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Review Article

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Exploiting biological tools for post-antibiotic era: novel sustainable strategies against antimicrobial resistance

Omnia Karem Riad*

Microbiology and Immunology Department, Faculty of Pharmacy, Al-Azhar University, Nasr city, Cairo, Egypt

*Correspondence E-mail: dr_omniakarem@azhar.edu.eg

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Abstract

Around the world, one of the biggest risks to public health is antimicrobial resistance (AMR). AMR poses substantial consequences on country economies and health systems. In the post-antibiotic era, searching for new cost-effective approaches is necessary to compensate for the continuous increase in AMR. The aims of this review are to explore the different biological and sustainable approaches that should be exploited to overcome the problem of AMR, discuss the mechanisms and advantages of different sustainable biological strategies, and introduce several strategies, including probiotic bacteria, predatory bacteria, and bacteriophages, which are powerful tools valuable to fighting resistant microorganisms with fewer chances of resistance development. Naturally synthesized products such as antimicrobial peptides and bacteriocins revealed successful treatment options. Additionally, the Clustered regularly interspaced short palindromic repeats (CRISPR-Cas) system is a gene-editing tool that can re-sensitize the resistant bacteria. Vaccination prevents infectious diseases and halts the emergence of resistant pathogens. Meanwhile, bacterial ghosts (BGs) and bacterial outer membrane vesicles (bOMVs) can be used to develop safe vaccines. bOMVs are also used as efficient tools for drug delivery due to their nanosizes. Additionally, antibody therapy and fecal microbial transplants are successful tools. Developing sustainable strategies to combat AMR through biological means looks promising. When compared to conventional antibiotics, these tactics have different mechanisms of action that may slow the emergence of antibiotic resistance.

Keywords: Probiotic and predatory bacteria, Bacteriophage therapy, Antimicrobial peptides, Bacterial ghosts, Bacterial outer membrane vesicles (bOMVs), Antibiotic resistance

1. Introduction

One of the biggest risks to public health worldwide is antimicrobial resistance (AMR), which contributed to the deaths of 4.95 million in 2019 and directly led to 1.27 million deaths worldwide (Antimicrobial Resistance Collaborators. 2022). Drug-resistant microorganisms are expanding, posing a threat to our ability to treat common diseases and perform life-saving procedures such as organ transplantation, replacements of hips, cancer chemotherapy, and cesarean sections. Drug-resistant infections also harm the plant and animal health, lower farm productivity, and jeopardize food security. AMR poses substantial consequences on country's economies and health systems. All nations, regardless of their income levels are affected by AMR (WHO. 2023). AMR not only causes death and disability but also incurs large financial consequences. According to World Bank reports, AMR could lead to US$ 3.4 trillion losses in annual gross domestic product (GDP) by 2030 and an additional US$ 1 trillion in healthcare expenses by 2050 (World Bank. 2024). The common antibiotic resistant bacteria include methicillin-resistant *Staphlococcus aureus* (MRSA), *Escherichia coli*, and *Pseudomonas aeruginosa* (Mousa *et al*., 2022). In 2017, the World Health Organization (WHO) released the first list of priority antibiotic resistant bacteria causing infection.

In the post-antibiotic era, searching for new cost-effective compounds is necessary. The objective of this review is to present a comprehensive discussion of the emerging biological approaches to avoid AMR. Beyond the traditional methods, we explore the untapped potential of BGs and bOMVs, offering a fresh perspective on combating this pressing global challenge. This comprehensive review also mentions the advantages and disadvantages of these biological approaches. By understanding these diverse strategies, researchers and policymakers can accelerate the development of sustainable solutions to mitigate the threat of AMR.

2. Biological approaches to overcome bacterial resistance

Different sustainable biological approaches can be used to find alternative antibacterial agents, including microorganisms e.g. bacteriophages probiotic and predatory bacteria, bacterial-derived elements e.g. bacteriocin and antimicrobial peptides (AMPs), bacterial outer membrane vesicles (bOMVs), and genetic elements e.g. clustered regularly interspaced short palindromic repeats-Cas (CRISPR-Cas) system (Vladkova *et al*., 2024).

2.1. Probiotic bacteria

The use of probiotic bacteria to overcome the continued rise in bacterial resistance cannot be neglected due to their high capabilities to perform different functions; especially in the field of antimicrobial resistance, availability, and sustainability (Ragan *et al*., 2022). There are four groups of probiotics: 1) viable and active probiotics e.g. Lactic acid bacteria (LAB), including *Lactobacillus* spp., *Pediococcus* spp., *Lactococcus* spp., and *Leuconotoc* spp. (Yin *et al*., 2023), 2) viable and inactive probiotics that can be found in the form of spores or vegetative cells, including *Bacillus* spp., which can be found in human diets and are used to treat intestinal and urinary disorders (*i.e*., *B. subtilis*, *B. clausii*, *B. coagulans*, and *B. licheniformis*) (Elshaghabee *et al*., 2017), 3) dead/non-viable probiotics (*i.e*., parabiotics, postbiotics, and prebiotics), which have multiple benefits, including being simpler to make and store, have unique mechanisms of action, have better accessibility of microbe-associated molecular pattern (MAMP), and have a higher chance of eliciting targeted reactions through particular ligand–receptor interactions (Nataraj *et al*., 2020), and 4) next-generation probiotics (NGP), where their majority are oxygen- and nutrition-sensitive gut bacteria, including strains of genetically modified (GE) bacteria and members of

the genera *Bacteroides*, *Clostridium*, *Faecalibacterium*, and *Akkermansia* (Saarela, 2019). Probiotic bacteria can be used as antibacterials against different pathogens e.g. *Streptococcus mutans* (Zaghloul *et al*., 2023), and/ or as antitoxins (Salem-Bekhit *et al*., 2023). Their biological qualities have been thoroughly studied; however, their antimicrobial qualities as cutting-edge antibiotic substitutes received less attention (Helmy *et al*., 2023). The antimicrobial activity of probiotic bacteria is primarily attributed to the secretion of antimicrobial substances e.g. bacteriocins, organic acids, hydrogen peroxide, carbon dioxide, diacetyl, and ethanol (Guan *et al*., 2023), thus competing with pathogens and/or blocking their adhesion sites. Besides, probiotics have a great role in immune modulation (Helmy *et al*., 2023).

Bacteriocins are cationic and ribosomally synthesized antimicrobial peptides that were first discovered nearly a century ago. With no negative side effects, bacteriocins might be a good option to replace antibiotics. They can act in various ways such as blocking the synthesis of cell walls and protein, blocking DNA gyrase and RNA polymerase, and/ or more frequently form holes in the target microbial cell membrane. Due to their extensive and safe history in food industry, lactic acid bacteria are excellent choices for producing bacteriocins, which are used to biocontrol certain bacterial spp. (Darbandi *et al*., 2022).

2.2. Antimicrobial peptides (AMPs)

Antimicrobial peptides are becoming increasingly popular as they are active at micromolar concentrations and act as broad-spectrum antimicrobials that do not readily cause resistance in microorganisms. AMPs are mainly a family of small peptides that are widely distributed in nature and are crucial components of an organism's innate immune system. Apart from that, microbial fermentation or enzymatic hydrolysis can be used to produce AMPs from a range of protein sources, including milk. Several food and pharmaceutical industries have used AMPs as bio-preservatives (Singh *et al*., 2023). Several AMPs are approved by the Food and Drug Administration (FDA) e.g. bacitracin for local skin and ocular infections; Dalbavancin, Daptomycin, Oritavancin, Telavancin, and Teicoplanin for bacterial skin infections; Enfuvirtide for Human immunodeficiency virus-1 (HIV-1) infection; and Telaprevir for Hepatitis C infection (Lei *et al*., 2019). AMPs are sustainable and can be derived from several sources, including plants, milk, and microorganisms. For example, Defensin- α, Drosomycin from insects; Cathelicidins and A defensin from human neutrophils; bactenecin from bovine neutrophils; purothionins from wheat endospore; nisin from *Lactococcus lactis*, enterocin from *Enterococcus*; ericin from *B. subtilis*; plantaricin from *L. plantarum*, and hominicin from *S. hominis* (Boparai and Sharma, 2020).

2.2.1. Mechanisms of antibacterial potential of the antimicrobial peptides

Unlike conventional antibiotics, AMPs interact with bacterial cell membranes by electrostatic interactions that prevent bacteria from developing resistance. These peptides are divided into membrane-acting and non-membrane-acting peptides according to their functions. Non-membrane peptides can pass from the membrane without harming it, but the membrane-acting peptides primarily contain cationic peptides that disrupt the membranes (Hollmann *et al*., 2018). A small number of antibacterial peptides such as LL-37, againins, melittin, and defensin, forms trans-membrane pores in the target membrane. AMPs that translocate across the cell membrane and interfere with normal cell functioning include buforin II, dermaseptin, HNP-1, pleurocidin, indolicidin, pyrrhocidin, and mersacidin. They also hamper several processes, including protein synthesis, nucleic acid synthesis, cell wall synthesis, and enzymatic activities (Boparai and Sharma, 2020).

2.2.2. The processes of creating, identifying, and purifying the antimicrobial peptides

The release of AMPs that are encoded in an inactive form within proteins occurs through

enzymatic hydrolysis and/or microbial fermentation. Enzymatic hydrolysis is better than microbial fermentation due to its quicker reaction times and greater scalability. Numerous novel peptides with distinct bioactivities can originate from the microbial proteolytic enzymes. Microbial protease is one of the most crucial instruments used for changing the structure of proteins and creating novel protein hydrolysates that yield targeted peptides with commercial potentials (Shivanna and Nataraj, 2020).

The use of microbial fermentation to produce bioactive peptides is becoming more popular since it is a cheap, safe, and natural method. To make up for their lack of amino acids, LABs have evolved the capacity to hydrolyze proteins, along with producing free amino acids for their own needs. LABs also create a variety of biologically active peptides (Raveschot *et al*., 2018). The proteolytic system of LABs is made up of different intracellular peptidases, including endopeptidases, aminopeptidases, tripeptidases, and dipeptidases, which break down peptides into small molecules and produce free amino acids and cell wall-bound proteinases. They first break down casein into oligopeptides and peptide transporters, which move the oligopeptides into the cytoplasm (Venegas-Ortega *et al*., 2019).

In the medical sciences, recombinant DNA technology (RDT) is widely employed to produce proteins and hormones with a variety of uses (Mada *et al*., 2020). The preferred technique used for isolating and purifying peptides according to their molecular weights is membrane filtration technology, which is cheap, non-chemical, energy-efficient, and simple to set up; among its other benefits (Singh *et al*., 2023).

Numerous methods can be used to determine the antimicrobial peptide sequence, including electrospray ionization (ESI), nanostructure laser desorption/ionization (NALDI), and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). In recent years, the most popular technique used for identifying and profiling the peptides from various food sources such as milk, is liquid chromatography combined with mass spectrometry (LC-MS) (Singh *et al*., 2023).

Peptaibol database, PenBase, Defensins Knowledgebase, PhytAMP, BACTIBASE, CAMP, YADAMP, DAMPD, Milk AMP, CAMPR3, DBAASP, APD, MBPBD, FeptideDB, and FermFooDb, are just a few of the antimicrobial peptide databases that have been developed in recent years (Singh *et al*., 2023).

2.2.3. Stem cell-derived antimicrobial peptides

For many years, researchers have been studying mesenchymal stem cells (MSCs) in great details to create a therapeutic product that is both safe and effective in treating a variety of chronic illnesses (Kumar *et al*., 2021). MSCs have the potential to support tissue healing, immunomodulation, and control of excessive inflammation (Harman *et al*., 2017). Later studies showed that human MSCs generate AMPs, which inhibit the synthesis of bacterial cell walls, among other ways, to kill the bacteria (Marx *et al*., 2020). Because it significantly lowers bacterial infections such as MRSA, secretome derived from MSCs represents a viable strategy to combat various related infections. The antibacterial effects of conditioned medium containing AMPs known as lipocal, hepcidin, and LL-37 that are derived from human bone marrow (BM) and umbilical cord MSCs (hUCMSCs) have shown effectiveness against antibiotic-resistant clinical pathogens e.g. *Staphylococcus aureus*, *E. coli*, and *K. pneumonia* (McCarthy *et al*., 2020). Ren *et al*., (2020) reported that hUMSCs exhibited direct antibacterial efficacies against a strain of imipenem-resistant *P. aeruginosa* that was isolated from human infant. Recently, it has been confirmed that the infected bone abnormalities can be treated with a self-assembling peptide hydrogel scaffold that incorporates exosomes produced from the stem cells (Xu *et al*., 2024).

3. Predatory bacteria (living antibiotics)

Interaction between microorganisms frequently takes the form of predation. In the microbial world,

predation ability is a feature shared by bacteria, protists, nematodes, and bacteriophages (Ezzedine *et al*., 2022). Predatory bacteria are found in many natural (*i.e*., terrestrial and aquatic) and artificial environments, including freshwater, saltwater, soils, plant rhizosphere, sewage, activated sludge in wastewater treatment plants, and intestines of the humans and other animals. They lyse other bacteria and take up their cell-derived macromolecules as nutrients (Ezzedine *et al*., 2022). Higher eukaryotic organisms are not harmed by the bacteriolytic activities of predatory bacteria, which are effective against a variety of other planktonic and biofilm-forming bacteria (Mookherjee and Jurkevitch, 2022). Predatory bacteria belong to several phylogenetic groups such as *Actinobacteria*, *Proteobacteria*, *Chloroflexi*, and *Cytophagaceae*. Two major categories of predatory bacteria are commonly found in various environments; mainly *Bdellovibrio* and like organisms (BALOs) and Myxobacteria (Inoue *et al*., 2022).

Bdellovibrio bacteriovorus is a small Gram-negative bacterium that naturally invades and kills Gram-negative pathogens. They prey on Gram-negative bacteria by direct invasion (*i.e*., epibiotic or periplasmic predation) (Atterbury and Tayson, 2021), and it has antibacterial potentialy against *K. pneumoniae*, *Yersinia pestis*, and *Salmonella* infections. In addition, *B. bacteriovorus* possesses antibiofilm activity (Novick, 2021). Myxobacteria, on the other hand, are typical facultative predatory bacteria (Thiery and Kaimer, 2020). They are gregarious bacteria that feed on prey cells by using a group-hunting technique and secreting hydrolytic enzymes and secondary metabolites. Generally, Myxobacteria can consume nematodes, yeast, fungi, protozoa, and both Gram-positive and Gram-negative bacteria (Inoue *et al*., 2022).

The life cycle of predation of BALO microorganisms includes searching for the prey and then attaching and entering it (Lai *et al*., 2013). A pore is formed and the flagellum disappears. The predator enters the periplasmic space of its host and settles there. The pore then shuts and the predator begins consuming its prey's intracellular components. After a step of septation, the host cell is lysed and new *B. bacteriovorus* progenies are released. Once a suitable prey is found, the newly born predators can either resume in the Host-Independent state or initiate a new predation cycle through the Host-Dependent cycle (Cavallo *et al*., 2021).

4. Recent imaging and quantification techniques

Microscopy is essential for the detection and analysis of BALOs. Electron microscopy visualizes key structures e.g. flagella in *B. bacteriovorus*, while the Cryo-electron tomography can visualize novel structures within the predator cells. In addition to displaying protein structures, X-ray crystallography has produced models of individual *B. bacteriovorus* proteins, enabling the evaluation of interactions to shed light on the functions of these proteins (Lai *et al*., 2013). The classical methods used for quantifying BALOs include double-layer overlay plates and counting the plaques. Faster methods for quantification in a single hour can be achieved by using fluorescent resazurin or SYBR-green assay (Jang *et al*., 2022).

5. Bacteriophage therapy

A new tool that may be used to combat MDR bacterial infections is bacteriophage therapy. Modern sequencing tools combined with bacteriophage characterization have sped up the search for potential therapeutic bacteriophage targets (Mousavi *et al*., 2021). The FDA has approved the use of bacteriophages as a potential intravenous treatment alternative to antibiotics, as they are naturally occurring killers of bacteria (Voelker, 2019).

5.1. Mechanisms of bacteriophage antagonistic activity against the bacterial cells

A bacteriophage can replicate using either the lytic or lysogenic methods after attaching to its host. It delivers its genetic material into the cytoplasm of the

host cell upon infection. The lytic replication cycle involves the bacteriophage using the host ribosomes for protein synthesis, followed by host cell lysing, and releasing of fresh bacteriophages to infect new hosts. During the lysogenic replication cycle, the phage genome is either kept as an episomal element or integrated into the bacterial chromosome. Alterations in the surrounding environment may have the potential to shift the phenomena into the lytic replication cycle. Phage endolysins; also known as lysins, are bacteriophage-encoded peptidoglycan hydrolases that following replication in the bacteriophage life cycle, they lyse Gram-positive bacterial cell walls to release progeny phages (Mahmoud *et al*., 2021).

5.2. Bacteriophage antibacterial activity range

Phage therapy has been effectively used in numerous clinical settings to treat bacterial infections, including those that are resistant to antibiotics (Kortright *et al*., 2019). Bacteriophages expressed potent antibacterial potency against MRSA and vancomycin-resistant *Staphylococcus aureus* (VRSA) strains (Walsh *et al*., 2021). Several studies have shown the efficiency of bacteriophage therapy for treatment of resistant infections caused by *E. coli*. For example, in the study conducted by Alexyuk and his colleagues, they isolated a cocktail of lytic phages with strong lytic activity and wide host ranges; along with long shelf life and maintained activity (Alexyuk *et al*., 2022). Besides, *E. coli* O157:H7 in foods has been also the target for effective killing by the bacteriophages (Sjahriani *et al*., 2021). Moreover, bacteriophages have shown great activity against *P. aeruginosa* and its biofilm formation ability (Chegini *et al*., 2020). Numerous studies revealed the lytic activity of bacteriophages against several bacterial strains e.g. *Hylicobacter pylori*, resistant *Enterococcus faeciu*m, *Proteus mirabilis*, *Serratia* spp., and *Salmonella enterica* serovar *typhimurium* (Al-Fakhrany and Elekhnawy, 2023).

Reports stated that the phage activity may extend to an antiviral effect by affecting the immune system and producing antibodies by the host immune system against Severe acute respiratory syndrome coronavirus 2 (SARS-CoV2), hence minimizing the host's damage induced by COVID-19 infection (Jalili *et al*., 2022).

6. Clustered regularly interspaced short palindromic repeats (CRISPR-Cas)

With its enormous potentials as a gene-editing tool, Clustered regularly interspaced short palindromic repeats (CRISPR-Cas) has drawn lot of interests as a substitute antibacterial treatment. Research is primarily focused on strategies that aim to either eliminate pathogenic strains or restore sensitivity to antibiotics (Devi *et al*., 2023). In bacteria and archaea, CRISPR-Cas is a unique adaptive immune trait that guards against the bacteriophages. Spacers are bacteriophages or plasmids that contain short sequences which are inserted into the bacterial genome in the form of a CRISPR array. The Cas protein machinery uses the guide RNAs from the spacers to specifically target the invasive nucleic acid that carries the same sequence (Shabbir *et al*., 2019). CRISPR-Cas system has a major impact on the spread of phage infection and antibiotic resistance genes. Bacterial horizontal gene transfer (HGT) is thought to be impeded by CRISPR-Cas systems (Tao *et al*., 2022).

By specifically removing the plasmids containing antibiotic-resistance genes, the CRISPR-Cas system can be used to re-sensitize drug-resistant bacteria to antibiotics (Gholizadeh *et al*., 2020). Gene editing of prokaryotes and eukaryotes is carried out using the Type II CRISPR-Cas9 system, which has revolutionized the molecular biology in the past ten years. Selective elimination of AMR genes from the bacterial populations has been demonstrated in multiple studies through the application of CRISPR-Cas9. Delivery of CRISPR-Cas for specific targeting of AMR genes in plasmids and chromosomes has been demonstrated using phagemids and conjugative plasmids, respectively. Hence, according to Kumar *et al*., (2021), elimination of AMR genes causes the

bacteria to become more susceptible to antibiotics (Kumar *et al*., 2021).

The use of CRISPR-Cas systems increases the superbugs' susceptibility to the currently available antibiotics and facilitates treatment, and they are also used to give the bacteria desirable characteristics such as the capacity to produce antimicrobial compounds. Up till now, several studies that used different CRISPR-Cas systems to modify antibiotic-resistant microorganisms have been published (Zhang *et al*., 2024).

Delivery of CRISPR-Cas components can be achieved through a variety of techniques, including phage, extracellular vesicle, and nanoparticle-based delivery. The antibiotic-resistant genes (ARG) on bacterial plasmids can be removed by delivering the CRISPR-Cas systems into the bacterial cells, which re-sensitizes the bacteria to the antibacterial agents. Additionally, CRISPR-Cas systems display potent bactericidal activity by causing the cell to breakdown upon identification of the target site on the genome and/ or the target ARG on the plasmid (Tao *et al*., 2022). Furthermore, the removal of carbapenemase resistance genes (*i.e*. bla_{KPC} and bla_{NDM}), re-sensitization of the resistant pathogen to carbapenems, and better beneficial outcomes on the clinical isolates of carbapenem-resistant *Enterobacteriaceae*, all could be achieved by introducing a plasmid vector containing the pCasCure system into the carbapenem-resistant *Enterobacteriaceae* (Kang *et al*., 2017). The targeted antimicrobial plasmids (TAPs) have the potential to re-sensitize the recipient cells harboring pOXA48 and stop the spread of drug resistance, by delivering the CRISPR-Cas system to *E. coli* and the closely related Gram-negative *Enterobacteriaceae* (Gama *et al*., 2017). Conjugative CRISPR/Cas9 was developed by Dong *et al*., (2019) to target the colistin resistance gene (*mcr-1*) in *E. coli*. CRISPR/Cas9 can eliminate drug-resistant plasmids, re-sensitizes the recipient cell to antibiotics, and also confers immunity against the *mcr-1* gene. The recombinant plasmid pMBLcas9-sgRNA has been transferred into the clinical isolate of *E. coli* that harbors *mcr-1* plasmids and successfully eliminated the multidrug resistance plasmids (Wang *et al*., 2019). *S. typhimurium*, *Campylobacter jejuni*, and *E. coli* 0157:H7 are examples of the antibiotic-resistant foodborne human pathogens that can be overcome by CRISPR used as quorum quenching agents (Higuera-Ciapara *et al*., 2024).

7. The use of antibodies

An inventive method of treating bacterial infections is the creation of monoclonal antibodies (mAbs) and molecules derived from antibodies that target bacterial pathogens (Muteeb *et al*., 2023). With their ability to precisely identify and neutralize bacterial targets, these engineered antibodies provide highly focused and accurate modes of treatment. To fully utilize these antibodies, a number of tactics have been tried with encouraging outcomes. The discovery of monoclonal antibodies (mAbs) directed against *Clostridium difficile*; the causative agent of antibiotic-associated diarrhea and colitis, is one noteworthy instance (Bhattacharyya *et al*., 2020). A monoclonal antibody called bezlotoxumab has been approved to prevent recurrent *C. difficile* infections. To prevent the harmful effects of *C. difficile* toxin B and mitigate the risk of recurrent infections in patients with high-risk, bezlotoxumab antibody has been used to bind to this toxin (Navalkele and Chopra, 2018). This strategy decreases the needs for antibiotics, which can make *C. difficile* infections more serious, and is also offering a targeted therapy. The development of mAbs that specifically target *Staphylococcus aureus* is another innovative advancement. Specifically, this pathogen has been effectively neutralized by binding to its surface protein through the design of an antibody-derived molecule; named *Staphylococcus aureus* capsular polysaccharide immune globulin. Given that MRSA infections are known for being resistant to numerous antibiotics, this strategy is especially significant in the fight against them (Liu *et al*., 2017). Through the use of antibodies such as Altastaph and others, researchers hope to improve the immune system's capacity to identify and eradicate MRSA infections.

Newly developed antibody-derived molecules have gone beyond the standard mAbs. Lysibodies are a well-known example, which are made to attack the conserved components on the bacterial cell wall to target a wide range of bacteria (Raz *et al*., 2017). These molecules function similarly to the monoclonal antibodies (mAbs) but are designed to identify a broad variety of bacterial spp., eliminating the requirement for highly specific antibodies against each pathogen. This method has the potential to be a flexible and potent treatment plan for a range of bacterial infections. Additionally, the use of antibody-conjugates; as powerful antimicrobials created by combining antibodies with toxic agents has been recently investigated by the researchers. The application of antibody–toxin conjugates directed against *P. aeruginosa* is one instance of this (Kajihara *et al*., 2021). This approach effectively destroys the bacteria by guiding the toxin to the bacterial pathogen with the help of antibodies. It may be a promising alternative to conventional antibiotics; particularly in cases of multidrug-resistant strains (Muteeb *et al*., 2023). Despite the mAbs' undeniable effectiveness in treating a wide range of diseases, there are still certain challenges concerning them, such as their costly production. Because mAbs are prone to chemical and enzymatic degradation in the gastrointestinal tract, systemic administration is not appropriate for non-invasive administration routes such as oral, nasal, or pulmonary. Meanwhile, the hybridoma mAb application has an additional drawback in that it raises the risk of cancer (Helmy *et al*., 2023).

8. Vaccines

Vaccination is essential to reduce the need for antibiotics and control the emergence of AMR (Hoelzer *et al*., 2018). This occurs because the vaccine reduces the pathogen's ability to infect the host by granting immunity against these microorganisms. This reduces the likelihood of certain bacterial mutations and emergence and dissemination of resistant genes to other bacteria (Kennedy and Read, 2017). For example, a group of children in South Africa who received the pneumococcal conjugate vaccine 9 (PCV9) have shown a 67 % reduction in the circulation of penicillin-resistant invasive pneumococcal strains when compared to controls (Klugman *et al*., 2003). Vaccines stop the infection before it even has a chance to spread or grow. The use of vaccines has frequently resulted in the worldwide eradication of multiple diseases, where diphtheria, tetanus, and pertussis incidence have decreased by 95% (Helmy *et al*., 2023).

There are numerous types of vaccines such as live, attenuated vaccines, toxoid vaccines, killed whole-cell vaccines, and subunit vaccines (*i.e*., outer membrane vesicles (OMVs), protein-polysaccharide conjugates, recombinant viral and bacterial vector vaccines, and nano-vaccines). Vaccine development is challenging for the highly variable pathogens and vaccination failure may frequently occur. Maintaining the cold chain by preserving the required temperature during transportation or storage of vaccines is needed to keep the live-attenuated vaccine potency, which poses extra costs. However, booster doses are required for killed and toxoid vaccines (Helmy *et al*., 2023).

9. Bacterial outer membrane vesicles (bOMVs)

Gram-negative and Gram-positive bacteria secrete lipid particles called bacterial outer membrane vesicles (bOMVs) through lysis or blebbing processes (*i.e*., blebbing is a cellular process where a small membrane-enclosed protrusion is formed on the bacterial cell surface). The fact that bOMVs can both encourage antimicrobial resistance and offer a variety of opportunities for therapeutic exploitation is becoming increasingly apparent. bOMVs can contribute to intra- and interspecific communications. As being non-living versions of their parent bacteria, bOMVs can carry a variety of bioactive substances, including lipids, proteins, metabolites, and nucleic acids. Despite being energy-intensive, the release of bOMVs appears to improve bacterial fitness; particularly for pathogens (Thapa *et al*., 2023). bOMVs have nanosizes ranging from 20 to 500 nm and are encased in a membrane bilayer made of bacterial lipids and proteins (Bos *et al*., 2021). This

membrane provides protection, thus creating a safe microenvironment in the lumen of a bOMV that enables the transit of biological molecules, which might otherwise be rendered ineffective or inactivated by exposure to the environment (Villageliu and Samuelson, 2022). It may be challenging to isolate and purify bOMVs, as doing so frequently requires expensive tools and complicated purification procedures. Precipitation and ultrafiltration are frequently used methods for separating the bOMV, while ultracentrifugation, density gradient fractionation, and immunoaffinity are other methods for their purification (Amatya *et al*., 2021).

• Role of bacterial outer membrane vesicles in fighting bacterial resistance

Bacterial OMVs are sustainable and involved in many aspects of microbiota interactions, including interspecies competitiveness. Some groups of researchers have proposed using native OMVs as natural antibiotics due to their inherent antibiotic property (Pérez *et al*., 2020). bOMVs have been observed to have lytic activity; especially those derived from *P. aeruginosa* PAO1 that can lyse *E. coli*. The normal dose of antibiotics (*i.e*., gentamicin) could be decreased if loaded on bOMVs and the same effective results have been obtained as the higher normally used dose. Moreover, the targeted drug delivery of antibiotics is promising, as the bOMVs can carry different drugs and protect their cargo from enzymatic degradation, due to their hydrophilic lumen and hydrophobic membrane (Collins and Brown, 2021).

10. Bacterial ghosts (BGs)

Although vaccination effectively guards against bacterial infections, it isn't effective against infections brought on by the most troublesome multidrug-resistant pathogens. Bacterial ghost (BG) is one of the promising sustainable strategies to be used in vaccination (López-Siles *et al*., 2021). The bacterial-derived empty cell envelopes are called BGs. They produced by carefully regulated chemical or biological processing, and have special benefits such as antigenicity retention, structural stability, and adaptability in cargo loading (Park, 2023).

Two techniques have been developed to create microbial or BGs that can be utilized in vaccinations. The first one focuses on using the bacteriophage E lysis gene to activate the Gram-negative bacteria at the right moment and transform them into ghost cells. The other is called the Sponge-Like protocol, which uses the critical activity of certain enzymes or the critical concentration of certain chemical compounds to create ghost cells with the proper surface antigens and 3D structure (Amara, 2023). Several successful trials have prepared an effective BG vaccine for *P. aeruginosa*, *A. baumannii*, and Enterohemorrhagic *E. coli* (EHEC) strain O157:H7 (Sheweita *et al*., 2022).

One benefit of using non-living ghost bacteria over previously documented live attenuated vaccine vectors is their safety. Since BG mimics a natural bacterial infection, it has been found that oral delivery of BGs for oral transmitted infections is a successful approach for future vaccine use. BG can be administered *via* various administration routes with marked protections against candidate pathogens. Conversely, it has been confirmed that oral administration of non-living whole-cell preparations such as BGs or subunit bacterial vaccines, reduces the immunogenicity (Sheweita *et al*., 2022). The BG probiotic system holds a significant promise as a treatment for gastrointestinal disorders such as colon cancer (Chen *et al*., 2021). Using "bacterial ghosts" to improve the delivery of drugs, including antibiotics, is a recent strategy. The pathogen-associated molecular patterns; namely lipopolysaccharides "LPS", lipoprotein, and peptidoglycans, enable the transportation of drugs, nucleic acids, and antigens to the immune cells. BGs not only shield the ensnared cargoes but also strengthen the immune systems to fight off malignancies and infections (Chen *et al*., 2021).

Nevertheless, there are some difficulties with using BGs in clinical settings. For example, it is important

to look into the potential therapeutic benefits of BGs from various bacterial strains when paired with drugs or antigens, as the BG characteristics will differ depending on their parent bacterial source. Additionally, leaky BGs pores need to be sealed, and additional studies are needed on the mechanisms and stability of combining different medications or antigens with BGs. Because BGs are readily eliminated by the immune system *in vivo*, it's critical to enhance their targeting and stability (Chen *et al*., 2021).

11. Fecal microbial transplant (FMT)

Transferring the processed fecal material from a healthy donor's gut to a recipient patient's gut is known as fecal microbial transplant (FMT). A nasoduodenal tube, nasojejunal tube, colonoscopy, and retention enema are some of the ways by which processed feces can be given to the recipient (Chauhan *et al*., 2021). When *C. difficile*-infected children in Maryland (USA) underwent colonoscopy administration of fecal matter from stool banks into their cecum and colon, the infection has been completely resolved in the recipients, and their levels of AMR and multidrug resistance genes decreased (Hourigan *et al*., 2019). In another study conducted on mice, treatment with *S. typhimurium* in combination with FMT and lytic phages resulted in total removal of *S. typhimurium*, a decrease in inflammatory cytokines, and restoration of the intestinal microbial diversity (Wang *et al*., 2022). Moreover, resistant infections caused by *K. pneumoniae*, MRSA, and β-lactamase-producing *Enterobacteriaceae* have been resolved by the application of FMT (Helmy *et al*., 2023). While the exact mode of action of FMT treatment is still unknown; however, it has been observed that the improved recipients' health is correlated with restoration of the healthy gut microbiota, which includes Firmicutes. Restoration of gut-beneficial microbiota such as *Bacteroides ovatus* and *Roseburia hominis* has been proposed to either suppress the growth of pathogens (*i.e*., *C. difficile*) or compete with them for resources and growth environments. Through direct competitive niche exclusion or indirect competition *via* the synthesis of bacteriocins like thuricin CD, the restored commensal microbiota opposes *C. difficile* (Helmy *et al*., 2023).

Conclusion

Antimicrobial resistance has significant effects on health systems and national economies. In the post-antibiotic era, finding new and affordable strategies is essential to offset the ongoing rise in AMR. Biological approaches to overcome bacterial infections are sustainable and eco-friendly strategies, which comprise a wide range of microorganisms that are used to overcome AMR, including probiotics, predatory bacteria, and bacteriophages. Meanwhile, AMPs, antibody therapy, vaccines, BGs, and bOMVs are effective bio-control methods. Using CRISPR-Cas system can be used for re-sentizing the resistant bacteria. Biological strategies decrease reliance on the currently used antibiotics and mitigate AMR.

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Conflict of interests

The author declares that she has no conflicting or competing interests.

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