





# Revolutionizing Antimicrobial Solutions Nanotechnology, CRISPR-Cas9 and Innovative Approaches to Combat Drug Resistance in ESKAPE Pathogens

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## Abstract

Antimicrobial drug resistance within ESKAPE pathogens is a formidable global challenge necessitating innovative solutions. This review explores a multifaceted strategy incorporating nanotechnology, CRISPR/Cas9, and other cutting-edge approaches to effectively combat multidrug resistance in ESKAPE bacteria. Nanotechnology presents a promising avenue through targeted drug delivery systems like antibiotic nanoparticles and antibiotic-antibody conjugates (AACs). While these nanostructures aim to enhance therapeutic efficacy and mitigate resistance spread, challenges such as anti-PEG antibodies and optimal drug release must be considered. Inspired by successful anticancer nanomedicines, nanotechnology seeks to optimize drug penetration and retention within infected tissues. The revolutionary CRISPR/Cas9 gene-editing technology offers a precise and tailored approach by selectively targeting and modifying bacterial resistance genes. This holds the potential to reverse or eliminate drug resistance in ESKAPE pathogens, though challenges like off-target effects and efficient delivery mechanisms require attention for clinical translation. Additionally, alternative approaches such as fecal microbial transplantation, bacteriophage therapy, and probiotic bacterial replacement are actively explored in clinical trials. These strategies diversify the arsenal against antibiotic resistance by targeting unique vulnerabilities in ESKAPE pathogens. A comprehensive and multidisciplinary strategy is imperative to effectively address antimicrobial drug resistance in ESKAPE pathogens. Integration of nanotechnology, CRISPR/Cas9, and emerging approaches offers a synergistic solution, holding promise in overcoming the challenges posed by these resilient multidrug-resistant bacteria. This review provides insights into current research, challenges, and potential breakthroughs, emphasizing the urgency for collaborative efforts to safeguard global health.

**Keywords:** Antimicrobial Resistance, Biological Barriers, Carbohydrates, Immunological Barrier, Liposome Nanomedicine, Targeted Delivery

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## INTRODUCTION

A global danger to human health and economic growth, antimicrobial resistance (AMR) claims millions of lives annually.<sup>1,2</sup> This happens as a result of bacterial resistance genes acquiring DNA or naturally occurring DNA alterations. These alterations can restrict a drug's absorption, change the drug's target, make it inactive, or eliminate the medication entirely from the microbe. These problems are often associated with the improper use of antibiotics to cure and prevent infections and related diseases.<sup>2,3</sup> Additionally, antimicrobial-resistant bacteria's biofilms have significantly increased multidrug resistance. Although fungi, viruses, and protozoa have been found to exhibit antimicrobial resistance, the most critical problem is the widespread growth of antibiotic-resistant bacteria.<sup>1,2</sup> Alternatives to therapy are limited and higher doses are frequently required when confronted with bacteria that are resistant to various antibiotics.<sup>4,5</sup> To address antibiotic resistance, alternative and more efficient therapies are of the utmost importance. Effective vaccinations and novel antibiotics could help combat the danger of antimicrobial resistance (AMR); nevertheless, these developments have encountered many obstacles. The US FDA has granted approval for just a handful of antibacterial medications in recent years, despite intense research efforts that have moved several antibiotics into late-stage clinical trials.<sup>4,5</sup>

The World Health Organization (WHO) has discovered twelve extremely virulent and multidrug-resistant pathogens, including *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp. Collectively known as ESKAPE bacteria, these diseases are able to avoid being affected by antimicrobial treatments.<sup>6,7</sup> As of right now, ESKAPE bacteria lack an effective vaccine, and while many clinical trials have been carried out, the majority of research efforts have been directed toward creating *S. aureus* vaccine candidates. The outcomes thus far are a touch underwhelming. Conversely, using various tactics, ESKAPE bacteria have successfully grown resistant to a variety of medications.<sup>4,5</sup> These include the

reduction of protein and nucleic acid synthesis, the depolarization of the cell membrane, the disruption of cell wall development, and the disruption of other metabolic pathways.<sup>8</sup> Drug targets are mutated and modified, permeability decreases, efflux is enhanced, and bacterial enzymes inactivate the drug, among other processes that end up resulting in this resistance.<sup>5</sup> These microorganisms have the ability to form biofilms that efficiently impede immunological reactions and the effectiveness of antibiotics, resulting in decreased susceptibility to antibiotic therapy. Therefore, they are liable for an array of severe and recurring infections. The quantity of antibiotics that can be used to treat ESKAPE bacteria is decreasing over time. Combination antibiotics has been a well-researched technique to meet these urgent needs, with certain combinations actively undergoing clinical trials. Additionally, <https://clinicaltrials.gov/> lists clinical trials examining other strategies such fecal microbial transplantation, bacteriophage therapy, and probiotic bacterial replacement.<sup>6,7</sup> The purpose of these studies is to look into new approaches to treat infections caused on by microorganisms that are resistant to antibiotics and battling antibiotic resistance. If current medicines are administered specifically to the site of infection, enhanced antibacterial efficiency and safety may be achieved; nonetheless, treating AMR bacteria frequently requires large antibiotic dosages. Targeted delivery strategies for broad-spectrum antibiotics include the use of antibiotic nanoparticles and antibiotic-antibody conjugates (AACs). These strategies seek to treat patients more effectively while lowering the risk of antibiotic resistance spreading to additional microorganisms.<sup>9,10</sup> Notably, similar tactics have been applied to anticancer treatments with success, resulting in the creation of FDA-approved medications including antibody-drug conjugates (ADCs) and nanomedicines for anticancer therapy.<sup>11,12</sup> ADC's extremely potent payloads and uptake through endocytosis and drug release by lysosomal enzymes, which result in remarkable cytotoxicity against cancer cells, are caused by all of the processes that AAC lacks. Therefore, unless far more powerful antibacterial compounds are used and rapid drug release is achieved, which

would then deliver the anticipated therapeutic benefit, AACs are less likely to be successful as antibacterial medications.<sup>8,9</sup>

If current medicines are administered specifically to the site of infection, enhanced antibacterial efficiency and safety may be achieved; however, treating AMR bacteria frequently requires broad antibiotic dosages. Broad-spectrum antibiotics can be delivered specifically using antibiotic-antibody conjugates (AACs) and antibiotic nanoparticles.<sup>13</sup> These strategies seek to treat patients more effectively while lowering the risk of antibiotic resistance spreading to additional microorganisms.<sup>14</sup> Notably, similar tactics have been applied to anticancer treatments with success, leading to the development of FDA-approved medications including antibody-drug conjugates (ADCs) and nanomedicines for the treatment of cancer. In general, serum proteins bind to nanoparticles to form a protein corona that changes the particles size, shape, and surface features. This generally leads to the particles being quickly eliminated from the body. In order to counter this, certain cancer nanomedicines minimize interactions with serum proteins by utilizing PEGylated lipids, which improves stability and lengthens the duration that the drug is in circulation. The discovery that anti-PEG antibodies are possible, however, poses an entirely novel problem because of the elevated immune system clearance of nanoparticles following interaction. By making use of the porous capillaries that surround abnormal tumor tissues, cancer nanomedicines have the ability to pass through vascular barriers and improve their penetration and retention within the tumor.<sup>15</sup> The fundamental traits of nanomedicine, including the stealth effect and improved permeability and retention, are considered crucial. In addition to these characteristics, patients various cellular, intracellular, and microenvironmental barriers provide significant difficulties. Personalized chemotherapy nanomedicines have been proposed as a possible treatment option for cancer in the future in order to overcome these obstacles.<sup>9</sup> By attempting to address the constraints brought about by the unique characteristics of each patient and the intricacies of the cancer microenvironment, these tailored treatments may result in more beneficial therapeutic outcomes.<sup>15</sup>

A group of particularly alarming bacteria known as “ESKAPE” has developed resistance to a wide range of current treatments. Because of the challenges involved in treating these bacteria, they can be referred as “bad bugs with no drugs” in the world of medicine. *Enterococcus faecium*, *Staphylococcus aureus*, *K. pneumoniae*, *Acinetobacter baumannii*, *P. aeruginosa*, and *Enterobacter* species are among the Gram-positive and Gram-negative pathogens collectively referred to as ESKAPE.<sup>16</sup> The term “ESKAPE” has been used by the Infectious Disease Society of America to refer to a particular class of pathogens that have their origins in hospitals. These microorganisms often serve as the root of serious and potentially fatal infections acquired in hospitals, particularly in patients with compromised immune systems or those who are very ill.<sup>17</sup> According to Klevens, hospital-acquired infections (HAIs) impact about 1.7 million patients in US hospitals and cause almost 99,000 deaths yearly. According to a 2011 assessment on healthcare-associated infections (HAIs) in the US, there were roughly 722,000 recorded cases, and these illnesses were linked to 75,000 fatalities. According to research, hospitals that use antibiotics serve as havens for the growth of drug-resistant bacteria. For example, drug-resistant strains of *S. aureus* that were resistant to penicillin threatened civilian hospitals in London shortly after the drug's appear in the 1940s.<sup>17,18</sup>

In the classification of various bacterial strains, their resistance levels and corresponding treatment options were analyzed. *Acinetobacter*, identified as Gram-negative, exhibits a high level of multidrug resistance to ceftazidime, aminoglycoside, fluoroquinolones, and carbapenems. Treatment strategies include the use of carbapenems,  $\beta$ -Lactamase inhibitors, Tigecycline, Aminoglycosides, Polymyxin therapy, and Synergy, and combination therapy. Similarly, Gram-negative *E. coli*, *K. pneumoniae*, and *P. aeruginosa* all display a high level of multidrug resistance to various antibiotics, with suggested treatments involving specific compounds like POL7080 and ACHN-975.<sup>19</sup> *Enterococcus* spp., a Gram-positive strain, demonstrates high resistance to ampicillin, aminoglycosides, and glycopeptides, with treatment options focusing on the RX-04 lead series targeting the 50S ribosomal subunit. Lastly, the Gram-positive, multidrug-resistant

**Table 1.** Organisms causing antimicrobial resistance

Pathogen	Category	Common Resistant Mechanism	Resistant Antibiotics	Treatment Options	Current Challenge
<i>E. faecium</i>	E	Horizontal gene transfer, biofilm formation	Vancomycin, ampicillin, fluoroquinolones	Linezolid, daptomycin, tigecycline	Increasing resistance trends, limited treatment options
<i>S. aureus</i>	S	$\beta$ -lactamase production, efflux pumps	Methicillin, oxacillin, cephalosporins, fluoroquinolones	Vancomycin, daptomycin, ceftaroline	Methicillin-resistant strains (MRSA) widespread, evolving resistance
<i>K. pneumoniae</i>	K	ESBL production, carbapenemase production	Carbapenems, cephalosporins, fluoroquinolones	Tigecycline, aminoglycosides, colistin	Emergence of extensively drug-resistant strains
<i>A. baumannii</i>	A	Efflux pumps, biofilm formation	Carbapenems, cephalosporins, fluoroquinolones	Colistin, tigecycline, sulbactam	High intrinsic resistance, limited treatment options
<i>P. aeruginosa</i>	P	Efflux pumps, intrinsic resistance	Carbapenems, cephalosporins, fluoroquinolones	Piperacillin/tazobactam, ceftazidime, amikacin	Broad resistance mechanisms, challenges in treatment selection
<i>Enterobacter</i> spp.	E	AmpC $\beta$ -lactamase production	Ampicillin, cephalosporins, fluoroquinolones	Carbapenems, ceftazidime, piperacillin/tazobactam	Increasing resistance, limited treatment options

*S. aureus* exhibits high resistance to  $\beta$ -lactam antibiotics, macrolides, fluoroquinolones, and aminoglycosides. Treatment for *S. aureus* involves the use of the RX-04 lead series to inhibit translation by stabilizing a distorted mode of P-tRNA binding. The overall categorization reflects the challenging nature of antibiotic resistance across diverse bacterial strains.<sup>19,20</sup>

**The broad mechanism causing antibiotic resistance**

Many microbes live in complex colonies called biofilms in their native environments, which include the human body. These bacterial communities are able to adapt to environmental hazards and they are especially more resistant to antibiotics. Resistance at the cellular level can be up to 1000 times weaker than resistance attained by biofilm formation.<sup>21</sup> Resistance usually arises from spontaneous genetic alterations in cells and is horizontally transferred to other microbes by plasmids.<sup>22</sup> Beyond resistance, tolerance is another way to resist antibiotics; this is seen in persister cells, as was previously mentioned. The microbial community may become more resistant to antibiotics if both resistance mechanisms are active. Resistance to antibiotics can occur through various mechanisms, each affecting the efficacy of the drug. One common mechanism is the inactivation of the drug, which involves hydrolysis or modification. For instance,  $\beta$ -lactamase is employed for  $\gamma$ -lactam resistance, while acetyltransferases are utilized for aminoglycoside resistance. Another mechanism involves the alteration of the drug's target, leading to a reduction in the drug's binding affinity.<sup>22</sup> This alteration is often caused by mutations, such as those in DNA gyrase, contributing to fluoroquinolone resistance. Reducing drug influx is another strategy, achieved by lowering the permeability, particularly in Gram-negative outer membranes. This limits the drug's ability to penetrate the bacterial cell. Extrusion of the drug is facilitated by efflux pumps, with accessory membrane fusion proteins playing a crucial role in this process. These pumps actively remove the drug from the bacterial cell, preventing its accumulation and thereby conferring resistance. Horizontal gene transfer is a significant mechanism where bacteria acquire resistance determinants from other microorganisms. This

process allows the rapid spread of resistance traits among bacterial populations.<sup>23</sup>

In summary, antibiotic resistance can result from the inactivation of drugs through hydrolysis or modification, alteration of drug targets, reduction in drug influx by lowering permeability, extrusion of drugs via efflux pumps, and horizontal gene transfer, emphasizing the diverse strategies bacteria employ to develop resistance against antibiotics<sup>24</sup> (Table 1).

### **Antimicrobial resistance in ESKAPE pathogens Adverse effects of antibiotic resistance on health, wildlife, and environment**

Antibiotic resistance is a multifaceted issue with far-reaching consequences for human health, wildlife populations, and the environment. In human health, antibiotic-resistant infections pose a grave threat, leading to increased morbidity, mortality, and healthcare costs. According to the World Health Organization (WHO), antibiotic resistance is responsible for an estimated 700,000 deaths globally each year, with projections suggesting that this number could rise to 10 million deaths annually by 2050 if current trends continue.<sup>25</sup> Moreover, antibiotic resistance undermines the effectiveness of medical treatments such as chemotherapy, surgery, and organ transplants, potentially rendering these life-saving interventions less effective.<sup>26</sup>

The impact of antibiotic resistance extends beyond human health to encompass wildlife populations and the broader environment. Studies have demonstrated the transmission of antibiotic-resistant bacteria between humans, domestic animals, and wildlife, highlighting the interconnectedness of these ecosystems.<sup>27</sup> Additionally, the widespread use of antibiotics in agriculture, aquaculture, and veterinary medicine contributes to the dissemination of resistant genes into natural environments through soil, water, and wildlife.<sup>28</sup> This environmental reservoir of antibiotic resistance genes poses a risk of horizontal gene transfer to human pathogens, further exacerbating the problem.<sup>29</sup>

Addressing antibiotic resistance requires a multifaceted approach that encompasses surveillance, stewardship, and innovation across human health, agriculture, and environmental sectors. Effective strategies include promoting

judicious antibiotic use, implementing infection prevention and control measures, investing in research and development of new antibiotics and alternative therapies, and fostering interdisciplinary collaboration among healthcare professionals, veterinarians, ecologists, and policymakers.

### **Alternative mechanisms for combating multidrug resistance in ESKAPE pathogens**

#### **CRISPR-Cas9**

CRISPR, or clustered regularly interspaced short palindromic repeats, and the Cas proteins which come together are an ingenious technology with numerous applications. CRISPR has a capacity to disrupt specific bacterial genes, including those linked to resistance present on plasmids, by inducing double-strand breaks. Due to its unique quality, it is utilized for targeting certain genes for resistance.<sup>24</sup> The CRISPR/Cas system's multiplexing capabilities, which permits the simultaneous targeting of numerous resistance genes, is a significant advantage. It is uncertain if this approach will effectively eradicate resistance genes from multidrug-resistant (MDR) bacteria that inhabit the intestinal microbiota. among the primary challenges is the need for an array of temperate phages that are specially designed to target various resistance genes, in addition to the required knowledge of the resistance genes carried by the bacteria. The scenario as it stands today appears feasible for this to happen. Research indicates that phages taken oral are well tolerated, which contributes to the efficacy of phage therapy for intestinal tract bacteria.<sup>28</sup> However, precautions must be undertaken to prevent bacteriophage inactivation by stomach acid in order to properly use CRISPR/Cas methods. This means that once the phages enter the digestive tract, they must be kept active. This requires further studies to verify the phages activity and strategies for maintaining it. Determining the ideal dosage is also essential and requires more investigation.<sup>30</sup>

### **Utilizing nanotechnology and host-directed therapies to address multidrug resistance**

#### **Nanotechnology and nanoparticles in the fight against multidrug resistance**

The effectiveness of antibacterial nanomedicines hinges on their ability to persist

in the bloodstream, navigate vascular barriers, and evade swift clearance by the immune system for clinical success. This persistence facilitates the targeted delivery of antimicrobial drugs to infection sites. To enhance antibiotic absorption by bacteria and improve pharmacological qualities, nanomedicine must penetrate biofilms through fusion or diffusion.<sup>31,32</sup> Serum-stable nanoparticles can integrate potent yet toxic antimicrobial compounds, reducing side effects through focused delivery without premature release. Combining nanotechnology with antimicrobial agents emerges as a promising strategy to combat antimicrobial resistance (AMR).<sup>33</sup>

Notable examples include the FDA-approved Arikayce™, employing amikacin liposome inhalation suspension for treating pulmonary illnesses caused by *Mycobacterium avium* complex. While clinical studies on Arikayce™ against *Pseudomonas aeruginosa* in cystic fibrosis patients did not surpass control group outcomes, ongoing research explores other nanoagents such as silver nanoparticles. Clinical trials, detailed in on <https://clinicaltrials.gov/>, illustrate progress in the field, covering diverse nanosystems like organic nanoparticles (liposomes, lipid-based nanoparticles, polymeric micelles) and inorganic nanoparticles (silver, silica, magnetic, zinc oxide). Berini *et al.* explored metallic nanoparticles as carriers for antibiotics against multidrug-resistant bacteria, enhancing stability and antibacterial activity through coatings with biocompatible polymers.<sup>34,35</sup>

Despite silver nanoparticles broad-spectrum antibacterial properties, concerns arise about the development of silver-resistant bacteria, mirroring antibiotic resistance. Nanoparticles offer promise in addressing multidrug resistance by penetrating biofilms, particularly those formed by ESKAPE bacteria.<sup>36</sup> Antibiotics encapsulated in various nanoparticles exhibit promising antibacterial properties, preventing biofilm formation and enhancing intracellular delivery.<sup>37</sup> Metal oxide-based nanoparticles demonstrate potent antibacterial effects, especially against bacterial biofilms on medical devices, dental materials, and wound dressings.<sup>38,39</sup> However, safety issues restrict their use for systemic infections. Lipid-based nanoparticles, such as liposomes, emerge as popular vehicles for

systemic and topical antibacterial therapies. Despite positive scientific results, FDA approval for systemic bacterial infection treatment with lipid-based nanomedicine remains pending, and clinical outcomes have been largely unsatisfactory.<sup>40</sup>

Crommelin *et al.* critical review underscores the dominance of liposomes in nanomedicine, suggesting lipid-based nanoparticles as promising options for developing antibacterial nanomedicine, drawing parallels with the success of anticancer nanomedicine and mRNA-liposome vaccines.<sup>41</sup>

### Host-directed therapies

Enhancing germ elimination involves modulating the body's inflammatory responses via Pattern Recognition Receptors (PRRs), including Toll-like receptors (TLRs), NOD-like receptors (NLRs), and RIG-I-like receptors. TLRs, recognizing pathogen-associated molecular patterns, play crucial roles in initiating immune responses. Understanding distinct pathways, like TLR-2 detecting bacterial lipopeptides and TLR-9 identifying CpG islands, is vital for regulating immune responses to enhance bacterial eradication while preventing excessive inflammation.<sup>41</sup>

NLRs, such as NLR-P3 and NLR-C4, recognize PAMPs within the cell's cytoplasm. NLR-P3 induces inflammasomes' formation, activating procaspase-1 and converting pro-IL-1 and pro-IL-18 into active forms. The substance MCC950 inhibits ASC oligomerization induced by NLR-P3, showing promise for novel treatment approaches. Identifying small compounds selectively blocking cytokine release in response to NLR-P3 activation offers a potential avenue for treating infections, requiring further research for a comprehensive understanding of their role in regulating bacterial infections.<sup>13</sup>

### Development of Vaccines

Vaccines function by retraining the immune system to respond rapidly and effectively to various pathogens, thereby preventing illnesses and infections. Notably, due to the phenomenon known as herd immunity, certain vaccinations offer protection to both those who are directly vaccinated and those who are not. In large populations, herd immunity operates by hindering the spread of diseases to unvaccinated individuals

while also safeguarding those who have been vaccinated.<sup>4,41</sup> Research indicates that herd immunity can protect a significantly larger number of individuals compared to individual community vaccinations. The efficacy of herd immunity is exemplified by vaccines against diseases like *Streptococcus pneumoniae* and Haemophilus influenzae type b (Hib). These vaccines effectively prevent the corresponding infections from establishing in vaccinated individuals, reducing the potential for pathogens to spread within the community.<sup>20</sup>

The Hib vaccine, in particular, demonstrated high efficacy in preventing illness and reducing the need for antibiotics. Its ability to generate herd immunity was particularly beneficial for newborns and older children. Before the release of the conjugate vaccination against Hib in 1980, infants and children were at serious risk from Hib, with reported cases in children under five ranging from 3.5 to 601 cases per 0.1 million. Hib  $\beta$ -lactam resistance increased in the 1970s due to bacteria expressing  $\beta$ -lactamases and, to a lesser extent, altered penicillin-binding proteins resulting from increased antibiotic use. Global surveillance in 1999 and 2000 found that 16.6% of all Hib strains tested positive for  $\beta$ -lactamase, with significant variations between nations. Fortunately, the development and successful application of Hib conjugate vaccines have significantly altered antimicrobial resistance trends, mitigating the threat posed by such resistance.<sup>21</sup>

Hib vaccines were initially developed in the 1960s, utilizing the Hib polysaccharide capsule linked to carrier proteins. The introduction of these vaccines led to a rapid decrease in documented Hib cases, accompanied by a substantial reduction in the pathogen's nasopharyngeal carriage, which was crucial for the development of herd immunity. Following the vaccine's introduction in 1980, Hib incidence rates significantly decreased, from 2.6 cases per 100,000 (1986–1987) to 0.08 cases per 100,000 (2011–2015).<sup>10</sup> In the United Kingdom, shortly after the vaccine's introduction in 1992, Hib disease in children under five was nearly eliminated, and there was also a notable decrease in  $\beta$ -lactamase-positive Hib strains. This demonstrated the vaccine's effectiveness in reducing both the incidence of Hib disease and the prevalence of antibiotic-resistant strains.<sup>11</sup>

*Streptococcus pneumoniae*, a major global source of serious illness in both adults and children, causes an estimated 1.6 million deaths per year according to WHO in 2005. Before the introduction of pneumococcal vaccinations in the 1990s, the USA reported an annual average of 64,000 cases (limited to children).<sup>18</sup> Concurrently, invasive pneumococci were developing resistance to three or more medication classes, including penicillin and other antibiotics. The vaccine, with over 92% efficacy against invasive pneumococcal disease (IPD) in children under five, was a significant success upon its debut. The vaccination not only decreased bacterial colonization in children but also contributed to the development of herd immunity among those not directly targeted for vaccination.<sup>22</sup>

### Inhibition of quorum sensing

For microbial communities to coordinate their activities, quorum sensing—a kind of communication between them—through signal molecules is essential. Microorganisms are able to express harmful features, control infection, and create biofilms because of this signalling mechanism.<sup>16</sup> The development of biofilms and the activation of efflux pumps, which increase bacterial resistance to antibiotics, are major issues related to quorum sensing. Quorum sensing is triggered when infections congregate in the host environment, which causes the synthesis of biofilms and virulence factors.<sup>22</sup> One proposed method of countering the negative consequences of quorum sensing is to interfere with this bacterial communication. Anti-quorum sensing drugs have the ability to obstruct this communication, increasing the sensitivity of pathogens to antibiotics as well as host immunological responses. A number of tactics have been put out to prevent quorum sensing in an effort to sever and lessen the influence of the bacterial dialogue. These tactics focus on several facets of the quorum sensing procedure, including: Further investigation into these strategies could provide fresh perspectives on how to handle antibiotic resistance and fight bacterial illnesses.<sup>22</sup>

Signal degradation: Breaking down or interfering with the signaling molecules themselves to disrupt communication between microbes.<sup>23</sup>

Blocking signals with antibodies: Using antibodies to obstruct or interfere with the signalling molecules, preventing their interaction with microbial receptors.

Inhibiting signal synthesis: Interrupting the synthesis or production of signaling molecules essential for quorum sensing communication.<sup>24</sup>

These quorum sensing inhibition strategies, originating from diverse sources, hold promise as potential therapeutic targets to counteract microbial communication, reduce biofilm formation, and enhance the effectiveness of antibiotic treatments and host immunity. Continued research into these approaches may offer new avenues for combating bacterial infections and addressing antibiotic resistance.<sup>15</sup>

Antimicrobial resistance presents a intricate challenge, especially within ESKAPE pathogens, encompassing *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species. The escalating resistance to antimicrobials in these pathogens results from a multitude of factors, constituting a complex and multifaceted process. Research indicates that a variety of genetic mechanisms contribute to the development of resistance in ESKAPE pathogens.<sup>26</sup>

To address multidrug resistance in these pathogens, diverse strategies have been developed. These innovations span the creation of new antimicrobials, including nanomaterials and antimicrobial peptides, with the objective of overcoming the challenges posed by antimicrobial resistance.<sup>13</sup> Despite promising outcomes demonstrated by several new approaches against specific pathogens, clinical microbiologists remain watchful for even slight increases in the minimum inhibitory concentration of antimicrobials, as it can indicate the emergence of uncontrollable resistance.<sup>17</sup>

The mechanism by which antibiotics exert selective pressure is still not fully understood, emphasizing the necessity for specific recommendations to enhance studies on the natural selection of these organisms under antibiotic pressure.<sup>28</sup>

A thorough comprehension of these processes is pivotal in shaping a rational

framework for antibiotic practices. Such insights aim to optimize the effectiveness and longevity of existing antibiotics while minimizing the impact of resilient infections. The implementation of specific recommendations derived from an enhanced understanding will be crucial in combating antimicrobial resistance and preserving the efficacy of antibiotics in clinical settings.<sup>16</sup>

### Additional chemical processes

Understanding how infectious agents interact with host resistance mechanisms is essential for developing innovative strategies to prevent or treat human infectious diseases. The emergence of technologies like next-generation sequencing has transformed scientific research, offering researchers insights into the physiological responses of various organisms through genomics and transcriptomes.<sup>25</sup> This high-throughput approach has given rise to new tools for creating innovative antimicrobials, with a specific focus on antimicrobial peptides (AMPs) as potential weapons against antimicrobial resistance. For instance, the venom of the scorpion *Heterometrus petersii* has been examined using platform 454 sequencing, revealing four antimicrobial and cytosolic peptides. Transcriptome and proteomics technologies have provided fresh perspectives on the mode of action of AMPs in different organisms, such as the bivalve mollusk *Ruditapes philippinarum* and the American dog tick.<sup>29</sup>

Molecular surveillance, employing whole-genome sequencing (WGS), has become a valuable addition to antimicrobial resistance (AMR) phenotypic surveillance. WGS enables the determination and evaluation of the entire genome sequence of microorganisms at low costs and in a limited time, serving as a comprehensive tool for bacterial antimicrobial resistance surveillance.<sup>17</sup> Despite its challenges related to cost and complexity, WGS has the ability to expedite clinical decision-making and enhance antibiotic resistance prediction. With the creation of the online database BacWGSTdb 2.0, it is now easier to track antibiotic resistance worldwide, which makes WGS a useful tool in clinical microbiology labs.<sup>16</sup>

The prediction of antimicrobial peptides from DNA/RNA libraries has gained prominence



**Table 2.** Examples of Antimicrobial Peptides <sup>40</sup>

Source	Name	Activity	Mechanism of Action
Humans	LL-37	Broad-spectrum	Disrupts bacterial cell membranes
Frogs	Dermaseptin	Broad-spectrum	Membrane permeabilization
Insects	Defensins	Broad-spectrum	Disrupts cell membrane integrity
Plants	Plantaricin A	Bacteriocidal	Inhibits cell wall synthesis

**Table 3.** Examples of Bacteriophages Used in Therapy

Phage Name	Target Bacteria	Application
T4	<i>E. coli</i>	Gastrointestinal infections
MR11	<i>S. aureus</i>	Skin and wound infections
DAR1	<i>P. aeruginosa</i>	Respiratory infections

in pharmaceutical research, capitalizing on the relationship between the immune responses of organisms and the ability of AMPs to combat antibiotic-resistant microorganisms.<sup>10</sup> Bioinformatics tools, such as the Antimicrobial Peptide Database (APD2) and Collection of Antimicrobial Peptides (CAMP), assist in the search and identification of antimicrobial peptides. Additionally, web servers like iAMP-2L are employed to identify uncharacterized sequences with antimicrobial properties. The significance of continuing study in this area is highlighted by the fact that these developments in bioinformatics provide the foundation for modeling and the discovery of new medications and physiologically active substances.<sup>12</sup>

### Antimicrobial Peptides (AMPs)

Antimicrobial peptides (AMPs) are a diverse group of naturally occurring molecules with broad-spectrum antimicrobial activity against bacteria, fungi, viruses, and parasites.<sup>29</sup> They are found in various organisms, including humans, animals, plants, and microorganisms. AMPs play a crucial role in innate immunity and host defense mechanisms<sup>30</sup> (Table 2).

Antimicrobial peptides exert their antimicrobial effects through various mechanisms, including disruption of bacterial cell membranes, inhibition of cell wall synthesis, and interference with intracellular processes. One of the advantages of AMPs is their ability to target bacterial membranes, making it less likely for bacteria to

develop resistance compared to conventional antibiotics.<sup>31</sup>

### Bacteriophage therapy

Bacteriophages, or simply phages, are viruses that infect and replicate within bacterial cells. Bacteriophage therapy involves the use of these viruses to specifically target and kill pathogenic bacteria.<sup>32</sup> Phages are highly specific, typically targeting only a narrow range of bacterial species or strains (Table 3).

Bacteriophage therapy offers several advantages, including high specificity, minimal disruption to beneficial bacteria, and the potential for phages to evolve to overcome bacterial resistance.<sup>31</sup> However, challenges remain in terms of optimizing efficacy, safety, and scalability for widespread clinical use.

In summary, both AMPs and bacteriophage therapy represent promising alternatives to conventional antibiotics in combating bacterial infections. Further research and development efforts are needed to harness their full potential and integrate them into clinical practice.

### Immunomodulation

Immune modulation involves enhancing the body's immune response to better combat bacterial infections. This approach recognizes the importance of the host immune system in controlling and eradicating pathogens. Immune modulation strategies encompass a range of interventions aimed at bolstering various components of the immune system, including innate and adaptive immunity. One key aspect of immune modulation is the development of vaccines. Vaccines stimulate the immune system to produce specific antibodies or activate cellular immune responses against bacterial pathogens. Vaccination programs have been successful in controlling and even eradicating infectious

**Table 4.** Comparisons of strategies involved to overcome resistance

Strategy	Safety	Efficacy	Commercial Viability	Economic Aspect
CRISPR-Cas9	Off-target effects; unintended modifications	Laboratory efficacy demonstrated; clinical evaluation	Challenges in delivery methods, regulatory approval	High initial investments; potential long-term benefits
Nanotechnology	Potential toxicity; environmental impact	Targeted antimicrobial delivery; enhanced efficacy	Scalability; stability of nano-materials	Initial investments in R&D; potential cost-effectiveness
Host-Directed Therapies	Immunosuppression; autoimmune reactions	Bolstered innate immune defenses; variable efficacy	Individualized treatment regimens; limited market potential	Costs of personalized therapies; reimbursement challenges
Vaccine Development	Adverse reactions; antigen selection	Prevention of infections; efficacy against strains	Long-term commercial potential; widespread adoption	Investments in R&D; economic savings in healthcare costs
Inhibition of Quorum Sensing	Minimal impact on host cells; disruptions	Disruption of bacterial virulence; biofilm formation	Complementing existing therapies; scalability issues	Economic benefits through enhanced antimicrobial efficacy
Additional Chemical Processes	Toxicity concerns; safety assessments	Discovery of new antimicrobial agents	Development costs; regulatory approval; market demand	Potential economic benefits through novel compound discovery
Antimicrobial Peptides (AMPs)	Low toxicity to mammalian cells	Broad-spectrum activity; low production cost	Large-scale production challenges; formulation issues	Production costs; market demand for AMP-based products
Bacteriophage Therapy	Specific targeting; favorable safety profile	Efficacy against antibiotic-resistant infections	Regulatory approval; manufacturing challenges	Cost-effectiveness compared to traditional antibiotics
Immunomodulation	Immune response modulation; safety monitoring	Enhanced host defenses; reduced antibiotic reliance	Individualized treatment challenges; regulatory hurdles	Economic considerations include treatment costs

**Table 5.** Innovative approaches to overcome antimicrobial drug resistance in ESKAPE pathogens, highlighting their respective descriptions, synergies, and challenges

Approach	Description
Nanotechnology in Antimicrobial Treatment	Utilizing engineered nanoparticles for targeted drug delivery to enhance bioavailability and overcome traditional treatment barriers.
CRISPR/Cas9 Technology	Employing the revolutionary genome editing tool to precisely modify microbial DNA, targeting and disabling resistance genes.
Combination Therapies and Synergy	Implementing synergistic combinations of antibiotics, nanoparticles, and CRISPR/Cas9 treatments to maximize efficacy and minimize resistance emergence.
Other Innovative Approaches	Exploring alternative strategies, including phage therapy, new antibiotic development, immunotherapies, and drug repurposing, to combat antimicrobial drug resistance.
Challenges and Future Directions	Addressing challenges such as off-target effects, optimizing delivery mechanisms, and ethical considerations, while staying vigilant to the evolving landscape of resistance mechanisms in ESKAPE pathogens.

diseases such as smallpox, polio, and tetanus. Additionally, advancements in vaccine technology, such as the development of conjugate vaccines and novel adjuvants, hold promise for expanding the efficacy and scope of vaccine-mediated immunity.<sup>33</sup>

Furthermore, immunomodulatory drugs can be utilized to enhance or suppress immune responses selectively. For example, cytokines such as interferons and interleukins can be administered to boost immune activity against bacterial infections. Similarly, monoclonal antibodies targeting specific bacterial antigens or virulence factors can neutralize pathogens or enhance phagocytosis by immune cells. Immune modulators have been explored for the treatment of infections caused by multidrug-resistant bacteria, offering alternative therapeutic options when conventional antibiotics fail.<sup>34,37</sup>

### Heterocyclic compounds

Heterocyclic compounds represent a diverse class of organic molecules characterized by the presence of at least one heteroatom, such as nitrogen, oxygen, or sulfur, within their ring structure. These compounds exhibit a wide range of biological activities, including antimicrobial properties. The structural diversity of heterocyclic compounds allows for the rational design and synthesis of molecules with tailored antimicrobial activity and pharmacokinetic profiles.<sup>36,38</sup> One class of heterocyclic compounds that has garnered significant interest in antimicrobial drug discovery is quinolones. Quinolones inhibit bacterial DNA gyrase and topoisomerase IV, essential enzymes

involved in DNA replication and repair, thereby disrupting bacterial cell division and proliferation. Despite widespread clinical use, the emergence of quinolone-resistant bacteria has spurred efforts to develop next-generation quinolone derivatives with improved efficacy and reduced resistance.<sup>35</sup>

Additionally, oxazolidinones represent another class of heterocyclic compounds with potent antimicrobial activity against Gram-positive bacteria. These compounds inhibit bacterial protein synthesis by binding to the 50S ribosomal subunit, thereby preventing the initiation of translation. Linezolid, the first oxazolidinone antibiotic approved for clinical use, has demonstrated efficacy in treating infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE).<sup>36,39</sup>

In conclusion, immune modulation and the use of heterocyclic compounds represent promising strategies to combat antibiotic resistance. These approaches leverage the host immune system and innovative drug design principles to develop novel therapeutic interventions against bacterial infections.

### Strategies to combat multidrug resistance (MDR)

Multidrug resistance (MDR) in bacterial infections poses a significant challenge to public health, necessitating innovative strategies for effective management. Various approaches have emerged, each with distinct considerations regarding safety, efficacy, commercial viability, and economic impact. Here, we discuss several prominent strategies along with their key attributes (Table 4).<sup>40</sup>

### Future perspectives

Nanomedicine has been designed with a dual purpose, serving as both a vaccine and a therapeutic delivery system to improve effectiveness and reduce adverse effects. Despite the potential, only a limited number of nanomedicines in clinical development have met expectations, particularly in the realm of personalized cancer treatments. However, the urgency to address antimicrobial resistance (AMR) has redirected the focus towards enhancing accessibility and affordability in this field.<sup>12</sup>

The primary aim in developing anti-AMR nanomedicine is to prioritize simplicity, achievable through a deeper comprehension of immune responses to nanomedicine within the human body and the intricate interactions involving nanomedicine, diverse microbes, and various human systems.<sup>41</sup> Establishing a correlation between nanostructure and function is crucial for guiding the design of nanomedicine to achieve optimal outcomes.<sup>37</sup>

Future endeavors in nanomedicine engineering aim to create more robust nanoparticles, utilizing either synthetic or naturally biocompatible and biodegradable materials. These engineered nanoparticles should exhibit improvements in uniformity, size control, surface charges, encapsulation, and readiness for seamless integration into a multicomponent system, facilitating targeted delivery.<sup>4,38</sup> Overcoming the challenge of heterogeneous biofilms is identified as a critical factor in the effectiveness of antibacterial nanomedicine.<sup>41</sup>

In the clinical development realm, a suggested shift in focus regarding nanoparticle design is proposed. The emphasis should move towards leveraging the extracellular environment rather than fixating on specific molecular structures. Effective delivery of therapeutics to bacteria is envisioned through methods such as fusion or diffusion.<sup>42</sup>

Drawing parallels with virus-like particles, especially lipid-based nanoparticles, introduces the potential for creating microbe-like particles through a combination of biosynthesis and chemical synthesis.<sup>43</sup> This innovative approach not only holds promise for developing effective vaccines against AMR but also underscores the broader role of these particles as vaccine/

immunogen delivery systems. The integration of nanotechnology and medicine holds significant potential, particularly in the multifaceted battle against antimicrobial resistance (Table 5).<sup>44</sup>

Innovative strategies are essential in combating the escalating threat of antibiotic resistance, necessitating a multifaceted approach to address multidrug-resistant (MDR) microbes. Integration of antimicrobial peptides (AMPs) with the precision targeting of the CRISPR/Cas9 system offers a promising avenue to overcome resistance mechanisms (Getahun *et al.*). Nanotechnology emerges as a beacon of hope, facilitating targeted delivery of antimicrobial agents and minimizing toxicity concerns. Combining CRISPR/Cas9<sup>40</sup> with nanoparticles presents a novel strategy to combat resistance.<sup>39,40</sup> Understanding the origins and impacts of resistance is crucial for devising effective interventions.<sup>38</sup> Disrupting microbial communication pathways through quorum sensing inhibition shows promise in enhancing treatment efficacy.<sup>41</sup> Overall, integrating these innovative strategies offers hope in mitigating the global health threat posed by antibiotic resistance.

### CONCLUSION

The complex and evolving landscape of antimicrobial resistance (AMR) poses a formidable global challenge that requires a multifaceted and innovative response. The threat, epitomized by the rise of ESKAPE bacteria, demands a shift from conventional antibiotic therapies to alternative strategies and a deeper understanding of molecular mechanisms. The limited success in developing effective vaccines against ESKAPE bacteria underscores the urgency to explore alternative treatments, including CRISPR-Cas9 technology, nanotechnology, and host-directed therapies. Each of these approaches brings unique advantages and challenges, highlighting the need for continued research and collaboration in the fight against AMR. Vaccines, as demonstrated by successes like the Haemophilus influenzae type b (Hib) vaccine, remain a crucial pillar in preventing infectious diseases and curbing the spread of antibiotic-resistant strains. The concept of herd immunity showcases the broader impact of vaccination, protecting both the directly vaccinated individuals and unvaccinated members of the

community. Quorum sensing inhibition emerges as a promising avenue to disrupt bacterial communication, potentially enhancing the effectiveness of antibiotics and the immune responses of the host. Targeting various aspects of quorum sensing, such as signal degradation, antibody interference, and signal synthesis inhibition, provides a nuanced approach to combating bacterial infections. The integration of molecular surveillance through whole-genome sequencing and the identification of antimicrobial peptides using advanced bioinformatics tools further enriches our understanding of AMR and informs clinical decision-making. These tools empower researchers to predict antibiotic resistance more efficiently, potentially transforming the landscape of clinical microbiology. In essence, embracing the collective power of these innovative approaches and technologies offers hope in the battle against AMR. The quest for effective solutions demands ongoing commitment, collaboration, and a holistic understanding of the intricate interactions between pathogens, antibiotics, and the human immune system. Only through such comprehensive efforts can we hope to preserve the efficacy of antibiotics and mitigate the impact of resilient infections, safeguarding global health for generations to come.

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#### CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

#### AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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#### DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

#### ETHICS STATEMENT

Not applicable.

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