordinated strategies to address AMR, including risk assessment, identification of contributing factors, and the development of control measures. According to a 2019 CDC report, AMR threats are classified into several categories. Urgent threats include infections caused by carbapenem-resistant *Acinetobacter* spp., *Candida auris*, *Clostridioides difficile*, drug-resistant *Neisseria gonorrhoeae*, and carbapenem-resistant Enterobacterales. Serious threats include multidrug-resistant *Pseudomonas aeruginosa*, antibiotic-resistant *Campylobacter* spp., and vancomycin-resistant *Enterococcus* (VRE). Based on resistance profiles, microorganisms are further classified as multidrug-resistant (MDR), extensively drug-resistant (XDR), or pan-drug-resistant (PDR).[1,3]

In low- and middle-income countries, AMR is further exacerbated by limited healthcare infrastructure, insufficient funding, and inadequate regulation of antibiotic use. Multidrug-resistant Gram-negative bacteria pose a particularly serious threat, especially among neonates. These include members of the family Enterobacteriaceae, notably strains producing extended-spectrum β-lactamases (ESBLs) as well as carbapenem-resistant organisms. Key examples include *Klebsiella pneumoniae* producing carbapenemases such as KPC (*K. pneumoniae* carbapenemase) and strains harboring New Delhi metallo-β-lactamase (NDM), as well as NDM-producing *Escherichia coli*. In response to this growing threat, global initiatives such as NeoAMR have been launched to develop strategies for antibiotic management in neonatal sepsis, particularly in low- and middle-income settings. The NeoAMR initiative involves more than 40 partners across 25 countries and focuses on four main areas: identification of key pathogens responsible for neonatal sepsis, surveillance of resistance patterns, standardization of diagnostic protocols, and optimization of empirical antibiotic therapy. The project has collected over 36,000 clinical samples and conducted microbiological analyses and genotyping of isolates to identify dominant MDR strains. The findings revealed substantial geographical variation in pathogen distribution, along with an alarmingly high prevalence of resistance to first-line antibiotics, including aminopenicillins and third-generation cephalosporins. Based on these data, region-specific recommendations for the empirical treatment of neonatal sepsis were developed, taking into account local resistance patterns.[3] NeoAMR also serves as a platform for scientific and educational collaboration, supporting the development of local diagnostic capacity and the establishment of AMR surveillance networks in high-burden regions. This initiative represents a modern, integrated approach to addressing AMR in one of the most vulnerable patient populations – newborns – and may serve as a model for future AMR control programs.[4]

Objectives

Developing and implementing strategies to limit the emergence and spread of antibiotic resistance is essential, as bacteria employ a range of sophisticated molecular defense mechanisms. These include genetic mutations, acquisition of resistance genes, and the use of mobile genetic elements, all of which contribute to the persistence and dissemination of resistance. A comprehensive understanding of the molecular and genetic basis of MDR, the mechanisms underlying its mobilization, and the dynamics of its spread in both environmental and human populations is critical for designing effective strategies to combat AMR.[1–3]

Bacterial resistance mechanisms

Bacterial resistance to antimicrobial agents is a major factor limiting the effectiveness of infection treatment. Bacteria employ a variety of resistance strategies, both intrinsic and acquired, that enable them to survive and proliferate in the presence of antibiotics. Understanding these mechanisms is essential for the development of effective alternative therapies and for limiting the further spread of AMR. This section outlines the key mechanisms of bacterial resistance, with particular emphasis on the role of horizontal gene transfer in their dissemination.[5]

Main types of bacterial resistance mechanisms

Resistance mechanisms can be broadly classified into several categories based on their mode of action and spectrum of activity against different classes of antibiotics.

1. Enzymatic inactivation of antibiotics: This is one of the most common resistance mechanisms and involves the production of bacterial enzymes that degrade or structurally modify antibiotic molecules. Examples include β-lactamases (e.g., ESBL, KPC, NDM), which hydrolyze the β-lactam ring of penicillins and cephalosporins,[1,6,7] as well as enzymes such as acetyltransferases, phosphotransferases, and nucleotidyl transferases, which modify aminoglycosides.[8,9]
2. Modification of antibiotic target sites: This mechanism involves genetic mutations or structural alterations in bacterial molecules that serve as targets for antimicrobial agents. Examples include methylation of ribosomal RNA mediated by erm genes in Streptococcus spp., which reduces susceptibility to macrolides,[10] as well as alterations in penicillin-binding proteins (PBPs) that underlie β-lactam resistance in methicillin-resistant Staphylococcus aureus (MRSA).[11]
3. Reduced membrane permeability: This mechanism is particularly important in Gram-negative bacteria, where decreased permeability of the outer membrane limits antibiotic entry through porin channels. Mutations in genes encoding porins (e.g., OmpF and OmpC

in *E. coli*) can reduce susceptibility to β-lactams, including carbapenems.[12]

4. Active efflux of antibiotics (efflux pumps): Bacteria can actively expel antibiotics from the cell via membrane-associated transport proteins. Examples include the MexAB–OprM efflux system in *P. aeruginosa* and the NorA pump in *S. aureus*.[13] This mechanism is a major contributor to MDR.
5. Bypass of metabolic pathways: Bacteria may evade the effects of antibiotics by utilizing alternative enzymes or metabolic pathways that are not targeted by the drug. A classic example is resistance to trimethoprim–sulfamethoxazole in *E. coli*, which is mediated by the production of a modified dihydrofolate reductase (DHFR) enzyme encoded by altered *dhfr* genes.[14]

Table 1 summarizes the mechanisms of bacterial resistance and provides representative examples of their occurrence. Many multidrug-resistant strains, including MDR, XDR, and PDR phenotypes, exhibit multiple resistance mechanisms simultaneously, which significantly complicates the treatment of infections.[15]

Table 1. Mechanisms of bacterial resistance and their occurrence

Resistance mechanism	Description of action	Examples of bacteria/genes
Enzymatic inactivation	Enzymatic degradation or modification of the antibiotic	*E. coli* (ESBL, NDM), *K. pneumoniae* (KPC)[8,9]
Target modification	Mutations or structural modifications of target proteins	MRSA (PBP2a), *S. pneumoniae* (23S rRNA)[11]
Reduced permeability	Loss/change of porins reducing penetration of the biological membrane by the drug	*P. aeruginosa*, *E. coli* (OmpF, OmpC)[12]
Efflux pumps	Actively pumping the antibiotic out of the cell	*S. aureus* (NorA), *P. aeruginosa* (MexAB-OprM)[13]
Bypassing the metabolic pathway	Production of an alternative enzyme or pathway that is drug insensitive	*S. aureus* (mutation in the DHFR gene)[14]

The importance of gene transfer

Horizontal gene transfer (HGT) plays a key role in the spread of bacterial resistance. It allows for the rapid transfer of resistance genes between different species and strains of bacteria, regardless of phylogenetic relatedness. Horizontal gene transfer can occur according to one of the following patterns:

- Conjugation: Involves the direct transfer of genetic material, typically plasmids, between bacterial cells via cell-to-cell contact. This is the most common mechanism for the dissemination of genes encoding enzymes such as ESBLs, KPC, and NDM β-lactamases.[16]
- Transformation: The uptake of free extracellular DNA by competent bacteria. For example, *Streptococcus pneumoniae* can incorporate DNA fragments containing penicillin resistance genes into its genome.[17]
- Transduction: The transfer of genetic material mediated by bacteriophages. Although less common than conjugation, this mechanism can also contribute to the spread of resistance genes.[18]

Genetic determinants of antibiotic resistance may be located on plasmids, transposons, integrons, and complex mosaic genetic elements composed of multiple mobile DNA sequences. Plasmids are extrachromosomal, autonomously replicating DNA molecules, typically circular, while transposons are mobile elements capable of relocating within the genome. Integrons are genetic platforms that capture and express gene cassettes through site-specific recombination. For example, class 1 integrons, commonly found in Enterobacterales, contain gene cassettes encoding multiple resistance determinants, along with integrase genes that facilitate their integration and expression within the host genome.[19] The dissemination of resistance genes is strongly influenced by environmental factors, including selective pressure exerted by antibiotic use, co-selection mechanisms (e.g., the co-occurrence of antibiotic resistance and heavy metal tolerance genes on the same plasmid), and the formation of biofilms, which promote horizontal gene transfer.[20] Importantly, metagenomic studies have demonstrated that antibiotic resistance genes are also present in microorganisms inhabiting environments with minimal human impact. These findings suggest that such genes are part of the natural resistome; however, anthropogenic activity significantly enhances their mobilization and spread.[20]

The aim of this work

The increasing resistance of microorganisms to antimicrobial agents represents one of the most serious public health challenges of the 21st century. It is estimated that antibiotic-resistant bacterial infections directly caused approx. 1.27 million deaths worldwide in 2019, exceeding those attributed to HIV/AIDS or malaria, while deaths associated with AMR reached 4.95 million globally.[20] Projections suggest that by 2050, infections caused by antibiotic-resistant bacteria could result in up to 10 million deaths annually, potentially surpassing cancer as a leading cause of mortality.[20] The primary drivers of this crisis include the overuse and misuse of antibiotics in human medicine, veterinary practice, and agriculture, as well as the limited development of new antimicrobial classes.[21] In response to these challenges, there is an urgent need to explore innovative therapeutic strategies. Modern biotechnology offers several promising approaches to combating infections caused by drug-resistant microorganisms. This review focuses on 4 such strategies: phage therapy, antimicrobial

peptides (AMPs), CRISPR-Cas9-based genome editing, and nanotechnology.[22] Each approach is characterized by a distinct mechanism of action and is currently at a different stage of clinical development. This paper aims to present and discuss biotechnological strategies for combating antibiotic resistance and to evaluate their potential clinical applications.

Methodology of literature search and selection

The literature review was conducted using the MEDLINE (via PubMed), Scopus, and Web of Science databases, with a particular focus on publications from 2010 to 2025. The search strategy was based on the following keywords: AMR, phage therapy, CRISPR-Cas9, AMPs, and nanotechnology. Both review articles and original studies reporting preclinical and clinical data were included in the analysis.

Results

Phage therapy

Phage therapy involves the use of bacteriophages – viruses that specifically infect bacteria – to treat infections. A key characteristic of phages is their high host specificity, as a given phage typically targets only particular bacterial species or strains. This specificity enables the elimination of antibiotic-resistant bacteria without harming human cells or disrupting the normal microbiota.[23]

The clinical potential of phage therapy has been demonstrated in numerous case reports and preliminary studies. For example, successful treatment has been reported in infections caused by *Acinetobacter baumannii*, *P. aeruginosa*, MRSA, and *Klebsiella pneumoniae*. Importantly, phages are capable of infecting and disrupting biofilm-forming bacteria.[23,24] In addition, both in vitro and in vivo studies have demonstrated potential synergistic effects between bacteriophages and conventional antibiotics.[25]

At present, novel strategies are being developed to enhance the efficacy of phage therapy. One such approach is the use of phage cocktails, which consist of combinations of multiple bacteriophages capable of targeting different strains of a given bacterial species or even multiple species. This strategy is particularly advantageous in the treatment of polymicrobial infections. The use of phage cocktails may improve therapeutic efficacy while reducing the risk of resistance to individual phages.[25]

A growing body of evidence supports the effectiveness of phage therapy using phage cocktails. One of the first randomized clinical trials in this field was conducted by Jault et al. as part of the PhagoBurn project. The study evaluated a cocktail of twelve natural lytic bacteriophages targeting *P. aeruginosa* (PP1131) in patients with burn wound infections. Although the clinical efficacy was limited – primarily due to insufficient concentrations of active phages – the study demonstrated the safety of phage therapy and laid the groundwork for the development of standardized clinical protocols.[26] More recent studies have focused on optimizing the design of phage cocktails. For example, Vaitekenas et al. emphasized the importance of phage diversity within therapeutic mixtures. The inclusion of phages targeting different bacterial receptors – such as lipopolysaccharides (LPS), type IV pili (T4P), outer membrane proteins, or efflux pump structures – can reduce the likelihood of resistance development while enhancing therapeutic efficacy. This approach is particularly relevant in infections caused by *P. aeruginosa* in patients with cystic fibrosis, where complex biofilm structures are commonly observed.[27] In addition, Rastegar et al. investigated combination therapy using bacteriophages and antibiotics against biofilms formed by XDR *A. baumannii*. Their study evaluated a four-phage cocktail (SA1, Eva, Ftm, Gln) in combination with antibiotics (ampicillin/sulbactam, meropenem, and colistin). Phages were selected based on activity against clinical biofilm-forming isolates. The results demonstrated that phage–antibiotic combination therapy significantly enhanced biofilm eradication, indicating a synergistic effect. These findings highlight the potential of phage therapy as an adjunctive strategy in the treatment of infections caused by XDR *A. baumannii*, particularly in the context of increasing resistance to conventional therapies.[28]

Another promising direction in the development of phage therapy is the use of genetically engineered bacteriophages. These phages can be modified to enhance lytic activity, broaden host range, or introduce additional therapeutic functions. For example, some engineered phages can be equipped with CRISPR-Cas9 systems, enabling the targeted disruption of resistance genes within the bacterial genome. Other modifications include the ability to degrade biofilms or to evade bacterial defense mechanisms, such as restriction–modification systems.[21]

In a study by Selle et al., recombinant bacteriophages were engineered to selectively target *C. difficile* using a CRISPR-Cas3 system. These engineered phages, referred to as crPhage, contained customized CRISPR sequences designed to target the bacterial genome. Both in vitro and in vivo (murine) models demonstrated that crPhage more effectively reduced *C. difficile* burden compared to wild-type phages, while also limiting intestinal tissue damage. Importantly, these modifications decreased the risk of lysogeny and associated adverse effects, such as increased expression of bacterial toxins.[29,30]

Recombinant bacteriophages are also being developed as engineered constructs that combine features of multiple natural phages or are synthetically designed based on genomic sequences of known bacteriophages. Such phages may exhibit enhanced stability, increased activity, and reduced susceptibility to neutralization by the host immune system.[31] Phages can be modified in vitro using approaches such as CRISPR-Cas9-mediated editing (discussed later

in this paper) or homologous recombination (HDR), allowing them to be tailored to specific therapeutic needs. These modifications enable not only efficient bacterial killing but also the selective modulation of the host microbiota. This strategy offers new opportunities for personalized phage therapy and the treatment of diseases associated with microbiota dysbiosis.[32] A compelling example of this approach is provided by Peters et al., who investigated short-term co-evolution of two bacteriophages (LP-048 and LP-125) and their interactions with phage-resistant *Listeria monocytogenes*. The study demonstrated that phage exposure exerted selective pressure on bacterial populations, leading to the emergence of phage-resistant strains. Importantly, co-infection with both phages resulted in spontaneous genetic recombination, giving rise to novel phage variants capable of infecting bacterial strains resistant to the original phages. These recombination events involved genes encoding proteins likely associated with host recognition and DNA interaction. These findings suggest that phage co-evolution and recombination may serve as natural mechanisms to expand host range and enhance the effectiveness of phage therapy against resistant bacterial populations.[33]

The advantages of phage therapy include the ability of bacteriophages to self-replicate at the site of infection, high tolerability in humans, and effectiveness against antibiotic-resistant strains.[23,24] However, several challenges remain. These include the narrow host range of individual phages, the need for personalized therapy, and the associated high costs, as well as the potential for bacteria to develop resistance to phages. Additional limitations include possible host immune responses to phages and the lack of standardized regulatory frameworks governing their clinical use.[25,34]

An example of a clinical facility offering phage therapy is the Phage Therapy Center at the Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, in Wrocław. Operating since 2005, the center provides treatment within the framework of a therapeutic experiment, primarily for patients with chronic infections unresponsive to conventional antibiotic therapy. The treatment process begins with patient qualification, including the evaluation of medical history, prior treatments, and microbiological documentation. A key step is phage typing, which involves the laboratory selection of bacteriophages active against the bacterial strain isolated from the patient. The center offers treatment for infections caused by pathogens such as *S. aureus* (including MRSA), *P. aeruginosa*, *E. coli*, *K. pneumoniae*, *Enterococcus faecalis*, and *Proteus mirabilis*. Therapy is initiated after obtaining informed consent and may be administered via oral, rectal, or topical routes. Treatment typically lasts 6–8 weeks and is closely supervised by a multidisciplinary team. Reported effectiveness reaches approx. 40% in patients with chronic infections.[34]

Antimicrobial peptides

Antimicrobial peptides are small bioactive molecules that constitute a key component of the innate immune system in many organisms. Their antimicrobial activity primarily involves disruption of microbial cell membranes, leading to rapid pathogen elimination. In addition, many AMPs interfere with intracellular processes, including protein, RNA, and DNA synthesis. Antimicrobial peptides exhibit a broad spectrum of activity against Gram-positive and Gram-negative bacteria, fungi, viruses, and certain parasites. Due to their distinct mechanism of action, resistance to AMPs develops less frequently compared to conventional antibiotics.[21,22] Antimicrobial peptides can be classified into several categories based on their origin, biosynthesis, structural characteristics, and biological functions (Table 2).

Certain clinically used antimicrobial agents, such as polymyxins (e.g., colistin), daptomycin, and glycopeptides (e.g., vancomycin), share functional similarities with AMPs, although they are not always classified as classical AMPs. Current research focuses on modifying naturally occurring peptides (e.g., melittin) and designing synthetic peptides with enhanced stability, selectivity, and reduced toxicity. An example of such an approach is murepavadin, a synthetic antimicrobial peptide with high specificity against *P. aeruginosa*. Murepavadin exerts its activity by binding to the outer membrane protein LptD, which is involved in LPS transport, leading to membrane destabilization and bacterial cell death. In addition, it demonstrates the ability to penetrate and disrupt bacterial biofilms, representing a significant advantage over many conventional antibiotics. Preclinical and clinical studies have demonstrated rapid bactericidal activity, high efficacy, and favorable safety profiles. As a result, murepavadin is considered a promising therapeutic candidate for the treatment of infections caused by MDR Gram-negative bacteria, particularly in the context of increasing resistance to existing antibiotics.[35]

Antimicrobial peptides may originate from natural sources (e.g., colistin, gramicidin) or be synthetically designed (e.g., pexiganan, omiganan). Natural AMPs are produced by a wide range of organisms, including bacteria (which produce bacteriocins such as colicins), fungi (e.g., copsin, plectasin), plants (e.g., plant defensins), insects (e.g., cecropins and insect defensins), and mammals, including humans (e.g., cathelicidins and defensins).[35–37] Increasing research efforts are focused on the development of peptide hybrids and AMP-based conjugates with nanoparticles or antibodies. These approaches aim to improve peptide stability, bioavailability, and target specificity. An example of such a strategy is the use of targeted nanocarriers, such as melittin-loaded nanoparticles, which have been investigated in early-stage studies, particularly in oncology. This approach enables controlled peptide release while reducing toxicity, including hemolytic effects.[35]

Table 2. Classification of antimicrobial peptides

Criterion	Categories	Characteristics/Examples
1. Origin	Eukaryotic	Mammals (human – LL-37, HBD-3), amphibians, fish, plants (γ-thionine), invertebrates (drosomycin)
	Prokaryotic	Polymyxins, gramicidins, bacitracin (products of bacterial metabolism)
2. Method of biosynthesis	Ribosomal	Coded in DNA, synthesized in a cell, e.g., cathelicidins, defensins
	Non-ribosomal	Synthesis by enzymes, e.g., polymyxins, bacitracin
3. Structure	Linear α-helical	LL-37, cecropine B
	β-sheet	Defensins α, β, θ
	Cyclic	Defensins θ, bactenecin
	Rich in specific amino acids	Indolicidin (tryptophan), OaBac (proline and arginine)
4. Molecular family	Defensins – α-defensins – β-defensins – θ-defensins – of plants/invertebrates	3 disulfide bridges, molecular weight 3–5 kDa – HNP-1 to -4 (neutrophils), HD-5 and HD-6 (Paneth cells) – HBD-1 to -4, secreted by epithelium – RTD (macaques), BTD (baboons) – cyclic, absent in humans – γ-thionins (plants), drosomycin (insects)
	Cathelicidins – LL-37 – other	Precursor: pre-propeptide with cathelin domain – The only human cathelicidin identified, active throughout the body – CAP-18 (rabbit), SMAP29 (sheep), Saha-CATH (Tasmanian devil)
5. Biological function	Direct action	Destruction of the cell membrane, disruption of DNA, RNA, and wall synthesis
	Immunomodulation	Cytokine induction, chemotaxis, activation of immune cells
	Neutralization of toxins	Anti-endotoxin activity (e.g., LPS)
	Anti-cancer activity	Magainin 2, chrysofsin 1, cecropin B
6. Unusual examples (species)	Komodo dragon	cAMPs homologous to histones (VK6–VK25)
	Giant panda	Cathelicidin-AM – fast antibacterial effect
	Tasmanian devil	Saha-CATH cathelicidins 1 to 6, broad spectrum of action

LPS – lipopolysaccharide; cAMP – cationic antimicrobial peptides.

Similarly, Xuan et al. described a synthetic antimicrobial peptide, SAAP-148, which exhibited potent antibacterial activity against pathogens such as *S. aureus* and *Acinetobacter baumannii*. The peptide demonstrated the ability to penetrate biofilms and eliminate bacteria in regions that are difficult to access with conventional antibiotics. Importantly, SAAP-148 showed enhanced synergistic effects when combined with tetracyclines, highlighting the potential of AMPs as components of combination therapies for drug-resistant infections.[38] Research has also focused on the topical application of AMPs, including their incorporation into antimicrobial dressings and bioactive implant coatings.[35,36] For example, Liu et al. developed a hydrogel coating containing WR and Bac2A peptides, which demonstrated durability, bioactivity in vivo, and strong antibacterial and anticoagulant properties in animal models. Such coatings may be applied to medical devices, including catheters, stents, and vascular grafts, to prevent infection and thrombosis.[39]

Another distinguishing feature of AMPs is their immunomodulatory activity. In addition to direct antimicrobial effects, AMPs can modulate the host immune response by promoting immune cell migration, regulating cytokine production, and enhancing wound healing. For example, LL-37 and defensins have been shown to play key roles in these processes.[35] As a result, AMPs are increasingly recognized as multifunctional molecules with both antimicrobial and therapeutic properties. Preclinical studies indicate that AMPs can enhance the efficacy of conventional antibiotics, for instance by facilitating their penetration into bacterial biofilms. Some peptides, such as IDR-1018, not only inhibit bacterial growth but also reduce biofilm formation and inflammation, making them promising candidates for the treatment of chronic wounds and skin infections.[40] Antimicrobial peptides can also be incorporated into combination therapies with other non-conventional approaches, such as bacteriophages or nanomaterials, enabling the development of multimodal treatment strategies with enhanced efficacy. Another promising direction involves antibody–peptide conjugates, which allow targeted delivery of AMPs to specific pathogens.[36] Despite their therapeutic potential, the clinical application of AMPs remains limited by challenges such as susceptibility to enzymatic degradation, short half-life, and potential cytotoxicity. Consequently, significant research efforts are focused on improving their stability and safety through strategies such as peptide cyclization, incorporation of D-amino acids, and encapsulation in nanocarriers (e.g., liposomes or hydrogels). These approaches enhance delivery efficiency and may facilitate broader clinical implementation of AMP-based therapies.[35,36]

CRISPR-Cas9 and genome editing

The CRISPR-Cas9 system is an adaptive defense mechanism in bacteria that has been transformed into a precise tool for genome editing. Its action is based on a complex of the Cas9 protein and a synthetic single-stranded guide RNA, known as single guide RNA (sgRNA). This RNA contains two functional parts: a crRNA sequence, responsible for recognizing a specific DNA fragment, and trans-activating CRISPR RNA (tracrRNA), which stabilizes the complex with the Cas9 protein.

After the Cas9–sgRNA complex is formed, the genome is searched for a sequence complementary to the guide RNA (gRNA), i.e., the editing target. An important condition for its recognition is the presence of a short DNA motif called the protospacer adjacent motif (PAM), which most often takes the form of the trinucleotide sequence "NGG." When the Cas9 protein locates the correct target site with an adjacent PAM motif in the DNA, a local fragment of the double helix unfolds. The gRNA hybridizes with one of the DNA strands, allowing the enzyme to precisely align itself. Once the match is confirmed, Cas9 activates its two cutting domains – HNH, which cleaves the strand complementary to the RNA, and RuvC, which cleaves the opposite strand. The result of the enzyme's action is a double-strand break (DSB). Damaged DNA must be repaired by endogenous cellular mechanisms. Most often, non-homologous end joining (NHEJ) occurs, which involves rapid joining of the cut ends without the use of a template. This process is efficient, but often introduces minor errors, such as insertions or deletions, which can lead to gene inactivation. The second possible scenario is repair by HDR, which requires the presence of an externally supplied DNA template. This allows for precise modification, e.g., repair of a mutation or introduction of a new sequence.[41] In therapeutic applications, this strategy can be used to eliminate resistance genes or selectively destroy pathogenic bacteria.[21,42]

In clinical practice and preclinical studies, CRISPR-Cas9 systems are primarily used in two therapeutic strategies. The first involves the precise excision of genes encoding antibiotic resistance factors, such as *mcr-1* or *blaNDM*, which in turn restores bacterial sensitivity to conventional antibiotics. The second strategy involves the complete destruction of the pathogen's genome, leading to bacterial cell death. Both methods use modified bacteriophages – phagemids – as vectors for the selective delivery of CRISPR-Cas9 elements to target cells. This approach allows for the effective elimination of resistant strains without affecting the rest of the microbiome.[21,42]

In 2024, the first-ever clinical trial using CRISPR-Cas3 therapy, delivered via a phage vector, was conducted, targeting *E. coli* strains in uncomplicated urinary tract infections (UTIs) in women. The trial, called ELIMINATE, was conducted by Locus Biosciences and aimed to evaluate the safety, tolerability, and efficacy of a preparation containing a cocktail of engineered phages integrated with the CRISPR-Cas3 system. The study group consisted of 39 adult women aged 18–70. All participants had confirmed active UTI of bacterial etiology (*E. coli*) and a history of at least 1 previous episode of UTI within the past year. The goal of the treatment was to reduce bacterial colonization and clinical symptoms. The therapeutic design was based on a cocktail of three modified lytic bacteriophages that naturally infect *E. coli*. Each phage was additionally equipped with a construct containing the CRISPR-Cas3 system, targeting highly conserved regions of the bacterial genome. After entering the bacterial cell, the phages induced a lytic cycle of infection, and Cas3 initiated extensive DNA degradation, which ultimately led to the complete destruction of the bacterial cell. The observed mechanism was significantly more effective than the precise DNA cleavage characteristic of the Cas9 protein. Importantly, no serious adverse effects were reported. No bacterial resistance to phages or CRISPR-Cas3 was observed either. The described study provides the first clinical evidence of the effectiveness of CRISPR-Cas3 as a therapeutic tool in the treatment of bacterial infections in humans. This technology combines the selectivity of bacteriophages with precise destruction of the microbial genome, making it a potentially groundbreaking solution in the fight against antibiotic resistance.[43] Further clinical trials are currently underway using CRISPR-Cas9 systems as an alternative approach for the treatment of infections caused by resistant strains, including *P. aeruginosa* and *K. pneumoniae*.[21,42]

The advantages of this approach include high precision, the ability to adapt the therapy to new strains, and minimal impact on the microbiome.[23] The challenges, however, include difficulties in delivering CRISPR-Cas9 systems to sites of infection, the possibility of immune reactions, and the risk of so-called off-target effects, i.e., unintended DNA cleavage outside the target sequence. This phenomenon results from the fact that gRNA can bind not only to a perfectly matched site in the genome but also to other highly similar sequences, leading to unintended genetic modifications. Such mutations can disrupt the function of important genes, increasing the risk of, among other things, cellular toxicity and carcinogenesis. A study conducted by Feng Zhang's team using the GUIDE-seq method demonstrated that Cas9 can cleave DNA at numerous unintended locations, raising concerns about the safety of this technology.[44] Similar conclusions were reported by Schaefer et al., who observed unexpected mutations in mice treated with CRISPR-based therapy.[45] In response to these challenges, more precise variants of Cas9 are being developed, along with improved gRNA design strategies aimed at minimizing off-target effects.[46] Nevertheless, CRISPR-Cas9 remains one of the most promising strategies in addressing the growing problem of AMR.[23]

Nanotechnology

Nanotechnology in antimicrobial medicine is developing dynamically as an innovative approach to combating antibiotic resistance. The use of nanomaterials such as metal nanoparticles (silver, gold, copper), metal oxides (e.g., ZnO, TiO_2), and polymer and lipid carriers enables the delivery of drugs to hard-to-reach sites of infection, penetration of bacterial biofilms, and direct bactericidal action. These nanoparticles are characterized by sizes ranging from 1 to 100 nm, which allows them to readily penetrate cell membranes and interact with microbial macromolecules at the molecular level.[47,48] Silver nanoparticles (AgNPs) exhibit strong antimicrobial activity against Gram-positive and Gram-negative bacteria, acting by damaging cell membranes, generating reactive oxygen species (ROS), and disrupting metabolic processes.[47,49] Their effectiveness against antibiotic-resistant strains makes them a promising tool for combating MDR pathogens. Furthermore, nanosilver can be combined with conventional antibiotics, leading to a synergistic effect – reducing the required doses and potentially delaying the development of resistance.

An example of this is the study by Panáček et al., which evaluated the effectiveness of combining AgNPs with various antibiotics (including ampicillin, gentamicin, and streptomycin) against selected strains of *E. coli* and *S. aureus*. It was shown that the combinations of antibacterial agents significantly increased antimicrobial activity, especially when compared to antibiotics used in monotherapy. The observed synergistic effect allowed the minimum inhibitory concentration (MIC) to be reduced severalfold. On this basis, the authors indicated that nanosilver may enhance the activity of conventional drugs and constitute an effective antimicrobial strategy against drug-resistant strains.[50] Gold nanoparticles (AuNPs) also exhibit high antibacterial activity, as demonstrated by the study of Muhammad Ilyas et al.[51] Their aim was to investigate the antimicrobial properties of AuNPs against selected bacterial pathogens. The authors used AuNPs synthesized biologically using *Delphinium denudatum* leaf extract and then assessed their effects on *E. coli*, *S. aureus*, *P. aeruginosa*, and *Salmonella typhi*. The results showed that AuNPs exhibited clear bactericidal activity, with effectiveness dependent on particle concentration and size. The authors also noted that nanoparticles likely interact with the bacterial cell membrane, causing damage and disruption of metabolic processes. This study confirms the potential of AuNPs as an alternative or complement to conventional antimicrobial therapy.[51]

Nanocarriers such as liposomes, dendrimers, or polymer nanoparticles (e.g., made of poly(lactic-co-glycolic acid) (PLGA)) can function as drug delivery systems while protecting active substances against degradation, increasing their bioavailability, and directing them to sites of infection. Examples include nanocarriers of colistin or vancomycin, which improve antibiotic penetration into bacterial biofilms and reduce drug toxicity. A study conducted by Zhang et al. assessed the effectiveness of colistin-loaded PLGA nanoparticles in the treatment of infections caused by MDR strains of *P. aeruginosa*. To conduct this study, nanoparticles were prepared by emulsification and solvent evaporation and then evaluated in vitro and in vivo (in a mouse model). It was found that colistin encapsulated in nanocarriers exhibited increased biofilm penetration and antibacterial effectiveness while reducing renal toxicity compared to the free form of the drug. These results confirm the potential of nanotechnology to treat antibiotic-resistant bacterial infections more safely and effectively.[44]

An innovative approach is the so-called "intelligent nanomaterials", which respond to environmental conditions such as pH, the presence of bacterial enzymes, or light. These allow controlled drug release in response to stimuli.[47] Nanotechnology is also widely used in diagnostics, offering fast, sensitive, and specific methods for detecting pathogens and resistance markers. Knowledge of their occurrence is extremely valuable because it enables rapid adaptation of pharmacotherapy to the properties of pathogens. An example of the effective use of nanotechnology in diagnostics is a study conducted by Hong-zhi Pan et al.[52] It describes an electrochemical nanosensor based on AuNPs and graphene-modified electrodes, used to detect the presence of a resistance gene encoding *Klebsiella pneumoniae* carbapenemase. To conduct this study, the authors used hybridization of specific DNA probes with the target gene fragment and detection of the electrochemical signal, which enabled detection of the gene with high sensitivity and a short analysis time. The results showed that this platform enables rapid detection of resistance genes without the need for DNA amplification, making it a promising tool in microbiological diagnostics, especially in hospital settings.[52]

The advantages of nanotechnology include increased therapeutic effectiveness, the ability to deliver drugs to hard-to-reach sites, the ability to overcome biological barriers, and the potential reduction of antibiotic doses. Challenges include the toxicity of some nanomaterials, tissue accumulation, the lack of long-term safety studies, and the need for legal regulations regarding their use.[47,48]

Discussion

Summarizing the main findings, all discussed strategies – phage therapy, AMPs, CRISPR-Cas9 gene editing, and nanotechnology – are characterized by high therapeutic potential. Their strength lies in the possibility of selective action against MDR pathogens and their effectiveness against biofilms. The weakness lies in the limited number of clinical trials, difficulties in standardizing methods, and issues related to safety and legal regulations. Compared to previous studies, there is an increasing emphasis

on personalized therapy and the combination of different strategies in a multimodal approach. Differences in research results arise mainly from variations in experimental models and the limited scale of clinical trials. It is important for clinicians that these innovative methods may complement or replace conventional antibiotics in the future, especially in the treatment of infections caused by MDR and XDR strains. Further clinical trials are necessary to determine the long-term effectiveness and safety of these new therapies. Challenges also include unanswered questions regarding interactions with the microbiome, the risk of immunogenicity, and the potential for secondary resistance.

Conclusions

Microbial resistance to conventional antibiotics necessitates the introduction of innovative treatment methods. Biotechnological strategies such as phage therapy, AMPs, CRISPR-Cas9 systems, and nanotechnology open up new therapeutic possibilities. Their development may significantly affect clinical practice in the future but requires further research, funding, and the establishment of an appropriate legislative framework. The implementation of these methods should go hand in hand with public education, the rational use of antibiotics, and interdisciplinary collaboration. Shortening the path from basic research to clinical practice is crucial for effectively combating the growing problem of AMR.

Use of AI and AI-assisted technologies

Not applicable.

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