Journal of Controlled Release 369 (2024) 25–38

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Review article

Antimicrobial sensitisers: Gatekeepers to avoid the development of multidrug-resistant bacteria

Hanfei Wang [a], Yingying Yang [a], Simeng Wang [a], Sara Badawy [b,c], Irma Ares [d], Marta Martínez [d], Bernardo Lopez-Torres [d], María-Rosa Martínez-Larrañaga [d], Xu Wang [a,b,**], Arturo Anadón [d,*], María-Aránzazu Martínez [d]

[a] National Reference Laboratory of Veterinary Drug Residues (HZAU) and MAO Key Laboratory for Detection of Veterinary Drug Residues, Huazhong Agricultural University, Wuhan, Hubei 430070, China
[b] MAO Laboratory for Risk Assessment of Quality and Safety of Livestock and Poultry Products, Huazhong Agricultural University, Wuhan, Hubei 430070, China
[c] Pathology Department of Animal Medicine, Faculty of Veterinary Medicine, Benha University, Egypt
[d] Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Universidad Complutense de Madrid (UCM), and Research Institute Hospital, 12 de Octubre (i+12), 28040 Madrid, Spain

ARTICLE INFO

Keywords:
Multidrug-resistant bacteria
Antibiotic adjuvant
Antimicrobial sensitizer
Indirect antibacterial

ABSTRACT

The resistance of multidrug-resistant bacteria to existing antibiotics forces the continued development of new antibiotics and antibacterial agents, but the high costs and long timeframe involved in the development of new agents renders the hope that existing antibiotics may again play a part. The "antibiotic adjuvant" is an indirect antibacterial strategy, but its vague concept has, in the past, limited the development speed of related drugs. In this review article, we put forward an accurate concept of a "non-self-antimicrobial sensitisers (NSAS)", to distinguish it from an "antibiotic adjuvant", and then discuss several scientific methods to restore bacterial sensitivity to antibiotics, and the sources and action mechanism of existing NSAS, in order to guide the development and further research of NSAS.

Abbreviations: AACs, acetyltransferases; *AcpP*, acyl carrier protein; AcrAB-TolC, acridine resistance proteins A and B coupled to the outer membrane channel TolC; ADP, adenosine diphosphate; AMP, antimicrobial peptides; AMR, antimicrobial resistance; ANTs, nucleotidyltransferases; APHs, phosphotransferases; ATP, adenosine triphosphate; CA, clavulanic acid; CEP-136, H-[NLys-^{t}BuAla]$_6$-NH$_2$; Cmr, cas repeat-associated mysterious protein module; CO_2, carbon dioxide; CoA, coenzyme A; crRNA, CRISPR RNA; CRISPR/Cas9, clustered regularly interspaced short palindromic repeat/CRISPR-associated 9; CSE, cystathionine γ-lyase; DHFR, dihydrofolate reductase; DHPS, dihydropteranoic acid synthase; DNA, deoxyribonucleic acid; DRB, drug-resistant bacteria; ESBLs, extended-spectrum beta-lactamases; FAD, flavin adenine dinucleotide; $FADH_2$, reduced flavin dinucleotide; FeHCC, iron-hexacyanocobaltate; *ftsZ*, filamentous temperature-sensitive protein Z; GOx, glucose oxidase; H_2O, water; H_2O_2, hydrogen peroxide; H_2S, hydrogen sulfide; LPS, lipopolysaccharide; *marA*, multiple antibiotic resistance activator; *marR*, multiple antibiotic resistance regulators; MBL, metallo-β-lactamases; *mcr1*, mobile colistin resistance gene; MDR, multi-drug resistant; MIC, minimum inhibitory concentration; mRNA, messenger ribonucleic acid; MRSA, methicillin-resistant *Staphylococcus aureus*; NAD^+, oxidized nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; NADPH, nicotinamide adenine dinucleotide phosphate; NDH, NADPH dehydrogenase complex; NDM-1, New Delhi metallo-β-lactamase 1; NO, nitric oxide; NSAS, non-self-antimicrobial sensitisers; OM, outer membrane; PABA, p-aminobenzoic acid; PBP2, penicillin-binding protein 2; PBP2a, penicillin-binding protein 2a; PGLA, AMP glycine leucine amide; PMF, proton motive force; PMQR, plasmid-mediated quinolone resistance; PPMO, peptide-conjugated phosphorodiamidate morpholino oligomer; PPMOs, peptide phosphodiamide morpholine oligomers; PSMαs, α-phenol-soluble modulins; R & D, antibiotic research and development; RNA, ribonucleic acid; RNAi, RNA interference; ROS, reactive oxygen species; *rpsJ*, 30S ribosomal protein S10; SARs, structure-activity relationships; SBL, serine-β-lactamases; TAN, taniborbactam; TCA, tricarboxylic acid; tracr, trans-activating CRISPR; *waaY*, lipopolysaccharide core heptose (II) kinase..

* Correspondence to: A. Anadón, Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Universidad Complutense de Madrid, 28040 Madrid, Spain.
** Correspondence to: X. Wang, National Reference Laboratory of Veterinary Drug Residues (HZAU) and MAO Key Laboratory for Detection of Veterinary Drug Residues, Wuhan, Hubei 430070, China.
E-mail addresses: wangxu@mail.hzau.edu.cn (X. Wang), aanadon@ucm.es (A. Anadón).

https://doi.org/10.1016/j.jconrel.2024.03.031
Received 24 October 2023; Received in revised form 23 February 2024; Accepted 16 March 2024
Available online 21 March 2024
0168-3659/© 2024 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY-NC license (http://creativecommons.org/licenses/by-nc/4.0/).

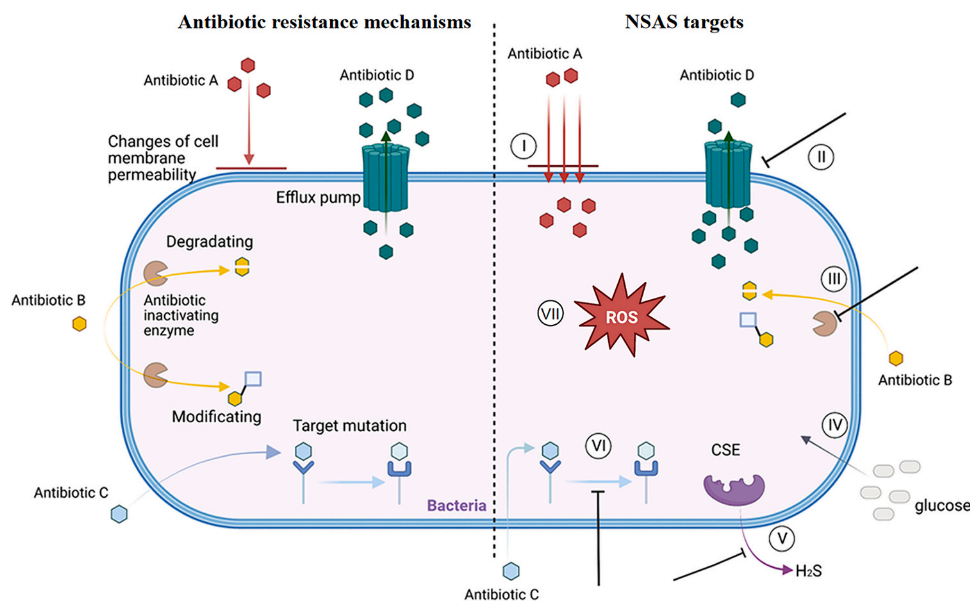

Fig. 1. Three ways for a single bacterium to develop antibiotic resistance, and seven ways to increase the sensitivity of the antibiotic to the AMR. The mechanisms of AMR include: synthesising degrading enzymes, lowering or modifying antibiotics to make antibiotics inactive; changing the binding target of antibiotics so that antibiotics play a role; changing the permeability of the cell membrane and preventing antibiotics from entering bacterial cells; the antibiotics in the bacterial cells are discharged from the cells through the efflux pump to reduce the enrichment of antibiotics in the bacterial cells. The sensitisation methods of "NSAS" include: (I) Changing the permeability of the bacterial cell membrane so that antibiotics can enter the interior of the bacteria; (II) Inhibiting the efflux pump of the bacteria, so that antibiotics can be enriched in the bacteria; (III) Inhibiting the activity of antibiotic degrading enzymes; (IV) Using externally supplemented carbon sources (such as glucose) to make bacteria sensitive to antibiotics again by stimulating the metabolic process of bacteria; (V) Inhibiting bacterial defence systems, such as CSE regulating H_2S production; (VI) Inhibiting mutations in antibiotic targets; (VII) Promote the generation of ROS. Abbreviations: AMR, antimicrobial resistance; CSE, cystathionine γ-lyase; H_2S, hydrogen sulfide; NSAS, non-self-antimicrobial sensitisers; ROS, reactive oxygen species.

1. Introduction

Antimicrobial resistance (AMR) has become a global concern [1]. Based on the latest report by the United States Centers of Disease Control and Prevention in 2019, 35,000 people still die from drug-resistant bacteria (DRB) infections every year in the United States [2]. According to the latest report from the European Centre for Disease Prevention and Control, the estimated burden of DRB infections in the European Union and the European Economic Area is also considerable, especially in the eastern and southern countries or regions of Europe, where infections caused by drug-resistant *Mycobacterium tuberculosis*, *Salmonella* spp. and *Neisseria gonorrhoeae* dramatically increases treatment costs and threatens people's lives, health and safety [3]. Meanwhile, Asia faces a greater burden of Gram-negative DRB infections, with the devastation caused by carbapenem-resistant Enterobacteriaceae and carbapenem-resistant *Acinetobacter baumannii* exacerbating the treatment burden in East and Southeast Asia [4]. Antibiotic resistance is not a new problem. The equilibrium between the development of antibiotics and the resistance of bacteria to antibiotics in the past has been disrupted as the prevalence of multidrug-resistant (MDR) bacteria has increased while the development of new antibiotics has declined. The facts and statistics jointly raise a crucial question: is the "post-antibiotic era" coming? [5]. Although antibiotics have played a good role over the past 80 years or so, the rapid evolution of bacteria makes it urgent for us to find better coping strategies [5].

Bacteria can develop resistance to antibiotics in a variety of ways [6]. Antibiotic resistance can be developed by single bacterial cells using three main mechanisms [7] (Fig. 1). The first mechanism produces inactivating enzymes that destroy the molecular structure of the antibiotics, making them lose antibacterial activity [8]. The second mechanism changes or hides the target-sites of the antibiotics by changing their conformation, making it difficult for antibiotics to bind, or producing special proteins [9]. The third mechanism reduces the accumulation of antibiotics in the bacteria by enhancing the permeability of their outer membrane (OM) or restraining the active efflux system [10]. The micro mutation of bacteria has a severe effect on the activity of a class or family of antibiotics, bringing great challenges in the use of antibiotics, especially single target antibiotics. In addition to the antibiotic resistance mechanisms of single bacterium, a colony effect can also cause bacterial resistance to antibiotics, such as biofilm formation and quorum sensing/quorum signalling (i.e., the ability to detect and respond to cell population density by gene regulation), and bacteria can escape the pursuit of antibiotics by hiding in host cells [7]. There are two main antibacterial strategies against AMR, one direct and the other indirect. The direct antibacterial strategy is a method to develop new and efficient antibiotics that can directly kill or inhibit bacterial growth.

Table 1
Differences between "Non-self-antimicrobial sensitizer" and "antibiotic adjuvants".

Comparison	Non-self-antimicrobial sensitizer	Antibiotic adjuvant
Core of the concept	Restore the sensitivity of drug-resistant bacteria or fungal to antibiotics or antifungal drugs.	Treatment of infectious diseases.
Including	It is a compound with a well-defined composition that may have little or no antimicrobial activity against a particular bacterium or fungus. Typically, these compounds have a MIC value greater than or equal to 16 μg/mL.	All substances with or without antibacterial activity.
Antibiotics included	No	Yes
Mode of action	(1) Reduce the MIC values of one or more antibiotics or antifungal drugs. (2) Only used in combination with one or a class of antibiotics or antifungal drugs.	By combining with one or more antibiotics.

Abbreviations: MIC, minimum inhibitory concentration.

However, developing a new and efficient antibacterial drug takes decades and involves extremely high costs. An economical and efficient indirect antibacterial strategy is to make existing antibiotics play a role again [11,12]. Although some scientific reports have mentioned the use of antibiotic adjuvants, the research on indirect antibacterials still lacks purpose and order [12,13].

Here, we redefine two concepts, the "non-self-antimicrobial sensitisers (NSAS)" and "antibiotic adjuvant", in order to make a clearer distinction in subsequent studies (Table 1). Although NSAS are also used to treat bacterial or fungal infections, the main research and development purpose of these compounds is the restoration of susceptibility of DRB or fungi to potent antibiotics or antifungal drugs that have lost their edge. They may have no or low anti-microbial activity but can reduce the minimum inhibitory concentration (MIC) value of one or more antibiotics or antifungal drugs. In practice, NSAS are only used in combination with one or a class or family of antibiotics or antifungals. "Antibiotic adjuvants" are developed to treat infectious diseases and can therefore include all substances with or without antibacterial activity, including antibiotics, playing a role in the treatment of infectious diseases by combining with one or more antibiotics. However, due to the vague concept of the "antibiotic adjuvant", researchers have often confused non-drug indirect treatment methods (e.g., faecal transplantation, probiotic substances, and innate immunity with Toll like receptor agonists, among others) with "antibiotic adjuvants", lacked a systematic distinction between the "antibiotic adjuvant" and the NSAS, and omitted an explanation of the mechanism, making it difficult to accurately characterise and apply basic and clinical research [12]. Defining NSAS can enable the definitive screening and identification of this kind of active substance and improve the speed of development of this type of drug. In this research review, we focus on NSAS for bacteria and do not discuss NSAS for fungi. We first summarise the methods of restoring bacterial sensitivity to antibiotics and then introduce several sources and mechanisms of NSAS. We also highlight the progress of basic and clinical research involving NSAS and their development prospects. This review article systematically introduces and summarises NSAS for the first time, with the intention of providing guidance and suggestions for their research and development.

2. How to increase the sensitivity of bacteria to antibiotics

From the current scientific research, there are six ways to increase the sensitivity of DRB to antibiotics: (i) inhibit the activity of the antibiotic-inactivating enzyme, (ii) reverse the change of antibiotic targets, (iii) change the accumulation of drugs in bacteria, (iv) attack the bacterial defence system, (v) stimulate bacterial metabolism, and (vi) weakening of DRB by reactive oxygen species (ROS). These techniques may provide inspiration for the development of NSAS.

2.1. Inhibit the activity of the antibiotic-inactivating enzyme

To reduce the effectiveness of antibiotics, some DRBs have evolved to directly destroy or alter their structure. Enzymatic degradation is one such means. Hydrolysis by DRB hydrolases has been reported to be associated with the inactivation of several major classes of antibiotics, including β-lactams and aminoglycosides [7,8]. β-lactamase is an enzyme that degrades β-lactam antibiotics, including penicillin's, cephalosporins, and carbapenems. It was first identified in Gram-negative bacteria. The enzyme opens the β-lactam core ring through serine nucleophilic attack or metal activation of water molecules, rendering the antibiotic ineffective [7]. β-lactamases can be extended-spectrum (ESBLs) or narrow-spectrum. ESBLs are of particular concern due to their ability to hydrolyze multiple β-lactam antibiotics. β-lactamases are classified into four molecular classes, A, B, C and D, based on their amino acid motifs. There are two main ways to classify them based on their mode of action. Classes A, C and D share a common mechanism for substrate hydrolysis through the production of acyl enzymes from the serine that serves as the active site. Class B β-lactamases, also known as metallo-β-lactamases (MBL), are classified as metalloenzymes. At least one zinc is used as the active site to facilitate hydrolysis of the β-lactam ring [14]. Recent research studies have shown that α-phenol-soluble modulins (PSMαs), a functional bacterial amyloid secreted by *Staphylococcus aureus*, is another protein that catalyses the hydrolysis of β-lactams in addition to β-lactamases. Specifically, PSMαs catalyse the hydrolysis of the amide bond of the four-membered β-lactam ring of nitrofen, an antibiotic β-lactam substitute [15].

Another way to aganist antibiotics is through covalent modification of key structural features, which prevents them from interacting with drug targets in Gram-negative bacteria. For example, aminoglycoside-modifying enzymes can alter hydroxyl/amino groups on the antibiotic, resulting in a significant decrease in its binding to the targets [14]. Aminoglycoside antibiotics are easily modifiable due to the aminocyclic alcohol nucleus surface attached to the amino sugar, which is rich in amide and hydroxyl groups, in comparison to other antibiotics. There are three main classes of aminoglycoside-modifying enzymes that catalyse the modification of different amide or hydroxyl groups of the 2-deoxystreptamine nucleus or the sugar moiety of cationic: nucleotidyltransferases (ANTs), phosphotransferases (APHs), and acetyltransferases (AACs) [7]. AACs catalyse the acetylation of -amino groups using acetyl coenzyme A as a donor substrate for the acceptor molecule. ANTs inhibit the action of aminoglycosides by transferring the adenosine monophosphate molecule from the adenosine triphosphate (ATP) to the -OH group on the antibiotic. APHs add a phosphate group to the aminoglycoside, altering the charge distribution of the drug and inhibiting its interaction with the ribosome [14]. These enzymes disrupt the chemical structure of aminoglycosides, reducing their affinity for the target site or preventing binding to the ribosome, rendering the antibiotic ineffective.

Horizontal transmission of antibiotic inactivating enzyme genes and aminoglycoside modifying enzyme genes between ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and Enterobacter spp.) pathogens has long been widely reported [14]. This acquired antibiotic resistance can be blocked by inhibitors. However, it is of concern that some *Streptococci* and *Stenotrophomonas maltophilia* carry antibiotic inactivation genes themselves [16,17]. Their evolution is accelerated under the stress of an antibiotic-containing environment, making it difficult to combat infections caused by these pathogens. Hence, there is an urgent need to develop drugs that target antibiotic-inactivating enzymes or intervene in the genes responsible for these enzymes, so that DRB becomes sensitive to antibiotics again.

2.2. Reverse the change of antibiotic targets

DRB develops resistance to antibiotics by changing the target of the antibiotics. These changes include: (i) structural changes of the target protein: for example, mutations in bacterial ribonucleic acid (RNA) polymerase and deoxyribonucleic acid (DNA) gyrase lead to resistance to rifamycins and quinolones; (ii) the production of new functional proteins: when bacteria come into contact with antibiotics, they produce new functional proteins that are not possessed by the original sensitive bacteria, which can compensate or replace the original functional proteins that were inhibited by the antibiotics and have low affinity, or even no affinity, with antibiotics, resulting in high AMR as the antibiotics cannot be combined with them; (iii) an increase in the number of target-proteins: even if there are enough target-proteins in the drugs, the normal function and morphology of bacteria can be maintained, and the bacteria can continue to grow and reproduce, leading to AMR.

Different types of bacteria modify antibiotic targets in different ways. Since many types of antibiotics target the ribosome, such as aminoglycosides, chloramphenicol and tetracyclines, DRB can use methyltransferases to methylate ribosomal proteins to defend against antibiotic attack [7]. Amino acid substitutions in quinolone-resistant bacteria caused by mutations in chromosomal genes encoding DNA rotamase

and/or topoisomerase IV, resulting in a progressive increase in quinolone MIC value with accumulation of mutations, have been observed in isolates of Gram-positive and Gram-negative pathogens of human and animal origin. Determinants of plasmid-mediated quinolone resistance (PMQR) have been described: AAC(6′)-Ib-cr, Qnr, QepA, OqxAB and CrpP. Although PMQR determinants result in low levels of resistance, they have been shown to reduce ciprofloxacin activity in models of urinary and respiratory tract infections [18]. In addition, DRBs can produce alternative targets that mimic the target of the antibiotic, thereby interfering with the binding of the antibiotic to the target. For example, methicillin-resistant *Staphylococcus aureus* (MRSA) carries the mecA gene, which encodes penicillin-binding protein 2a (PBP2a), an alternative trans-peptidase with low binding affinity for most β-lactam antibiotics. As a result, it is resistant not only to methicillin but to all β-lactam antibiotics [19]. In conclusion, recognising the strategies of DRBs to modify antibiotic targets may help in the development of drugs against these new targets.

2.3. Change the accumulation of drugs in bacteria

The reduced accumulation of antibacterial drugs in bacteria is an important mechanism of AMR and is considered to be one of the most common reasons for bacterial AMR. The decrease in drug accumulation is related to the efflux pump system of the bacteria and the cell membrane permeability.

Gram-negative bacteria have a thick extracellular membrane, which can provide a natural barrier for bacteria and prevent hydrophobic extracellular substances from entering the cell, such as detergents, dyes and antibiotics, among others.

This is why drug-resistant Gram-negative bacteria are difficult to kill. Gram-negative bacteria have two cell membranes, of which remodelling of the OM is an important antibiotic resistance process observed in several Gram-negative bacteria, reducing the influx of antibiotics and leading to their survival. OM remodelling is a process by which bacteria regulate or remove specific membrane components, such as lipids and proteins, to adapt to a new environment. During this process, new proteins, such as porins, can integrate into the cell membrane and degrade existing proteins. This mechanism has recently been shown to have a major impact on the rate of AMR, a bacterial trait that urgently requires further research and targeted therapy [20]. Bacterial OM pore proteins are the main conduits for antibiotic influx. A number of regulatory proteins are responsible for the transcriptional and post-transcriptional regulation of genes encoding different pore proteins. These proteins include the two-component signalling systems CpxR and OmpR and small non-coding RNA proteins (e.g. *micF*, *micA* and *micC*). These two-component signalling systems, such as the EnvZ-OmpR regulatory system, are able to detect changes in osmolality or the presence of various antimicrobial drugs in the external environment. In this way, the protein composition of the OM can undergo ‘remodelling’ changes. In addition, CpxR and other proteins in the Cpx coat can also detect external changes in osmolarity and antimicrobial drugs that trigger OM remodelling. This system works in conjunction with the small non-coding RNA protein *micC*, which is upregulated in the presence of antimicrobial drugs, particularly β-lactams, to initiate OM remodelling [20].

These complex transporter proteins are normally found in the inner membrane and OM of gram-negative bacteria and are responsible for extruding harmful substances, such as antibiotics, from the cell wall. This results in a lower concentration of antibiotics inside the cell, meaning that the bacteria can tolerate high concentrations of antibiotics. There are six structural families of drug efflux pumps in MDR bacteria, including (i) small multidrug-resistance family; (ii) major facilitator superfamily; (iii) resistance nodule dissection family; (iv) ATP binding cassette superfamily; (v) multidrug and toxic compound extrusion family; and (vi) the proteobacterial antimicrobial compound efflux family [14]. These active efflux systems play a critical role in generating resistance to a wide range of chemical antibiotics and antimicrobial agents, and their occurrence in clinical and environmental bacterial strains is rapidly increasing. Importantly, these systems are virtually ubiquitous in all bacterial communities, not just Gram-negative bacteria, and generate antibiotic resistance through common resistance mechanisms [21]. Overall, efflux pumps can be divided into two categories based on their energy source, one using ATP (i.e. primary efflux pumps) and the other using proton motive force (i.e. secondary efflux pumps). In summary, inhibition of efflux pumps can effectively promote the increase of antibiotic concentration in bacteria, and only when the antibiotic reaches a certain concentration in the bacteria, it can effectively kill the bacteria.

2.4. Attack the bacterial defence system

Nitric oxide (NO) and hydrogen sulfide (H_2S), which have long been considered as secondary metabolites of bacteria, have also now been found to be two key factors of the bacterial defence system. It has been confirmed that endogenous H_2S can protect the growth of bacteria, its production being regulated by three enzymes, cysteine sulphide cystathionine β-synthase, cystathionine γ-lyase (CSE) and 3-mercaptopyruvate sulfurtransferase [22]. Recently, Shatalin et al. found that the ability of *Staphylococcus aureus* and *Pseudomonas aeruginosa* to produce H_2S decreased when the CSE gene was knocked out, and the therapeutic effect of gentamicin on *Staphylococcus aureus* and *Pseudomonas aeruginosa* improved, although the therapeutic effect of gentamicin was minimised after exogenous compensation of H_2S. This evidence indicates that CSE is involved in the endogenous production of H_2S and that H_2S has protective effects on bacteria under antibiotic treatment [23]. Shatalin et al. designed CSE-targeted drugs based on structure-activity relationships (SARs), significantly enhancing the therapeutic effects of various antibiotics (gentamicin, norfloxacin, and vancomycin) on *Staphylococcus aureus* and *Pseudomonas aeruginosa* in the presence of these antibiotic drugs [23]. This research study provides new insight into increasing bacterial sensitivity: it is also possible to restore the sensitivity of MDR bacteria to antibiotics by inhibiting the bacterial defence system. Recent research studies have shown that NO synthase, the key protein of endogenous NO produced by bacteria, plays a key role in the electron transfer and colonisation of *Staphylococcus aureus*, suggesting that NO synthase has great potential as a target for the development of antibiotics or NSAS [24,25]. To summarise, the targets related to the production of bacterial endogenous NO and H_2S can be used as the sensitisation targets of DRB. We can look forward to the discovery of more targets related to bacterial defence systems and the development of drugs to attack bacterial defence systems to restore the sensitivity of DRB to antibiotics.

2.5. Stimulate bacterial metabolism

In addition to AMR, drug tolerance (also called phenotypic AMR) [6] is also a major cause of antibiotic treatment failure and repeated infection [26]. AMR is characterised as a change of the MIC value, while antibiotic tolerance is characterised as a change of the killing kinetics and the existence of time-dependent biphasic killing [27]. The emergence of antibiotic tolerance is due to the fact that sensitive bacterial subsets survive by sacrificing at the metabolic level and avoiding antibiotic-induced death under a lethal dose of antibiotics, especially in high-density bacteria. New therapeutic strategies are urgently required for combating antibiotic tolerance [28]. Tolerance is considered to be a physiological state that promotes the development of AMR [26,29]. The formation of bacterial tolerance is attributed to impaired drug uptake and can therefore be reversed by metabolic stimulation [30].

Density dependent persistence is the main reason for the failure of quinolone treatment. For high-density bacteria exposed to quinolones, with the failure of metabolites, some bacteria enter a dormant state [27]. On the other hand, quinolones kill bacteria by destroying their DNA, but

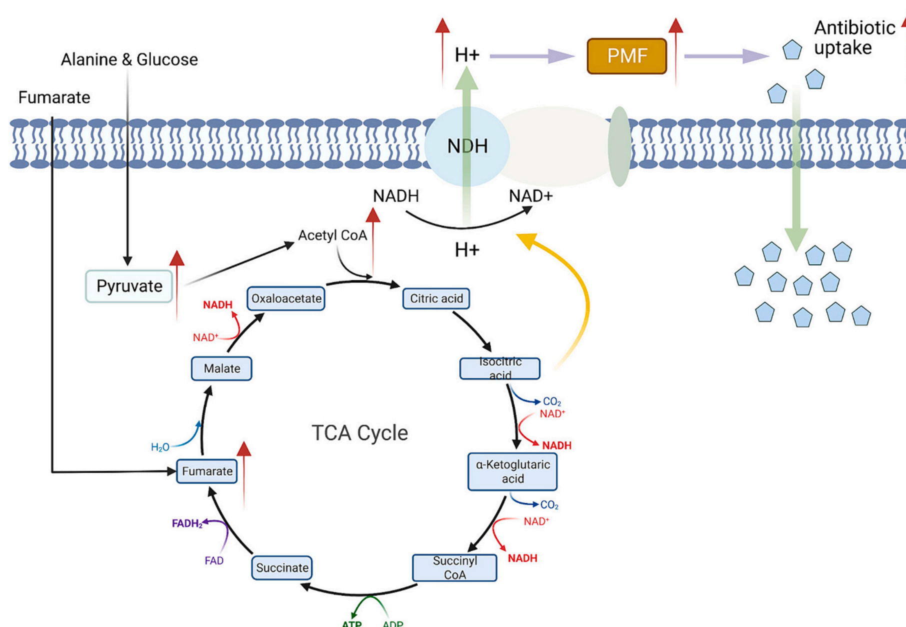

Fig. 2. Recovery of antibiotic sensitivity of DRB by metabolic stimulation. Supplement exogenous carbon sources, such as alanine, glucose or fumaric acid, to stimulate the bacterial TCA cycle, accelerate the production of proton energy, restore the metabolism of dormant bacteria, and promote the absorption of antibiotics by tolerant bacteria. Abbreviations: ADP, adenosine diphosphate; ATP, adenosine triphosphate; CO_2, carbon dioxide; CoA, coenzyme A; DRB, drug-resistant bacteria; FAD, flavin adenine dinucleotide; $FADH_2$, reduced flavin dinucleotide; H_2O, water; NAD^+, oxidized nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; NADPH, nicotinamide adenine dinucleotide phosphate; NDH, NADPH dehydrogenase complex; PMF, proton motive force; TCA, tricarboxylic acid.

DNA damage activates a Salt Overly Sensitive gene network (a complex network consisting of 40 genes) that results in the production of repair proteins and the formation of bacterial tolerance [31]. Gutierrez et al. successfully re-sensitised high-density populations of *Escherichia coli*, *Staphylococcus aureus* and *Mycobacterium smegmatis* to quinolones by adding stationary phase cultures, glucose, and a suitable terminal electron acceptor to stimulate respiratory metabolism [27]. Similarly, the activity of amino glycoside antibiotics can be enhanced by metabolic stimulation [30,32]. Fumarate can stimulate tricarboxylic acid (TCA) cycles to generate proton energy, activate cell respiration and improve the sensitivity of *Pseudomonas aeruginosa* to aminoglycoside antibiotic tobramycin [30]. Exogenous glucose or alanine promotes the TCA cycle by substrate activation, which in turn increases the production of NADH and proton energy, stimulating the absorption of antibiotics and restoring the sensitivity of MDR to aminoglycoside antibiotic kanamycin [32]. The concept of bacterial tolerance shows that the bacterial growth environment can be used as a vital target for antibiotic sensitisation [26]. By supplementing carbon sources to stimulate bacterial metabolism, high-density non-mitotic DRB are sensitised to antibiotics again, thus achieving the therapeutic purpose (Fig. 2).

2.6. Reactive oxygen species attack

Reactive oxygen species (ROS) are by-products of oxygen metabolism and play an important role in cell signalling and homeostasis in the body. They include peroxides, superoxides, hydroxyl radicals, monoclinic oxygen and alpha-oxygen. Due to their powerful oxidative capacity, ROS cause genetic and structural damage to bacteria and biofilms, making it difficult for them to develop drug resistance and playing a crucial role in the body's defence against pathogen invasion. ROS-based antimicrobial strategies have been extensively studied in recent years and represent a different therapeutic strategy from antibiotics. Unlike antibiotics, which target specific bacterial targets, ROS attack leads to the overall weakening of bacteria, resulting in damage to the bacterial periplasm, cell membrane and other structures, as well as metabolic imbalance, which greatly mitigates the emergence of bacterial drug resistance [33]. For example, the nanomaterials mimic vanadium chloride peroxidase, which catalyses a reaction that produces abundant monovalent oxygen and hypochlorous acid to carry out the destruction of bacteria and biofilms when halide ions and hydrogen peroxide (H_2O_2) coexist [34]. The ROS produced by catalyzed H_2O_2 can effectively degrade the protein, polysaccharide and nucleic acid components of bacterial biofilms. There are research studies on the decomposition of H_2O_2 by the catalase-like active component of iron tetraoxide metal nanoparticles for cleaning and disinfection [35]. However, relatively high doses of ROS can cause unwanted damage. Therefore, in one study, glucose oxidase (GOx) was encapsulated in a carrier material. GOx produces gluconic acid by consuming glucose in the wound, and GOx regulates the pH microenvironment suitable for the antibacterial activity of the nanoenzymes to achieve a glucose-H_2O_2-hydroxyl radical cascade catalytic reaction [34]. Current studies have focused on the direct bactericidal production of materials via ROS, and there have been few studies of ROS synergising with antibiotics. We believe that the potential of ROS to damage bacterial cell membranes can greatly enhance the accumulation of antibiotics in bacterial cells and the re-sensitisation of DRB to antibiotics.

3. Sources of non-self-antimicrobial sensitisers and their mechanism of action

3.1. Natural small molecule compounds

Natural small molecule compounds are not only the source of most antibiotics, including bactrim, penicillin, hygromycin among others, but also the main source of non-self-antimicrobial sensitisers (NSAS). Among them, plants and micro-organisms are the biggest treasure trove of natural small molecule NSAS, which mainly include terpenoids, steroids, flavonoids and polyphenols. They can act synergistically with antibiotics to reduce the MIC values of antibiotics against DRBs, and their mechanisms of action mainly include (i) inhibiting the activity of antibiotic inactivating enzymes, (ii) inhibiting the modification of antibiotic targets, and (iii) promoting the accumulation of antibiotics in

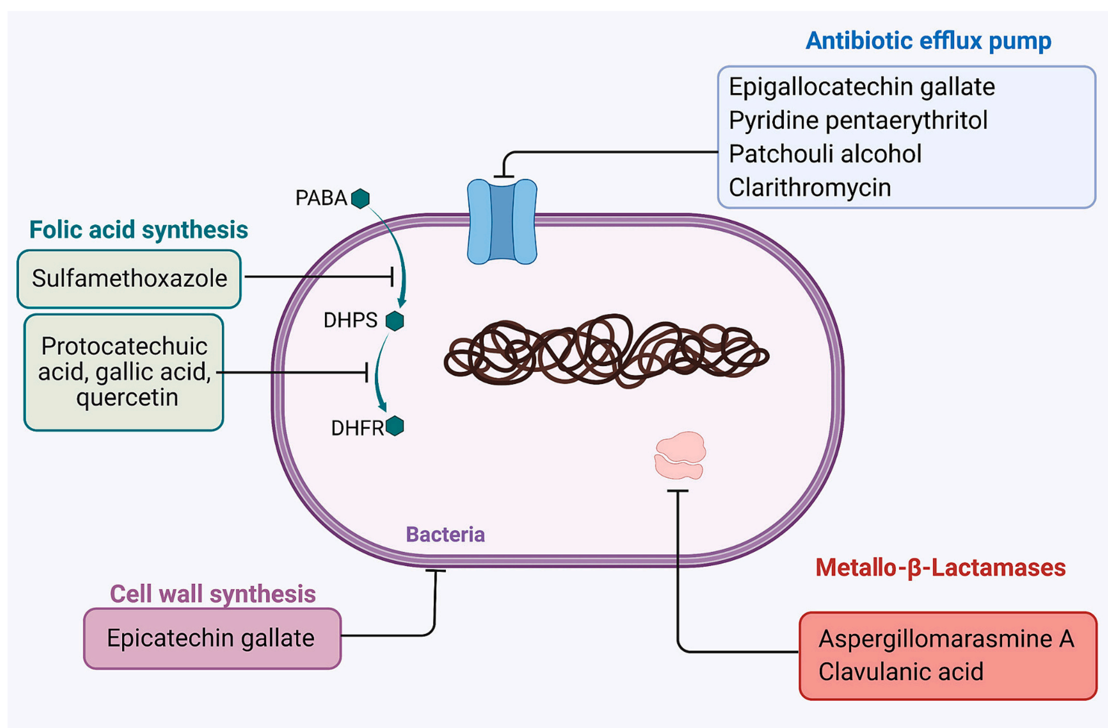

Fig. 3. Action mechanism of a natural small molecular compounds "NSAS". Natural small molecule compounds from plants can restore bacterial sensitivity to antibiotics by inhibiting folic acid synthesis pathway, inhibiting bacterial cell wall synthesis and inhibiting the antibiotic efflux pump. A bacterial source can inhibit β-lactamase to reverse AMR. Abbreviations: AMR, antimicrobial resistance; DHFR, dihydrofolate reductase; DHPS, dihydropteranoic acid synthase; NSAS, non-self-antimicrobial sensitisers; PABA, p-aminobenzoic acid.

bacteria (Fig. 3) [36].

Microbes are the largest source of antibiotics, and almost all natural antibiotics are secondary metabolites of microorganisms [37]. However, the standard methods for finding these natural antibiotics focus on single molecules and often ignore natural products with low antibacterial activity, even though these natural products can improve the antibacterial activity of antibiotics, i.e., they are "NSAS". King et al. found a natural product from fungi called aspergillomarasmine A, which inhibited various bacterial MBL and fully restoring the susceptibility of Enterobacteriaceae, *Acinetobacter* spp. *and Pseudomonas* spp. genera, which are resistant to β-lactam antibiotics, to meropenem, reducing the MIC values of meropenem to 2 μg/mL [38]. More famous β-lactam antibiotic is clavulanic acid (CA), which was first discovered in 1976 from *Streptomyces clavuligerus* (ATCC27064) fermentation broth [37]. CA is also a β-lactamase inhibitor, although its antibacterial activity is very weak (CA inhibits most Gram-negative and Gram-positive bacteria at a concentration greater than 30 μg/mL), and it can irreversibly bind to β-lactamases, especially those produced by *Staphylococcus aureus*, *Klebsiella aerogenes* and *Proteus mirabilis,* thereby inhibiting the β-lactamase from decomposing β-lactam antibiotics and restoring the sensitivity of DRB to penicillin and cephalosporin antibiotics [39]. Small molecule compounds of plant origin are also known to have antibiotic-inactivating enzyme inhibitory activity. Baicalin, a flavonoid found in *Scutellaria amoena*, also inhibits the enzyme β-lactamase and is therefore an interesting compound for inhibiting antibiotic resistance. This natural compound, if studied, could play a key role in the fight against infectious diseases caused by bacteria [40]. These researches prove the value of a continuous investment in antibiotic-inactivating enzyme inhibitors and the importance of finding NSAS as partners of known antibiotics, in order to expand the use of antibiotics [41,42].

A number of natural small molecules of plant origin show activity in inhibiting antibiotic target modification in bacteria. Alternative PBP2a binds to β-lactam antibiotics, reducing their affinity for the bacteria and rendering DRB resistant to antibiotics. Corilagin, a tannin isolated from *Arctostaphylos uva-ursi*, has an MIC value of 128 μg/mL. However, when a lower concentration of corilagin (16 μg/mL) was used in combination with oxacillin and other β-lactams against MRSA, the MIC values were reduced by 100–2000 folds. No significant reduction in MICs was observed for methicillin-sensitive *Staphylococcus aureus* strains. The MIC values of the other antibiotics used (fosfomycin, erythromycin, vancomycin) were also unaffected. This suggests that corilagin may have increased the susceptibility of MRSA to β-lactams via inhibiting of the target PBP2a. A compound, tellimagrandin I, was isolated from red rose (*Rosa canina*) and its synergistic activity with oxacillin and other β-lactam antibiotics was investigated. Tellimagrandin I reduced the MIC value of β-lactam antibiotics by 128–512 fold. The fractional inhibitory concentration index was 0.39, indicating a good synergistic effect [40]. The flavonoid myricetin increases the sensitivity of *Pseudomonas aeruginosa* to sulfamethoxazole, which may be related to its ability to inhibit DNA gyrase [36].

The bacterial efflux pump can affect the enrichment of antibiotics in bacterial cells, thus greatly reducing the activity of antibiotics. Epigallocatechin gallate enhances the sensitivity of drug-resistant *Staphylococcus* to tetracycline by impairing the tetracycline efflux pump and increasing the intracellular retention of the antibiotic drug [43]. Pentaerythritol (a simple five-carbon tetraol used in the fabrication of resins, alkylated resins, varnishes, polyvinyl chloride stabilisers, tall oil esters and olefin antioxidants) can increase the accumulation of antibiotics in bacteria by inhibiting *NorA* efflux pumps [44]. The combined application of patchouli alcohol (a sesquiterpene alcohol found in patchouli oil) and clarithromycin (macrolide antibiotic) promotes the inhibition and sterilisation of *Helicobacter pylori*, which may be related to the fact that patchouli alcohol can inhibit the expression of efflux pump genes (namely *hp0605*, *hp1327* and *hp1489*) of *Helicobacter pylori*, reducing the drug-resistance of *Helicobacter pylori* [45]. 4-Hydroxybenzaldehyde, a natural compound derived from *Gastrodia elata* B1 (Orchidaceae), was originally found to have antioxidant and neuromodulatory activities in rat brain studies, and shows a synergistic effect

when combined with antibiotics, including chloramphenicol, thiamphenicol and gentamicin [46,47]. The MIC values of chloramphenicol, thiamphenicol, gentamicin and rifampicin were reduced 4, 8, 4 and 4 fold, respectively by 4-hydroxybenzaldehyde using *Acinetobacter baumannii* as indicator bacteria. 4-Hydroxybenzaldehyde does not support the growth of *Acinetobacter baumannii*, but can promote the influx of chloramphenicol by up-regulating PCA metabolism-related genes (*pca* genes) and osmotic stress (*bet* genes) [47]. 4-Hydroxybenzaldehyde has an LD50 value of 3980 mg/kg bw in rats and is regarded as safe and not toxic and is used as a food flavouring agent in Europe [48]. Although the antibacterial effect of small molecule compounds in these plants is weak, the "synergistic antibacterial effect" between small molecule compounds ensures that plants will not be infected by bacteria.

3.2. Synthetic small molecule compounds

Synthetic small molecular compounds have also occupied half of the history of antibiotic development, typically such as quinolone antibiotics, sulfonamides and some chloramphenicol-like, among others. However, most current research focuses on these small molecules for direct antibacterial activity, which can be abandoned if the antibacterial effect is poor. However, most of the current research studies focus on the direct antibacterial effect of these synthetic small molecules, which may be abandoned if the antibacterial effect is very poor, while ignoring a large number of potential NSAS [49,50]. The mechanisms of antibacterial sensitisation of synthetic small molecule NSAS under study are: (i) inhibition of antibiotic inactivating enzyme activity; (ii) promotion of antibiotic accumulation in bacteria.

Among the synthetic small molecule compounds the development of antibiotic inactivating enzyme inhibitors is of the highest interest. Bicyclic boronates has been developed early on as a β-lactamase inhibitor and has considerable clinical potential [51]. One such compound, the (predominantly) monocyclic boronic acid vaborbactam is approved for the treatment of complicated urinary tract infections in co-administration with meropenem [52]. The bicyclic boronates are proposed to work by binding in a manner analogous to that of the tetrahedral intermediate(s) likely involved in β-lactam catalyzed hydrolysis by both serine-β-lactamases (SBL) and MBL. Taniborbactam (TAN, formerly VNRX-5133) has passed phase 1 clinical testing and is currently in phase 3 clinical testing in combination with cefepime. TAN has been shown to successfully inhibit SBLs and MBLs in vitro, and to restore cefepime's activity in vivo against a class A SBL (CTX-M-14) producing strain of *Klebsiella pneumoniae* in a neutropenic lung model of infection as well as CTX-M-15 expressing *Escherichia coli* in ascending urinary tract infections in mice [53,54].

Some synthetic small molecule compounds also promote antibiotic accumulation in bacteria and reduce DRB resistance to antibiotics. The anti-protozoal drug pentamidine is a bacterial OM permeabilising agent that exerts effective synergistic effects with rifampicin, novobiocin and erythromycin [55]. In a mouse model of colistin-susceptible *Acinetobacter baumannii* infection, 10 mg/kg bw pentamidine and 5 mg/kg bw novobiocin affected 100% survival, while in a model of colistin-resistant *Acinetobacter baumannii*, 10 mg/kg bw pentamidine and 50 mg/kg bw novobiocin rescued 90% of mice [55]. Pentamidine caused a 40 nm fluctuation on the surface of treated *Escherichia coli* cells, bound purified lipid a with high affinity (dissociation constant Kd ~ 120 nm) *in vitro*, led to enhanced lipopolysaccharide (LPS) release similar to polymyxin, and its activity was hindered by exogenous LPS and Mg^{2+}, all of which suggest a direct correlation with LPS [55]. Nevertheless, pentamidine is plagued by adverseeffects, including nephrotoxicity, hypotension and hypoglycaemia, limiting its clinical potential [56].

The diamidine MD-124 is a derivative developed by Yu et al. in the study of the antimicrobial sensitizer MD-100, a scaffold identified from a screening of cationic compounds for rifampicin potentiation in *Escherichia coli* [57]. MD-124 sensitised *Escherichia coli*, *Acinetobacter baumannii* and *Klebsiella pneumoniae* to rifampicin and clarithromycin with strain-specific activity. MD-124 was able to sensitize *Escherichia coli* to rifampicin 512-fold at 5 μg/mL (~10 μM), reducing the MIC value of rifampicin from 10 μg/mL to 0.02 μg/mL [57]. At the same concentration, MD-124 itself had no effect on bacterial growth. MD-124 bound to the hydrophilic portion of LPS and was more selective for bacterial LPS than eukaryotic phospholipids. In addition, MD-124 exhibited low cytotoxicity against human embryonic kidney HEK293 and embryonic mouse fibroblast NIH3T3 cells [57].

There have been countless compounds studied and developed, and it is worth considering how to efficiently screen effective chemical synthetic NSAS. Structure-based ligand screening may be a highly feasible method. Effective NSAS can be found by screening compounds that target drug resistance genes or bacterial efflux pumps [58,59]. One example found through high-throughput screening is the β-lactamase inhibitor Of1 (a tricyclic indoline), which can significantly inhibit β-lactamase activity and restore the sensitivity of MRSA to β-lactam antibiotics, but has no effect on methicillin-sensitive *Staphylococcus aureus* [60]. The method of targeted screening as a strategy to find new uses for old antibiotic drugs not only shortens the development time but also reduces the high cost of drug research and development. Many joint studies are in progress to identify chemical agents that can improve the efficiency of antibiotics and reduce their toxicity and adverse/side effects [47].

3.3. Antimicrobial peptides

While plants and microorganisms have been described as the main sources of small molecule NSAS, there is also a class of NSAS in animals that are antimicrobial peptides (AMPs). Of the 3940 AMPs identified to date, more than 60% are of animal origin (data from the Antimicrobial Peptide Database, https://aps.unmc.edu/) [61,62]. With the exception of a few short peptides that act on internal bacterial targets, most AMPs interfere with the permeability of bacterial cell membranes through their unique amphiphilicity and interesting charge arrangements [63]. Some AMPs are potent antibacterial agents in their own right, while others, although weakly antibacterial, alter the permeability of bacterial cell membranes, increasing the accumulation of antibiotics inside the bacteria and achieving a sensitizing effect. For example, AMP glycine leucine amide (PGLA) used in combination with antibiotics produces up to 30-fold decrease in the antibiotic resistance level of resistant bacteria, which may be related to the fact that PGLA alters the cell membrane permeability of the DRB and facilitates the accumulation of antibiotics within the bacterial cell, and it is thought that PGLA can be used to eradicate DRB and inhibit the evolution of resistance from scratch [64]. The proline hinge peptide KL-L9P (sequence Ac-KLLKLLKKPLKLLK-NH_2) reduced the MIC of linezolid, rifampicin and clarithromycin against MDR *Escherichia coli* by more than 32-fold and is active in a murine model of cutaneous *Acinetobacter baumannii* infection. KL-L9P does not compromise the OM integrity, but causes morphological changes in the OM, resulting in a surface substantial increase in roughness and mobility. The authors suggested that KL-L9P binds to lipid A as a hinge helix and produces a stiff gel-like LPS structural domain that affects the arrangement of OM [65].

In addition to natural AMPs, peptide derivatives are also very promising attempts as NSAS. The polymyxin derivatives, polymyxin B nonapeptide, deacylpolymyxin B, deacylcolistin, polymyxin B octapeptide, polymyxin B heptapeptide, guanidylated-colistin, guanidylated-polymyxin B among others, all retain the membrane permeability of polymyxin and greatly improved the susceptibility of resistant bacteria to polyantibiotics [66]. NAB7061 (linear portion of the peptide, threonyl-aminobutyryl) and NAB739 (a cyclic peptide portion identical to that of polymyxin B), in which the diaminobutyryl residue of the linear portion of polymyxins was replaced by a neutral aminobutyryl or D-Ser residue, respectively, both of which synergised effectively with rifampicin, clarithromycin and vancomycin [67]. SPR741, previously known as NAB741, carries three positive charges and has an acetyl

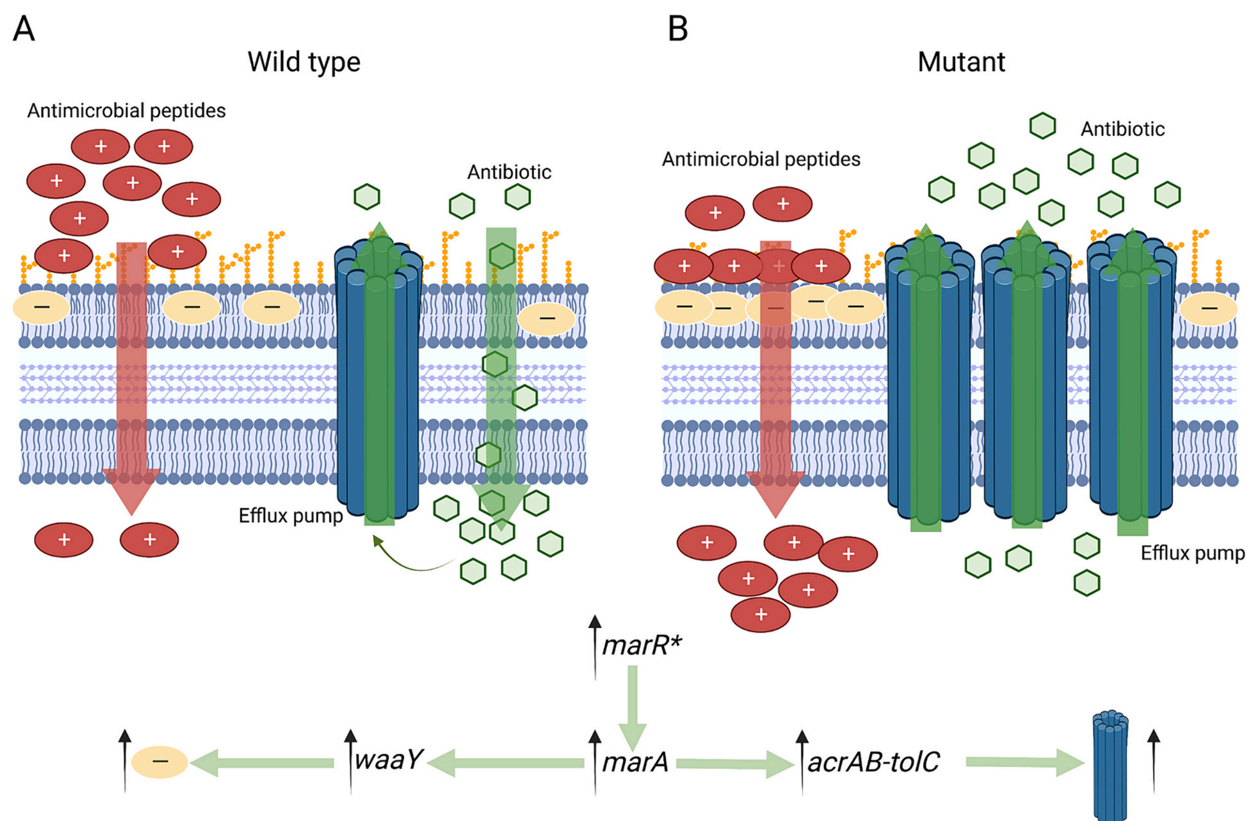

Fig. 4. Bacterial resistance to antibiotics increases the lateral branch sensitivity of antimicrobial peptides. (A) *marR* gene wild-type bacteria and (B) *marR* gene mutant bacteria. The mutation of the *marR* gene leads to the up-regulation of *marA* gene expression, further promotes the expression of the efflux pump related gene *acrAB-tolC*, and leads to an increase of the efflux pump. The efflux pump expels the antibiotics enriched in the bacterial cells to make the bacteria resistant to antibiotics. On the other hand, mutation of the *marR* gene will also lead to the up-regulation of the *waaY* gene, which will increase the number of anionic phospholipids on the bacterial cell membrane, thus absorbing a large number of cationic AMPs, changing the permeability of the bacterial cell membrane, and enabling the antimicrobial peptides to enter and kill the bacteria. Abbreviations: *acrAB-tolC*, acridine resistance proteins A and B coupled to the outer membrane channel TolC; *marA,* multiple antibiotic resistance activator; *marR*, multiple antibiotic resistance regulators; *waaY*, lipopolysaccharide core heptose (II) kinase.

Table 2
Antimicrobial peptides used in clinical trials as anti-infective therapies.

Peptide	Clinical application	Target and mechanism of action	Route of administration	Developmental stage (decision)	Company	Reference
Human lactoferrin-derived peptide hLF1–11	LPS-mediated diseases and fungal infections	DNA-binding	Intravenous	Phase 1 (completed)	AM-Pharma	
Histatin-1 and 3, P-113 (histatin derivatives)	Chronic *Pseudomonas aeruginosa* infections, gingivitis, and periodontal diseases	Reactive oxygen species generation	Topical (gel mouthwash)	Phase 1, Phase 2–3	Demgen	
Lytixar (LTX-109)	Uncomplicated Gram-positive skin infections. Methicillin-resistant *Staphylococcus aureus* and methicillin-sensitive *Staphylococcus aureus* nasal carriage	Membrane permeabilization	Topical	Phase 2	Lytix Biopharma	
Opebacan (rBPI21, neuprex)	Meningococcal wound, and burn infections	Membrane permeabilization	Intravenous	Phase 2 (completed)	Xoma	[75]
Opebacan rBPI21, neuprex)	Post-traumatic infections	Membrane permeabilization	Intravenous	Phase 2 (failed)	Xoma	
Omiganan (MB1–226, MX-594AN)	Catheter-associated infection	Membrane permeabilization	Topical	Phase 3 (completed)	Migenix	
EA-230	Sepsis, endotoxemia	Immunomodulation	Intravenous	Phase 2 (recruiting)	Exponential Biotherapies	
Dusquetide (IMX942)	Oral mucositis	Immunomodulation	Topical	Phase 3 (recruiting)	Inimex Pharmaceuticals	
Brilacidin (PMX-30063)	Acute bacterial skin infections	Membrane permeabilization	Intravenous or topical	Phase 2	Innovation Pharmaceuticals	

Abbreviations: DNA, deoxyribonucleic acid; LPS, lipopolysaccharide.

group substituted for a hydrophobic octanoyl residue. SPR741 was initially reported to sensitize *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae* and *Acinetobacter baumannii*. The combination of 4 μg/mL SPR741 and 1 μg/mL rifampicin inhibited 27 broadly resistant clinical isolates of *Acinetobacter baumannii* [68]. SPR741 act primarily on OM and has little depolarising effect on *Escherichia coli* cytoplasmic membrane, while causing fluctuations of 10–15 nm at the cell surface, enough to allow antibiotics that are rejected by OM to pass through the cell membrane, and it is the DRB that is re-sensitised to these antibiotics. SPR741 entered phase 1 clinical trials in 2016 but was discontinued in January 2020 and replaced by SPR206 (a different polymyxin analog from the potentiator platform). While SPR741 was developed as an antibiotic adjuvant, SPR206 has antibacterial activity as a standalone therapy and boasts potentially superior safety and efficacy profiles to those of SPR741 [69–71].

Peptide-based modified analogues, synthetic mimics of AMPs, have also attracted great interest from researchers in the field of antimicrobial sensitization. Moon et al. performed SARs analysis on 17 linear lipopeptide paenipeptin identified analogues 9 and 16, which retained strong *Pseudomonas aeruginosa*-specific antibacterial activity. When combined analogues 9 and 16 at 4 μg/mL with clarithromycin, erythromycin and vancomycin a more than 64-fold increase in their activity against *Acinetobacter baumannii* and *Klebsiella pneumoniae*, with biofilm eradication potential and non-haemolytic properties [72]. The mimetic peptide H-[NLys-^{t}BuAla]$_6$-NH$_2$ (CEP-136) sensitised laboratory strains and clinical isolates of *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* to rifampicin, clarithromycin and azithromycin, and showed an enhanced effect on rifampicin and azithromycin treatment in a murine model of peritonitis. Although sensitizing activity was reduced in LPS-deficient *Escherichia coli*, the MIC of CEP-136 was unaffected, suggesting that the compound does not bind LPS. The synergistic effect of CEP-136 with antibiotics was particularly strong in Δ*rfaG* mutants without an outer core sugar, suggesting that CEP-136 promotes antibiotic uptake through core instability within LPS. CEP-136 has a low cytotoxicity and safety profile and could be used to further develop effective adjuvants for repurposing antibiotics for use against MDR Gram-negative infections [73].

Although there are examples of bacterial resistance to AMPs, interestingly, bacterial resistance to antibiotics increases the collateral sensitivity to AMPs such as cationic antimicrobial peptide 18 kDa [64,74]. Lázár et al. found that the inhibitory effect of multiple antibiotic resistance activator (*marA*) in Gram-negative MDR bacteria decreased significantly after multiple antibiotic resistance regulators (*marR*) gene mutation, resulting in the up-regulation of the acridine resistance proteins A and B coupled to the outer membrane channel TolC (AcrAB-ToLC) efflux pump, thus increasing the AMR to a variety of antibiotics. On the other hand, *marA* also promotes the up-regulation of lipopolysaccharide core heptose (II) kinase (*waaY*), a kinase responsible for LPS core phosphorylation, which can increase the net negative charge of the bacterial OM [64]. This in turn enhances the sensitivity to cationic AMPs interacting with the membrane (Fig. 4). Compared with traditional antibiotics, antibacterial peptides have slower drug resistance, broad-spectrum antibacterial membrane activity and can regulate the host immune response [64,75]. So far, there are few AMPs used as drugs for clinical infectious diseases (Table 2), which may be due to the obstacles in clinical application, including poor antibacterial activity, high toxicity and high production costs [76–78]. However, as a kind of NSAS, AMPs have great development and utilisation value [79–81].

3.4. Metal ions

Metal ions such as iron, gallium, zinc, copper and silver have long been found to have antimicrobial activity, but the cytotoxicity associated with these metal ions limits their use, but the preparation of these metal ions into materials that can be used as NSAS is very promising [82,83]. The main mechanisms of antimicrobial sensitisation by metal ions include (i) inhibition of bacterial efflux pumps and promotion of antibiotic enrichment within bacteria; (ii) inhibition of biofilm formation to inhibit pathogens; (iii) promotes the production of ROS; (iv) inhibition of antibiotic-inactivating enzyme activity [84]. For example, a ferrous ion-based material, iron-hexacyanocobaltate (FeHCC) can cause an imbalance of the bacterial membrane potential, increasing the permeability of the bacterial cell membrane, as well as promoting the production of ROS by bacteria, resulting in the growth inhibition of *Escherichia coli* and *Staphylococcus aureus* and also restores the susceptibility of DRB to antibiotics. Due to its high stability, FeHCC has the potential to be used on self-cleaning surfaces or food packaging [85].

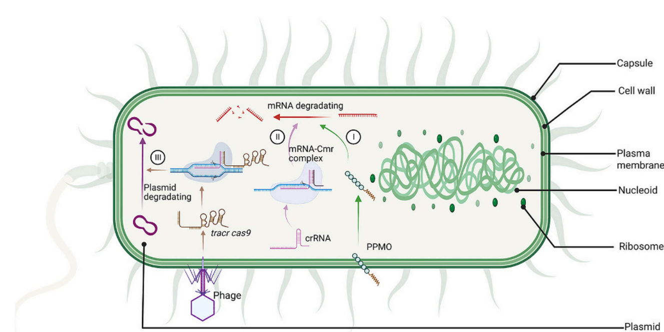

Fig. 5. Three gene therapy methods increase the sensitivity of drug-resistant bacteria to antibiotics. (I) PPMOs cross the bacterial cell membrane and bind to drug resistance genes or proteins to degrade them. (II) After crRNA enters the bacteria, it binds with the mRNA expressed by the drug resistance gene and then degrades the target mRNA, so that the drug resistance gene of the bacteria cannot be expressed. (III) The plasmid is degraded by phage injection of cas9 to inhibit the expression and transmission of drug resistance genes in bacterial plasmids. Abbreviations: Cmr, cas repeat-associated mysterious protein module; crRNA, CRISPR RNA; mRNA, messenger ribonucleic acid; PPMOs, peptide phosphodiamide morpholine oligomers; tracr, trans-activating CRISPR.

The oxidation of metal ions or combination with nanotechnology can not only reduce the cytotoxicity of these metal ions but can also enable them to play the role of NSAS [84,85]. For example, iron oxide and zinc oxide nanoparticles improve the therapeutic effect of antibiotics on DRB by combining them with antibiotics [84]. Because of the iron-based and zinc-based materials promote local accumulation of antibiotics while inhibiting the function of bacterial efflux pumps, resulting in a significant increase in the concentration of antibiotics in the bacterial cell. In addition, some iron-based or zinc-based materials also promote the production of large amounts of ROS by bacteria, which reduces the vitality of the DRB [86]. A copper coordination complex can increase the sensitivity of MDR *Escherichia coli* to β-lactam antibiotics ertapenem (the first of a new group of carbapenems) and meropenem, this is due to its ability to inhibit the activity of the New Delhi metallo-β-lactamase 1 (NDM-1), which restores the sensitivity of β-lactam-resistant antibiotics to β-lactam antibiotics [87]. As a kind of NSAS, metal ions also have great application potential in specific fields because of their wide source.

3.5. Gene technology

Gene technology can also be applied to sensitize MDR bacteria, the principle being to use targeted complementary antisense oligonucleotides to target messenger ribonucleic acid (mRNA) or DNA, to silence essential genes or antibiotic resistance genes and restore the sensitivity of MDR bacteria to antibiotics. In infectious diseases, ribonucleic acid interference (RNAi), peptide phosphodiamide morpholine oligomers (PPMOs) and clustered regularly interspaced short palindromic repeat/CRISPR-associated 9 (CRISPR/Cas9) are all widely applied technologies (Fig. 5).

3.5.1. RNAi silences resistance genes in drug-resistant bacteria

RNAi can silence the drug resistance genes of MDR bacteria [88]. It is a process in which double stranded RNA silences target genes by interfering with the expression of target RNA at the translation level [89]. After double stranded CRISPR RNA (crRNA) enters the bacteria, it cleaves the target mRNA at two clear sites, starting from the 3′ end of prokaryotes by binding to the repeat-associated mysterious protein module (or Cmr) complex [90]. For example, *Pyrococcus furiouses* cleaves target RNA through two different sites of crRNA, to resist the invasion of viruses and foreign particles [90]. The crRNA degradation process of target mRNA is also reported in *Sulfolobus solfataricus* and *Synechocystis* sp. [91,92]. This characteristic of crRNA can also be used for the drug resistance gene silencing of MDR bacteria, such as the β-lactamase gene. A crRNA consisting of 37 nucleotides complementary guide sequences and 5′-tags was designed for cleavage of β-lactamase mRNA. The cleavage product showed that β-lactamase was cleaved at 14 nucleotides downstream of the 3′ end of the engineered crRNA [93]. However, at present, all clinical trials of RNAi treatment, such as human immunodeficiency virus treatment and viral immunity, focus on transferring transgene expression to the target cells. RNAi has not received extensive attention regarding antibiotic treatment, particularly regarding the infection of MDR bacteria [94].

3.5.2. Peptide phosphodiamide morpholine oligomers inhibit the expression of related proteins in drug-resistant bacteria

The synthetic peptide phosphodiamide morpholine oligomers (PPMOs) are usually designed to inhibit the expression of specific genes in Gram-negative bacteria, such as acyl carrier protein (*acpP*), filamentous temperature-sensitive protein Z (*ftsZ*) and 30S ribosomal protein S10 (*rpsJ*) genes in *Acinetobacter baylyi* [95]. PPMOs are synthetic oligomers simulating the structure of nucleic acid and have four bases, similar to DNA, where the ends are connected with membrane penetrating peptides. Therefore, PPMOs can pass through the OM of Gram-negative bacteria [95]. For MDR, PPMOs can also target drug-resistant genes to restore the sensitivity of bacteria to antibiotics. A peptide-conjugated phosphorodiamidate morpholino oligomer (PPMO) designed by Sully et al. to inhibit NDM-1 restored the sensitivity of drug-resistant *Escherichia coli* and *Acinetobacter baumannii* to the β-lactam antibiotic meropenem and improved the survival rate of mice infected with DRB in *Escherichia coli* sepsis models [96]. The PPMO studied by Sturge et al. targeted the efflux pump gene of Gram-negative bacteria and inhibited the efflux pump activity by inhibiting the expression of *acr* in the AcrAB-TolC complex, enhancing the antibacterial effect of levofloxacin (a third-generation quinolone) and azithromycin (an advanced-generation macrolide, highly concentrated within polymorphonuclear leucocytes, which gravitate to sites of infection by chemotactic mechanisms) on clinical *Enterobacteriaceae* strains [97]. In addition, the inhibition of mobile colistin resistance gene (*mcr1*) by PPMO was able to restore the sensitivity of *Escherichia coli* to polymyxin [98]. However, a report that bacteria gradually develop resistance to PPMO is worrisome: Puckett et al. reported that a strain of *Escherichia coli* resistant to $(RFF)_3$RXB-AcpP (a PPMO, where R is arginine, F, phenylalanine, X is 6-aminohexanoic acid, B is β-alanine, and AcpP is acyl carrier protein) was isolated from *Escherichia coli* mutants, and research studies have shown that the drug resistance may be related to the peptide fragment of PPMO [99]. Nevertheless, the emergence rate of PPMOs resistance is slower than that of antibiotics, and it has great potential as an NSAS.

3.5.3. Clustered regularly interspaced short palindromic repeat/CRISPR-associated 9 (CRISPR/Cas9) cleave drug resistance genes in multidrug-resistant bacteria

As one of the landmark technologies of this century, CRISPR/cas9 was proposed for the treatment of infections caused by MDR bacteria [100–104]. Cas9 is a double stranded DNA nuclease that can programmatically cut almost any DNA sequence [105,106]. In 2014, two research groups published their studies almost at the same time, removing the drug resistance genes in MDR bacteria using CRISPR/cas9 technology to treat MDR *Staphylococcus aureus* [107] and *Escherichia coli* [108], respectively. In Bikard's et al. research, the plasmid of *Staphylococcus aureus* was destroyed using CRISPR/cas9 to target antibiotic resistance genes, immunised with non-toxic *Staphylococcus* using a plasmid, so as to prevent the transmission of drug-resistant genes carried by the plasmid and achieve good therapeutic effects on the in vivo infection models [107]. Citorik et al. used the same method to remove carbapenem-resistant Enterobacteriaceae and enterohemorrhagic *Escherichia coli* NDM-1 and SHV-18 genes to eliminate these DRB [108]. Yosef et al. adopted a more far-reaching strategy to selectively eliminate DRB by combining CRISPR/cas9 technology and lytic phase selection of antibiotic sensitive bacteria [109]. This review study has reversed the current situation of antibiotic resistance, so that existing antibiotics can still play a role. However, there are too many drug-resistance gene mutations, and the clinical value of a single drug-resistance gene mutation is limited. Kim et al. found a conserved sequence in all mutants using the CRISPR/cas9 system, which not only made MDR re-sensitive, but also reduced drug resistance to other antibiotics [110].

It is undeniable that CRISPR/cas9 has great potential for the treatment of human genetic diseases [111]. However, there are still many hurdles to be overcome in the clinical application of CRISPR/cas9, such as off-target effects, homology-directed repair efficacy, efficiency and specificity, translatability of the in vivo drug delivery methods, the immunogenicity of drug delivery carriers and cas9 peptides, and the applicability of editing cells [112]. Zohra et al. evaluated the challenges faced by the application of CRISPR/cas9 technology in MDR bacteria [113]. The advantage of the CRISPR/cas9 system is that it can target different antibiotic resistance genes at the same time, but the disadvantage is that it is impossible to determine whether the phage still has activity and the optimal dose when it reaches the intestine [113]. On the other hand, in addition to the targeting effect and low efficiency, we believe that the CRISPR/cas9 system is more likely to accelerate the formation of "superbacteria" (superbugs) in the treatment of AMR. When CRISPR/cas9 is used to remove drug-resistant genes, bacteria will also fight it by hiding target genes or mutating new drug resistance genes [7]. Fortunately, CRISPR/cas9 has not been widely used in DRB. With new antibiotics being developed, the combination of CRISPR/cas9 and antibiotics can inhibit the formation of AMR to a certain extent.

4. Conclusions and future directions

Antibiotics are among the most important tools of modern medicine, but their efficacy is threatened by the evolution of resistance [114]. However, the cost of redeveloping a new antibiotic is prohibitively high, and it takes about 10–15 years for a new drug to go from basic research to clinical use [115]. In addition, the increasingly strict control of antibiotics adds to the development cost and risk, making it impossible for many pharmaceutical companies to choose to continue to develop new antibiotics [116]. Newly developed antibiotics are often introduced into the infectious disease clinic, and AMR also soon appears [117]. DRB resistance to antibiotics can be divided into two main modes. One is classic 'selective' resistance to antibiotics, which includes inactivation of antibiotic degradation, modification of antibiotic targets and resistance to antibiotic accumulation in the cell. The other type of resistance is non-selective, the way bacteria naturally survive in response to external environmental stresses. These modes are passively activated or enhanced by external antibiotic coercion to resist antibiotic attack. To address these two modes of bacterial resistance, we summarise and provide two types of 'sensitisation' strategies: the first is to restore the original efficacy of the antibiotic, such as inhibiting antibiotic inactivating enzymes, reversing changes in antibiotic targets and altering the permeability to enhance antibiotic accumulation. The second class is to weaken the viability of DRB and indirectly increase the susceptibility of DRB to antibiotics. For instance, attacking the defence system of the DRB, metabolic stimulation to reverse the 'tolerant' state of the bacteria,

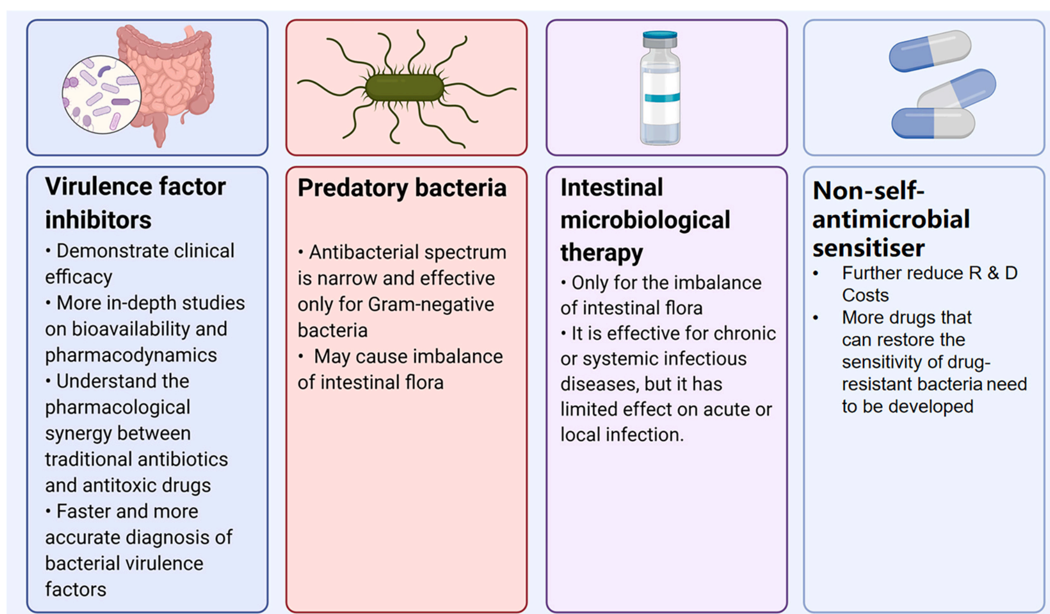

Fig. 6. Challenges of antibiotic substitution strategies. Abbreviations: R & D, antibiotic research and development.

and ROS attack to accelerate bacterial weakening. In conclusion, these strategies represent a promising and feasible direction for reversing bacterial resistance [118].

In the "post-antibiotic era", we still don't know how long the life of antibiotics will last. The unified understanding is that the era of completely relying on antibiotics is now gone. New alternative strategies, such as intestinal microbial therapy, predatory bacteria and bacterial virulence factor inhibitors, have great application potential, but it will take time to achieve large-scale clinical application in infectious diseases (Fig. 6) [119,120]. How to slowly withdraw antibiotics from the centre of the historical stage and undertake new alternative treatments is an urgent problem to be considered. NSAS can play a critical role in this process, NSAS offer the opportunity to reverse the selective dominance of AMR and the development of antibiotic resistance; they are the "gatekeepers" that prevent the development of AMR. The combined use of NSAS and antibiotics can minimise the development of bacterial resistance and buy time for the development of new antibiotics and other alternative strategies [121–123]. NSAS come from a variety of sources and have received increasing attention from researchers over the past four years. In the future, NSAS will need to be used wisely and monitored effectively. Although the development of NSAS can effectively delay the development of AMR, the rational and wise use of antibiotics can limit the selection pressure of antibiotics on DRB, and strict health measures can prevent the spread of DRB [117]. By limiting the application field of antibiotics and increasing their cost, it is possible to ensure that antibiotics are used properly and that the right patients receive the right medications at the right time [124]. Finally, we look forward to a more in-depth study of NSAS.

CRediT authorship contribution statement

Hanfei Wang: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Yingying Yang:** Writing – original draft, Visualization, Supervision, Investigation, Formal analysis, Conceptualization. **Simeng Wang:** Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Sara Badawy:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Irma Ares:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Marta Martínez:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Bernardo Lopez-Torres:** Writing – original draft, Visualization, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **María-Rosa Martínez-Larrañaga:** Writing – original draft, Visualization, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Xu Wang:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Arturo Anadón:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Investigation, Conceptualization. **María-Aránzazu Martínez:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

All authors have none conflicts to declare.

Data availability

Data will be made available on request.

Acknowledgments

This work was supported by Major Special Science and Technology Plan (202302AA310020), National Natural Science Foundation of China (NSFC) (32072925) and Project Ref. PID2020-115979RR-C33 from Ministerio de Ciencia e Innovación, Spain (Project/AEI/10.13039/501100011033).

References

[1] O. Cars, S.J. Chandy, M. Mpundu, A.Q. Peralta, A. Zorzet, A.D. So, Resetting the agenda for antibiotic resistance through a health systems perspective, Lancet Glob. Health 9 (2021) E1022–E1027, https://doi.org/10.1016/S2214-109X(21)00163-7.

[2] CDC, Antibiotic resistance threats in the United States, 2019. https://stacks.cdc.gov/view/cdc/82532, 2019 (accessed 15 June 2022).

[3] A. Cassini, L.D. Högberg, D. Plachouras, A. Quattrocchi, A. Hoxha, G.S. Simonsen, M. Colomb-Cotinat, M.E. Kretzschmar, B. Devleesschauwer, M. Cecchini, D. A. Ouakrim, T.C. Oliveira, M.J. Struelens, C. Suetens, D.L. Monnet, Burden of AMR Collaborative Group, Attributable deaths and disability-adjusted life-years

caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: A population-level modelling analysis, Lancet Infect. Dis. 19 (2019) 56–66, https://doi.org/10.1016/S1473-3099(18)30605-4.
[4] Y. Akeda, Current situation of carbapenem-resistant Enterobacteriaceae and Acinetobacter in Japan and Southeast Asia, Microbiol. Immunol. 65 (2021) 229–237, https://doi.org/10.1111/1348-0421.12887.
[5] J.H. Kwon, W.G. Powderly, The post-antibiotic era is here, Science 373 (2021) 471, https://doi.org/10.1126/science.abl5997.
[6] S.M. Schrader, J. Vaubourgeix, C. Nathan, Biology of antimicrobial resistance and approaches to combat it, Sci. Transl. Med. 12 (2020) eaaz6992, https://doi.org/10.1126/scitranslmed.aaz6992.
[7] Y. Liu, S. Ding, J. Shen, K. Zhu, Nonribosomal antibacterial peptides that target multidrug-resistant bacteria, Nat. Prod. Rep. 36 (2019) 573–592, https://doi.org/10.1039/C8NP00031J.
[8] Y. Liu, R. Li, X. Xiao, Z. Wang, Molecules that inhibit bacterial resistance enzymes, Molecules 24 (2019) 43, https://doi.org/10.3390/molecules24010043.
[9] T.M. Uddin, A.J. Chakraborty, A. Khusro, B.R.M. Zidan, S. Mitra, T.B. Emran, K. Dhama, M.K.H. Ripon, M. Gajdács, M.U.K. Sahibzada, M.J. Hossain, N. Koirala, Antibiotic resistance in microbes: history, mechanisms, therapeutic strategies and future prospects, J. Infect. Public Health 14 (2021) 1750–1766, https://doi.org/10.1016/j.jiph.2021.10.020.
[10] A. Crabbé, P.Ø. Jensen, T. Bjarnsholt, T. Coenye, Antimicrobial tolerance and metabolic adaptations in microbial biofilms, Trends Microbiol. 27 (2019) 850–863, https://doi.org/10.1016/j.tim.2019.05.003.
[11] G. Annunziato, Strategies to overcome antimicrobial resistance (AMR) making use of non-essential target inhibitors: a review, Int. J. Mol. Sci. 20 (2019) 5844, https://doi.org/10.3390/ijms20235844.
[12] H. Douafer, V. Andrieu, O. Phanstiel IV, J.M. Brunel, Correction for Douafer, et al., Antibiotic adjuvants: make antibiotics great again! J. Med. Chem. 62 (2019) 8665-8681, J. Med. Chem. 63 (2020) 1440, https://doi.org/10.1021/acs.jmedchem.9b02109.
[13] J. Bailey, L. Gallagher, W.T. Barker, V.B. Hubble, J. Gasper, C. Melander, C. Manoil, Genetic dissection of antibiotic adjuvant activity, mBio 13 (2022), https://doi.org/10.1128/mbio.03084-21 e03084–21.
[14] A. Gauba, K.M. Rahman, Evaluation of antibiotic resistance mechanisms in gram-negative bacteria, Antibiotics 12 (2023) 1590, https://doi.org/10.3390/antibiotics12111590.
[15] E. Arad, K.B. Pedersen, O. Malka, S. Mambram Kunnath, N. Golan, P. Aibinder, B. Schiøtt, H. Rapaport, M. Landau, R. Jelinek, Staphylococcus aureus functional amyloids catalyze degradation of β-lactam antibiotics, Nat. Commun. 14 (2023) 8198, https://doi.org/10.1038/s41467-023-43624-1.
[16] C. Uruén, C. García, L. Fraile, J. Tommassen, J. Arenas, How *Streptococcus suis* escapes antibiotic treatments, Vet. Res. 53 (2022) 91, https://doi.org/10.1186/s13567-022-01111-3.
[17] T. Gil-Gil, J.L. Martínez, P. Blanco, Mechanisms of antimicrobial resistance in *Stenotrophomonas maltophilia*: a review of current knowledge, Expert Rev. Anti-Infect. Ther. 18 (2020) 335–347, https://doi.org/10.1080/14787210.2020.1730178.
[18] A. Muggeo, E. Cambau, M. Amara, M. Micaëlo, B. Pangon, O. Bajolet, H. Benmansour, C. de Champs, T. Guillard, Phenotypic and genotypic quinolone resistance in *Escherichia coli* underlining GyrA83/87 mutations as a target to detect ciprofloxacin resistance, J. Antimicrob. Chemother. 75 (2020) 2466–2470, https://doi.org/10.1093/jac/dkaa189.
[19] M.M. Idrees, K. Saeed, M.A. Shahid, M. Akhtar, K. Qammar, J. Hassan, T. Khaliq, A. Saeed, Prevalence of mecA- and mecC-associated methicillin-resistant *Staphylococcus aureus* in clinical specimens, Punjab, Pakistan, Biomedicines 11 (2023) 878, https://doi.org/10.3390/biomedicines11030878.
[20] N.C. Rosas, T. Lithgow, Targeting bacterial outer-membrane remodelling to impact antimicrobial drug resistance, Trends Microbiol. 30 (2022) 544–552, https://doi.org/10.1016/j.tim.2021.11.002.
[21] A.B. Lorusso, J.A. Carrara, C.D.N. Barroso, F.F. Tuon, H. Faoro, Role of efflux pumps on antimicrobial resistance in *Pseudomonas aeruginosa*, Int. J. Mol. Sci. 23 (2022) 15779, https://doi.org/10.3390/ijms232415779.
[22] K. Shatalin, E. Shatalina, A. Mironov, E. Nudler, H_2S: a universal defense against antibiotics in bacteria, Science 334 (2011) 986–990, https://doi.org/10.1126/science.1209855.
[23] K. Shatalin, A. Nuthanakanti, A. Kaushik, D. Shishov, A. Peselis, I. Shamovsky, B. Pani, M. Lechpammer, N. Vasilyev, E. Shatalina, D. Rebatchouk, A. Mironov, P. Fedichev, A. Serganov, E. Nudler, Inhibitors of bacterial H_2S biogenesis targeting antibiotic resistance and tolerance, Science 372 (2021) 1169–1175, https://doi.org/10.1126/science.abd8377.
[24] T.L. Kinkel, S. Ramos-Montañez, J.M. Pando, D.V. Tadeo, E.N. Strom, S.J. Libby, F.C. Fang, An essential role for bacterial nitric oxide synthase in *Staphylococcus aureus* electron transfer and colonization, Nat. Microbiol. 2 (2017) 16224, https://doi.org/10.1038/nmicrobiol.2016.224.
[25] J.K. Holden, H. Li, Q. Jing, S. Kang, J. Richo, R.B. Silverman, T.L. Poulos, Structural and biological studies on bacterial nitric oxide synthase inhibitors, Proc. Natl. Acad. Sci. USA 110 (2013) 18127–18131, https://doi.org/10.1073/pnas.1314080110.
[26] A. Harms, E. Maisonneuve, K. Gerdes, Mechanisms of bacterial persistence during stress and antibiotic exposure, Science 354 (2016) aaf4268, https://doi.org/10.1126/science.aaf4268.
[27] A. Gutierrez, S. Jain, P. Bhargava, M. Hamblin, M.A. Lobritz, J.J. Collins, Understanding and sensitizing density-dependent persistence to quinolone antibiotics, Mol. Cell 68 (2017) 1147–1154, https://doi.org/10.1016/j.molcel.2017.11.012.
[28] Y. Liu, K. Yang, H. Zhang, Y. Jia, Z. Wang, Combating antibiotic tolerance through activating bacterial metabolism, Front. Microbiol. 11 (2020) 577564, https://doi.org/10.3389/fmicb.2020.577564.
[29] I. Levin-Reisman, I. Ronin, O. Gefen, I. Braniss, N. Shoresh, N.Q. Balaban, Antibiotic tolerance facilitates the evolution of resistance, Science 355 (2017) 826–830, https://doi.org/10.1126/science.aaj2191.
[30] S. Meylan, C.B.M. Porter, J.H. Yang, P. Belenky, A. Gutierrez, M.A. Lobritz, J. Park, S.H. Kim, S.M. Moskowitz, J.J. Collins, Carbon sources tune antibiotic susceptibility in *Pseudomonas aeruginosa* via tricarboxylic acid cycle control, Cell. Chem. Biol. 24 (2017) 195–206, https://doi.org/10.1016/j.chembiol.2016.12.015.
[31] T. Dörr, K. Lewis, M. Vulić, SOS response induces persistence to fluoroquinolones in *Escherichia coli*, PLoS Genet. 5 (2009) e1000760, https://doi.org/10.1371/journal.pgen.1000760.
[32] B. Peng, Y.B. Su, H. Li, Y. Han, C. Guo, Y.M. Tian, X.X. Peng, Exogenous alanine and/or glucose plus kanamycin kills antibiotic-resistant bacteria, Cell Metab. 21 (2015) 249–262, https://doi.org/10.1016/j.cmet.2015.01.008.
[33] Y. Long, L. Li, T. Xu, X. Wu, Y. Gao, J. Huang, C. He, T. Ma, L. Ma, C. Cheng, C. Zhao, Hedgehog artificial macrophage with atomic-catalytic centers to combat drug-resistant bacteria, Nat. Commun. 12 (2021) 6143, https://doi.org/10.1038/s41467-021-26456-9.
[34] F. Mo, M. Zhang, X. Duan, C. Lin, D. Sun, T. You, Recent advances in nanozymes for bacteria-infected wound therapy, Int. J. Nanomedicine 17 (2022) 5947–5990, https://doi.org/10.2147/IJN.S382796.
[35] S. Cao, Z. Zhao, Y. Zheng, Z. Wu, T. Ma, B. Zhu, C. Yang, X. Xiang, L. Ma, X. Han, Y. Wang, Q. Guo, L. Qiu, C. Cheng, A library of ROS-catalytic metalloenzyme mimics with atomic metal centers, Adv. Mater. 34 (2022) 2200255, https://doi.org/10.1002/adma.202200255.
[36] P. Jayaraman, M.K. Sakharkar, C.S. Lim, T.H. Tang, K.R. Sakharkar, Activity and interactions of antibiotic and phytochemical combinations against *Pseudomonas aeruginosa* in vitro, Int. J. Biol. Sci. 6 (2010) 556–568, https://doi.org/10.7150/ijbs.6.556.
[37] P.S. Saudagar, S.A. Survase, R.S. Singhal, Clavulanic acid: a review, Biotechnol. Adv. 26 (2008) 335–351, https://doi.org/10.1016/j.biotechadv.2008.03.002.
[38] A.M. King, S.A. Reid-Yu, W. Wang, D.T. King, G. De Pascale, N.C. Strynadka, T. R. Walsh, B.K. Coombes, G.D. Wright, Aspergillomarasmine A overcomes metallo-β-lactamase antibiotic resistance, Nature 510 (2014) 503–506, https://doi.org/10.1038/nature13445.
[39] C. Reading, M. Cole, Clavulanic acid: a beta-lactamase-inhibiting beta-lactam from *Streptomyces clavuligerus*, Antimicrob. Agents Chemother. 11 (1977) 852–857, https://doi.org/10.1128/AAC.11.5.852.
[40] M. Ayaz, F. Ullah, A. Sadiq, F. Ullah, M. Ovais, J. Ahmed, H.P. Devkota, Synergistic interactions of phytochemicals with antimicrobial agents: potential strategy to counteract drug resistance, Chem. Biol. Interact. 308 (2019) 294–303, https://doi.org/10.1016/j.cbi.2019.05.050.
[41] K.J. Meyer, J.R. Nodwell, Biology and applications of co-produced, synergistic antimicrobials from environmental bacteria, Nat. Microbiol. 6 (2021) 1118–1128, https://doi.org/10.1038/s41564-021-00952-6.
[42] V.A. López-Agudelo, D. Gómez-Ríos, H. Ramirez-Malule, Clavulanic acid production by *Streptomyces clavuligerus*: insights from systems biology, strain engineering, and downstream processing, Antibiotics 10 (2021) 84, https://doi.org/10.3390/antibiotics10010084.
[43] A. Sudano Roccaro, A.R. Blanco, F. Giuliano, D. Rusciano, V. Enea, Epigallocatechin-gallate enhances the activity of tetracycline in staphylococci by inhibiting its efflux from bacterial cells, Antimicrob. Agents Chemother. 48 (2004) 1968–1973, https://doi.org/10.1128/AAC.48.6.1968-1973.2004.
[44] B. Marquez, L. Neuville, N.J. Moreau, J.P. Genet, A.F. dos Santos, M.C. Caño de Andrade, A.E. Sant'Ana, Multidrug resistance reversal agent from *Jatropha elliptica*, Phytochemistry 66 (2005) 1804–1811, https://doi.org/10.1016/j.phytochem.2005.06.008.
[45] Y. Zhong, L. Tang, Q. Deng, L. Jing, J. Zhang, Y. Zhang, F. Yu, Y. Ou, S. Guo, B. Huang, H. Cao, P. Huang, Y. Xu, Unraveling the novel effect of patchouli alcohol against the antibiotic resistance of *Helicobacter pylori*, Front. Microbiol. 12 (2021) 674560, https://doi.org/10.3389/fmicb.2021.674560.
[46] J.H. Ha, D.U. Lee, J.T. Lee, J.S. Kim, C.S. Yong, J.A. Kim, J.S. Ha, K. Huh, 4-Hydroxybenzaldehyde from Gastrodia elata B1. Is active in the antioxidation and GABAergic neuromodulation of the rat brain, J. Ethnopharmacol. 73 (2000) 329–333, https://doi.org/10.1016/s0378-8741(00)00313-5.
[47] B. Shin, C. Park, J.A. Imlay, W. Park, 4-Hydroxybenzaldehyde sensitizes *Acinetobacter baumannii* to amphenicols, Appl. Microbiol. Biotechnol. 102 (2018) 2323–2335, https://doi.org/10.1007/s00253-018-8791-1.
[48] EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF), Scientific opinion on flavouring group evaluation 20, revision 4 (FGE.20Rev4): benzyl alcohols, benzaldehydes, a related acetal, benzoic acids, and related esters from chemical groups 23 and 30, EFSA J. 10 (2012) 2994, https://doi.org/10.2903/j.efsa.2012.2994.
[49] L. Gao, H. Wang, B. Zheng, F. Huang, Combating antibiotic resistance: current strategies for the discovery of novel antibacterial materials based on macrocycle supramolecular chemistry, Giant 7 (2021) 100066, https://doi.org/10.1016/j.giant.2021.100066.
[50] C. González-Bello, Recently developed synthetic compounds with anti-infective activity, Curr. Opin. Pharmacol. 48 (2019) 17–23, https://doi.org/10.1016/j.coph.2019.03.004.
[51] J.P. Werner, J.M. Mitchell, M.A. Taracila, R.A. Bonomo, R.A. Powers, Exploring the potential of boronic acids as inhibitors of OXA-24/40 β-lactamase, Protein Sci. 26 (2017) 515–526, https://doi.org/10.1002/pro.3100.

[52] M.W. McCarthy, T.J. Walsh, Meropenem/vaborbactam fixed combination for the treatment of patients with complicated urinary tract infections, Drugs Today 53 (2017) 521–530, https://doi.org/10.1358/dot.2017.53.10.2721815.
[53] P.A. Lang, A. Parkova, T.M. Leissing, K. Calvopiña, R. Cain, A. Krajnc, T. D. Panduwawala, J. Philippe, C.W.G. Fishwick, P. Trapencieris, M.G.P. Page, C. J. Schofield, J. Brem, Bicyclic boronates as potent inhibitors of AmpC, the class C β-lactamase from *Escherichia coli*, Biomolecules 10 (2020) 899, https://doi.org/10.3390/biom10060899.
[54] A. Krajnc, J. Brem, P. Hinchliffe, K. Calvopina, T.D. Panduwawala, P.A. Lang, J.J. A.G. Kamps, J.M. Tyrrell, E. Widlake, B.G. Saward, T.R. Walsh, J. Spencer, C. J. Schofield, Bicyclic boronate VNRX-5133 inhibits metallo- and serine- β -lactamases, J. Med. Chem. 62 (2019) 8544–8556, https://doi.org/10.1021/acs.jmedchem.9b00911.
[55] J.M. Stokes, C.R. MacNair, B. Ilyas, S. French, J.P. Côté, C. Bouwman, M.A. Farha, A.O. Sieron, C. Whitfield, B.K. Coombes, E.D. Brown, Pentamidine sensitizes gram-negative pathogens to antibiotics and overcomes acquired colistin resistance, Nat. Microbiol. 2 (2017) 17028, https://doi.org/10.1038/nmicrobiol.2017.28.
[56] C.R. MacNair, M.A. Farha, M.H. Serrano-Wu, K.K. Lee, B. Hubbard, J.P. Cote, L. A. Carfrae, M.M. Tu, J.L. Gaulin, D.K. Hunt, D.T. Hung, E.D. Brown, Preclinical development of pentamidine analogs identifies a potent and nontoxic antibiotic adjuvant, ACS Infect. Dis. 8 (2022) 768–777, https://doi.org/10.1021/acsinfecdis.1c00482.
[57] B. Yu, M. Roy Choudhury, X. Yang, S.L. Benoit, E. Womack, K. Van Mouwerik Lyles, A. Acharya, A. Kumar, C. Yang, A. Pavlova, M. Zhu, Z. Yuan, J.C. Gumbart, D.W. Boykin, R.J. Maier, Z. Eichenbaum, B. Wang, Restoring and enhancing the potency of existing antibiotics against drug-resistant gram-negative bacteria through the development of potent small-molecule adjuvants, ACS Infect. Dis. 8 (2022) 1491–1508, https://doi.org/10.1021/acsinfecdis.2c00121.
[58] K.C. Kouman, M. Keita, R. Kre N'Guessan, L.C. Owono Owono, E. Megnassan, V. Frecer, S. Miertus, Structure-based design and in silico screening of virtual combinatorial library of benzamides inhibiting 2-trans enoyl-acyl carrier protein reductase of *Mycobacterium tuberculosis* with favorable predicted pharmacokinetic profiles, Int. J. Mol. Sci. 20 (2019) 4730, https://doi.org/10.3390/ijms20194730.
[59] L.N. Alberca, M.L. Sbaraglini, J.F. Morales, R. Dietrich, M.D. Ruiz, A.M. Pino Martínez, C.G. Miranda, L. Fraccaroli, C.D. Alba Soto, C. Carrillo, P.H. Palestro, A. Talevi, Cascade ligand- and structure-based virtual screening to identify new trypanocidal compounds inhibiting putrescine uptake, Front. Cell. Infect. Microbiol. 8 (2018) 173, https://doi.org/10.3389/fcimb.2018.00173.
[60] J.D. Podoll, Y. Liu, L. Chang, S. Walls, W. Wang, X. Wang, Bio-inspired synthesis yields a tricyclic indoline that selectively resensitizes methicillin-resistant *Staphylococcus aureus* (MRSA) to β-lactam antibiotics, Proc. Natl. Acad. Sci. USA 110 (2013) 15573–15578, https://doi.org/10.1073/pnas.1310459110.
[61] M. Erdem Büyükkiraz, Z. Kesmen, Antimicrobial peptides (AMPs): a promising class of antimicrobial compounds, J. Appl. Microbiol. 132 (2022) 1573–1596, https://doi.org/10.1111/jam.15314.
[62] C. Shao, Y. Zhu, Z. Lai, P. Tan, A. Shan, Antimicrobial peptides with protease stability: progress and perspective, Future Med. Chem. 11 (2019) 2047–2050, https://doi.org/10.4155/fmc-2019-0167.
[63] M.N. Melo, R. Ferre, M.A.R.B. Castanho, Antimicrobial peptides: linking partition, activity and high membrane-bound concentrations, Nat. Rev. Microbiol. 7 (2009) 245–250, https://doi.org/10.1038/nrmicro2095.
[64] V. Lázár, A. Martins, R. Spohn, L. Daruka, G. Grézal, G. Fekete, M. Számel, P. K. Jangir, B. Kintses, B. Csörgő, Á. Nyerges, Á. Györkei, A. Kincses, A. Dér, F. R. Walter, M.A. Deli, E. Urbán, Z. Hegedűs, G. Olajos, O. Méhi, B. Bálint, I. Nagy, T.A. Martinek, B. Papp, C. Pál, Antibiotic-resistant bacteria show widespread collateral sensitivity to antimicrobial peptides, Nat. Microbiol. 3 (2018) 718–731, https://doi.org/10.1038/s41564-018-0164-0.
[65] S. Hyun, Y. Choi, D. Jo, S. Choo, T.W. Park, S.J. Park, S. Kim, S. Lee, S. Park, S. M. Jin, D.H. Cheon, W. Yoo, R. Arya, Y.P. Chong, K.K. Kim, Y.S. Kim, Y. Lee, J. Yu, Proline hinged amphipathic α-helical peptide sensitizes gram-negative bacteria to various gram-positive antibiotics, J. Med. Chem. 63 (2020) 14937–14950, https://doi.org/10.1021/acs.jmedchem.0c01506.
[66] R.J. Melander, A.E. Mattingly, A.M. Nemeth, C. Melander, Overcoming intrinsic resistance in gram-negative bacteria using small molecule adjuvants, Bioorg. Med. Chem. Lett. 80 (2023) 129113, https://doi.org/10.1016/j.bmcl.2022.129113.
[67] M. Vaara, J. Fox, G. Loidl, O. Siikanen, J. Apajalahti, F. Hansen, N. Frimodt-Møller, J. Nagai, M. Takano, T. Vaara, Novel polymyxin derivatives carrying only three positive charges are effective antibacterial agents, Antimicrob. Agents Chemother. 52 (2008) 3229–3236, https://doi.org/10.1128/AAC.00405-08.
[68] D.V. Zurawski, A.A. Reinhart, Y.A. Alamneh, M.J. Pucci, Y. Si, R. Abu-Taleb, J. P. Shearer, S.T. Demons, S.D. Tyner, T. Lister, SPR741, an antibiotic adjuvant, potentiates the in vitro and in vivo activity of rifampin against clinically relevant extensively drug-resistant *Acinetobacter baumannii*, Antimicrob. Agents Chemother. 61 (2017), https://doi.org/10.1128/AAC.01239-17 e01239–17.
[69] M. Vaara, O. Siikanen, J. Apajalahti, J. Fox, N. Frimodt-Møller, H. He, A. Poudyal, J. Li, R.L. Nation, T. Vaara, A novel polymyxin derivative that lacks the fatty acid tail and carries only three positive charges has strong synergism with agents excluded by the intact outer membrane, Antimicrob. Agents Chemother. 54 (2010) 3341–3346, https://doi.org/10.1128/AAC.01439-09.
[70] S. French, M. Farha, M.J. Ellis, Z. Sameer, J.P. Côté, N. Cotroneo, T. Lister, A. Rubio, E.D. Brown, Potentiation of antibiotics against gram-negative bacteria by polymyxin B analogue SPR741 from unique perturbation of the outer membrane, ACS Infect. Dis. 6 (2020) 1405–1412, https://doi.org/10.1021/acsinfecdis.9b00159.
[71] N.K. Prasad, I.B. Seiple, R.T. Cirz, O.S. Rosenberg, Leaks in the pipeline: a failure analysis of gram-negative antibiotic development from 2010 to 2020, Antimicrob. Agents Chemother. 66 (2022), https://doi.org/10.1128/aac.00054-22 e00054–22.
[72] S.H. Moon, X. Zhang, G. Zheng, D.G. Meeker, M.S. Smeltzer, E. Huang, Novel linear lipopeptide paenipeptins with potential for eradicating biofilms and sensitizing gram-negative bacteria to rifampicin and clarithromycin, J. Med. Chem. 60 (2017) 9630–9640, https://doi.org/10.1021/acs.jmedchem.7b01064.
[73] E.H. Mood, L. Goltermann, C. Brolin, L.M. Cavaco, A.J. Nejad, N. Yavari, N. Frederiksen, H. Franzyk, P.E. Nielsen, Antibiotic potentiation in multidrug-resistant gram-negative pathogenic bacteria by a synthetic peptidomimetic, ACS Infect. Dis. 7 (2021) 2152–2163, https://doi.org/10.1021/acsinfecdis.1c00147.
[74] D.I. Andersson, D. Hughes, J.Z. Kubicek-Sutherland, Mechanisms and consequences of bacterial resistance to antimicrobial peptides, Drug Resist. Updat. 26 (2016) 43–57, https://doi.org/10.1016/j.drup.2016.04.002.
[75] M. Magana, M. Pushpanathan, A.L. Santos, L. Leanse, M. Fernandez, A. Ioannidis, M.A. Giulianotti, Y. Apidianakis, S. Bradfute, A.L. Ferguson, A. Cherkasov, M. N. Seleem, C. Pinilla, C. de la Fuente-Nunez, T. Lazaridis, T. Dai, R.A. Houghten, R.E.W. Hancock, G.P. Tegos, The value of antimicrobial peptides in the age of resistance, Lancet Infect. Dis. 20 (2020) e216–e230, https://doi.org/10.1016/S1473-3099(20)30327-3.
[76] S. Gera, E. Kankuri, K. Kogermann, Antimicrobial peptides - unleashing their therapeutic potential using nanotechnology, Pharmacol. Ther. 232 (2022) 107990, https://doi.org/10.1016/j.pharmthera.2021.107990.
[77] Y. Han, M. Zhang, R. Lai, Z. Zhang, Chemical modifications to increase the therapeutic potential of antimicrobial peptides, Peptides 146 (2021) 170666, https://doi.org/10.1016/j.peptides.2021.170666.
[78] L. Chen, T. Shen, Y. Liu, J. Zhou, S. Shi, Y. Wang, Z. Zhao, Z. Yan, C. Liao, C. Wang, Enhancing the antibacterial activity of antimicrobial peptide PMAP-37 (F34-R) by cholesterol modification, BMC Vet. Res. 16 (2020) 419, https://doi.org/10.1186/s12917-020-02630-x.
[79] R. Cebrián, C. Xu, Y. Xia, W. Wu, O.P. Kuipers, The cathelicidin-derived close-to-nature peptide D-11 sensitises *Klebsiella pneumoniae* to a range of antibiotics in vitro, ex vivo and in vivo, Int. J. Antimicrob. Agents 58 (2021) 106434, https://doi.org/10.1016/j.ijantimicag.2021.106434.
[80] J.L. da Silva, S. Gupta, K.N. Olivier, A.M. Zelazny, Antimicrobial peptides against drug resistant *Mycobacterium abscessus*, Res. Microbiol. 171 (2020) 211–214, https://doi.org/10.1016/j.resmic.2020.03.001.
[81] K.R. Baker, B. Jana, A.M. Hansen, K.J. Vissing, H.M. Nielsen, H. Franzyk, L. Guardabassi, Repurposing azithromycin and rifampicin against gram-negative pathogens by combination with peptide potentiators, Int. J. Antimicrob. Agents 53 (2019) 868–872, https://doi.org/10.1016/j.ijantimicag.2018.10.025.
[82] C.S. Vinagreiro, R. Lopes, B. Royo, G.J. Da Silva, M.M. Pereira, Synthesis of iron (II)-N-heterocyclic carbene complexes: paving the way for a new class of antibiotics, Molecules 25 (2020) 2917, https://doi.org/10.3390/molecules25122917.
[83] A. Evans, K.A. Kavanagh, Evaluation of metal-based antimicrobial compounds for the treatment of bacterial pathogens, J. Med. Microbiol. 70 (2021) 001363, https://doi.org/10.1099/jmm.0.001363.
[84] Q. Ye, W. Chen, H. Huang, Y. Tang, W. Wang, F. Meng, H. Wang, Y. Zheng, Iron and zinc ions, potent weapons against multidrug-resistant bacteria, Appl. Microbiol. Biotechnol. 104 (2020) 5213–5227, https://doi.org/10.1007/s00253-020-10600-4.
[85] M. Ciabocco, P. Cancemi, M.L. Saladino, E. Caponetti, R. Alduina, M. Berrettoni, Synthesis and antibacterial activity of iron-hexacyanocobaltate nanoparticles, J. Biol. Inorg. Chem. 23 (2018) 385–398, https://doi.org/10.1007/s00775-018-1544-x.
[86] M.J. Miller, R. Liu, Design and syntheses of new antibiotics inspired by nature's quest for iron in an oxidative climate, Acc. Chem. Res. 54 (2021) 1646–1661, https://doi.org/10.1021/acs.accounts.1c00004.
[87] K.Y. Djoko, M.E.S. Achard, M.D. Phan, A.W. Lo, M. Miraula, S. Prombhul, S. J. Hancock, K.M. Peters, H.E. Sidjabat, P.N. Harris, N. Mitić, T.R. Walsh, G. J. Anderson, W.M. Shafer, D.L. Paterson, G. Schenk, A.G. McEwan, M. A. Schembri, Copper ions and coordination complexes as novel carbapenem adjuvants, Antimicrob. Agents Chemother. 62 (2018), https://doi.org/10.1128/AAC.02280-17 e02280–17.
[88] J.P. Hegarty, D.B. Stewart Sr., Advances in therapeutic bacterial antisense biotechnology, Appl. Microbiol. Biotechnol. 102 (2018) 1055–1065, https://doi.org/10.1007/s00253-017-8671-0.
[89] G.J. Hannon, RNA interference, Nature 418 (2002) 244–251, https://doi.org/10.1038/418244a.
[90] C.R. Hale, P. Zhao, S. Olson, M.O. Duff, B.R. Graveley, L. Wells, R.M. Terns, M. P. Terns, RNA-guided RNA cleavage by a CRISPR RNA-cas protein complex, Cell 139 (2009) 945–956, https://doi.org/10.1016/j.cell.2009.07.040.
[91] Z. Zebec, A. Manica, J. Zhang, M.F. White, C. Schleper, CRISPR-mediated targeted mRNA degradation in the archaeon *Sulfolobus solfataricus*, Nucleic Acids Res. 42 (2014) 5280–5288, https://doi.org/10.1093/nar/gku161.
[92] K. Abe, Y. Sakai, S. Nakashima, M. Araki, W. Yoshida, K. Sode, K. Ikebukuro, Design of riboregulators for control of cyanobacterial (Synechocystis) protein expression, Biotechnol. Lett. 36 (2014) 287–294, https://doi.org/10.1007/s10529-013-1352-x.
[93] C.R. Hale, S. Majumdar, J. Elmore, N. Pfister, M. Compton, S. Olson, A.M. Resch, C.V.C. Glover III, B.R. Graveley, R.M. Terns, M.P. Terns, Essential features and rational design of CRISPR RNAs that function with the Cas RAMP module

complex to cleave RNAs, Mol. Cell 45 (2012) 292–302, https://doi.org/10.1016/j.molcel.2011.10.023.
[94] J.A. Edson, Y.J. Kwon, RNAi for silencing drug resistance in microbes toward development of nanoantibiotics, J. Control. Release 189 (2014) 150–157, https://doi.org/10.1016/j.jconrel.2014.06.054.
[95] B.L. Geller, K. Marshall-Batty, F.J. Schnell, M.M. McKnight, P.L. Iversen, D. E. Greenberg, Gene-silencing antisense oligomers inhibit Acinetobacter growth in vitro and in vivo, J. Infect. Dis. 208 (2013) 1553–1560, https://doi.org/10.1093/infdis/jit460.
[96] E.K. Sully, B.L. Geller, L. Li, C.M. Moody, S.M. Bailey, A.L. Moore, M. Wong, P. Nordmann, S.M. Daly, C.R. Sturge, D.E. Greenberg, Peptide-conjugated phosphorodiamidate morpholino oligomer (PPMO) restores carbapenem susceptibility to NDM-1-positive pathogens in vitro and in vivo, J. Antimicrob. Chemother. 72 (2017) 782–790, https://doi.org/10.1093/jac/dkw476.
[97] C.R. Sturge, C.F. Felder-Scott, R. Pifer, C. Pybus, R. Jain, B.L. Geller, D. E. Greenberg, AcrAB–TolC inhibition by peptide-conjugated phosphorodiamidate morpholino oligomers restores antibiotic activity in vitro and in vivo, ACS Infect. Dis. 5 (2019) 1446–1455, https://doi.org/10.1021/acsinfecdis.9b00123.
[98] S.M. Daly, C.R. Sturge, C.F. Felder-Scott, B.L. Geller, D.E. Greenberg, MCR-1 inhibition with peptide-conjugated phosphorodiamidate morpholino oligomers restores sensitivity to polymyxin in *Escherichia coli*, mBio 8 (2017) e01315–e01317, https://doi.org/10.1128/mBio.01315-17.
[99] S.E. Puckett, K.A. Reese, G.M. Mitev, V. Mullen, R.C. Johnson, K.R. Pomraning, B. L. Mellbye, L.D. Tilley, P.L. Iversen, M. Freitag, B.L. Geller, Bacterial resistance to antisense peptide phosphorodiamidate morpholino oligomers, Antimicrob. Agents Chemother. 56 (2012) 6147–6153, https://doi.org/10.1128/AAC.00850-12.
[100] M. Alves-Bezerra, N. Furey, C.G. Johnson, K.D. Bissig, Using CRISPR/Cas9 to model human liver disease, JHEP Rep. 1 (2019) 392–402, https://doi.org/10.1016/j.jhepr.2019.09.002.
[101] L. Cai, A.L. Fisher, H. Huang, Z. Xie, CRISPR-mediated genome editing and human diseases, Genes Dis. 3 (2016) 244–251, https://doi.org/10.1016/j.gendis.2016.07.003.
[102] M. Goren, I. Yosef, U. Qimron, Sensitizing pathogens to antibiotics using the CRISPR-cas system, Drug Resist. Updat. 30 (2017) 1–6, https://doi.org/10.1016/j.drup.2016.11.001.
[103] S. Tao, H. Chen, N. Li, W. Liang, The application of the CRISPR-Cas system in antibiotic resistance, Infect. Drug Resist. 15 (2022) 4155–4168, https://doi.org/10.2147/IDR.S370869.
[104] M.U. Javed, M.T. Hayat, H. Mukhtar, K. Imre, CRISPR-Cas9 system: a prospective pathway toward combatting antibiotic resistance, Antibiotics 12 (2023) 1075, https://doi.org/10.3390/antibiotics12061075.
[105] J. van der Oost, E.R. Westra, R.N. Jackson, B. Wiedenheft, Unravelling the structural and mechanistic basis of CRISPR-cas systems, Nat. Rev. Microbiol. 12 (2014) 479–492, https://doi.org/10.1038/nrmicro3279.
[106] M. Behr, J. Zhou, B. Xu, H. Zhang, In vivo delivery of CRISPR-cas9 therapeutics: progress and challenges, Acta Pharm. Sin. B 11 (2021) 2150–2171, https://doi.org/10.1016/j.apsb.2021.05.020.
[107] D. Bikard, C.W. Euler, W. Jiang, P.M. Nussenzweig, G.W. Goldberg, X. Duportet, V.A. Fischetti, L.A. Marraffini, Exploiting CRISPR-cas nucleases to produce sequence-specific antimicrobials, Nat. Biotechnol. 32 (2014) 1146–1150, https://doi.org/10.1038/nbt.3043.
[108] R.J. Citorik, M. Mimee, T.K. Lu, Sequence-specific antimicrobials using efficiently delivered RNA-guided nucleases, Nat. Biotechnol. 32 (2014) 1141–1145, https://doi.org/10.1038/nbt.3011.
[109] I. Yosef, M. Manor, R. Kiro, U. Qimron, Temperate and lytic bacteriophages programmed to sensitize and kill antibiotic-resistant bacteria, Proc. Natl. Acad. Sci. USA 112 (2015) 7267–7272, https://doi.org/10.1073/pnas.1500107112.
[110] J.S. Kim, D.H. Cho, M. Park, W.J. Chung, D. Shin, K.S. Ko, D.H. Kweon, CRISPR/cas9-mediated re-sensitization of antibiotic-resistant *Escherichia coli* harboring extended-spectrum β-lactamases, J. Microbiol. Biotechnol. 26 (2016) 394–401, https://doi.org/10.4014/jmb.1508.08080.
[111] G. Sharma, A.R. Sharma, M. Bhattacharya, S.S. Lee, C. Chakraborty, CRISPR-cas9: a preclinical and clinical perspective for the treatment of human diseases, Mol. Ther. 29 (2021) 571–586, https://doi.org/10.1016/j.ymthe.2020.09.028.
[112] W.J. Dai, L.Y. Zhu, Z.Y. Yan, Y. Xu, Q.L. Wang, X.J. Lu, CRISPR-cas9 for in vivo gene therapy: promise and hurdles, Mol. Ther. Nucleic Acids 5 (2016) e349, https://doi.org/10.1038/mtna.2016.58.
[113] T. Zohra, M. Numan, A. Ikram, M. Salman, T. Khan, M. Din, M. Salman, A. Farooq, A. Amir, M. Ali, Cracking the challenge of antimicrobial drug resistance with CRISPR/cas9, nanotechnology and other strategies in ESKAPE pathogens, Microorganisms 9 (2021) 954, https://doi.org/10.3390/microorganisms9050954.
[114] M. Baym, L.K. Stone, R. Kishony, Multidrug evolutionary strategies to reverse antibiotic resistance, Science 351 (2016) aad3292, https://doi.org/10.1126/science.aad3292.
[115] D. Altarac, M. Gutch, J. Mueller, M. Ronsheim, R. Tommasi, M. Perros, Challenges and opportunities in the discovery, development, and commercialization of pathogen-targeted antibiotics, Drug Discov. Today 26 (2021) 2084–2089, https://doi.org/10.1016/j.drudis.2021.02.014.
[116] E. Power, Impact of antibiotic restrictions: the pharmaceutical perspective, Clin. Microbiol. Infect. 12 (2006) 25–34, https://doi.org/10.1111/j.1469-0691.2006.01528.x.
[117] L.D. Högberg, A. Heddini, O. Cars, The global need for effective antibiotics: challenges and recent advances, Trends Pharmacol. Sci. 31 (2010) 509–515, https://doi.org/10.1016/j.tips.2010.08.002.
[118] I. Djinni, W. Djoudi, S. Souagui, F. Rabia, S. Rahmouni, I. Mancini, M. Kecha, *Streptomyces thermoviolaceus* SRC3 strain as a novel source of the antibiotic adjuvant streptazolin: a statistical approach toward the optimized production, J. Microbiol. Methods 148 (2018) 161–168, https://doi.org/10.1016/j.mimet.2018.04.008.
[119] G.S. Canfield, A. Chatterjee, J. Espinosa, M.R. Mangalea, E.K. Sheriff, M. Keidan, S.W. McBride, B.D. McCollister, H.C. Hang, B.A. Duerkop, Lytic bacteriophages facilitate antibiotic sensitization of *Enterococcus faecium*, Antimicrob. Agents Chemother. 65 (2021), https://doi.org/10.1128/AAC.00143-21 e00143–21.
[120] C. Ghosh, P. Sarkar, R. Issa, J. Haldar, Alternatives to conventional antibiotics in the era of antimicrobial resistance, Trends Microbiol. 27 (2019) 323–338, https://doi.org/10.1016/j.tim.2018.12.010.
[121] G. Cox, A. Sieron, A.M. King, G. De Pascale, A.C. Pawlowski, K. Koteva, G. D. Wright, A common platform for antibiotic dereplication and adjuvant discovery, Cell Chem. Biol. 24 (2017) 98–109, https://doi.org/10.1016/j.chembiol.2016.11.011.
[122] C. González-Bello, Antibiotic adjuvants - a strategy to unlock bacterial resistance to antibiotics, Bioorg. Med. Chem. Lett. 27 (2017) 4221–4228, https://doi.org/10.1016/j.bmcl.2017.08.027.
[123] H. Huang, W. Geng, X. Wu, Y. Zhang, L. Xie, T. Ma, C. Cheng, Spiky artificial peroxidases with V−O−Fe pair sites for combating antibiotic-resistant pathogens, Angew. Chem. Int. Ed. Eng. 63 (2024) e202310811, https://doi.org/10.1002/anie.202310811.
[124] M.R. McKellar, A.M. Fendrick, Innovation of novel antibiotics: an economic perspective, Clin. Infect. Dis. 59 (2014) S104–S107, https://doi.org/10.1093/cid/ciu530.