Review

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Therapeutic and Unconventional Strategies to Contrast Antimicrobial Resistance: A Literature Review

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The fast emergence and spread of drug-resistant infectious pathogens and the resulting increase in associated and attributable deaths is a major health challenge globally. Misuse of antibiotics, insufficient infection prevention and control (IPC) in hospitals, food, animal feed, and environmental contamination due to drug-resistant microbes and genes have been the main drivers for antimicrobial resistance (AMR). AMR can lead to ineffective drug treatment, persistence of infection, and risk of severe disease especially in frail, immunocompromised, elderly patients. It is estimated that AMR will cause around 10 million deaths every year after 2050, the same number of deaths due to cancer occurring every year in present times. AMR affects the progress towards the Sustainable Development Goals (SDGs) and is crucial for pandemic preparedness and response. Therefore, the international authorities such as G7 and G20, the World Bank, the World Health Organization (WHO), the General Assembly of the United Nations, and the European Union call for innovative antibiotics and strategies to combat this health threat. To underline this emergency, two lists of resistant "priority pathogens" and a global research agenda for AMR in human health have been published by the WHO. Although investigation of safe and effective treatments remains a top priority, the pipeline for new antimicrobials is not promising, and alternative solutions are needed urgently. In recent times, the interest in fighting AMR has increased, and a number of preventive or therapeutic options have been explored. In this literature review, we discuss the scientific evidence and the limits of the main proven unconventional strategies to combat the AMR phenomenon in the human sector.

Keywords: antimicrobial resistance; drug-resistant microorganisms; microbiota modulators; vaccines; phages; monoclonal antibodies; antibiotic combination; therapeutics

Introduction

Antimicrobial Resistance (AMR)

The ability of microorganisms to resist antimicrobial drug treatments eliminating them or halting their growth is defined as 'antimicrobial resistance' (AMR). These microbes can be responsible for human, animal, and/or plant infections, representing a major health threat worthy of discussion at the highest international fora; i.e., G7 and G20 summits [1,2] in recent years. AMR, of which antibiotic resistance (ABR) is undoubtedly the prevalent core, is a phenomenon that can occur either naturally or induced by anthropic activities. After Sir Alexander Fleming discovered penicillin in 1928, the use of antibiotics has enabled the collapse of mortality caused by bacterial infectious diseases observed in the last century. However, their continued use over the years has led to a substantial change in the bacterial ecosystem, with both Gram-positive and Gram-negative bacterial species appearing with reduced chemosensitivity to antibiotic treatments. The improper use, prescription abuse, and incorrect disposal of antibiotics are among the main causes of the fast growth and spread of microorganisms resistant to their action, resulting in a loss of effectiveness of the pharmacological therapies available and serious risks to public health. Epidemiological estimates indicated that, in 2019, the infections caused by multi-resistant bacteria were responsible directly for 90,100–188,000 deaths in the World Health Organization (WHO) European region and for nearly 1.3 million deaths globally, AMR accounting for the third leading cause of death globally in 2019, after ischaemic heart disease and stroke [3]. Considerably, the number of AMR-associated deaths was around four times these estimates in 2019. Therefore, AMR has become one of the major public health problems world-wide, with severe implications (increased morbidity, mortality, and hospitalization length, possible disability and clinical complications, escalation in the number of diagnostic tests and medical procedures needed, increase in the cost of healthcare, occurrence of epidemics, animal, food and environmental contamination) and economic impact, often concerning healthcare-associated infections (HAI) [4]. AMR and HAI were identified as special health issues under Article 2 of Regulation (EU) 2022/2173 on serious cross-border threats to health. For these reasons, on June 2023 the Quadripartite organizations (Food and Agriculture Organization of the United Nations—FAO, United Nations Environment Programme—UNEP, World Health Organization—WHO, and the World Organisation for Animal Health—WOAH), have established the Quadripartite Technical Group on the Economics of Antimicrobial Resistance (QTG-EA) [5]. Particularly, in 2008 Rise and colleagues named the ESKAPE pathogens (*Enterococcus faecium* (*E. faecium*), *Staphylococcus aureus* (*S. aureus*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Acinetobacter baumannii* (*A. baumannii*), *Pseudomonas aeruginosa* (*P. aeruginosa*), Enterobacterales or *Enterobacter* spp.) a group of opportunistic pathogenic bacteria demonstrating multidrug resistance and virulence capable of "escaping" the biocidal effect of antibiotic drugs [6,7]. Then, in 2017, the WHO classified into three priority groups 12 families of antibiotic-resistant pathogens for which new antibiotics are needed urgently [8]. Drug-resistant microbes represent a real threat to the global health of the planet, as they have developed forms that are generally multidrug-resistant (MDR), extensively drug-resistant (XDR) and pan-drug-resistant (PDR) [9,10]. Recently, the WHO published also the fungal priority pathogens list to guide research and development, and take targeted public health action [11]. Over the years, the number of antimicrobial chemotherapeutics active and effective against these micropathogens has been decreasing. In addition, scarce availability of new antimicrobial chemotherapeutics results from insufficient financial incentives for manufacturers to invest in developing new antimicrobials. The annual WHO pipeline analysis showed a decline in the number of antibiotics under development from 31 in 2017 down to 27 in 2021 [12]. In the preclinical stage, the number of products has remained relatively constant over the last few years. Therefore, the use of alternative therapeutic strategies to antimicrobial chemotherapeutics is an important avenue to counter AMR. Here we discuss the main proven clinical options, i.e., the use of microbiota modulators, vaccines, bacteriophages, monoclonal antibodies, and antibiotic combinations, being aware that more opportunities exist and can be effective in reducing drug-resistant infections, like phytochemicals and essential oils, nanoparticles, peptides, metals, predatory microorganisms, and repositioning of non-antibiotic drugs.

Main Unconventional Options against Antimicrobial-Resistant Pathogens

AMR is a silent pandemic. However, antimicrobial chemotherapy is the one area of science that has not seen much research progress in recent years. Overall, the clinical pipeline is insufficient to tackle the increasing emergence and spread of AMR [12]. A great deal of research is underway to rapidly identify new innovative therapeutic solutions to combat the phenomenon of AMR. In March 2023, the WHO launched the first-ever Paediatric Drug Optimization (PADO) priority list for antibiotics to facilitate research and development efforts targeted to infants and children [13]. In June 2023, the WHO published a policy brief on the AMR global research agenda in human health to prioritize 40 research topics for evidence-based information by 2030 [14]. Although some alternative strategies have long been known and proven in several models *in vivo* or through clinical trials, these options are still unconventional and not routinely applied to combat AMR due to several challenges.

Microbiota Modulators

The human microbiota plays a key role in the response to infectious agents. Therapies that aim to modulate the intestinal microbiota may have a dual objective, both preventing pathogen colonisation and promoting pathogen elimination, contributing to combat bacterial resistant infections [15]. These microbiota-modulating therapies include prebiotics, probiotics and fecal microbiota transplantation (FMT). There are multiple mechanisms by which modulating the human microbiota can avoid infections. In the clinical setting, prebiotics can result in the expansion of targeted species within the community and in the inhibition of the growth of pathogens through their exclusion from the niche. Prebiotics promote the growth of certain members of the endogenous microbiota and correct the microbiota composition by acting as a substrate for fermentation [16]. Besides, prebiotics can modulate the innate immune system, enhance the gut barrier functions, and prevent pathogen colonization. Also, prebiotics can enhance the individual immunologic response to seasonal influenza vaccination in adults by raising antibody titers [17]. Probiotics are live microorganisms whose oral administration is capable of bringing about beneficial effects on human health through stimulation of both mucosal and systemic immunity. Probiotics are hypothesised to prevent or treat infections by competing with pathogens for nutritional and functional resources [18]. Evidence showed that non-toxigenic *Clostridioides difficile* (*C. difficile*) spores can prevent the colonisation of toxigenic *C. difficile* and significantly reduce recurrent infections [19,20]. In addition, probiotics can produce antimicrobial substances such as bacteriocins that inhibit the bacterial growth [21]. The association of a prebiotic with a probiotic is called symbiotic. The simultaneous administration of at least two of these active ingredients increases

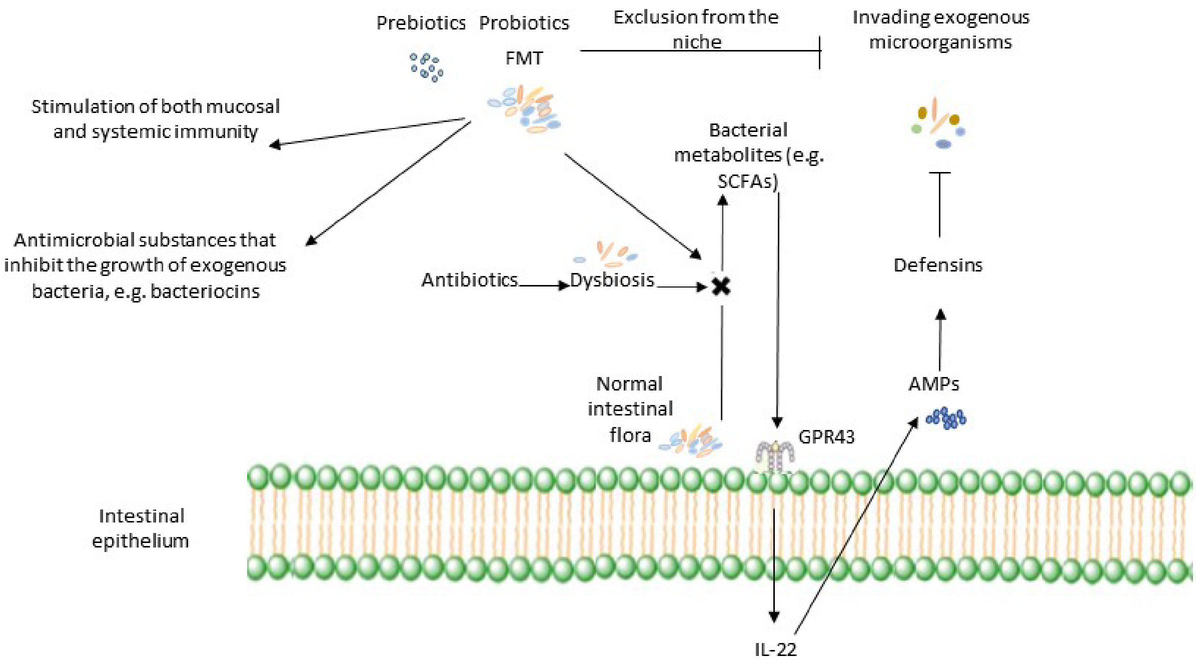

Fig. 1. Microbiota modulators can counteract infection or colonization due to pathogenic microorganisms by several mechanisms. Supplementation with prebiotics, probiotics or fecal transplantation of microbiota (FMT) can stimulate both mucosal and systemic host immunity, restore the physiological flora and its immunological functions in the gut, and induce the production of antimicrobial substances. Probiotics and FMT can exclude exogenous microorganisms from the niche. Black cross represents inhibitory regulation. The programs used for the image are Microsoft PowerPoint 2019 MSO version 16.0.10401.20025 (Forethought, Inc., Silicon Valley, California) and Microsoft Paint 3D (V. 6.2203.1037.0, Microsoft Corporation, Redmond, Washington, United States).

the survival of the probiotic microorganism, as it makes the specific substrate immediately available for its fermentation. Clinical evidence has shown that the administration of a symbiotic orally in infants can contribute to the reduction of death from sepsis [22]. In recent years, significant attention has been given to FMT, i.e., the administration of a fecal suspension from a healthy donor to a recipient, with the aim of microbiota modulation in an attempt to respond to an infection. This technique originated centuries ago and it has been revived in recent times to counter the increasing prevalence and virulence of *C. difficile* infection (CDI), as well as its resistance to common antimicrobials, being proven to be a highly effective treatment for recurrent or refractory CDI [23,24]. FMT has shown some success in intestinal decolonisation of multi-resistant pathogenic microorganisms to resolve the infection, including vancomycin-resistant Enterococci (VRE), *S. aureus* and β-lactamase-producing Enterobacterales [25]. On the contrary, it is well established that the gut microbiota is perturbed by the use of antibiotics and that these changes in the physiological microbial composition—named dysbiosis—can lead to negative health effects. Dysbiosis causes the reduction of short-chain fatty acids (SCFAs, such as propionic acid and butyric acid) released from the intestinal flora in physiological conditions, decreasing the production of mucins and antimicrobial peptides (AMPs) that can protect against non-indigenous microorganisms (via cascading). Indeed, the interaction of SCFAs on the microbiota through binding to G-protein-coupled receptor 43 (GPR43) leads to an increase in interleukin-22 (IL-22), which promotes the production of defensins [17]. In Fig. 1 we represented the main mechanisms by which the microbiota modulators can act against invading microorganisms.

Vaccines

A preventive strategy to combat infections caused by multidrug-resistant bacteria comes from the use of vaccines. Vaccines are associated with few resistance events compared to antimicrobials [26,27] preventing the start of infection, while drugs work therapeutically on an ongoing infection where bacteria can mutate. The prophylactic use of vaccines can avoid the need to treat bacterial infections and then the use of antimicrobial chemotherapeutics with the risk of increasing the emergence of multidrug-resistant bacterial species. Recently, numerous trials are underway to test effective vaccines against drug-resistant bacteria [28]. Thanks to vaccination strategies, bacterial infections due to diphtheria or tetanus have never become resistant to antibiotic treatments. However, in recent years examples of wide and non-clonal expansion of vaccine-induced isolates with diverse phenotype and virulence factors have been reported for *Bordetella pertussis* (*B. pertussis*) [29] by several countries, with an increase in cases affected by mutant forms that escape vaccination. Specifically, some evidence has shown genetic variability in the two key antigens pertactin and pertussis toxin in bacterial forms circulating after vaccination [29–31]. Even if there have been cases of resistance to vaccination, the long-term result has been a reduction in the number of infected cases, in the severity of the disease, and in the use of antibiotics. Recently, vaccines designed with different methodologies against some bacterial species such as *Escherichia coli* and *Staphylococcus aureus* [32] are being tested. Influenza vaccination significantly reduces antibiotic use both in the European and American regions, while the effect of pneumococcal vaccination is less pronounced [33]. The use of vaccines for mitigation of AMR and consequent economic saving is a major issue, particularly in settings where these formulations are not widely used. The use of pneumococcal conjugate vaccine (PCV) has averted over 700,000 antibiotic treatment failures and 27.8% AMR-related deaths in Ethiopia between 2011 and 2017, resulting in savings of $32.7 million [34].

Bacteriophages

Bacteriophages, also named phages, are viruses that mainly infect bacterial cells. Generally, during a replication cycle, bacteriophages infect certain bacteria by adhering to their surface due to viral proteins that recognise specific bacterial receptors. Subsequently, the phage fixes its fimbriae on the host's membrane and injects its genome inside the bacterial cell by means of a contraction mechanism, while the proteic envelope remains on the outside. Once penetrated inside the host bacterium, the phage genome starts the lytic or the lysogenic cycle by using the bacterial ribosomes to produce its proteins. During the lysogenic cycle, the phage genome incorporates itself into the bacterial genome. The resulting prophage replicates within the bacterial cell until the lytic cycle is triggered. During the lytic cycle, every infected bacterial cell produces around 20,000 new virions secreting lytic enzymes (endolysins) that hydrolyze the bacterial cell wall and ensure viral release. Bacteriophages are an important part of the human microbiota, playing a key role in the genetic exchange between non-pathogenic and pathogenic bacteria through gene transfer from one bacterial strain to another [35]. A case report showed that a 15-year-old cystic fibrosis patient with disseminated *Mycobacterium abscessus* (*M. abscessus*) infection was treated with a cocktail of three phages [36]. The lytic phage derivatives were modified by genome engineering to effectively attack and kill the infectious strain of *M. abscessus*. Intravenous phage treatment was well tolerated and associated with clinical improvements [36]. Recent clinical trials have provided numerous indications, although with conflicting or complementary conclusions on the safety and usefulness of phage therapy [37,38]. One study demonstrated the efficacy of topical treatment with 12 anti-pseudomonas phages, being the most illuminating clinical trial of the last decade directed at *P. aeruginosa* infection in burn wounds [39]. However, some issues need to be considered carefully when it comes with bacterial phage therapy, i.e., phage narrow host range, possible emergence of bacterial resistance, phage stability in the gastrointestinal tract, potential eukaryotic immune response, phage-specific training, and existence of prophages in bacterial production strains [40]. Target bacteria may develop resistance to phage attack and adsorption over time by altering receptor sites. Resistance to phage treatment has been shown after administration for infection due to *A. baumannii*, that made it necessary to switch to different phages to which the resistant strain was susceptible [41]. While receptor variation is commonly involved in resistance, there are several antiphage mechanisms that enable bacteria to resist phage infection or actively limit phage growth, including restriction, abortive infection systems and phage-encoded defences. Furthermore, some bacterial species can produce endotoxins during the lysis induced by phages, which can cause septic shock in the patient. Besides, during therapeutic application there may be a significant decrease in phage concentrations caused by the reticuloendothelial system or by neutralisation due to antibodies [40–44].

Monoclonal Antibodies

In recent times, an alternative therapeutic strategy to antibiotic treatment of bacterial infections is the use of antibacterial monoclonal antibodies (mAbs) [45]. The advantage of treatment with mAbs is their potential for greater specificity towards bacterial molecular targets while allowing fewer off-target effects and less selective pressure for cross-resistance to other mAbs or antibiotics. Importantly, antibacterial mAbs do not damage the human microbiota. mAbs can act through specific and selective mechanisms that block the bacterial virulence factors, limiting the development of bacterial resistance. Several bacteria, such as *B. pertussis*, *Corynebacterium diphtheriae*, *Clostridium botulinum*, *Clostridium tetani*, and *C. difficile*, secrete toxins that cause infectious diseases to which mAbs can be used effectively with a bactericidal effect [45–50]. However, the mechanism of action does not provide for an immediate effect [47,48]. mAbs have also demonstrated efficacy against viral infections like SARS-CoV-2 during the recent COVID-19 pandemic [51–53]. To date, the use of mAbs remains one of the most promising technologies in the era of increasing health threats due to AMR.

Antibiotic Combinations

Some evidence has shown a certain contribution in combating AMR from the synergistic use of antibacterials belonging to different pharmacological families and having different molecular targets, or from the combined use of antimicrobials with bacteriostatic and bactericidal activity. The pharmacological rationale for the use of antibacterial drug synergism is that the inhibition of two molecular targets in the bacterial species, using lower dosages than with single administration, can reduce the development of resistant strains. The use of double antibiotic combinations in some cases has shown greater efficacy than single administration, with a reduction in the emergence of resistant pathogens. Several antibiotics in combination, both newly discovered and older antibiotics, have been used demonstrating good efficacy against ESKAPE pathogens. A combination of fosfomycin and daptomycin was tested successfully against the two Gram-positive members of ESKAPE, *E. faecium* and *S. aureus* [54]. However, combination therapy with antibiotics may be associated with dysbiosis and reduced diversity of the microbiota (Fig. 1). The potential effects of dysbiosis should be considered especially when prescribing antibiotics to children [55].

Conclusions

The fast spread and increase of AMR and its global impact calls for both preventive and therapeutic alternatives to the currently available antimicrobials. In view of this global health threat, we must pursue two parallel avenues urgently: both the optimal use and appropriate disposal of conventional antimicrobial therapies and the application of the most effective alternative strategies, such as the use of human microbiota modulators, monoclonal antibodies, antibiotic combinations, phages, and vaccines. Microbiota modulators are proved to be effective in maintaining and restoring the gut microbiota and its immunological and metabolic function, thus decreasing infection disease susceptibility [56]. While prebiotics and probiotics, used separately or as joint synbiotic therapy, can prevent the colonization of pathogens [57], FMT can clear the pathogen. FMT may also act through the transfer of bacteriophages. mAbs do not damage the human microbiota and can be very specific toward their target molecules, limiting adverse effects. Antibiotic combinations can have negative impact on the microbiota. However, the synergistic action of well-selected compounds broadens their action and promotes positive health outcomes. Phages could represent an alternative to antibiotics for the treatment of bacterial infections mainly caused by the ESKAPE pathogens. Despite a number of promising pieces of scientific evidence in the last decades, including their ability to eradicate biofilms, their lytic spectrum is usually limited to a few bacterial strains or species combined with difficulties in the production and purification process, variable clinical efficacy and unclear safety profile [58], together with absence of national guidelines, make phage therapy an approved medicine just in a few selected countries, otherwise being used as compassionate therapy only [59,60]. Together with prebiotics and probiotics, vaccines can have a prophylactic approach to overcoming pharmacological barriers. However, vaccine formulations toward drug-resistant pathogens present a number of obstacles: different clinical manifestations induced by the same pathogen or multiple circulating serotypes associated with the same disease, commensal microbes showing pathogenic characteristics under certain circumstances only, short-term immunological protection, and ubiquitarity of many microorganisms combined with several host factors are complications that affect vaccine development [61]. Repositioning of non-antibiotic drugs offers a broad and interesting opportunity [62] as an alternative strategy to combat AMR. Other options aiming at restoring the microbial susceptibility to antibiotics and host immunity, i.e., Clustered Regularly Interspaced Short Palindromic Repeats and their associated Cas proteins (CRISPR/Cas system), antisense antimicrobial therapeutics, nanoparticles, antimicrobial peptides (AMPs), and essential oils, need be further explored in the clinical practice. Host-directed alternative therapies, i.e., regulation of pattern recognition receptors (PRRs) signaling pathways or inhibition of the microbial information system (named "quorum sensing") [63], are even more complex to be standardized in respect to subjective immune responses.

Focusing research on the most promising unconventional options for reducing the frequency of drug-resistant infections remains crucial to combat this silent pandemic as well as applying infection prevention and control (IPC) practices and repeated specific training of health professionals, evidence-based information and awareness on AMR targeted to the general public, overall reduction of antimicrobial use and correct disposal, routine practice of rapid diagnostic tools discriminating at least between viral and bacterial infections, together with the use of One Health integrated surveillance. AMR affects the progress towards the Sustainable Development Goals (SDGs) that focus on poverty reduction (SDG1), zero hunger (SDG2), good health and well-being (SDG3), decent work and economic growth (SDG8), and it is a substantive element of the international pandemic preparedness and response.

Availability of Data and Materials

All information included in the text refers to the references listed at the end of the manuscript.

Author Contributions

AV and MS designed the research study. AV, MB, AP, RL and AZ performed acquisition of data. AV and MS analyzed the data. AV, MB and AP wrote the original draft. MS, RL and AZ reviewed and edited the manuscript. MS, RL and AZ supervised the research study. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable. This review included information collected and published by other subjects.

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Conflict of Interest

The authors declare no conflict of interest.

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