Antibiotic Resistant Bacteria: Current Situation and Treatment Options To Accelerate The Development of a new antimicrobial arsenal

Journal:	*Expert Review of Anti-infective Therapy*
Manuscript ID	ERI-2022-ST-0012.R1
Manuscript Type:	Review
Keywords:	Antibiotic resistance, Antivirulent therapy, Drug repurposing, Mathematical prediction model, Microbiota therapy, POT, Phagotherapy

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Abstract

Introduction

Antibiotic resistance is one of the biggest public health threats worldwide. Currently, antibiotic-resistant bacteria kill 700,000 people every year. These data represent the near future in which we find ourselves, a "post-antibiotic era" where the identification and development of new treatments are key. This review is focused on the current and emerging antimicrobial therapies which can solve this global threat.

Areas covered

Through a literature search using databases such as Medline and Web of Science, and search engines such as Google Scholar, different antimicrobial therapies were analyzed, including pathogen-oriented therapy, phagotherapy, microbiota and antivirulent therapy. Additionally, the development pathways of new antibiotics were described, emphasizing on the potential advantages that the combination of a drug repurposing strategy with the application of mathematical prediction models could bring to solve the problem of AMRs.

Expert Opinion

This review offers several starting points to solve a single problem: reducing the number of AMR. The data suggest that the strategies described could provide many benefits to improve antimicrobial treatments. However, the development of new antimicrobials remains necessary. Drug repurposing, with the application of mathematical prediction models, is considered to be of interest due to its rapid and effective potential to increase the current therapeutic arsenal.

Keywords

Antibiotic resistance, antivirulent therapy, drug repurposing, mathematical prediction model, microbiota therapy, POT, phagotherapy.

Article Highlights Box

- The present and near future in which we find ourselves is a "post-antibiotic era", where the therapeutic arsenal we have is unable to combat the bacterial infections that are emerging
- Different techniques are being studied to act as adjuvants of traditional antibiotic treatments by prolonging the efficacy of current antibiotics and providing more time to discover new drugs and new effective alternatives to AMRs.
- Alternative treatments can provide many benefits to improve infection treatments but the development of new antimicrobials remains necessary.
- As for the development of new molecules, the costs, the low success rate and long periods of time make it impossible to obtain an effective therapeutic arsenal against current AMRs.
- Drug repurposing could contribute to the cost-effective search of new antibiotics in a faster and cost-effective way compared to traditional methods.

Plain Language Summary

What is the context?

- Antibiotic resistance is currently one of the biggest public health threats worldwide.
- Many of the available antibiotics are useless against drug resistant bacteria.
- The present and near future in which we find ourselves is a "post-antibiotic era", where the therapeutic arsenal we have is unable to combat the bacterial infections that are emerging

What is new?

- We accessed published studies to explore different techniques that are available to improve current treatment options. Currently, these strategies cannot replace antibiotic therapy. The existing knowledge leaves all these alternatives as adjuvant treatments to antibiotics. Therefore, research into new antibiotics remains important.
- This review exposes that drug repurposing could contribute to the cost-effective search of new antibiotics in a faster and cost-effective way compared to traditional methods.

What is the impact?

- The review emphasizes the urgency of identifying new targets that can aid in the development of new therapies.
- Drug repurposing can greatly shorten the time and cost of development of new antibiotics. This strategy adds to the value of certain commercialized molecules, recovering part of the investment. In addition, it provides greater knowledge about other therapies and about the mechanisms by which bacteria develop resistance.

1. Introduction

Antibiotic resistance is currently one of the biggest public health threats globally [1, 2]. The emergence of antimicrobial-resistant bacteria implies an increase in mortality and morbidity rates, as well as an increase in hospital admissions and their duration, causing a higher likelihood of nosocomial infections [1, 3]. Currently, antibiotic-resistant bacteria kill 700,000 people every year worldwide, and the annual number of deaths caused by antimicrobial resistance (AMR) is expected to be 10 million by 2050, surpassing diabetes, cancer, cardiovascular disease and traffic accidents, and assuming a cost of around 100 billion dollars worldwide [4, 5]. Therefore, this problem does not only affects the health sector, but is also associated with an increase in costs in terms of economic losses, presenting a significant impact on the world economy [1].

In 2017, the World Health Organization (WHO) published a list of pathogens of global priority and classifying these microorganisms as critical, high and medium priority bacteria depending on the urgency there is to search for new antibiotics against them [Table 1] [6, 7].

Table 1. WHO list of priority pathogens for the research for new antibiotics [6]

Priority 1: CRITICAL	Priority 2: HIGH	Priority 3: Medium
Acinetobacter baumannii, carbapenem-resistant. *Pseudomonas aeruginosa*, carbapenem-resistant. *Mycobacterium tuberculosis,* β-lactams resistant, macrolides resistant, aminoglycosides resistant, anphenicols resistant, quinolones resistant, tetracyclines resistant, isoniazid resistant, etambutol resistant, pyrazinamide resistant and rifampicin resistant. *Enterobacterales (Klebsiella pneumoniae, Escherichia coli, Enterobacter spp., Serratia spp., Proteus spp., Providencia spp, and Morganella spp.)*, carbapenem-resistant, 3rd generation cephalosporin-resistant and broad spectrum β-lactams resistant.	*Enterococcus faecium*, vancomycin resistant. *Staphylococcus aureus*, methicillin resistant, intermediate vancomycin susceptibility and vancomycin resistant. *Helicobacter pylori*, clarithromycin resistant. *Campylobacter spp.*, fluoroquinolones resistant. *Salmonella spp.*, fluoroquinolones resistant. *Neisseria gonorrhoeae*, cephalosporin resistant and fluoroquinolones resistant.	*Streptococcus pneumoniae*, penicillin resistant. *Haemophilus influenzae*, ampicillin resistant. *Shigella spp.*, fluoroquinolones resistant.

A group of bacterial species known as ESKAPE pathogens are responsible for most life-threatening nosocomial infections and can "escape" the bactericide action of antimicrobial agents through various resistance mechanisms [Figure 1] [1,8].

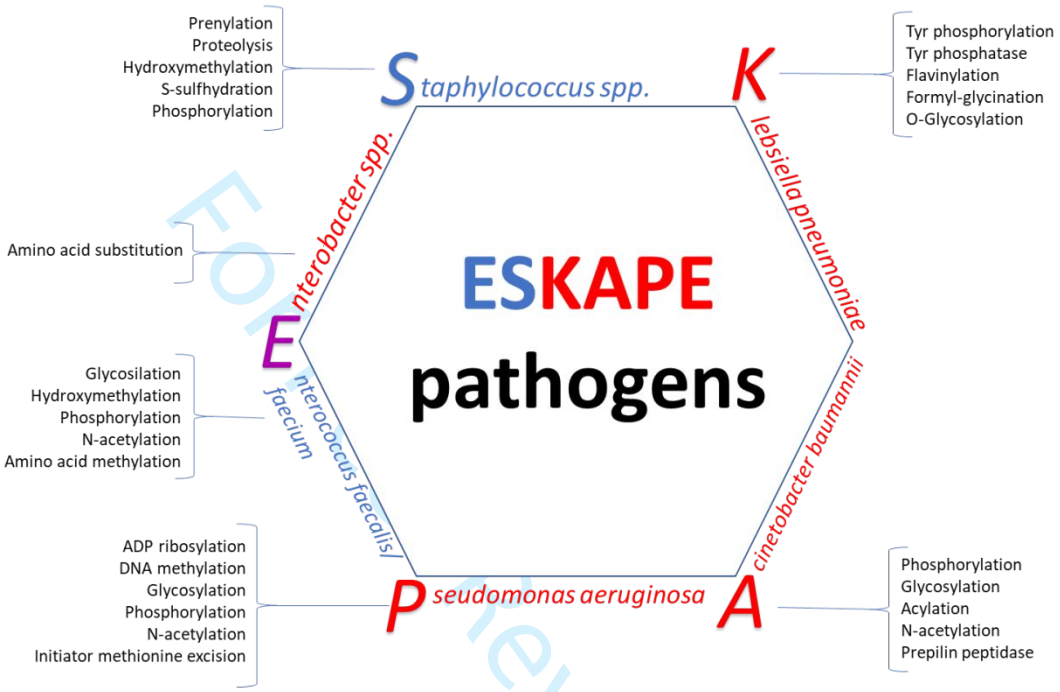


Figure 1. Diagram showing ESKAPE bacteria and their resistance mechanisms by which they inhibit the effects of antimicrobials [8].

One of the main pathogens producing nosocomial infections is the gram-negative coccobacillus *Acinetobacter baumannii,* classified in the WHO list of critical priority for finding new antibiotics. It has been estimated that infection rates for multidrug-resistant (MDR) strains of *A. baumannii* range from 47% in regions such as North America, to 93% in Europe and the Middle East [9]. This multiresistance capacity is due to a variety of well-documented mechanisms such as the production of chromosomal β-lactamase and intrinsic oxacillinase, in addition to the loss of expression of some porins and the overexpression of various active expulsion systems [9-11]. *A. baumannii* presents a set of enzymes involved in the process of protein phosphorylation to form the biofilm matrix [8]. Glycosylation mediated by glycosyltransferases and oligosaccharyltransferases, together with N-acylation also involved in biofilm synthesis, in adittion to having an important role in virulence, metabolic stress and antibiotic resistance Similarly, acylation, phosphorylation and glycosylation of lipid A confer

A. baumannii resistance to colistin due to modifications in the structure of lipopolysaccharides (LPS). Another mechanism of interest in the resistance is the specific prepilin peptidase that participates in the modifications of the pyline subunit, the main constituent of the fibers of the pili. The combination of these mechanisms provides *A. baumannii* with resistant properties, having observed strains with extreme resistance (resistance to 2 groups of antibiotics), multiresistance (resistance to 3 or more families of antibiotics) and even panresistance (resistance to all available antimicrobials) in different studies [6, 8, 11].

Pseudomonas aeruginosa is also listed as a critical priority because it is, along with *A. baumannii,* the most common human pathogen in nosocomial infections [6, 7, 12]. It is one of the main causes of disease and mortality in humans, causing acute infections and even prolonged infections in cases of patients with immunosuppressive or chronic diseases (such as cystic fibrosis, AIDS, burns, injuries and cancer) [11-13]. The natural resistance spectrum of *P. aeruginosa* encompasses most penicillins, first, second, and most third generation cephalosporins, tetracyclines, cotrimoxazole, rifampicin , carbapenems, aminoglycosides, and quinolones have also been observed [12, 14, 15]. This wide resistance capacity is due to the low permeability of the outer membrane, the existence of several excretion systems and the presence of different beta-lactamases and carbapenemases [11]. In addition, metabolic mechanisms such as ADP-ribosylation, DNA-methylation and glycosylation of target proteins, together with phosphorylation, N-acetylation and the initiator of methionine cleavage into host proteins, caused by various toxins and enzymes, improve its resistance and virulence [8, 15]. All the mechanisms that *P. aeruginosa* presents to resist most of the antibiotics available result in serious limitations in the therapeutic options against this agent.

Staphylococcus aureus is a human pathogen associated with high rates of infection and mortality, and is one of the leading causes of minor, nosocomial, and food poisoning infections, as well as serious health problems such as toxic shock syndrome, endocarditis, and osteomyelitis [2, 16-18]. This microorganism is classified as high priority due to its extraordinary ability to acquire resistance to many antibiotics, finding methicillin-resistant (MRSA), vancomycin-resistant (VRSA) strains as well as other antimicrobial agents of last resort such as linezolid and daptomycin [2, 16, 19]. Additionally, *S. aureus* may exhibit transient resistance to antibiotics by entering a latent state of non-division known as

persistent bacteria [2,4]. Persistent bacteria show high resistance to most current antibiotics. This is because they target proteins that participate in the biosynthetic processes of the pathogen, which are minimized or inactive in the latent state, preventing the effect of these drugs [2]. Other mechanisms that increase its resistant properties are those that participate in a post-translational way in the organism, such as proteolysis and prenylation. The latter consists of the addition of a sequence of amino acids in proteins and molecules, such as antibiotics, by means of a prenyl group, modifying its structure. Studies suggest that strains of *S. aureus* capable of prenylation have advantages in colonization, as well as improved resistance to antibiotics targeting the cell wall, antimicrobial peptides (AMP) and aminoglycosides [8]. Hydroxymethylation, S-sulfhydration and phosphorylation of certain surface proteins provide this agent with an increase in its virulence, in addition to preventing infection by phages and inhibition of the kinase activity produced by certain antibiotics, resisting the cell death that this would cause.

Streptococcus pneumoniae is a gram-positive bacteria classified as a medium priority in the search for new treatments for its ability to colonize the upper respiratory tract and cause infections such as meningitis, sinusitis, bronchitis, and pneumonia, among others. There are strains resistant to different antibiotics such as penicillin, erythromycin, tetracycline and/or trimethoprim-sulfamethoxazole, and resistance to tetracyclines, chloramphenicol and fluoroquinolones has been reported recently [16, 20]. Their resistance is due to changes in the target proteins of antibiotics, such as changes in the penicillin binding domains of transpeptidase, or by efflux pumps, such as the expulsion of fluoroquinolones mediated by the PmrA pump [20]. These modifications occur by chromosomal mutations or by the acquisition of genome from other bacteria by horizontal transfer. In addition, *S. pneumoniae* is also able to make post-translational changes in antibiotic target proteins. Processes such as phosphorylation and glycosylation improve the ability of this pathogen to generate biofilms, and consequently resist antibiotic treatment [21].

Mycobacterium tuberculosis is a long-established threat. Tuberculosis (TB) remains a serious public health issue worldwide, causing millions of deaths every year and an estimated one-third of the world's population is latently infected [22]. WHO has launched the 'End TB' strategy, which aims to reduce TB deaths by 95% and reduce new cases by 90% between 2015 and 2035. Although there has been a decline in incidence and mortality rates globally, the

current rate of said reduction is insufficient to meet the 2035 targets mainly due to several challenges, such as the increase in multiresistant TB and the extensive drug resistance [23]. Genetic resistance to drugs presented by *M. tuberculosis* is acquired mainly by spontaneous mutations in chromosomal genes that cause modification or overproduction of the drug target, providing the ability to inactivate drugs or decrease drug activation. This intrinsic resistance is the result of the interaction between the cell wall, which limits drug absorption, and the activity of efflux pumps, which transport a variety of substrates from the inside to the outside of the cell [23].

All these pathogens are proof of the present and near future in which we find ourselves, a "post-antibiotic era" where the therapeutic arsenal we have is unable to alleviate the bacterial infections that are emerging, and as time goes by, its effectiveness is declining. In order to avoid this situation, WHO promotes different prevention and control measures to stop the appearance of these resistances [6]. One such measure is to encourage the development of antimicrobial therapies that can combat current AMR. Throughout this review we will focus on analyzing current and emerging antimicrobial therapies and the development pathways of new existing antibiotics, emphasizing the drug repositioning strategy and the potential advantages it can bring to the AMR problem.

2. Search for solutions to the problem of AMR: new therapies and innovation in the search for new antibiotics

This review was carried out by performing a literature search using the databases PubMed and Web of Sciences along with Google Scholar. Initially, the search was carried out using the 2015-2022 time range. However, older references have been included for definition purposes where deemed necessary. Search terms such as “antimicrobial therapy”, “antivirulent therapy”, “alternative antimicrobial”, “phagotherapy”, “antimicrobial development” and “drug repurposing” were used. Both, reviews and original research papers were consulted, as well as different official web pages such as that of WHO.

2.1 Alternative therapies to antibiotics

Different techniques are currently being explored and developed to improve the effectiveness of the current therapeutic arsenal, such as the combination therapy that will be discussed

later on. However, these techniques do not prevent the loss of effectiveness due to the existence or emergence of resistance to current antibiotic families [2, 24-26]. As our available set of antimicrobial treatments becomes less effective, it is vital to identify new targets that can aid in the development of new treatments and strategies.

That is why one of the most interesting initiatives in recent years is the search for new alternative therapies to antibiotics. All these alternatives act as adjuvants of traditional antibiotic treatments. This does not only prolong the efficacy of current antibiotics, but also provides more time to discover new drugs and new effective alternatives to AMRs.

2.1.1 Pathogen-oriented therapy. Monoclonal antibodies, antimicrobial peptides, nanoparticles and combination therapy

Pathogen-Oriented Therapy (POT) consists of a set of strategies whose purpose is the application of antibacterial compounds or materials directly in infected regions to treat species or strains of pathogenic bacteria in a specific way. This specificity improves the efficacy of the drug while reducing its concentration, and therefore factors such as non-targeting, toxicity and the development of resistance are reduced [4]. Within this therapy we can find strategies such as the application of monoclonal antibodies (mcA), antimicrobial peptides (AMP), nanoparticles (NP) and combination therapy.

Before the discovery of antibiotics, the most widely used way to treat infectious diseases was serum therapy, which was based on the obtention of antibodies from the plasma of patients recovered from a specific infection to be administered to infected patients. With the development of antibiotics, this therapy became less important in the treatment of infections, because antibiotics have lower production costs [27]. However, the progressive emergence of antibiotic-resistant bacteria presents antibody therapy as a promising alternative to the use of antimicrobial molecules, especially after demonstrating the advantages that mcA can provide against this problem [28, 29]. The mcA are specifically directed at the antigens present on the surface of the pathogen or the toxins secreted by them [30, 31]. This specificity allows to treat the infection without affecting the microbiota of the organism, which differentiates it from broad-spectrum antibiotics. Therefore, it would be possible to treat the infection very specifically, decreasing the use of conventional antibiotics and thus reducing the occurrence of resistance [4]. Although the development of mcA has regained interest as

a treatment against antibiotic-resistant infections, many have only reached clinical trials due to its still high costs and the occurrence of adverse reactions at the immune level. Therefore, more research is needed to improve knowledge on this therapy against AMRs [4].

POT also includes AMP, which are an essential part of the innate response in humans and other higher organisms. AMPs are oligomers, from 8 to 50 amino acids, that have homogeneous structural groups, usually amphipathic sequences and cationic [32]. Due to their important antibacterial function, trials have been conducted that demonstrate promising candidates for combating AMR [33]. These peptides contribute to the first line of defense against infections, as they are directed against prokaryotes, providing direct antibacterial activity and a mediation of the inflammatory response (cytokine release, angiogenesis, cell proliferation, wound healing and chemotaxis) [16]. AMPs target the bacterial membrane or intracellular components to achieve an antibacterial, nonstick and antibiofilm effect. This effect is achieved by an increase in permeability and cell lysis after targeting the cytoplasmic membrane [34, 35]. Another effect produced by AMPs is the neutralization or disintegration of LPS, the main endotoxin responsible for gram-negative infections, so they have an interesting role as protectors against sepsis. An advantage of AMPs is that they do not interact with specific pathogen targets, which slows down the emergence of resistances [35].

An example of AMP is thyrotricin, a natural antimicrobial composed of two polypeptides, thyrocidin and gramicidin, which has been used for more than 60 years. Thyrotricin was isolated and identified in 1939 from *Bacillus brevis* and demonstrated antibacterial activity against gram-positive bacteria in guinea pig wounds as a substitute for synthetic antibiotics [36]. Several types of AMP have since been discovered, such as teixobactin, a polypeptide discovered in 2015 and produced by the bacterial species *Eleftheria terrae* [Figure 2] [37, 38]. Teixobactin has been shown to be effective against gram-positive bacteria such as *S. aureus,* VRSA and *M. tuberculosis* [37]. This is because teixobactin inhibits bacterial wall synthesis by binding to non-proteogenic molecules involved in plasma membrane formation [39]. Another recent example is a study where possible oligomers with antibiotic function were analyzed in the human proteome by searching for physicochemical determinants of AMPs [32]. A total of 2603 encrypted AMPs derived from plasma proteins, clotting factors or diuretic hormones were discovered. In the same study, 55 of the 2603 encrypted AMPs were synthesized and

characterized to evaluate their antimicrobial activity. In this way, it was found that encrypted AMPs had antibiotic activity against pathogens such as E. *coli, S. aureus, P. aeruginosa, K. pneumoniae, and A. baumannii,* in addition to acting as modulators of the human microbiota, an effect not exerted by traditional AMPs [32, 34, 35]. These new AMPs have turned out to be a great discovery in the search for new antimicrobials, since they present a structure and a mechanism of action that differs from the AMPs registered in the Peptide Activity and Structure Database (BDAEP) [40] and may be part of a new class of AMP.

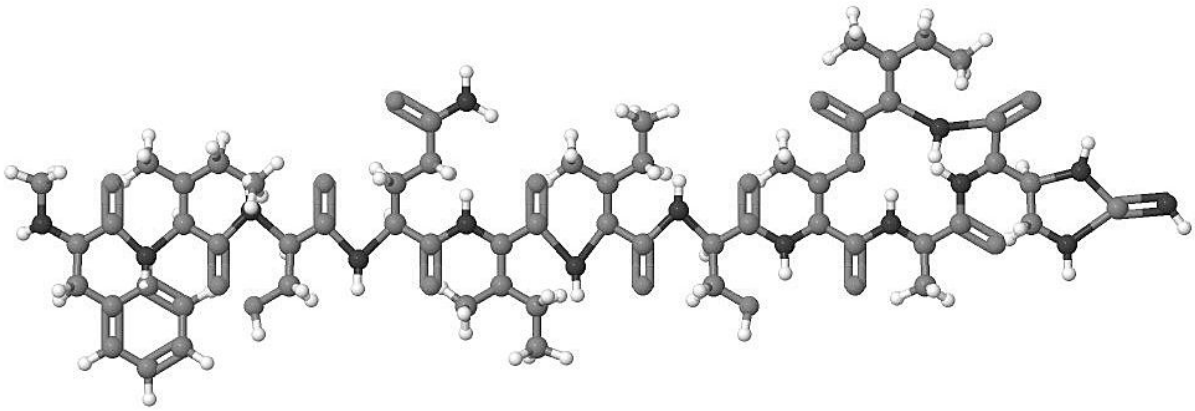


Figure 2. 3D structure of the AMP teixobactin, a polypeptide which has been shown antibiotic effects [37].

Treatment of bacterial infections with AMP appears to be a good option due to its favorable characteristics: broad spectrum of activity, low incidence of bacterial resistance, a specific mechanism of action, and rapid elimination kinetics [34, 35]. However, AMPs present several limiting factors: firstly, sensitivity to proteolytic digestion in different body fluids affects their stability and pharmacokinetic profile. In this way, most AMPs are restricted to topical or intravenous application due to their short half-life, as they are susceptible to proteolytic degradation. Secondly, the mechanisms of action for the inhibition of biofilm, cell adhesion, and interference of *quorum sensing* (QS) remain unclear [8]. This regulation is very important at the level of resistance production, as it triggers collective responses such as the formation of biofilms [13]. Finally, AMPs also entail high production costs and risk of cytotoxicity. Due to all these disadvantages, studies are being carried out aiming to improve the knowledge of their mechanisms of action and the development of more stable and specific PAMs (called peptidomimetics) in order to solve these limitations [1, 41].

The development of NP is another strategy that is part of the POT group. NPs are particles less than 100nm in at least one of their dimensions, but larger than atoms and molecules. This small size allows them to be absorbed by phagocytes and introduce the antibiotic into eukaryotic cells, targeting intracellular pathogens [42]. In addition, the versatility of NPs allows them a large drug dose, a high adaptability of these molecules, regardless of their hydrophobic or hydrophilic nature, and adequate stability in physiological fluids [43]. All this allows a correct administration of the antibiotic in the desired target, with a good bioavailability and therapeutic effect, in addition to a controlled biodegradation, minimizing adverse effects. Therefore, NP therapy has advantages in terms of lower resistance and side effects compared to traditional antimicrobial therapies [44]. Despite the advantages offered by nanoparticles, there are still challenges to be addressed, such as improving physicochemical properties, improved pharmacokinetic profiles, and comprehensive studies on long-term exposure in humans. These limitations mean that, despite their antibacterial potential, NPs are more often applied as a delivery system for other compounds, such as antibiotics, with which the combination has been shown to produce a synergy capable of treating infections caused by starins with emerging bacterial resistance (44, 45). In this regard, nanohybrids, also called metal-nanoparticles (MNP), are being designed to combine different metals with different antimicrobial agents. These MNPs (alone or in combination with other antimicrobial agents) provide improvements in this technique to combat the development of AMRs [46]. Examples of MNP are silver-NPs (AgNPs), which have been shown to be a potential candidate for treating infectious diseases [47]. This is due to its ability to alter the permeability of the bacterial membrane, interacting with different compounds and releasing silver ions, obtaining an antibacterial effect [48]. In the same way, gold (AuNP), zinc oxide (ZnONP) and titanium dioxide (TiO_2NP) have proven their effectiveness against gram-positive and gram-negative pathogen strains [49-51]. Currently NP are not widely used in clinical practice, but with appropriate studies, they may have great potential against the problem of AMRs, being able to improve current antimicrobial treatments [52].

Another alternative within this set of strategies is combination therapy, which consists in the combination of two antibiotics (also called antibiotic-antibiotic conjugates [AAC]) or an antibiotic and an antibiotic adjuvant (efflux pump inhibitors, antibiotic-modifying enzymes and/or mcA and AMP) or even two adjuvants [16]. This combination improves the efficacy of

individual antibiotics or adjuvants [1, 53]. Depending on the molecules present in this combination, it is possible to improve the inhibitory function of these drugs, in addition to alleviating pharmacokinetic problems such as the toxicity profile of the drugs. This improvement in the pharmacokinetic profile is due to the advantage of achieving an optimal antibiotic effect with lower doses of the combined compounds, which could also reduce the occurrence of resistance [1, 54]. Along these lines, quinolones/fluoroquinolones with oxazolidinone have demonstrated efficacy against gram-positive pathogens, as well as the combination of aminoglycosides with ciprofloxacin against gram-negative bacteria [54, 55]. In their study, Liu *et al.* [25] highlight the importance of antibiotic adjuvants in combination therapies, where both molecules are not antibiotic compounds and target non-essential but synthetically lethal gene functions. In this way, immune enhancers could present mild antivirulent or antibiotic activity, since they treat infection and minimize the appearance of resistance due to their selective low pressure against microbial populations.

Despite the advantages of the combination therapy, this strategy pays more attention to whether it has equal or higher antibacterial activity compared to classic antibiotic treatments. However, most studies lack systematic research on the mechanism and occurrence of resistances that appear or may appear after treatment with this therapy [4]. More studies are needed to consolidate combination therapy as an equally effective alternative to traditional antimicrobial therapies without increasing cases of resistance to these drugs.

2.1.2 *Microbiota therapy. Probiotics*

Interest in the role of the microbiota in human health has been growing in recent years. The role of bacteria present at the intestinal level in various infectious diseases has been widely proven, as well as the effect of their manipulation or therapeutic care in the evolution of certain diseases. It is also important to note that recent studies have found that antibiotic resistance can also be transmitted in the intestinal environment even without the use of these drugs [56]. This is due to the already named persistent bacteria, which, by their ability to enter a state of latency in which they minimize their metabolism, avoid the effect of antibiotics. Once they leave this state and start to colonize and grow the ability to enter a latent state is transferred through resistance genes to other bacteria of the same or even different species. To avoid the risk of resistance caused by antibiotic treatment, fecal microbiota transplant therapy is used, which consists of the recovery of an optimal intestinal microbiota for the

patient by transplanting intestinal microorganisms from human feces [57]. Therefore, it is of interest to study the changes or corrections that can be made in the human microbiota, not only as a treatment for bacterial infections, but also as a strategy to reverse the appearance of AMR.

It is also possible to modify the microbiota by administering probiotics. Probiotics are microorganisms that, when administered in adequate amounts, are able to inhibit or exclude the growth of pathogenic microorganisms in the microbiota, conferring benefits for host health [24]. The main objective of probiotics is to contribute to the maintenance of the composition of the microbiota for which they are designed. Probiotics achieve this beneficial effect by competing for nutrients or adhesion space, reducing the coaggregation of pathogens that are antagonized by producing fatty acids. It also secretes molecules with antimicrobial effects (bacteriocins, hydrogen peroxide, nitric oxide), stimulate the immune system (increase the production of macrophages, cytokines, interleukins, and tumor necrosis factor) and carry out a barrier function that stimulates the tissue reducing pathogenic bacterial populations and altering biofilms [58]. Several studies show that the use of probiotics as prophylactics reduces the risk of infectious diseases in both humans and animals, which in turn reduces the use of antibiotics [59–61]. In addition, the treatment has little chance of causing the appearance of resistance, as it neither destroys the human microbiota nor increases the risk of reinfection. There are studies where the efficacy of this therapy has been demonstrated in cases of diarrhea due to *Clostridioides difficile,* and even as a prophylactic and therapeutic treatment in *P. aeruginosa* infections [62, 63]. Another advantage of the use of probiotics is the reduction in the incidence, duration and/or severity of antibiotic-associated diarrhea, improving adherence to treatment and consequently reducing the occurrence of resistance [16]. Although microbiota therapy is an alternative that can prevent and/or resolve the onset of AMRs, the direct role of probiotics in the treatment of antibiotic-resistant infections has not yet been established. In fact, several clinical trials have shown mixed results on the overall benefit of probiotics, many of them without significant differences between the application or not of different probiotics in different patient populations (64-78). This demonstrates the need for more studies that evaluate and better understand the mechanisms of action involved in the fight against AMRs, in addition to acquiring a deeper knowledge of the role that the microbiota has in the human being [58, 63].

In addition, the process of manufacturing and designing probiotic products is expensive and complex, with few effective models. It must be noted that probiotics must be specifically selected so as not to be carriers of antibiotic resistance [16]. Further research into the potential of this therapy is needed in order to become an optimal pathway for the treatment of AMRs.

2.1.3 Phagotherapy

Bacteriophages were discovered by Félix d'Herelle in the early twentieth century, observing the existence of viruses with exclusive bactericidal activity [79]. Bacteriophages are viruses that infect bacteria and can replicate inside them through the lytic or lysogenic cycle. Regardless of the cycle, bacteriophages attach to the host bacterial cell and inject DNA into the cell. During the lysogenic cycle, the DNA of the virus is integrated into the DNA of the bacterium and, therefore, replicates according to it and is called prophage. When conditions are right, the lysogenic cycle changes to the lytic cycle [79]. During lytic infection, the virus uses the bacteria's machinery for its own replication and assembly, followed by cell lysis and the release of progeny bacteriophages. This progeny is able to infect new bacteria, repeating the process of bacteriophage replication and death of infected bacterial cells.

Bacteriophage therapy usually takes advantage exclusively of bacteriophages with lytic cycles because the therapeutic goal is the rupture of the bacterial cell. Although the idea of using bacteriophages as a treatment in bacterial infections was initially rejected after the discovery of antibiotics, it is currently receiving great interest due to the crisis of resistance to these drugs. Thus, there is growing interest in the discovery and development of new bacteriophages, especially with the availability of genome sequencing [80-82]. Bacteriophages self-amplify, kill bacteria by penetrating them and altering many or all bacterial processes without affecting the patient's microbiota [83]. At the same time, they are unable to penetrate eukaryotic cells, providing safety for human use.

Bacteriophages are especially effective for the eradication of bacterial biofilms, penetrating them and exploiting water channels or altering the matrix of extracellular biofilm through the expression of depolymerases, and are amplified while targeting latent bacteria [84]. An example of this is the study conducted by Waters *et al.* which demonstrated the efficacy of the bacteriophage PELP20 against biofilms produced by *P. aeruginosa* in patients with cystic

fibrosis [85]. In addition, bacteriophages increase the effect of antibiotics and have a high potential to be used as combination therapy. Thus, Kuraman *et al.* [86] studied the effect of bacteriophage SATA-8505 and antibiotic-bacteriophage treatment against *S. aureus* biofilms. It was found that there was a significant reduction in viable cells associated with the biofilm when treatment with bacteriophages preceded antibiotics, providing proof that bacteriophages can increase antibiotic activity against *S. aureus* biofilms.

Another interesting contribution of this therapy is its use in the CRISPR-Cas system of bacteria. The CRISPR system is an acquired immune defense mechanism that has evolved from the constant attack of viruses [81]. This system presents different Cas proteins that participate in the processes of crRNA synthesis, the integration of spacer sequences and the recognition and cleavage of exogenous DNA. In the case of DNA cleavage, bacteria are unable to repair such cleavage, triggering cell death. Therefore, the use of the CRISPR-Cas system may be an interesting line of development as an antibacterial therapy, as it can specifically cut the DNA fragments of the bacterium that are of interest (such as resistance genes or genes essential for the survival of the bacterium). Citorik *et al. [*82] used bacteriophages that activated the CRISPR-Cas9 system in such a way that they specifically eliminated resistance genes to certain beta-lactams. With the same procedure, they were able to eliminate pathogenic *E. coli* strains without altering the integrity of other bacteria. However, bacteriophage-based release systems remain inadequate in terms of efficacy and safety [83]. Efficacy is impaired by the ability of bacteria to neutralize bacteriophages, and safety by issues such as the possible rapid emergence of bacterial endotoxins as a result of bacterial lysis [84]. More well-designed randomized research and clinical trials are needed to define the role of bacteriophages as a new treatment option and to better understand the interaction between bacteriophages and antibiotics [24, 84].

2.1.4 Antivirulent therapy

Bacteria infect and spread within hosts using a multitude of virulence factors, such as efflux pumps or adhesion proteins [13]. Antivirulent therapy consists in the alteration of virulence factors through interaction with molecules that have the ability to inhibit or modify them. The advantage of this therapy is that it does not try to kill the pathogen but to reduce its virulence by inhibiting factors not essential for its survival [13]. This causes a decrease in the selective

pressure of the bacteria, making it possible to treat the infection while hindering the appearance of resistance [85].

Several studies have shown that antivirulent therapy could be effective against multiresistant bacteria, not only as an individual therapy, but also as an adjuvant to current antibiotic treatments [13, 23, 87-93]. One study showed compounds, such as verapamil, that acted as inhibitors of efflux pumps in *M. tuberculosis* infections, proving the ability of this therapy to improve existing treatments against this pathogen [Figure 3] [23]. In addition to inhibiting efflux pumps, they could help overcome the intrinsic resistance of *M. tuberculosis* and prevent the occurrence of mutations that confer antibiotic resistance [23]. Several studies also talk about the attenuation of the virulence factors of *S. aureus* and/or immunity modulation, demonstrating antivirulent effects in models of *Caenorhabditis elegans* [88-93]. A part of this therapy highlights the importance of acting on QS since there are studies that have shown the ability to block virulence, and even lead to the metabolic suicide of several pathogens by activating QS regulatory enzymes [13, 87]. However, this therapy has drawbacks when interacting with QS, due to its complexity and high degree of plasticity. In addition, molecules that have exhibited antivirulent activity often present toxicity problems [23]. Therefore, it is necessary to carry out more studies that improve this technique, since it could be an alternative to traditional antibiotics or return the susceptibility of pathogens to the current therapeutic arsenal [87].

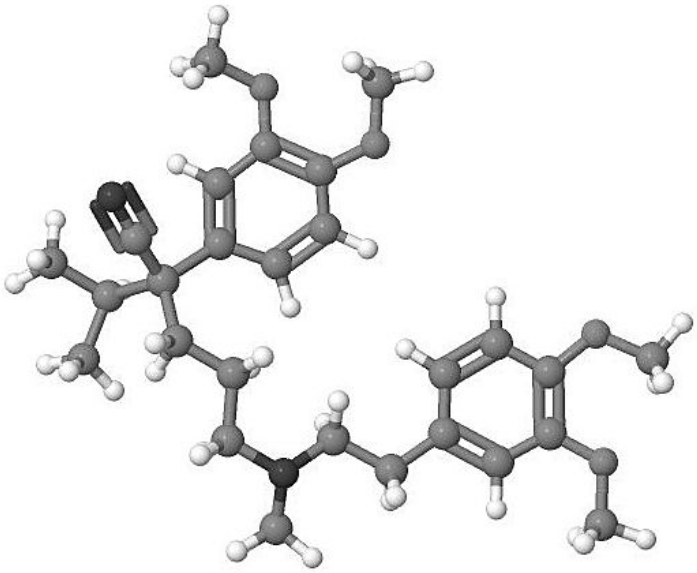


Figure 3. 3D structure of Verapamil, a well-known antihypertensive drug that has been shown to decrease virulence and the development of resistance in *M. tuberculosis*. [23].

The information presented in this section demonstrates the importance of exploring therapies based on AMP, antibodies, probiotics, bacteriophages and antivirulents to discover their full potential against the problem of antibiotic resistance, since they could become alternatives capable of replacing antibiotics and/or reducing the appearance of resistances. Despite the variety of therapies, these strategies cannot completely replace traditional antibiotic therapy. The lack of studies and the presence of disadvantages in terms of efficacy or safety leaves all these alternatives as adjuvant treatments to antibiotics. That is, these treatment pathways are still under development and require a lot of time, resources and efforts to move forward [Table 2] [4].

Table 2. Advantages and disadvantages of alternative therapies to antibiotics

		Examples	General characteristics	Advantages	Disadvantages	References
POT			Application of antibacterial compounds or materials directly to infected regions.	Specific application, which improves the effectiveness of the drug at a lower concentration. Reduction of the development of resistances.	Depending on the molecules: production problems, toxicity, instability, and inefficacy.	
	Monoclonal antibodies	17H12, 8F12, 2C7, SA-13, SA-15 and SA-17.	Application of antibodies that specifically target the external antigens of the pathogen.	Specific strategy without adverse effects on the body's microbiota. Reduction of the development of resistances.	High cost of production and adverse reactions at the immune level.	29, 30
	Antimicrobial Peptides (AMP)	Thyrotricin, gramicidine, teixobactin.	Oligomers that target the bacterial membrane or intracellular components performing an antibacterial effect.	They do not interact with specific targets, slowing down the emergence of resistances.	Pharmacokinetic limitations, high production costs and risk of cytotoxicity.	33, 34
	Nanoparticles (NP)	AgNP, AuNP, ZnONP, TiO_2NP.	Small particles that can penetrate eukaryotic cells and target intracellular pathogens.	They have versatility in the loading and adaptability of the drug and adequate stability in physiological fluids. Improving the effectiveness of the drug and slowing down the emergence of resistance.	Physicochemical properties and unfavorable pharmacokinetic profiles. Need for studies on long-term exposure in humans.	48-51
	Combination therapy	MCB3681, cadazolid, zaviceft.	Combination of molecules (antibiotics or not) that have an antibiotic effect.	Improving the effectiveness of current antibiotics. Better toxicity profile and efficacy of the molecules involved. Decrease in the appearance of resistance.	Lack of studies on the mechanism and appearance of resistance after therapy.	53, 54

Microbiota therapy	Fecal microbiota transplant, modified *E. coli* strains.	Administration of beneficial microorganisms for the reestablishment of a healthy microbiota.	Antimicrobial effect, immunostimulant effect and improvement of the barrier function of the body's tissue. Low chances of emergence of resistances. Harmless to the human microbiota.	Lack of studies regarding the mechanisms of action involved and high production costs.	57, 63
Phagotherapy	ΦRGN_{ndm-1} and ΦRGN_{shv-18}.	Application of bacteriophages that target and penetrate pathogenic bacteria.	Specificity. Harmless to eukaryotic cells. Effectiveness in the eradication of biofilms. Improvement of the effectiveness of antibiotics.	Small number of patients studied, lack of trials and studies explaining the phage-antibiotic interaction.	82
Antivirulent therapy	Thioridazine, verapamil, and closantel.	Drug interaction in targets not essential for the pathogenic microorganism.	Improvement of current antibiotic treatments. Ability to decrease virulence and the appearance of resistance.	Lack of studies and knowledge about this therapy and risk of toxicity of known molecules.	23, 91-93

2.2 Development of new antibiotics. *De novo* synthesis and drug repurposing

Since the discovery of Salvarsan in 1912, the pharmaceutical industry has been involved in the search and development of new molecules that would prevent the spread of infectious diseases [94]. Techniques such as lead design and computational design were developed and attained great importance in the search for such molecules. It was through these techniques that it was possible to increase the therapeutic arsenal against bacterial infections in the Golden Age of antibiotics [94, 95]. Unfortunately, bacteria have been developing resistance to these antibiotics, reducing their effectiveness, which has decreased the treatment options available against infectious diseases [5]. Molecules such as colistin, whose application had been banned for human use due to its toxicity, are reintroduced into the therapeutic arsenal as a last resort in resistant infections like carbapenemase-producing *Enterobacteriaceae.* Due to the emergence of resistance, the industry has lost interest in searching for and developing new antimicrobial molecules. In fact, almost all the antibiotics used today are based on discoveries made more than 30 years ago. In addition, the *de novo* development of new antibiotics is a slow process due to the progressive increase in unsatisfactory clinical data, such as unexpected pharmacokinetic parameters, poor stability, low permeability, and lack of *in vivo* activity and efficacy [26, 94]. Historical data show that the success rate of clinical drug development is low and that only one-fifth of the products studied will be approved for Phase I clinical trials [96]. All these difficulties have an impact on one of the limiting factors in the development of antibacterial compounds, the economic one. Currently, commercializing a new molecule costs between 2000 and 3000 million dollars and is a process that lasts between 13 and 15 years [5, 94, 97]. However, in the case of antibiotics this time can be extended to 20 years and reach costs between 568 and 700 million dollars. In addition, the success rates are quite hopeless; among antibiotics from existing classes in preclinical development, only 1 in 15 will be approved and reach patients. In the case of new classes of antibiotics, only 1 in 30 will be a success [5]. This is because the fundamental problem with the development of new antimicrobials is that the market is inherently limited by their design. In order to slow down and control continued antimicrobial resistance, newer medicines have restricted uses. This makes it challenging for biopharmaceutical research companies to recoup research and development costs in subsequent sales [5]. To this we must add the problem of the speed at which bacterial resistance appears; the growth rate of bacterial drug resistance tends to be

underestimated and is much faster than the rate of development of new antibiotics [2-4, 26]. In fact, the generation of resistant bacteria by horizontal transfer of resistance genes between bacteria or chromosomal mutation takes an average of 2 years [25]. This is due to the excessive and inappropriate use of antibiotics not only in human consumption, but also in livestock and agriculture. Consequently, there is a widening gap between the clinical need for new antibiotics and the discovery and development of new drugs [3, 4, 25]. The search for a new generation of antimicrobials to mitigate the spread of antibiotic resistance is urgent [1, 12]. In order to address this need, several organizations have proposed to promote innovation, research and development of new drugs to combat resistant pathogens [98-101]. This fund aims to bring 2-4 antimicrobials to the market by 2030, in addition to promoting new reimbursement models and establishing incentives to enable improvements in R&D and marketing of antimicrobials. However, the above-mentioned limitations make investment in the development of these drugs unattractive to the pharmaceutical industry due to their lack of profitability. Declining profits, coupled with antimicrobial efficacy issues, have led numerous pharmaceutical companies to go bankrupt or abandon antibacterial drug discovery lines [5, 13, 94].

Due to the existing scientific and commercial challenges in drug development, it is increasingly difficult to find new antibiotics for clinical application using traditional methods [2, 25]. An interesting methodology that could not only improve current therapies, but could also contribute to the search for new molecules with antimicrobial activity in a faster and, therefore, more cost-effective way compared to traditional methods, is the drug repositioning/repurposing, which consists in the generation of new clinical opportunities for molecules already known and/or approved, providing a new indication to the usual one [102-106]. The repurposing of drugs, specifically non-antibiotic drugs that have undergone extensive toxicological and pharmacological analysis, is an effective method of reducing the time, cost and risks associated with conventional antibiotic innovation by moving directly to preclinical testing and clinical trials [23, 25]. Several studies have demonstrated the usefulness of this method to identify a new clinical use as antibiotics against different pathogenic microorganisms in molecules already known or marketed [103-107]. In addition, new molecules have been detected through *in silico* homology studies that could be reused as new antimicrobial treatments against several microorganisms such as viruses and bacteria,

highlighting the possibility of becoming the new seeds from which to obtain new and better molecules with antimicrobial activity [18, 23, 103]. Therefore, drug repurposing allows the discovery of new therapeutic opportunities for molecules already known and/or commercialized. An important feature of this method is that these repositioned molecules can also be found to be those considered as failures, adding value to a lost investment by providing new input to these drugs [94]. Another advantage of this strategy is that it can participate in the therapies listed above. In the case of combination therapy, we have talked about antibiotic adjuvants, that is, molecules that improve antibiotic treatments by combining them with the latter or among adjuvants themselves. Drug repurposing has shown that it can identify antibiotic adjuvants that could work in combination with current antibiotic treatments, regaining the effectiveness they had before the emergence of resistance. We have also talked about antivirulent therapy, which provides a new approach that allows to reduce the frequency with which resistance appears. However, one of the disadvantages of molecules with this activity is toxicity. Drug repositioning could provide the tools needed to identify safe anti-virulent molecules using molecules with an already known toxicity profile [23]. Several studies show that drug repositioning increases knowledge and combines the advantages that these therapies can provide [Table 3] [107-113]. An example of this is the study carried out by García-Fernandez *et al.,* which demonstrated the ability to obtain resistance inhibitors against pathogens such as MRSA by repositioning statins such as zaragozic acid [107]. Another example of MRSA is the study of El-Halfawy *et al.*,where, through mathematical prediction models, they found molecules with adjuvant and antivirulent capacity simultaneously[108].

Table 3. Drugs with antimicrobial effect discovered by repositioning drugs

Drug	Usual clinical function	Antimicrobial function discovered by repositioning	References
Zaragozic acid	Antihypercholesterolemic	Inhibition of membrane lipid synthesis in MRSA strains	107
Captopril	Antihypertensive	Inhibition of metallo-β-lactamases in Gram-negative bacteria	109
Disulfiram	Treatment of alcoholism		110

		Inhibition of metallo-β-lactamases in Gram-negative bacteria	
Ticlopidine	Antiplatelet	Inhibition of teicoic acid biogenesis in MRSA strains. Antibiotic adjuvant in cefuroxime treatments.	111, 112
Pentamidine	Antiprotozoal	Increased membrane permeability by interaction with LPS of antibiotics restricted to Gram-positive bacteria in Gram-negative bacteria.	25, 113
Melatonin	Dietary supplement for sleep disorders	Increased outer membrane permeability, promoter of oxidative damage and inhibition of efflux pumps in Gram-negative bacteria.	25
Closantel, Niclosamide and Oxycloanide	Anthelmintics	Increased membrane permeability and inhibition of biofilm formation in Gram-positive bacteria.	2
Suramine	Anthelmintic	Inhibition of the SOS repair system, increasing membrane permeability and inhibiting the formation of biofilms in *M. tuberculosis* strains.	3
Curcumin	Food additive		
Loperamide	Antidiarrheal	Increased membrane permeability and inhibition of efflux pumps in Gram-negative bacteria	25
Azidothymidine	Antiretroviral	Inhibition of efflux pumps and SOS response in Gram-negative bacteria resistant to colistin and carbapenems.	

Mathematical prediction models, which characterize biomolecular structural, physical, chemical, and biological properties, are key to the success of machine learning models for both drug design and repurposing [114-116]. Within these models we can find different methodologies, such as quantitative structure-activity relationship (QSAR) models which link chemical structures and pharmacological activities (or other properties) quantitatively for a series of compounds through mathematical relationships [106, 116, 117]. Another method is molecular docking, which consists of predicting ligand-molecule conformities and/or residue conformation at the ligand binding site [118]. We can also find topological data analysis (TDA), which applies geometry and topology to develop tools that allow us to study the qualitative

characteristics of the data we have on molecules [119, 120]. All of them are based on the fundamental principle that molecules with similar structures/properties have similar pharmacological activities [121, 122].

Using mathematical prediction methods such as TDA, it is possible to study and calculate the structures, dimensions and geometries of proteins. This methodology could help the repositioning of already known drugs, since bacterial proteins of interest could be analyzed to verify their similarity with target proteins of known drugs [114, 123]. Thus, if the topological similarity between the target and bacterial proteins is optimal, the active substance could exert an antibiotic effect when interacting with the bacterial protein. Therefore, the development of a prediction model using TDA to analyze the similarity between original target proteins and bacterial proteins could be an alternative capable of providing new therapeutic approaches to already known molecules, with all that it would entail in terms of economic savings and speed in the discovery and development of new antibiotics [114, 123].

All of these examples demonstrate that combining these therapies and strategies along with drug repositioning could provide many benefits in improving antimicrobial treatments. In addition, the trials that can be carried out through the repositioning of drugs could reveal new therapeutic targets and improve the knowledge of antimicrobial therapies and the mechanisms by which bacteria obtain the capacity of resistance.

3. Conclusions

Humanity is facing a situation where current antibiotic treatments are unsatisfactory in proportion to existing AMRs, and the rate at which these resistances appear is greater than the discovery of new effective antimicrobials. Strategies such as POT, microbiota therapy, phagotherapy or antivirulent therapy represent a hope for the future, either as complementary treatments to antibiotics or as antibiotic-independent treatments. However, these strategies have a number of drawbacks, which require additional research to be able to bring them into clinical practice.

As for the development of new molecules, the costs, the low success rate and long periods of time make it impossible to obtain an effective therapeutic arsenal against current AMRs. The

strategy of reusing or repositioning previously approved drugs greatly shortens the time and cost of development, while ensuring the safety of said drugs. This strategy adds to the value of certain commercialized molecules, recovering part of the investment. In addition, it not only benefits the increase of the current therapeutic arsenal, it is also able to provide greater knowledge about therapies such as antivirulent therapy or combination therapy, and about the mechanisms by which bacteria develop resistance.

4. Expert opinion

The literature reviewed suggests that research dedicated to the discovery of alternative treatments to traditional antibiotics for AMR is a field of great interest. Being able to treat infections that are becoming difficult to cure and even untreatable due to AMR is something that, according to the Pharmaceutical Research and Manufacturers of America (PhRMA), will become increasingly common [5]. There are different causes that accelerate the emergence of resistance, including the overuse of these drugs, as well as a lack of knowledge about their target in population. In 2015, O'Neill wrote a report outlining these causes in different sectors such as agriculture, healthcare and the general population [124]. Due to this situation, the emergence of resistance to the current therapeutic arsenal is accelerating, which means that finding alternatives capable of matching the effectiveness and safety provided by antibiotics would be a major breakthrough for this worldwide health problem.

The alternative therapies named in this paper prove that, with the necessary scientific knowledge and research, it is possible to find a therapeutic equivalent to current antibiotics, as well as to reduce the appearance of resistance [2]. However, while emerging strategies are still being developed to be as effective and safe as antibiotics, new antibiotics and antibiotic families need to be found.

Another factor that must be taken into account is the economic one, which limits both the development of de novo antibiotics and the improvement and development of the emerging strategies mentioned. Pharmaceutical companies do not consider it promising to invest in the research of new antibiotic molecules due to the acceleration of the appearance of AMR itself, which makes investment in this field less and less profitable [5]. Therefore, it is necessary to find ways to develop new antimicrobials in a time and cost-effective manner.

The data show that drug repurposing makes it possible to obtain new antibiotics from known molecules. Since the pharmacokinetic data, the toxic dose and the dosage of the active compound are already known, the development process is accelerated. When applied to research, production costs and investment are reduced, as well as error rates, as the process moves directly to preclinical phases or clinical trials [23, 25]. This benefits pharmaceutical companies, as many of them have had to reject these lines of research because they do not provide a profitable return on investment. Drug repurposing together with the application of mathematical models provides a fast, cost-effective and safe way to obtain a new therapeutic arsenal with a low failure rate. Therefore, drug repurposing could be applied to save time against the appearance of AMR, while other alternative therapies achieve satisfactory results in terms of efficacy, safety and costs compared to the development of antibiotics.

We are currently in the era of big data, in which it is difficult to control the enormous amount of information that we have to provide quality scientific knowledge. Improving the management of this data with the help of fields such as machine learning will allow us to better understand issues such as emerging resistance mechanisms, as well as speed up the process of obtaining new molecules with biocidal capacity, in addition to reducing costs and failure rates of these processes [115, 118].

As discussed in this work, infections caused by bacteria resistant to antibiotics will be one of the most important causes of death in the world in the future. For this reason, the need for new antimicrobial therapies is and will be a field in continuous evolution. The line dedicated to the search for these therapies will continue to advance, probably with improvements in the therapies mentioned in this review in terms of safety and efficacy. It is also likely that, due to the need for new families of antibiotics, special attention will be paid to the vast microbial variety present on earth, as it has proven to be a promising field for the development of new antibiotic molecules, as well as new techniques to identify microbial biodiversity from these samples [37, 38]. Along with the improvement of the mathematical prediction models and drug repurposing, it is possible to achieve a new therapeutic arsenal capable of curbing the severity of the problem.

This review offers several starting points for a solution to a single problem: reducing the number of AMR. It also points to gaps in potential alternative therapies, justifying further

research in this field. We consider drug repurposing to be of interest due to its rapid and effective potential to increase the current therapeutic arsenal, in addition to continuing to develop possible emerging strategies.

Author contribution statement

A.T-P., B.S-G., and M.T.P-G. contributed to the implementation of the research, to the analysis of the results and to the writing of the manuscript. B.S-G. and M.T.P-G. designed and directed the project.

Funding details

A.T-P. was supported by CEINDO-SANTANDER (Spain). Work cited in this review from the author's laboratory was supported in part by grants from the Universidad CEU Cardenal Herrera (INDI18/34, INDI 19/39, INDI20/38 and INDI21/44).

Disclosure statement

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

References

[1] León-Buitimea A, Garza-Cárdenas CR, Garza-Cervantes JA, et al. The demand for new antibiotics: antimicrobial peptides, nanoparticles, and combinatorial therapies as Future strategies in antibacterial agent design. Front Microbiol. 2020;11:1669.

[2] Kim SM, Escorbar I, Lee K, et al. Anti-MRSA agent discovery using Caenorhabditis elegans-based high-throughput screening. J Microbiol. 2020;58(6):431-444.

[3] Memar MY, Yekani M, Celenza G, et al. The central role of the SOS DNA repair system in antibiotics resistance: A new target for a new infectious treatment strategy. Life Sci. 2020;262:118562.

[4] Shang Z, Chan SY, Song Q, et al. The Strategies of Pathogen-Oriented Therapy on Circumventing Antimicrobial Resistance. Research (Wash DC). 2020;28:2016201.

[5] PhRMA. Fact Sheet on Challenges with AMR. 2021 [cited 2021 Oct 20]. Available from: https://phrma.org/-/media/Project/PhRMA/PhRMA-Org/PhRMA-Org/PDF/A-C/AMR-Ecosystem-Challenges-Backgrounder_PhRMA.pdf

**** This report sets out the current and future problems of antibiotic resistance in terms of deaths and costs worldwide.**

[6] WHO. Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. 2017 [cited 2021 Oct 6]. Available from: https://www.who.int/medicines/publications/WHO-PPL-Short_Summary_25Feb-ET_NM_WHO.pdf

***This summary highlights the bacteria with the highest priority in the search for new antibiotics.**

[7] WHO. WHO publishes the list of bacteria for which new antibiotics are urgently needed. 2017 [cited 2021 Oct 3]. Available from: https://www.who.int/es/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed"

[8] Tiwari V. Post-translational modification of ESKAPE pathogens as a potential target in drug discovery. Drug Discov Today. 2019;24(3):814-822.

[9] Gallagher P, Baker S. Developing new therapeutic approaches for treating infections caused by multi-drug resistant *Acinetobacter baumannii: Acinetobacter baumannii* therapeutics. J Infect. 2020;81(6):857-861.

[10] ECDC. Antimicrobial resistance in Europe 2014. Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net). 2015 [cited 2021 Oct 7]. Available in: https://www.ecdc.europa.eu/sites/default/files/media/en/publications/Publications/antimicrobial-resistance-europe-2014.pdf

[11] Fariñas MC, Martínez-Martínez L. Infections caused by multi-resistant gram-negative bacteria: enterobacteria, *Pseudomonas aeruginosa, Acinetobacter baumannii* and other non-fermenting gram-negative bacilli. Enferm Infecc Microbiol Clin. 2013;31:402-409.

[12] Murugan N, Malath, J, Therese KL, et al. Application of six multiplex PCR's among 200 clinical isolates of Pseudomonas aeruginosa for the detection of 20 drug resistance encoding genes. Kaohsiung J Med Sci. 2018;34:79-88.

[13] Dolan SK. Current Knowledge and Future Directions in Developing Strategies to Combat Pseudomonas aeruginosa Infection. J Mol Biol. 2020;432(20):5509-5528.

[14] Vila J, Marco F. Interpretive reading of the non-fermenting gram-negative bacilli antibiogram. Enferm Infecc Microbiol Clin. 2010;28:726–36. 43.

[15] Lister PD, Wolter DJ, Hanson ND. Antibacterial-resistant Pseudomonas aeruginosa: clinical impact and complex regulation of chromosomally encoded resistance mechanisms. Clin Microbiol Rev. 2009;22:582–610.

[16] Karaman R, Jubeh B, Breijyeh Z. Resistance of Gram-Positive Bacteria to Current Antibacterial Agents and Overcoming Approaches. Molecules. 2020;25(12):2888.

[17] Tong SY, Davis JS, Eichenberger E, et al. Staphylococcus aureus infections: Epidemiology, pathophysiology, clinical manifestations, and management. Clin. Microbial. Rev. 2015; 28, 603–661. DOI: 10.1128/CMR.00134-14

[18] Troeman DPR, Van Hout D, Kluytmans JAJW. Antimicrobial approaches in the prevention of Staphylococcus aureus infections: a review. J. Antimicrob. Chemother. 2019;74, 281–294.

[19] Foster T. Antibiotic resistance in Staphylococcus aureus. Status and prospects. FEMS Microbiol Rev. 2017;41:430–449.

[20] Kim L, McGee L, Tomczyk S, et al. Biological and Epidemiological Features of Antibiotic-Resistant Streptococcus pneumoniae in Pre- and Post-Conjugate Vaccine Eras: A United States Perspective. Clin. Microbial Rev. 2016;29:525–552.

[21] Arya T, Kishor C, Saddanapu V, et al. Discovery of a new genetic variant of methionine aminopeptidase from Streptococci with possible post-translational modifications: biochemical and structural characterization. PLoS One. 2013;8(10):e75207.

[22] Terreni M, Taccani M, Pregnolate M. New Antibiotics for Multidrug-Resistant Bacterial Strains: Latest Research Developments and Future Perspectives. Molecules. 2021;26,2671.

[23] Rodrigues L, Cravo P, Viveiros M. Efflux pump inhibitors as a promising adjunct therapy against drug resistant tuberculosis: a new strategy to revisit mycobacterial targets and repurpose old drugs. Expert Rev Anti Infect Ther. 2020;18(8):741-757. ****This article proves the potential of drug repurposing as a way to obtain safe and effective antivirulents.**

[24] O'Sullivan JN, Rea MC, Hill C, et al. Protecting the outside: biological tools to manipulate the skin microbiota. FEMS Microbiol Ecol. 2020;96(6):fiaa085.

[25] Liu Y, Tong Z, Shi J, et al. Drug repurposing for next-generation combination therapies against multidrug-resistant bacteria. Theranostics. 2021;11(10):4910-4928.

[26] Knoblauch R, Geddes CD. Carbon Nanodots in Photodynamic Antimicrobial Therapy: A Review. Materials. 2020;13(18):4004.

[27] Shukra AM, Sridevi NV, Chandran D, et al. Production of recombinant antibodies using bacteriophages. Eur J Microbiol Immunol. 2014;4(2):91–98.

[28] McConnell MJ. Where are we with monoclonal antibodies for multidrug resistant infections? Drug Discov Today. 2019; 24(5):1132-1138.

[29] Diago-Navarro E, Motley MP, Ruiz-Peréz G, et al. Novel, Broadly Reactive Anticapsular Antibodies against Carbapenem-Resistant Klebsiella pneumoniae Protect from Infection. mBio. 2018;9(2):e00091-18.

[30] Gulati S, Beurskens FJ, de Kreuk BJ et al. Complement alone drives efficacy of a chimeric antigonococcal monoclonal antibody. PLoS Biol. 2019;17(6).

[31] Thomsen IP, Sapparapu G, James DBA, et al. Monoclonal Antibodies Against the Staphylococcus aureus Bicomponent Leukotoxin AB Isolated Following Invasive Human Infection Reveal Diverse Binding and Modes of Action. J Infect Dis. 2017; 215(7):1124-1131.

[32]Torres MDT, Melo MCR, Crescenzi O, et al. Mining for encrypted peptide antibiotics in the human proteome. Nat Biomed Eng . 2021;6(1):67-75. doi: 10.1038/s41551-021-00801-1

[33] Pacios O, Blasco L, Bleriot I, et al. Strategies to Combat Multidrug-Resistant and Persistent Infectious Diseases. Antibiotics. 2020;9(2):65.

[34] Vila J, Moreno-Morales J, Ballesté-Delpierre C. Current landscape in the discovery of novel antibacterial agents. Clin Microbiol Infect. 2020;26(5):596-603.

[35] de Oliveira Júnior NG, Franco OL. Promising strategies for future treatment of Klebsiella pneumoniae biofilms. Future Microbiol. 2020;15:63-79.

[36] Lang C, Staiger C. Tyrothricin–an underrated agent for the treatment of bacterial skin infections and superficial wounds?. Pharmazie. 2016;71(6):299-305.

[37] Ling LL, Schneider T, Peoples AJ, et al. A new antibiotic kills pathogens without detectable resistance. Nature. 2015;517:455-459.

[38] Lewis K. At the Crossroads of Bioenergetics and Antibiotic Discovery. Biochemistry. 2020; 85(12):1469-1483.

[39] Shukla R, Medeiros-Silva J, Parmar A, et al. Mode of action of teixobactins in cellular membranes. Nat Commun. 2020;11(1):2848.

[40] Pirtskhalava M, Gabrielian A, Cruz P, et al. DBAASP v.2: an enhanced database of structure and antimicrobial/cytotoxic activity of natural and synthetic peptides. Nucleic Acids Res. 2016;44,(D1):D1104–12. doi: 10.1093/nar/gkv1174

[41] Marlon HC, Elizabete SC, Karen GN, et al. Peptides containing d-amino acids and retro-inverso peptides: General applications and special focus on antimicrobial peptides. In: Peptide Applications in Biomedicine, Biotechnology and Bioengineering. Elsevier Science. 2018;131-155.

[42] Buzea C, Pacheco II, Robbie K. Nanomaterials and nanoparticles: sources and toxicity. Biointerphases. 2007;2(4):MR17–MR71.

[43] Akagi T, Baba M, Akashi M. Preparation of nanoparticles by the self-organization of polymers consisting of hydrophobic and hydrophilic segments: potential applications. Polymer. 2007; 48(23):6729–6747.

[44] Lee NY, Ko WC, Hsueh PR. Nanoparticles in the treatment of infections caused by multidrug-resistant organisms. Front Pharmacol. 2019;10:1153.

[45] Sharma V, Kumar A, Dhawan A. Nanomaterials: exposure, effects and toxicity assessment. Proc Natl Acad Sci India Sect B Biol Sci. 2012;82:3-11.

[46] Shaikh S, Nazam N, Rizvi SMD, et al. Mechanistic insights into the antimicrobial actions of metallic nanoparticles and their implications for multidrug resistance. Int J Mol Sci. 2019;20:2468.

[47] Borthagaray G, Mondelli M, Facchin G, et al. Silver-containing nanoparticles in the research of new antimicrobial agents against ESKAPE pathogens. In: Inorganic Frameworks as Smart Nanomedicines. Elsevier Science. 2018;317–386.

[48] Morones-Ramirez JR, Winkler JA, Spina CS, et al. Silver enhances antibiotic activity against gram-negative bacteria. Sci Transl Med. 2013;5:190ra81.

[49] Kumar R, Shukla SK, Pandey M, et al. Synthesis and antimicrobial effects of colloidal gold nanoparticles against prevalent waterborne bacterial pathogens. Cogent Chem. 2016;2:1192522.

[50] Tiwari V, Mishra N, Gadani K, et al. Mechanism of anti-bacterial activity of zinc oxide nanoparticle against carbapenem-resistant Acinetobacter baumannii. Front Microbiol. 2018 9:1218.

[51] de Dicastillo CL, Patiño C, Galotto MJ, et al. Novel hollow titanium dioxide nanospheres with antimicrobial activity against resistant bacteria. Beilstein J Nanotechnol. 2019;10 1716–1725.

[52] Barhoum A, García-Betancourt ML, Jeevanandam J, et al. Review on natural, incidental, bioinspired, and engineered nanomaterials: history, definitions, classifications, synthesis, properties, market, toxicities, risks, and regulations. Nanomaterials (Basel). 2022;12(2):177.

[53] Shapiro S. Speculative strategies for new antibacterials: all roads should not lead to Rome. J Antibiot. 2013;66(7):371-86.

[54] Bradley JS, Broadhurst H, Cheng K, et al. Safety and efficacy of ceftazidime-avibactam plus metronidazole in the treatment of children ≥3 months to <18 years with complicated intra-abdominal Infection. Pediatr Infect Dis J. 2019;38(8):816–824.

[55] Kali A, Charles MVP, Srirangaraj S. Cadazolid: a new hope in the treatment of Clostridium difficile infection. Australas Med J. 2015;8(8):253–262.

[56] Bakkeren E, Huisman JS, Fattinger SA, et al. Salmonella persisters promote the spread of antibiotic resistance plasmids in the gut. Nature. 2019;573(7773):276–280.

[57] Bilinski J, Grzesiowski P, Sorensen N et al. Fecal microbiota transplantation in patients with blood disorders inhibits gut colonization with antibiotic-resistant bacteria: results of a prospective, single-center study. Clin Infect Dis. 2017;65(3):364–370.

[58] Hols P, Ledesma-García L, Gabant P, et al. Mobilization of Microbiota Commensals and Their Bacteriocins for Therapeutics. Trends Microbiol. 2019;27(8):690-702.

[59] Mignolet J, Fontaine L, Sass A, et al. Circuitry Rewiring Directly Couples Competence to Predation in the Gut Dweller *Streptococcus salivarius*. Cell Rep. 2018;22(7):1627-1638.

[60] Vilà B, Fontgibell A, Badiola I, et al. Reduction of *Salmonella enterica* var. Enteritidis colonization and invasion by *Bacillus cereus* var. toyoi inclusion in poultry feeds. Poult Sci. 2009;88(5):975-979.

[61] Bories G, Brantom P, de Barberá JB, et al. Opinion of Scientific the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) on a request from the European Commission on the safety and efficacy of the product Toyocerin® (*Bacillus cereus* var. toyoi) as feed additive for rabbit breeding does. EFSA J. 2008;912, 1-13

[62] Borody TJ, Brandt LJ, Paramsothy S, et al. Fecal microbiota transplantation: a new standard treatment option for *Clostridium difficile* infection. Expert Rev Anti-Infect Ther. 2014;11(5):447–449.

[63] Hwang IY, Koh E, Wong A, et al. Engineered probiotic Escherichia coli can eliminate and prevent Pseudomonas aeruginosa gut infection in animal models. Nat Commun. 2017;8(1, article 15028).

[64] Forssten S, Evans M, Wilson D, et al. Influence of a probiotic mixture on antibiotic induced microbiota disturbances. World J Gastroenterol. 2014;20:11878-11885.

[65] Warrack S, Panjikar P, Duster M, et al. Tolerability of a probiotic in subjects with a history of methicillin-resistant *Staphylococcus aureus* colonisation. Benef Microbes. 2014;5:389–395.

[66] Hua XT, Tang J, Mu DZ. Effect of oral administration of probiotics on intestinal colonization with drug-resistant bacteria in preterm infants. Zhongguo Dang Dai Er Ke Za Zhi. 2014;16:606–609.

[67] Doron S, Hibberd PL, Goldin B, et al. Effect of *Lactobacillus rhamnosus* GG administration on vancomycin-resistant *Enterococcus* colonization in adults with comorbidities. Antimicrob Agents Chemother. 2015;59:4593–4599.

[68] Randomized controlled study of probiotics containing *Lactobacillus casei* (Shirota strain) for prevention of ventilator-associated pneumonia. J Med Assoc Thai. 2015;98:253–259.

[69] Kwon JH, Bommarito KM, Reske KA, et al. Randomized controlled trial to determine the impact of probiotic administration on colonization with multidrug-resistant organisms in critically ill patients. Infect Control Hosp Epidemiol. 2015;36:1451–1454.

[70] Warrack S, Ziegler M. A pilot randomized trial to determine the tolerability of a probiotic in patients colonized with vancomycin-resistant *Enterococcus.* J Probiotics Health. 2016;4.

[71] Eggers S, Barker AK, Valentine S, et al. Effect of *Lactobacillus rhamnosus* HN001 on carriage of *Staphylococcus aureus*: results of the impact of probiotics for reducing infections in veterans (IMPROVE) study. BMC Infect Dis. 2018;18:129.

[72] Esaiassen E, Hjerde E, Cavanagh JP, et al. Effects of probiotic supplementation on the gut microbiota and antibiotic resistome development in preterm infants. Front Pediatr. 2018;6:347.

[73] Mahmoodpoor A, Hamishehklar H, Asghari R, et al. Effect of a probiotic preparation on ventilator-associated pneumonia in critically ill patients admitted to the intensive care unit: a prospective double-blind randomized controlled trial. Nutr Clin Pract. 2019;34:156–162.

[74] Dall LB, Lausch KR, Gedebjerg A, et al. Do probiotics prevent colonization with multi-resistant Enterobacteriaceae during travel? A randomized controlled trial. Trav Med Infect Dis. 2019;27:81–86.

[75] Ljungquist O, Kampmann C, Resman F, et al. Probiotics for intestinal decolonization of ESBL-producing *Enterobacteriaceae*: a randomized, placebo-controlled clinical trial. Clin Microbiol Infect. 2020;26:456–462.

[76] Buyukeren M, Yigit S, Buyukcam A, et al. A new use of *Lactobacillus rhamnosus* GG administration in the NICU: colonized vancomycin-resistant enterococcus eradication in the gastrointestinal system. J Matern Fetal Neonatal Med. 2020:1–7.

[77] Lopez de Toro Martin-Consuegra I, Sanchez-Casado M, Pérez-Pedrero Sánchez-Belmonte MJ, et al. The influence of symbiotics in multi-organ failure: randomised trial. Med Clin (Barc). 2014;143:143–149.

[78] Salomao MCC, Heluany-Filho MA, Menegueti MG, et al. A randomized clinical trial on the effectiveness of a symbiotic product to decolonize patients harboring multidrug-resistant Gram-negative bacilli. Rev Soc Bras Med Trop. 2016;49:559–566.

[79] Sulakvelidze A, Alavidze Z, Morris JG Jr. Bacteriophage therapy. Antimicrob Agents Chemother. 2001;45(3):649-659.

[80] Hsu MN, Chang YH, Truong VA. CRISPR technologies for stem cell engineering and regenerative medicine. Biotechnol Adv. 2019;37(8):p. 107447.

[81] Pickar-Oliver A, Gersbach CA. The next generation of CRISPR–Cas technologies and applications. Nat Rev Mol Cell Biol. 2019;20(8):490–507.

[82] Citorik RJ, Mimee M, Lu TK. Sequence-specific antimicrobials using efficiently delivered RNA-guided nucleases. Nat Biotechnol. 2014;32(11):1141–1145.

[83] Mirski Lidia M, Nakonieczna A, Gryko R. Bacteriophages, phage endolysins, and antimicrobial peptides - the possibilities for their common use to combat infections and in the design of new drugs. Ann Agric Environ Med. 2019;26(2):203-209.

[84] Pang Z, Raudonis R, Glick BR, et al. Antibiotic resistance in Pseudomonas aeruginosa: mechanisms and alternative therapeutic strategies. Biotechnol Adv. 2019;37(1):177-192.

[85] Waters EM, Neill DR, Kaman B, et al. Phage therapy is highly effective against chronic lung infections with Pseudomonas aeruginosa. Thorax. 2017;72:666-667.

[86] Kumaran D, Taha M, Yi Q, et al. Does Treatment Order Matter? Investigating the Ability of Bacteriophage to Augment Antibiotic Activity against Staphylococcus aureus Biofilms. Front. Microbial. 2018;9:127.

[87] Defoirdt T. Quorum-Sensing Systems as Targets for Antivirulence Therapy. Trends in Microbiol. 2017;26 (4).

[88] Irazoqui JE, Troemel ER, Feinbaum RL, et al. Distinct pathogenesis and host responses during infection of *C. elegans* by *P. aeruginosa* and *S. aureus*. PLoS Pathog. 2010;6: e1000982.

[89] Irazoqui JE, Urbach JM, Ausubel FM. Evolution of host innate defence: insights from Caenorhabditis elegans and primitive invertebrates. Nat. Rev. Immunol. 2010;10, 47–58.

[90] Peterson ND, Pukkila-Worley R. Caenorhabditis elegans in high-throughput screens for anti-infective compounds. Curr. Opin. Immunol. 2018;54, 59–65.

[91] Rajamuthiah R, Fuchs BB, Conery AL, et al. Repurposing salicylanilide anthelmintic drugs to combat drug resistant Staphylococcus aureus. PLoS One. 2015;10, e0124595.

[92] Rajamuthiah R, Fuchs BB, Jayamani E, et al. Whole animal automated platform for drug discovery against multidrug resistant Staphylococcus aureus. PLoS One. 2014;9, e89189.

[93] Rajamuthiah R, Jayamani E, Majed H et al. Antibacterial properties of 3-(phenylsulfonyl)-2-pyrazinecarbonitrile. Bioorg. Med. Chem. Lett. 2015; 25, 5203–5207.

[94] Natalie KB, Chengwen T, Christopher RF. "Brief Overview of Approaches and Challenges in New Antibiotic Development: A Focus On Drug Repurposing." Front. Cell. Infect. Microbiol. 2021;11 684515.

**** This article discusses the potential of drug repurposing as a way to obtain new drugs from molecules that are classified as failures.**

[95] Mohr KI. History of Antibiotics Research. Current Topics in Microbiology and Immunology. 2016;499.

[96] Hay M, Thomas DW, Craighead JL, et al. Clinical development success rates for investigational drugs. Nat Biotechnol. 2014;32(1):40–51.

[97] Scannell JW, Blanckley A, Boldon H, et al. Diagnosing the decline in pharmaceutical R&D efficiency. Nat Rev Drug. 2012;11(3):191–200.

[98] United Nations. Call to Action on Antimicrobial Resistance (AMR)-2021. 2021 [cited 2022 Mar 14]. Available from: https://www.un.org/pga/75/wp-content/uploads/sites/100/2021/04/Call-to-Action-on-Antimicrobial-Resistance-AMR-2021.pdf

[99] O'Neill J. Review on Antimicrobial Resistance. Tackling Drug-Resistant Infections Globally: Final Report and Recommendations. 2016 [cited 2022 Mar 14]. Available from: https://amr-review.org/sites/default/files/160518_Final%20paper_with%20cover.pdf

[100] Priorities of the global leaders group on AMR for 2021-2022. 2021 [cited 2022 Mar 14]. Available from: Https://cdn.who.int/media/docs/default-source/antimicrobial-resistance/glg-action-plan-july-2021_final.pdf?sfvrsn=daalbd02_5&download=true

[101] Plan Nacional Resistencia Antibióticos. 2016 [cited 2022 Mar 14]. Available from: https://www.resistenciaantibioticos.es/es/system/files/content_images/folleto_pran.pdf

[102] SuayGarcia B, Pérez-Gracia MT. Present and Future of Carbapenem-resistant *Enterobacteriaceae* (CRE) Infections. Antibiotics-Basel. 2019;122.

[103] Pérez-Moraga R, Forés-Martos J, Suay-García B, et al. A COVID-19 Drug Repurposing Strategy through Quantitative Homological Similarities Using a Topological Data Analysis-Based Framework. Pharmaceutics. 2021;13(4):488.

[104] Pushpakom S, Iorio F, Eyers PA, et al. Drug repurposing: progess, challenges and recommendations. Nat Rev Drug Discov. 2018;18, 41-58.

[105] Langedijk J, Mantel-Teeuwisse AK, Slijkerman DS, et al. Drug repositioning and repurposing: terminology and definitions in literature. Drug Discov Today. 2015;20(8):1027-1034. doi: 10.1016/j.drudis.2015.05.001.

[106] Suay-Garcia B, Falcó A, Bueso-Bordils JI, et al. Tree-Based QSAR Model for Drug Repurposing in the Discovery of New Antibacterial Compounds Against Escherichia coli. Pharmaceuticals. 2020;13(12):431.

[107] Garcia-Fernandez E, Koch G, Wagner RM, et al. Membrane microdomain disassembly inhibits MRSA antibiotic resistance. Cell. 2017;171:1354-1367.

[108] El-Halfawy OM, Czarny TL, Flannagan RS, et al. Discovery of an antivirulence compound that reverses β-lactam resistance in MRSA. Nat Chem Biol. 2020;16:143-149.

[109] Brem J, van Berkel SS, Zollman D, et al. Structural basis of metallo-β-lactamase inhibition by captopril stereoisomers. Antimicrob Agents Chemother. 2016;60:142-150

[110] Chen C, Yang KW, Wu LY, et al. Disulfiram as a potent metallo-β-lactamase inhibitor with dual functional mechanisms. Chem Commun. 2020;56:2755-2758

[111] Farha MA, Leung A, Sewell EW, et al. Inhibition of WTA synthesis blocks the cooperative action of PBPs and sensitizes MRSA to β-lactams. ACS Chem Biol. 2013;8:226-233

[112] Farha MA, Czarny TL, Myers CL, et al. Antagonism screen for inhibitors of bacterial cell wall biogenesis uncovers an inhibitor of undecaprenyl diphosphate synthase. Proc Natl Acad Sci USA. 2015; 112:11048-11053.

[113] Stokes J, MacNair C, Ilyas B, et al. Pentamidine sensitizes Gram-negative pathogens to antibiotics and overcomes acquired colistin resistance. Nat Microbiol. 2017;2,17028.

[114] Meng Z, Xia K. Persistent spectral based machine learning (PerSpect ML) for drug design. Sci. Adv. 2020;7:19.

[115] Ericksen SS, Wu H, Zhang H, et al. Machine Learning Consensus Scoring Improves Performance Across Targets in Structure-Based Virtual Screening. J Chem Inf Model. 2017;57:1579–1590.

[116] Suay-Garcia B, Bueso-Bordils JI, Falcó A, et al. Quantitative structure-activity relationship methods in the discovery and development of antibacterials. Wiley Interdiscip Rev-Comput Mol Sci. 2020;e1472.

[117] Singh S, Supuran CT. 3D-QSAR CoMFA studies on sulfonamide inhibitors of the Rv3588c beta-carbonic anhydrase from Mycobacterium tuberculosis and design of not yet synthesized new molecules. J Enzym Inhibit Med Chem. 2014;29:449–455.

[118] Lima, AN, Philot EA, Goulart T, et al. Use of machine learning approaches for novel drug discovery. Expert Opin Drug Discov. 2016;17460441.2016.1146250.

[119] Macalino SJY, Billones JB, Organo VG, et al. In Silico Strategies in Tuberculosis Drug Discovery. Molecules. 2020;25(3):665.

[120] Otter N, Porter MA, Tillmann U, et al. A roadmap for the computation of persistent homology. EPJ Data Sci. 2017;6:17

[121] van Laarhoven T, Marchiori E. Predicting Drug-Target Interactions for New Drug Compounds Using a Weighted Nearest Neighbor Profile. PLoS One. 2013;8(6):e66952. ***This article outlines the foundations on which mathematical prediction models are based.**

[122] Farha MA, Brown ED. Drug repurposing for antimicrobial discovery. Nat Microbiol. 2019;4:565-577.

[123] Wang R, Li S, Cheng L, et al. Predicting associations among drugs, targets and diseases by tensor decomposition for drug repositioning. BMC Bioinformatics. 2019;20(Suppl 26):628.

[124] O'Neill, J. Antimicrobials in agriculture and the environment: Reducing unnecessary use an waste. https://amr-review.org/sites/default/files/Antimicrobials%20in%20agri-culture%20and%20the%20environment%20%20Reducing%20unnecessary%20use%20and%20waste.pdf 2015 (cited 2019 Nov 15)

Table 1. WHO list of priority pathogens for the research for new antibiotics [6]

Priority 1: CRITICAL	Priority 2: HIGH	Priority 3: Medium
Acinetobacter baumannii, carbapenem-resistant. *Pseudomonas aeruginosa*, carbapenem-resistant. *Mycobacterium tuberculosis,* β-lactams resistant, macrolides resistant, aminoglycosides resistant, anphenicols resistant, quinolones resistant, tetracyclines resistant, isoniazid resistant, etambutol resistant, pyrazinamide resistant and rifampicin resistant. *Enterobacterales (Klebsiella pneumoniae, Escherichia coli, Enterobacter spp., Serratia spp., Proteus spp., Providencia spp, and Morganella spp.)*, carbapenem-resistant, 3rd generation cephalosporin-resistant and broad spectrum β-lactams resistant.	*Enterococcus faecium*, vancomycin resistant. *Staphylococcus aureus*, methicillin resistant, intermediate vancomycin susceptibility and vancomycin resistant. *Helicobacter pylori*, clarithromycin resistant. *Campylobacter spp.*, fluoroquinolones resistant. *Salmonella spp.*, fluoroquinolones resistant. *Neisseria gonorrhoeae*, cephalosporin resistant and fluoroquinolones resistant.	*Streptococcus pneumoniae*, penicillin resistant. *Haemophilus influenzae*, ampicillin resistant. *Shigella spp.*, fluoroquinolones resistant.

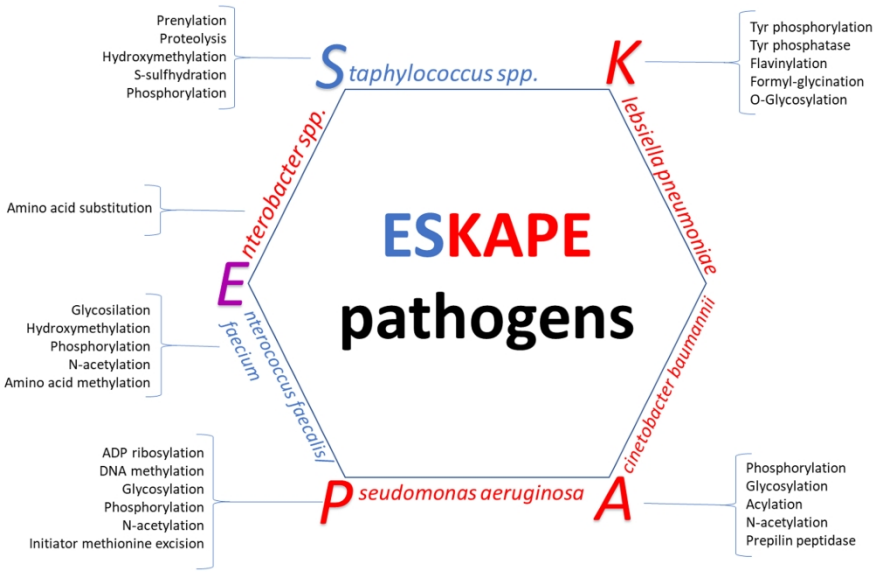
Table 2. Advantages and disadvantages of alternative therapies to antibiotics

		Examples	General characteristics	Advantages	Disadvantages	References
POT			Application of antibacterial compounds or materials directly to infected regions.	Specific application, which improves the effectiveness of the drug at a lower concentration. Reduction of the development of resistances.	Depending on the molecules: production problems, toxicity, instability, and inefficacy.	
	Monoclonal antibodies	17H12, 8F12, 2C7, SA-13, SA-15 and SA-17.	Application of antibodies that specifically target the external antigens of the pathogen.	Specific strategy without adverse effects on the body's microbiota. Reduction of the development of resistances.	High cost of production and adverse reactions at the immune level.	29, 30
	Antimicrobial Peptides (AMP)	Thyrotricin, gramicidine, teixobactin.	Oligomers that target the bacterial membrane or intracellular components performing an antibacterial effect.	They do not interact with specific targets, slowing down the emergence of resistances.	Pharmacokinetic limitations, high production costs and risk of cytotoxicity.	33, 34
	Nanoparticles (NP)	AgNP, AuNP, ZnONP, TiO_2NP.	Small particles that can penetrate eukaryotic cells and target intracellular pathogens.	They have versatility in the loading and adaptability of the drug and adequate stability in physiological fluids. Improving the effectiveness of the drug and slowing down the emergence of resistance.	Physicochemical properties and unfavorable pharmacokinetic profiles. Need for studies on long-term exposure in humans.	48-51

Combination therapy	MCB3681, cadazolid, zaviceft.	Combination of molecules (antibiotics or not) that have an antibiotic effect.	Improving the effectiveness of current antibiotics. Better toxicity profile and efficacy of the molecules involved. Decrease in the appearance of resistance.	Lack of studies on the mechanism and appearance of resistance after therapy.	53, 54
Microbiota therapy	Fecal microbiota transplant, modified *E. coli* strains.	Administration of beneficial microorganisms for the reestablishment of a healthy microbiota.	Antimicrobial effect, immunostimulant effect and improvement of the barrier function of the body's tissue. Low chances of emergence of resistances. Harmless to the human microbiota.	Lack of studies regarding the mechanisms of action involved and high production costs.	57, 63
Phagotherapy	ΦRGN_{ndm-1} and ΦRGN_{shv-18}.	Application of bacteriophages that target and penetrate pathogenic bacteria.	Specificity. Harmless to eukaryotic cells. Effectiveness in the eradication of biofilms. Improvement of the effectiveness of antibiotics.	Small number of patients studied, lack of trials and studies explaining the phage-antibiotic interaction.	82
Antivirulent therapy	Thioridazine, verapamil, and closantel.	Drug interaction in targets not essential for the pathogenic microorganism.	Improvement of current antibiotic treatments. Ability to decrease virulence and the appearance of resistance.	Lack of studies and knowledge about this therapy and risk of toxicity of known molecules.	23, 91-93

Table 3. Drugs with antimicrobial effect discovered by repositioning drugs

Drug	Usual clinical function	Antimicrobial function discovered by repositioning	References
Zaragozic acid	Antihypercholesterolemic	Inhibition of membrane lipid synthesis in MRSA strains	107
Captopril	Antihypertensive	Inhibition of metallo-β-lactamases in Gram-negative bacteria	109
Disulfiram	Treatment of alcoholism	Inhibition of metallo-β-lactamases in Gram-negative bacteria	110
Ticlopidine	Antiplatelet	Inhibition of teicoic acid biogenesis in MRSA strains. Antibiotic adjuvant in cefuroxime treatments.	111, 112
Pentamidine	Antiprotozoal	Increased membrane permeability by interaction with LPS of antibiotics restricted to Gram-positive bacteria in Gram-negative bacteria.	25, 113
Melatonin	Dietary supplement for sleep disorders	Increased outer membrane permeability, promoter of oxidative damage and inhibition of efflux pumps in Gram-negative bacteria.	25
Closantel, Niclosamide and Oxycloanide	Anthelmintics	Increased membrane permeability and inhibition of biofilm formation in Gram-positive bacteria.	2
Suramine	Anthelmintic	Inhibition of the SOS repair system, increasing membrane permeability and inhibiting the formation of biofilms in *M. tuberculosis* strains.	3
Curcumin	Food additive		
Loperamide	Antidiarrheal	Increased membrane permeability and inhibition of efflux pumps in Gram-negative bacteria	25
Azidothymidine	Antiretroviral	Inhibition of efflux pumps and SOS response in Gram-negative bacteria resistant to colistin and carbapenems.	



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