

ALTERNATIVE APPROACHES TO ANTIBIOTICS FOR TARGETING BACTERIAL PATHOGENS

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ABSTRACT

Antibiotics once regarded as magic bullets are no more considered so. Overuse of antibiotics in humans, agriculture, and animal husbandry has resulted in the emergence of a wide range of multidrug-resistant (MDR) pathogens which are difficult to treat. Antimicrobial resistance (AMR) is a serious global health problem associated with high mortality in the era of modern medicine. AMR has led to depletion of the antibiotic pipeline and developing new antibiotics is extremely challenging due to technical and financial issues and also resistance emerges as soon any new antibiotic is introduced. Meanwhile, to reduce dependence on antibiotics, other alternatives such as Probiotics, Prebiotics, Bacteriocins, Antimicrobial peptides, Bacteriophages, Essential oils are being explored. This review provides an overview of various promising, potential and under investigative strategies as alternatives to antibiotics.

Key words: AMR, Probiotics, Prebiotics, Bacteriocins, Amp, Bacteriophages, EO's

1. INTRODUCTION

Antimicrobial resistance (AMR) is a rapidly emerging worldwide health concern. Currently, antibiotic resistance causes 700 000 patient deaths worldwide each year (AMR). According to estimates, this death toll will rise to 10 million by 2050, resulting in a minimum 2.5 % decline in the GDP. Without effective antibiotics, treating infections has become extremely difficult. Overuse/misuse of antibiotics in humans, animals, and agriculture, inadequate infection control practices, increased use of invasive devices, inadequate facilities for rapid diagnosis of infections, increased national and international travel are the key contributing factors for the rapid emergence of drug-resistant pathogens [1]. Bacteria have developed AMR by several mechanisms. Among those are enzymatic drug inactivation/modification, altered target production, decreased drug permeability, increased efflux due to over-expression of efflux pumps, bypassing of metabolic pathway/overproduction of target, target mimicry, and others [2].

Antibiotics are no more regarded as magic bullets. There is an urgent need to explore innovative alternatives to antibiotics acting differently by preventing infections, reducing the emergence of resistance by targeting different mechanisms of action (MOAs) or increasing the effectiveness of existing antibiotics [3].

2. REVIEW OF LITERATURE

Novel Strategies to Combat AMR

Probiotics

Probiotics are microorganisms that live in a symbiotic relationship with the host. They provide health benefits and perform several biological functions when provided in adequate amounts. Probiotics were discovered and selected based on certain criteria, which ensure safety and effectiveness requirements. These include organisms such as Lactobacillus, Bacillus, Bifidobacterium, Saccharomyces boulardii, non-pathogenic strains of E.coli, Clostridioides, Veillonella [4]. Probiotics have been found to help with a variety of pathological conditions, including constipation, diarrhea, polycystic ovarian syndrome, ulcerative colitis, stress and anxiety, inflammatory bowel disease, breast cancer, and diabetes.

The mechanism of action of probiotics are probiotics competitively exclude pathogens by a variety of mechanisms, including competing with them for nutrients, blocking the epithelial adhesion sites, and decreasing the intestinal lumen pH [5]. Production of antibacterial compounds. Compounds that are produced in the metabolome of probiotics include organic acids (butyric, lactic, and acetic acids), bacteriocins, hydrogen peroxide, amines and peptides, which not only antagonize opportunistic pathogens but also play a crucial role in regulation of host cellular proliferation, differentiation, inflammation, angiogenesis, and metastasis [6]. Promoting the synthesis and secretion of mucus by intestinal goblet cells to form a protective layer against pathogens [7].

Prebiotics

Prebiotics are defined as "non-digestible food materials that beneficially impact the host by selectively enhancing the growth and/or metabolism of bacterial species inhabiting the GIT, and thus improve the host health" [8]. Prebiotics are non-digestible oligosaccharides (fructans, inulins, xylans, galactans, and mannan), fibers (pectin, non-starch

polysaccharides (such as β -glucan), xylooligosaccharides, and isomaltooligosaccharide), and seeds containing gums. To use prebiotics as alternatives to antibiotics, specific criteria must be met. Prebiotics should have a well-identified source chemical composition and structure, be a pure product, be at a suitable dose, and have been assessed in animal models or 3D cells to confirm their safety and beneficial health impact on the microflora [9]. When used as feed additives for livestock and poultry, prebiotics have shown an ability to improve host health and productivity via selective stimulation of proliferation and metabolism of the gut microbiota, such as *Akkermansia* spp., *Christensenella* spp., *Propionibacterium* spp., *Faecalibacterium* spp. and *Roseburia* spp., *Lactobacillus* spp., and *Bifidobacterium* spp. [10]. Prebiotics have also shown potential to eliminate harmful bacteria, such as *Salmonella*, *Campylobacter*, *Clostridium* and *E. coli* [11]. It was reported that the activity of probiotic *Bifidobacterium* strains against *C. difficile* was significantly stimulated in the presence of five prebiotics (oligosaccharides) [12]. Similarly, the activity of *Pediococcus acidilactici* was enhanced against *E. coli*, *Salmonella*, *E. fecalis* and *S. aureus* in the presence of garlic and basil (natural prebiotics) [13].

Bacteriocins

Bacteriocins are a specific kind of ribosomally-synthesized AMPs of a length of 20–60 amino acids, cationic and hydrophobic, produced by many species of bacteria and archaea [14]. Studies have noted that antimicrobial peptides can act as bioprotectors against spoilage and pathogen contamination since they have shown excellent antimicrobial activity against gram-positive and gram-negative bacteria. Additionally, they prevent the proliferation of thermophilic, spore-forming microorganisms [15]. Nowadays, one of the most relevant safety problems in the food industry is cross-contamination with bacteria such as *Salmonella* spp., *Shigella* spp., *Micrococcus* spp., *Enterococcus faecalis*, *Bacillus licheniformis*, *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Campylobacter jejuni*, *Yersinia enterocolitica*, *Vibrio parahemolyticus*, *Escherichia coli* 0157:H7, and *Clostridium botulinum*. Nisin, a bacteriocin produced by *Lactococcus lactis*, is a legally approved natural preservative for dairy products, canned vegetables, juice, alcoholic beverages, meat, and fish used to prevent food-spoilage caused by *Lactobacillus* spp, and prevents the growth of *L. monocytogenes*, *S. aureus* and *Clostridium* spp. [16], also increases shelf-life without changing the flavor, texture or aroma, particularly does not alter the physical, chemical and biological properties [15].

Antimicrobial Peptides

Anti-microbial peptides (AMPs) also regarded as cationic host defense peptides are a highly diverse family of small proteins with a varying number of amino acids [17]. These AMPs found to have a variety of biological activities such as antitumor, anti-inflammatory, antibacterial, antifungal, antiviral, and antimitogenic activity, in addition to their ability to act as immune modulators. AMPs are proven to be effective against MDR pathogens, hence they are potential candidates for combating AMR. AMPs possess several advantages; produce microbicidal activity in the micromolecular range, rapidly kill bacteria, and have low resistance selection. Furthermore, they demonstrate antibacterial action by interfering with multiple targets; alter the cell membrane, interfere with the formation of protein and cell wall, and others [18]. Some examples of AMPs are Clavanin A, α -helical peptides, Amyloidogenic peptides, Tachyplesin-1, thermore, hepcidin etc Microcin J25 was found to bind to the secondary channel of the RNA polymerase and block trigger-loop folding. LL-37 was shown to inhibit *E. coli* via stopping the activity of palmitoyl transferase PagP [19]. NP-6 was found to inhibit the β -galactosidase activity of *E. coli* [20].

Bacteriophages

Phages or bacterial viruses are obligate parasites that infect bacteria and archaea. Phages are classified according to their size, shape, type of nucleic acid and mechanism of action in the host bacterial cell [21]. In vitro trials showed that phages have many advantages over antibiotics, including high host specificity (phages can target one strain of bacteria without perturbing the human or animal gut microbiota, while antibiotics do not distinguish between pathogenic and beneficial bacteria); cost effectiveness and time saving; inhibition of multi-drug-resistant bacteria while antibiotics increase them; ease of delivery to the target site and ability to penetrate the blood–brain barrier; no antagonistic effect detected between phages when given as a cocktail (mixture of different phages); that phages could prevent biofilm formation [22].

The majority of phages are virulent (lytic) with a negligible probability of becoming temperate (lysogenic) [23]. The lytic cycle of virulent phages starts by attachment of tailed phage to the cell surface receptors of a host. This interaction is two steps, a reversible phase followed by an irreversible phase. After the irreversible attachment, the lysis enzymes break down the host cell wall and the phage ejects its genetic material into the host cell with the assistance of processive host enzymes. The phage genome then manipulates the host cell metabolism by redirecting it for DNA replication and protein biosynthesis to the production of phage particles (nucleic acids and capsids); the host cell genome is degraded at this stage, followed by phage particle assembly and host cell lysis. The new viral particles are then released to re-

attach to a new host cell. The lytic cycle of bacteriophages is performed by key phage proteins (holins) that permeabilize the host cell membrane and endolysins that degrade cell wall peptidoglycan. Consequently, the bacterial cells lose their cell wall integrity and the selective permeability of cell membranes, eventually resulting in cell lysis due to osmotic disruption [24].

Bacteriophages have also been approved for prophylaxis and treatment of infections due to *Salmonella* (PLSV-1™) and *Clostridium perfringens* (INT-401™) in poultry. Fixed phage mixtures are commercially available for biocontrol of food-borne pathogens such as *E. coli*, *Salmonella* serotypes and *Listeria monocytogenes*, *Shigella* and for surface disinfection [25].

Essential Oils (EOs)

Essential oils are volatile, aromatic, and oily liquids extracted from plant parts, such as seeds, leaves, buds, twigs, flowers, bark, herbs, wood, fruits, and roots [26]. Plants generate EOs as a natural defense against pathogens and herbivore feeding by reducing the appetite of herbivores. EOs are complex natural mixes that contain anywhere from 20 to 60 distinct components in various proportions. The antibacterial effects of EOs are dictated by their primary ingredients (85%), which include terpenes, terpenoids, and aromatic and aliphatic groups from different natural source [27]. EOs and their components are characterized by their hydrophobic nature that allows them to interact with the lipids of the microbial cell membrane [28]. They can sensitize cells and cause severe membrane damage, resulting in leaking of essential intracellular contents, bacterial cytoplasmic membrane collapse, and bacterial cell death. Cell wall breakdown, cytoplasmic membrane damage, cytoplasm coagulation, and membrane protein degradation are the common causes of the leakage [29].

Menthol, pulegone, linalool, thymol and camphor, extracted from *Salvia lavandulifolia*, *Lavandula angustifolia*, *Mentha piperita*, *Mentha pulegium*, and *Satureja montana*, respectively, have shown antagonistic effects against *P. aeruginosa*, *S. pyogenes*, *S. mutans*, *S. sanguis*, *S. salivarius*, and *E. faecalis* [30]. *Epilobium parviflorum*, *Salvia desoleana*, *S. sclarea*, and *Allium sativum* were reported to produce palmitic acid, linoleic acid and α -linolenic acid, which have shown an ability to inhibit *E. faecalis*, *S. aureus*, *P. aeruginosa*, *S. epidermidis*, and *E. coli* [31]. Moreover, cinnamomum was reported to produce cinnamaldehyde, which was shown to inhibit *E. coli*, *S. aureus*, and *S. typhimurium* [32]. *Dipterocarpus gracilis* was reported to produce elemicin and geranyl acetate, which were shown to suppress *B. cereus* and *Proteus mirabilis* [33].

3. CONCLUSION

Antimicrobial resistance in the number of pathogens is considered one of the most serious global health concerns. Infections caused by MDR pathogens are difficult to treat, often require expensive and sometimes toxic drugs for therapy. Numerous alternatives to conventional antibiotics have been developed to combat antimicrobial resistance and treat bacterial infections. Research efforts have been made to limit AMR in both humans and animals by exploring various interventions, including probiotics, prebiotics, phage therapy, EOs, AMPs, as potential replacements for antibiotics.

Despite the promising role of most of these strategies in promoting host immunity and in antagonizing a range of human and animal pathogens, their variable effects, combined with their limited spectrum, safety concerns, and poor efficacy, are among the potential limitations to their use. Nonetheless, the exuberant development of molecular technologies may improve the efficacy of existing strategies and reduce their limitations.

With millions of people travelling around the world and the uncontrollable spread of AMR, holistic AMR control requires global solidarity to expand and implement robust antimicrobial stewardship programs in both medical and veterinary practices.

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