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Novel approaches to overcome antibiotic resistance: Phage therapy, nanoparticles, and natural antimicrobials

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Abstract

The escalating crisis of antibiotic resistance worldwide poses considerable dangers to public health, emphasizing the ineffectiveness of traditional antibiotics against multidrug-resistant bacterial strains. Consequently, scientists are working on new strategies that serve as substitutes or complementary techniques to conventional antibacterial therapeutics. Three potential approaches include: phage therapy, nanoparticles, and natural antimicrobials. Phage therapy uses bacteriophages, viruses that particularly target and destroy bacteria, to offer an extremely exclusive, versatile, and self-replicating treatment alternative. Both polymeric and metal-based nanoparticles demonstrate promising antibacterial activities through different mechanisms, including cell membrane degradation, oxidative stress generation, biofilm synthesis inhibition, and improved drug delivery by working synergistically with other antibiotics. Nanoparticles can also be manipulated to enhance selectivity and decrease toxic effects. Plant, algae, and animal-derived natural antimicrobials comprise a wide variety of structurally distinct compounds offering antimicrobial activities against a broad spectrum of bacteria, including interfering with the mechanisms of resistance development. Progress in genomics, biotechnology, and nanoscience is opening new pathways of innovation, improvement, and administration of these new substances. This review highlights the modes of action, recent clinical studies, and possible therapeutic applications of these alternative strategies, underlining their role in overcoming the problems of antibiotic resistance.

1. Introduction

One of the most pressing concerns in global public healthcare is the growing challenges posed by antibiotic resistance (Gulumbe et al. 2025). Antibiotic resistance is defined as the ability of microbes to resist the harmful effects of antimicrobial components, thereby sabotaging years of healthcare advancements and undermining the efficacy of current treatments (Mehrotra et al. 2024). According to the World Health Organization (WHO), multidrug-resistant pathogens are behind approximately 700,000 fatalities every year; moreover, according to an assessment, this number may be elevated to ten million by 2050 if necessary initiatives have not been taken (Ahmed et al. 2024). Several factors contribute to the development of this silent pandemic, including the excessive misuse of antibiotics in both healthcare and agricultural settings, inadequate infection prevention techniques, and slow progress in improving antibiotic quality (Manyi-Loh et al. 2018). Furthermore, rapid evolution and modification are the distinct properties of bacteria that contribute to their progressive resistance mechanisms. For instance, stimulation of efflux pumps, beta-lactamase enzyme synthesis, and changes in molecular targets (Ahmed et al. 2024). Furthermore, healthcare systems have to face severe economic losses due to antibiotic resistance, including escalated expenses due to prolonged hospitalization, compulsion for high-priced and harmful procedures, and extended diagnostic analysis (Baciu et al. 2024). Additionally, at the global level, food security and biosafety have also been affected severely by antibiotic resistance, as MDR microorganisms progressively impact both livestock and aquatic animals (Founou et al. 2021). Regardless of global projects, including the Global Action Plan on Antimicrobial Resistance, an immediate requirement for the development of advanced strategies should be met to overcome this

escalating dilemma (Talebi et al. 2019).

Conventional antibiotic treatments are regarded as the foundation of mainstream medicine. However, they have been increasingly unsuccessful in controlling resistant pathogens. Conventional antibiotics' ability to demonstrate bacteriostatic or bactericidal effects by targeting protein synthesis, cell wall formation, and nucleic acid replication is also responsible for the emergence and multiplication of resistant variants (Uddin et al. 2021). Moreover, horizontal gene transfer amplifies the propagation of drug-resistant genes in bacterial communities and reduces the efficiency of many frontline and final-line antibiotics (Amábile-Cuevas and Lund-Zaina 2024). Similarly, the inability of conventional antibiotics to break through the biofilms is another considerable limitation. Biofilms are defined as complex bacterial communities enclosed in a protective intercellular matrix and primarily contribute to chronic infections. They show approximately 1000 times more resistance to antibiotics than their planktonic forms (Sharma et al. 2023; Lenchenko et al. 2024). The major factors behind the emergence of this resistance include the ability of persister cells to survive the antimicrobial invasion, restricted penetration of antibiotics, and modifications in the microenvironment of the biofilm (Uruén et al. 2020). Furthermore, scientific, financial, and supervisory challenges have slowed down the development of novel antibiotics. Additionally, drug manufacturers have to deal with extravagant costs and an extensive timeframe for antibiotic development, combined with limited economic outputs, as the typical application of antibiotics is limited to reducing resistance (Muteeb et al. 2023). Therefore, medical professionals are currently left with limited alternatives for tackling resistant infections, owing to the gap in the development of a diverse range of efficient antibiotics. Conventional antibiotics have problems that exceed resistance. The vague nature of various antibiotics mostly leads to off-target delivery, resulting in the deterioration of probiotic microflora. Additionally, this may cause ecological imbalances at a broader level and lead to secondary infections, including Clostridioides difficile colitis (Shim 2023). These challenges emphasize the necessity of a substitute therapeutic modality to overcome the complicated antibiotic resistance processes. This review article highlights the medicinal potential of phage therapy, nanotechnology, and natural antimicrobials to fight against MDR bacterial strains.

2. Phage Therapy

Traditional antibiotics have been successfully substituted by phage therapy (Pal et al. 2024). It is an intervention that was developed before antibiotics; however, it has been gaining attention due to its potential applications against antibiotic resistance (Malik et al. 2021). Bacteriophages are the viruses that solely attack and spoil bacteria (Ranveer et al. 2024). Frederick Twort and Félix d'Hérelle were the first ones to individually discover bacteriophages in 1915 and 1917, respectively (Gordillo Altamirano and Barr 2019). Bacteriophages are known for their extremely peculiar nature of infecting a particular species or even a specific bacterial strain, compared with the miscellaneous nature of antibiotics targeting a broad spectrum of bacterial species (Sanz-Gaitero et al. 2021). This destructive mechanism of the phage is fueled by its ability to recognize and bind to specific receptors on the bacterial cell membranes (Hatfull et al. 2022).

2.1 Life Cycle of Bacteriophage

Lysogenic and lytic cycles are the life cycles of bacteriophage, counted among the most fundamental facets of its antibacterial mechanisms (Kortright et al. 2019). The direct association of the lytic cycle with the deterioration of the bacteria confirms its therapeutic importance (Dong et al. 2021). The lytic cycle is initiated by the attachment of the tail fibers of the phage to the surface of the exposed bacteria by identifying unique receptors on the cell wall (Ouyang et al. 2024). After adhering, the phage hijacks the bacterial biological machinery by injecting its genetic material into bacterial cells (Wang et al. 2024). Consequently, the inserted DNA triggers the cellular machinery to synthesize viral elements, such as nucleic acids and proteins needed to reproduce new phages (Pfeifer et al. 2022). As a result, continuous replication of new phages filled the bacterial cell. Fresh phages are released into the surroundings after the accumulation of phage particles ultimately causes the bacterial cell to burst (Dennehy and Abedon 2021). These released phages can target neighboring bacteria, transmitting the loop of infection and disintegration (Taati Moghadam et al. 2020; Zalewska-Piątek and Piątek 2020). The bactericidal properties of the lytic cycle demonstrate the effective defense of the bacteriophages, particularly against MDR bacteria (Hasan and Ahn 2022).

2.2 Application of Phage Therapy

The application of phage therapy in established medical practice consists of four main steps, as mentioned in Fig. 1: Firstly, the identification of the bacterial pathogen involves recognition of the pathogen using procedures like PCR to determine the particular bacterial strain triggering the infection after the diagnosis of the patient (Gerace et al. 2022). Secondly, the phage screening or engineering process led to the selection of phages based on the antibacterial activities and, if needed, engineered them for improved results. *In vivo* and *in vitro* settings are utilized to establish the safety and validity (Łobocka et al. 2021). The third step is bacteriophage preparation that involves proliferation, identification, and isolation of competent phages, consequently providing better preparation for therapeutic purposes (João et al. 2021). And lastly, application and monitoring include the administration of the phage via the most effective procedure, assessment of the therapy outcomes, and observation for any future alterations (Briot et al. 2022).

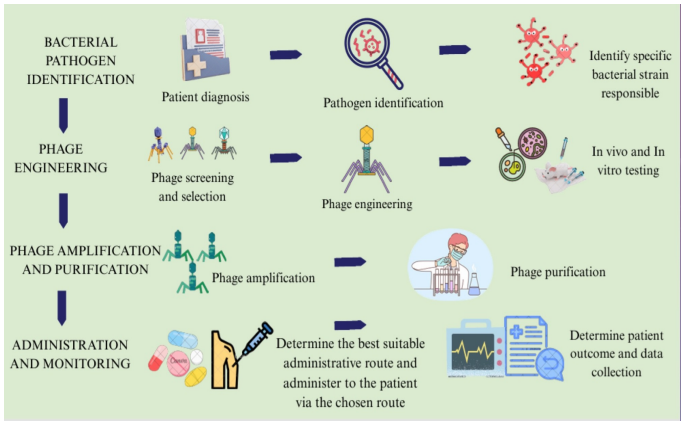

***Fig. 1**. Steps involved in the preparation and application of phage therapy*

Recently, to evaluate the efficacy and understand the combating mechanism of phage therapy against MDR bacteria, several clinical trials and scientific investigations have been conducted by microbiologists, as mentioned in Table 1. For example, Kaabi et al. (2020) and Wittebole and Opal (2020) performed a case study to find

out the efficacy of phage therapy against *Pseudomonas aeruginosa* induced chronic otitis. Complete recovery with a significant reduction in the bacterial loads in the patient was the outcome. Wittebole and Opal (2020) demonstrated a 70 percent recovery in patients with diabetic foot ulcer triggered by MDR *Staphylococcus aureus,* both in the presence and absence of standard care. Moreover, according to Law et al. (2019) and Tamma et al. (2022), patients with cystic fibrosis induced by *P. aeruginosa* exhibited complete recovery, characterized by a reduction in bacterial count and increased pulmonary activity. Furthermore, Onallah et al. (2023) emphasized the importance of phage therapy as a potential last-resort medication in a clinical trial where it was administered as compassionate treatment to patients with sepsis caused by *Acinetobacter baumannii.* Another investigation led by Jault et al. (2019) showed the high effectiveness of phage therapy through a considerable reduction in the burn wound infection and rapid healing triggered by MDR *P. aeruginosa, Escherichia coli, and Klebsiella pneumoniae.* Similarly, Leitner et al. (2021) revealed that direct administration of phage therapy into the bladder resulted in the full recovery and considerable reduction in signs and symptoms in patients suffering from urinary tract infection caused by MDR bacteria. Taha et al. (2019) demonstrated that after failure of several antibiotic courses, improved limb activity and full recovery in patients with diabetic leg infection caused by *K. pneumoniae.* Lastly, Ooi et al. (2019) showed that phage therapy led to a significant reduction in bacterial count and sinus symptoms in patients with chronic rhinosinusitis caused by *S. aureus.*

3. Nanoparticles (NPs)

To overcome the resistant mechanisms of bacterial strains, NPs have been introduced as a promising cutting-edge technique in medical systems, along with new approaches to overcome the challenges of conventional therapeutics (Mehwish et al. 2024; Shaban et al. 2025). NPs are defined as nano-sized materials with unique physical and chemical properties, such as enhanced surface area, highly reactive, atomic-level associations with biological systems, and controlled surface chemistries (Khan and Hossain 2022). These unique characteristics of NPs are the reasons behind the elevated efficiency of traditional antibiotics, the

Table 1 The efficacy and efficiency of phage therapy against multiple MDR bacteria in clinical trials and case studies

Clinical trial/ case study	Antibiotic-resistant bacteria	Obsolete antibiotic	Condition treated	Phage therapy results	Observations	References
Chronic Otitis	*P. aeruginosa*	Carbapenem	Chronic Otitis Media	Bacterial load reduction and significant clinical improvement	Direct injection of phages into the infected zone indicated high efficacy	(Kaabi et al. 2020; Wittebole and Opal 2020; Mohammed et al. 2024)
Compassionate use	*A.baumannii*	Carbapenem	Sepsis	Complete recovery was ensured by phage therapy	Phage therapy acted as a promising last-resort therapeutic agent in emergencies	(Onallah et al. 2023; Dorgham et al. 2024)
UTI Treatment	*E. coli*	Penicillins, cephalosporins	Urinary tract infection	80% of patients showed complete recovery	Persistent Antibiotic-resistant UTIs were recovered by phage therapy when administered directly into the bladder	(Leitner et al. 2021)
Chronic Rhinosinusitis	*S. aureus*	Methicillin, vancomycin	Chronic rhinosinusitis	Significant decrease in bacterial colonization and sinus signs and symptoms	High efficacy of phage therapy against biofilm-related chronic infections	(Ooi et al. 2019; Bessembayeva et al. 2024)
Diabetic Foot Ulcer	*S. aureus*	Methicillin, vancomycin	Diabetic foot ulcers	Up to 70% of patients made a complete recovery	High efficacy exhibited by phage therapy in the treatment of MDR bacterial infection with or without standard care	(Wittebole and Opal 2020)
Diabetic Leg Infection	*K. pneumoniae*	Carbapenem, cephalosporins, ciprofloxacin	Diabetic leg infection	Complete recovery after multiple unsuccessful antibiotic courses	Emphasis on the administration of phage therapy for deep treatment and chronic infections resistant to traditional antibiotics	(Taha et al. 2019)
Burn Wound Infection	*E. coli, K. pneumoniae, P. aeruginosa*	Carbapenem, penicillins, cephalosporins, ciprofloxacin	Infected burn wounds	Significant decrease in the intensity of infection and rapid wound recovery	High efficacy of phage therapy in complicated wound infection, and is commonly intolerant to traditional antibiotics	(Jault et al. 2019)
Cystic Fibrosis	*P. aeruginosa*	Carbapenem	Chronic respiratory infection	Reduced bacterial count and enhanced pulmonary function	Phages were utilized as precision medicine, suppressing biofilm-related resistance mechanisms	(Law et al. 2019; Tamma et al. 2022)

modification of the resistant mechanism, and offer novel bactericidal approaches (Tabassum et al. 2024; Elshobary et al. 2025).

3.1 Synthesis and Characterization of NPs

The formation and characterization of NPs involve a cascade of precise biological, physical, and chemical operations to control the composition, shape, and size measurements based on nanoscale (Joudeh and Linke 2022; Akhreim et al. 2024). Synthesis of NPs is led by several techniques such as the sol-gel process, chemical reduction, green synthesis, and hydrothermal (Bokov et al. 2021; Patil et al. 2021). Agglomeration is inhibited, and conformity is ensured during the process of NPs formation; moreover, practicality, along with chemical and physical properties, are characterized after synthesis (Joudeh and Linke 2022). For characterization of NPs, technologies, such as Transmission electron microscope (TEM), which is used to determine the size and shape, Dynamic light scattering (DLS) for particle size analysis and measure zeta potential, X-ray diffraction (XRD) to study crystalline structure, and Fourier-transform infrared microscopy to determine surface functional groups of NPs have been used (Mongkolsuttirat and Buajarern 2021; Eid 2022; Filippov et al. 2023; Shukla et al. 2023). Therefore, these techniques serve an in-depth comprehension of NPs properties, ensuring their utilization in ecosystem restoration, medical systems, electronics, energy, and other fields.

3.3 Mechanisms of Action

Many fundamental mechanisms make the NPs a more promising therapeutic agent against antibiotic-resistant bacteria than conventional antibiotics, as mentioned in Fig. 2. For instance, the unique mechanism of traditional antibiotics to target the formation of bacterial cell membranes or protein synthesis increased their efficacy at first, but later became less effective due to gene transfer, and the adaptable nature of bacteria led to the development of resistance (Baran et al. 2023). On the other hand, nanoparticle-dependent strategies represent a wide range of non-specific mechanisms of action, including the induction of oxidative stress and membrane degradation, as mentioned in Table 2, with little probability of blockage by resistance mechanisms (Anand et al. 2022). Moreover, weak penetration and modified bacterial conditions make traditional antibiotics less effective against biofilms (Singh et al. 2022). However, target delivery and the power to pass through the extracellular matrix enhanced the biofilm-degradation capabilities of nanomaterials (Birk et al. 2021). Furthermore, traditional antibiotics are limited by non-flexible chemical structures and exclusive function, whereas high chemical reactivity, composition, and tunable size make the nanoparticles multifunctional (Cheesman et al. 2017; Coates et al. 2020; Kamble et al. 2022; Siwal et al. 2022; Zhu et al. 2022). The excessive production of reactive oxygen species (ROS) is one of the main mechanisms induced by nanoparticles to combat antibiotic-resistant bacterial infections. Nanoparticles trigger the synthesis of highly reactive oxygen species, including hydrogen peroxide (H_2O_2), hydroxyl radicals (•OH), and superoxide ions, causing biological membranes, proteins, and DNA degradation and, ultimately, bacterial cell death (Mammari et al. 2022). For instance, silver nanoparticles (Ag NPs) and Titanium dioxide nanoparticles (TiO_2 NPs) oxidized and disrupted the lipid bilayers of bacteria through changes in permeability and discharge of cytoplasmic components (Al-Sa'ady and Hussein 2020; Bekele and Alamnie 2022). Similarly, zinc oxide nanoparticles (ZnO NPs) inhibited the activity of bacterial enzymes essential for metabolic processes through the oxidation of amino acids, especially methionine, cysteine, and tyrosine (Krishnamoorthy et al. 2022; Iqbal et al. 2024a).

Moreover, Nanoparticles can disrupt the biological membranes of MDR bacteria through electrostatic interactions, physical disruption, lipid peroxidation, and fluidity modification. For example, the positive surface charges of silver nanoparticles (Ag NPs) interacted electrostatically with the negatively charged teichoic acids of Gram-positive bacteria and lipopolysaccharides of Gram-negative bacteria, leading to enhanced permeability, discharge of cellular contents, and ultimately, bacterial death (Dakal et al. 2016; Häffner and Malmsten 2017; Iqbal et al. 2024b). In another study, sharp edges or cylindrical configuration of graphene oxide (GO) and carbon nanotubes (CNTs) degraded the lipid bilayer by creating pores that ultimately led to osmotic imbalance, discharge of cellular content, and bacterial cell rupture (Baek et al. 2019). Furthermore, unique properties of nanoparticles enable them to degrade or block biofilm synthesis. For instance, Ag NPs and copper nanoparticles (Cu NPs) degraded the extracellular matrix of biofilms, altered structural integrity, and removed the shield against antibiotics at 200 ppm (Lange et al. 2021). Moreover, GO directly disrupted bacterial cell membranes through penetrating biofilms and, hence, increased antibiotic permeability in *E. coli*, *S. aureus*, and *C. albicans* by 90.36%, 91.72%, and 91.17%, respectively (Elbasuney et al. 2023).

Additionally, the application of traditional antibiotics in combination with nanoparticles represents beneficial impacts, including biofilm elimination, lessening the standard antibiotic doses, and reducing the development of resistance mechanisms (León-Buitimea et al. 2022). Clinical investigations indicate that nanoparticles are employed as carriers for antibiotic delivery, enhancing their chemical stability and controlled release, thus reducing the required concentrations and also the off-target side effects (Nazli et al. 2022). For example, synergistic effects of GO NPs and ciprofloxacin caused significant biofilm disruption in *P. aeruginosa* (Li et al. 2024). Furthermore, ampicillin disrupted the bacterial cell membrane, resulting in increased drug delivery and its effectiveness in MDR *P. aeruginosa* when combined with Ag NPs (Khalil et al. 2021). Hence, continued experimentation and technological advancements in nanomaterials for multidrug-resistant bacteria signify a paradigm shift in tackling the challenges of conventional antibiotics, and hence demonstrate their potential to transform the future of antibacterial therapeutic regimens.

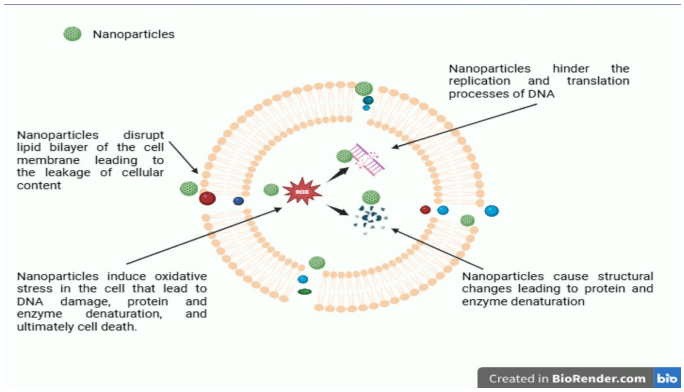

Fig. 2. *Mechanisms of action of nanoparticles against MDR bacteria, including cell membrane degradation, oxidative stress generation, DNA fragmentation, and protein and enzyme denaturation*

Table 2 Mechanisms of action of various NPs against a broad range of MDR bacteria

Nanoparticles (NPs)	Antibiotic-resistant bacteria	Mode of action	References
Ag NPs	*S. pneumoniae*	Oxidative stress generation, bacterial membrane degradation	(Al-Sa'ady and Hussein 2020; Bekele and Alamnie 2022)
ZnO NPs	*E. coli, P. aeruginosa, Salmonella typhi, Serratia marcescens, K. pneumoniae, Proteus mirabilis*	Enzyme activity inhibition by the oxidation of amino acids	(Krishnamoorthy et al. 2022)
GO NPs	*P. aeruginosa*	Oxidative stress generation, DNA fragmentation	(El-Kaliuoby et al. 2025)
Ag NPs	*E. coli*	Bacterial membrane disruption through electrostatic interactions	(Dakal et al. 2016; Häffner and Malmsten 2017)
GO NPs	*E. coli*	Bacterial cell membrane degradation through pore formation	(Baek et al. 2019)
Au NPs	*E. coli*	Bacterial cell membranes' functional disability	(Chavan et al. 2020)
Ag NPs	*E. coli, S. aureus*	Biofilm degradation	(Lange et al. 2021)
GO NPs	*S. aureus, C. albicans, E. coli*	Biofilm degradation	(Elbasuney et al. 2023)
Au NPs	*S. aureus*	Bacterial cell wall degradation	(Sadeghi et al. 2024)
Ag NPs	*P. aeruginosa*	Bacterial cell membrane degradation	(Khalil et al. 2021)
GO NPs	*P. aeruginosa*	Biofilm synthesis inhibition	(Li et al. 2024)
Ag NPs	*K. rosea, S. sciuri, S. lentus*	Biofilm synthesis inhibition, quorum sensing disruption, oxidative stress generation, bacterial cell membrane degradation, and efflux pumps disruption	(Ali et al. 2024)

4. Natural Antimicrobials

Natural antimicrobials are biologically and chemically active components that originate from natural sources and are known for their antimicrobial properties against a wide variety of microorganisms (Abdallah et al. 2023; Deshmukh and Gaikwad 2024). These bioactive substances can be derived from heterogeneous origins, comprising animals (lactoferrin, magainins, cecropins), plants (phenols, flavonoids, alkaloids, terpenes, essential oils), microorganisms (penicillin, bacteriocins, nisin), and minerals (Gupta et al. 2021; Abd El-Hack et al. 2023; Cheruvari and Kammara 2024; Hilal et al. 2024; Hendiani et al. 2025). Natural antimicrobials have been an integral part of conventional healthcare practices employed for control and prevention of infection, wound repair, for packaging, cleaning, and sterilizing procedures, decades before synthetic antibiotics became known (Qadri et al. 2022; Biswal et al. 2023; Guo et al. 2023). However, their medicinal restorative properties have also been recognized in advanced healthcare systems, especially with the escalating crisis of antibiotic resistance (Huang et al. 2021). These natural antimicrobials have exhibited many different mechanisms to overcome multidrug-resistant bacteria, including degradation of bacterial membranes, metabolic disruption, suppressing biofilm formation, and hindering quorum sensing (Nourbakhsh et al. 2021; Chaieb et al. 2022; Shamim et al. 2023). Furthermore, they reduce the possibility of the emergence of resistance through multiple modes of action. Natural antimicrobials can be utilized as an efficient alternative or complementary therapy for controlling and preventing resistant infections, when administered alone or in combination with traditional antibiotics.

4.1 Plant-based antimicrobials

There is a wide range of bioactive components in the medicinal plants that promise significant bacteriostatic and bactericidal activities against various MDR bacterial strains due to their promiscuous nature, as mentioned in Fig. 3 (Silva et al. 2021; Sharifi-Rad et al. 2022; Díaz-Puertas et al. 2023). This multifaceted ability of phytochemicals interferes with the development of resistant mechanisms in the bacteria and signifies their application as precursors for the production of antibiotics to handle such infections (Gadisa et al. 2019; Álvarez-Martínez et al. 2020; Rashid et al. 2024). The antibacterial mechanisms exhibited by phytochemicals are different from those of antibiotics, including inhibition of quorum-sensing, adhesion, penetration, motility, induction of oxidative stress, and degradation of the bacterial

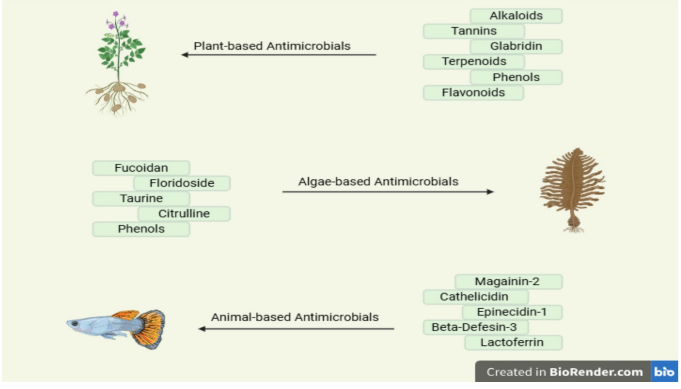

Fig. 3. *Plant-based, algae-based, and animal-based antimicrobials that exhibit bactericidal and bacteriostatic activity against MDR bacteria*

membrane, as mentioned in Table 3. Moreover, the synergistic effects of phytochemicals and antibiotics also proved beneficial in controlling and managing resistant bacterial infections (Gonçalves et al. 2023). Biofilm is one of the main factors developing resistance to antibiotics and is associated simultaneously with many other resistance processes, including interference with penetration and inactivation of antibacterial components in the biofilm, reduced bacterial growth, regulated efflux pump, and apoptosis (Saxena et al. 2019; Bagheri et al. 2024).

MDR bacteria are targeted by phytochemicals through inhibiting the synthesis of biofilms. For example, 20 mg/ml aqueous extract of *Aloe vera* hindered the growth of biofilm in methicillin-resistant *S. aureus* (Saddiq and Al-Ghamdi 2018). Similarly, the synthesis of biofilm and its related proteins was hindered in *S. aureus* when treated with Aloe-emodin, which is a component originating from *Aloe vera* (Xiang et al. 2017). Furthermore, plant bioactive components inhibit the growth of MDR bacteria through cell membrane disruption. For instance, Phenols derived from *Psidium guajava* L. cause the breakdown of lipid bilayers by making holes in them and promote the discharge of intracellular content. This breakdown led to considerable inhibition of biofilm growth, enzyme activity, nucleic acid and toxin synthesis in *Bacillus subtilis, S. aureus, E. coli, P. aeruginosa*, and *S. faecalis* (Soliman et al. 2016; Takó et al. 2020). Similarly, oxidative stress generation, protein and enzyme denaturation, and biofilm suppression were induced by glabridin in methicillin-resistant *S. aureus*.

4.2 Algae-based antimicrobials

Nowadays, researchers focus on the aquatic sources to identify their antimicrobial activities, especially against MDR bacteria (Song et al. 2021). For instance, marine algae serve as a vital food source for aquatic animals and a renewable resource for *Homo sapiens* (Hannan et al. 2020). Moreover, the bioactive substances of marine algae make it a promising therapeutic agent (Song et al. 2021). There are two types of marine algae: microalgae and macroalgae, inhabiting almost all of the ecosystems of the world. It is estimated that 164,000 species of macroalgae and microalgae are present, and 9,800 of them are marine inhabitants (El-Beltagi et al. 2022). However, macroalgae are reported as a potential antibiotic alternative to combat both gram-positive and gram-negative bacteria, both in clinical studies and living environments (Lauritano and Ianora 2018). Red algae, brown algae, and green algae are also known as *Rhodophyta, Phaeophyta,* and *Chlorophyta,* respectively, are the three main categories of marine algae classified based on types of pigment (de Borba Gurpilhares et al. 2019). Marine algae are the source of a broad spectrum of antimicrobial substances and minerals, particularly brown algae such as fucoidans, laminarins, and alginates. Moreover, a diverse range of polyphenols is also present, such as benzoic acids, catechins, cinnamic acid, flavonoids, ligands, anthraquinones, quercetin, and phlorotannins (Pina-Pérez et al. 2017; Gómez-Guzmán et al. 2018; Tahir et al. 2024).

Targeting bacterial cells and biofilms are the main bactericidal strategies demonstrated by marine algae against MDR bacteria. For example, 80% suppression of biofilm synthesis and bacterial growth was reported in methicillin-resistant *S. aureus* when treated with brown algae extracts of two species, *Fucus serratus* and *Fucus vesiculosus* (Higgins et al. 2019). Similarly, poultry-associated foodborne pathogens responsible for salmonellosis are *S. Typhimurium*, as well as *S. Enteritidis*. The abundant use of antibiotics has been attributed to the development of resistance by the new drug-resistant strains of Salmonella (Kulshreshtha et al. 2020). Methanolic extract from *Padina gymnospora* (brown algae) greatly inhibited *S. typhimurium* with zones of 27 mm (Pina-Pérez et al. 2017), while red seaweed aqueous extracts from *Chondrus crispus* and *Sarcodiotheca gaudichaudii* potentially inhibited *S. Enteritidis* at a 15 μg/mL MIC value (Kulshreshtha et al. 2020). Moreover, extracts derived from *Padina* as well as *Ulva* species exhibited bacteriostatic activities against *Bacillus cereus, Listeria monocytogenes*, and *S. aureus* at concentrations below 500 μg/mL (Dussault et al. 2016). Strong antibacterial properties against *B. cereus, S. aureus, Enterococcus faecalis*, and *L. monocytogenes* were observed upon treatment with crude extracts derived directly from *Ulva intestinalis.* The range of the registered MIC and MBC values was from 256 to 512 μg/mL (Srikong et al. 2017).

4.3 Animals-based antimicrobials

Animal-based antimicrobials are naturally occurring substances originating from animals with the power to combat microbial pathogens, including antibiotic-resistant bacteria. Because of their ability to offer substitute or supplemental treatments to standard antibiotics, these antimicrobials are gaining a large amount of interest as several customary antibiotics are, to an increasing extent, becoming less effective due to resistance. Antimicrobial peptides (AMPs), a variety of enzymes such as lysozyme, along with several proteins like lactoferrin, are included within animal-based antimicrobial agents; all of these agents have shown potential in inhibiting the growth of antibiotic-resistant pathogens. For example, cathelicidins and defensins are among the AMPs synthesized by many animal species and represent bacteriostatic and bactericidal activity against multidrug-resistant bacteria. The mechanism of action exhibited by these antibacterial agents is damaging cell membranes, interfering with the synthesis of the cell wall, and inhibiting protein synthesis. Furthermore, there are some other distinct modes of action. It includes strengthening host immunity against infection and binding of antimicrobials to essential metals vital for the growth and proliferation of bacteria, for instance, binding of lactoferrin to iron. Hence, animal-based antimicrobials serve as a promising therapeutic agent and to develop new medicinal therapies to overcome the infections induced by resistant bacterial strains.

Magainin-2 is an animal-based AMP derived from the skin of *Xenopus laevis* that disturbs the integrity of the bacterial cell membrane by making toroidal pores in it, leading to leakage of intracellular contents and ultimately, cell death. Magainin-2 tested against *E. coli,* carbapenem-resistant *Klebsiella pneumoniae,* methicillin-resistant *S. aureus, P. aeruginosa*, and *A. baumannii* with an MIC range of 80-160 mg/L (Denardi et al. 2022). Similarly, cathelicidin isolated from the *Alligator mississippiensis* exhibited strong bactericidal activity by altering the membrane permeability of multidrug-resistant *A. baumanii* and carbapenem-resistant *K. pneumoniae* at 300 μg/ml (Barksdale et al. 2017). Moreover, APs Epinecidin-1 isolated from *Epinephelus coioides* and human Beta-Defesin-3 showed strong antibacterial activity with MIC values ranging from 4-16 mg/L when tested against in carbapenem-resistant *K. pneumonia, K. aerogenes, P. aeruginosa,* and *A. baumanii* and methicillin-resistant *S. aureus.* Lastly, 5L and 6L are LfcinB6-derived peptides naturally present in Bovine lactoferrin showed significant antibacterial activity with MIC values of 4 μg/ml when tested against Tetracyclin-resistant strain (E007) and Vancomycin-resistant strains (WC176 and C68) of *Enterococcus faecium.* It caused cell membrane depolarization, ATP leakage, suppression of biofilm synthesis, and disruption in metabolic cycles (Mishra et al. 2022).

Table 3 Mechanisms of action of natural components of plant, algae, and animal-based antimicrobials against a broad range of MDR bacteria

Source	Natural chemicals	Target antibiotic-resistant bacteria	Mode of action	References
Plant-based Antimicrobials	Aloe-emodin	Methicillin-resistant *S. aureus*	biofilm synthesis inhibition	(Xiang et al. 2017; Saddiq and Al-Ghamdi 2018)
	Phenolics	*E. coli, S. aureus, P. aeruginosa, B. subtilis, S. faecalis*	Biofilm inhibition, cell membrane disruption, nucleic acid and enzyme degradation	(Soliman et al. 2016; Takó et al. 2020)
	Glabridin	Methicillin-resistant *S. aureus*	Oxidative stress generation, biofilm inhibition, protein denaturation	(Gangwar et al. 2020)
Algae-based Antimicrobials	Fucoidan	Methicillin-resistant *S. aureus*	Biofilm synthesis inhibition, bacteriostatic	(Higgins et al. 2019)
	Floridoside, Isethionic acid, Taurine, Citrulline	*S. Enteritidis*	Bacteriostatic	(Kulshreshtha et al. 2020)
	Phenols, Flavonoids	*S. Typhimurium*	Bacteriostatic	(Pina-Pérez et al. 2017)
	Phenols	*B. cereus, S. aureus, E. faecalis, L. monocytogenes*	Bacteriostatic	(Srikong et al. 2017)
Animal-based Antimicrobials	Magainin-2	*E. coli*, carbapenem-resistant *K. pneumoniae*, methicillin-resistant *S. aureus, P. aeruginosa, A. baumannii*	Bacterial cell membrane degradation	(Denardi et al. 2022)
	Cathelicidin	*A.baumanii*, carbapenem-resistant *K. pneumoniae*	Bacterial cell membrane degradation	(Barksdale et al. 2017)
	Epinecidin-1, human Beta-Defesin-3	*K. pneumonia, K. aerogenes, P. aeruginosa* and *A. baumanii*, methicillin-resistant *S. aureus*.	Bactericidal	(Bolatchiev 2022)
	lactoferrin	*E. faecium*	Cell membrane depolarization, ATP leakage, suppressed biofilm synthesis, and metabolic cycle disturbances	(Mishra et al. 2022)

5. Conclusion

The rapidly growing threats of antibiotic resistance call for prompt and novel treatment strategies. Phage therapy, NPs, and natural antimicrobials are potential substitutes to traditional antibiotics through a distinct mode of action to fight MDR bacteria. Phage therapy offers concrete antibacterial actions, while NPs promote improved absorption and targeted drug delivery. A broad range of bioactive components has the power to suppress the resistance mechanism of MDR bacteria. Collectively, crises associated with antibiotic resistance emphasize on increased demand for the implementation of these alternative therapeutic and multidisciplinary pathways to combat antibiotic resistance in the future. Long-term research, scientific validation, and administrative uphold are vital to transform these new techniques into pragmatic and efficient approaches, and defend the healthcare system worldwide in the post-antibiotic age.

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