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Alternative strategies for fighting multidrug resistant bacterial infections

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ABSTRACT

The unprecedented successes in antibiotic therapy led the scientists to consider that the war against infectious diseases has finished, but the emergence of resistant pathogenic bacteria has stopped this enthusiasm. Nowadays, the effectiveness of antibiotic therapy is continuously decreasing, due to the emergence and spread of antibiotic resistance among many important clinical pathogens, raising an acute need to find new, more effective antibiotics or other promising alternatives, to effectively treat the frequently occurring life-threatening infections. However, we are confronting an "innovation gap" in the development of novel antibiotics. The absence of measures and solutions to prevent the emergence of resistance and to combat pathogens will be felt both in the case of infectious diseases, whose treatments are based on antibiotics and in current routine medical practices, such as invasive diagnostic techniques, transplants, implantation of prosthetic devices. These side effects are difficult to quantify but threaten to cancel important progress in medical practice. Because of this, there is a great need for implementing national/worldwide policies for the rational use of antibiotics and also, to reinvigorate anti-infective strategies that involve the development or use of alternative methods including anti-infective compounds that act through new mechanisms of action. This minireview is presenting some of the currently proposed alternative strategies that could be used instead of antibiotics to prevent or treat bacterial infections produced by resistant strains.

Keywords: *antibiotic resistance, alternative anti-infectious strategies, antimicrobial peptides, essential oils, metal based antimicrobials.*

1. INTRODUCTION

The unprecedented successes in antibiotic therapies in the late 1960s led to the famous statement made by Dr. William H. Stewart: "It is time to close the book on infectious diseases and *declare* the *war against* pestilence *won*" [1]. The emergence of resistant pathogenic bacteria, at the same time with the widespread use of antibiotics, has made the magic bullets to lose their efficacy and the euphoria of victory to last no longer. Penicillin resistant strains were highlighted in 1940, following the success of penicillin use for treating bacterial infections among soldiers wounded during the Second World War. The effectiveness of antibiotic therapy has been decreasing lately, due to the emergence and spread of antibiotic resistance among pathogens. This justifies trying to find new, more promising and more effective antibiotics against various life-threatening infections. But, unfortunately, only a new class of antibiotics has been introduced into the clinical treatment over the last 20 years, namely oxazolidinones, and the other ones were only modified variants of the existing ones [2].

The absence of measures and solutions to prevent the emergence of resistance and to combat pathogens will be felt both in the isolated cases whose treatments are based on antibiotics and in current routine medical practices, such as diagnosis interventions, surgery and transplants. These side effects are difficult to quantify but threaten to dramatically cancel important progress in medical practice.

Most surgery will lead to an increased risk of mortality because these techniques are dependent on the efficacy and availability of antibiotic prophylaxis to combat infections that occur despite good medical practice.

The rapid evolution of pathogens has allowed for the rapid development of antibiotic resistance. Resistance is "the ability of an organism to survive and multiply in the presence of a high level of antimicrobial agent"[3]. There are five general strategies whereby a pathogen can develop antibiotic resistance: modifying the target molecule of the antibiotic, producing enzymes that destroy/modify the drug, passive impermeability mediated by loss of porins, pumping the drug out of the bacterial cell by efflux pumps and circumventing certain metabolic pathways inactivated by the antibiotic.

The emergence of drug resistance has been a constant threat since the beginning of the antibiotic discovery era. The gap between the introduction of a new antibiotic in clinical treatment and the emergence of resistance to the respective antibiotic is only a few years. For example, penicillin-resistant *Staphylococcus aureus* strains were isolated only two years after the introduction of the penicillin antibiotic into the market [4].

Currently, due to the depletion of antibiotic reserves, it can be argued that the presence of multiresistant bacteria is a frightening problem. It is estimated that about 700,000 people currently die from bacterial infections caused by multidrug-resistant pathogens [5]. *Mycobacterium tuberculosis*multidrug-resistant strains lead to increased mortality rates. In India, neonatal infections with resistant strains cause the death of over 60,000 newborns annually. It is estimated that in three decades, the number of deaths due to antibiotic resistance worldwide could

exceed the number of cancer deaths [6].Because of this, there is a great need for an antibiotics rational use policyworldwide / nationally. On the other hand, it is critical to reinvigorating anti-infective defense strategies that involve the development or use of alternative methods including anti-infective compounds that act through new mechanisms of action.

2. ANTIMICROBIAL PEPTIDES

Currently, antimicrobial peptides (AMP) are increasingly viewed as an alternative to conventional antibiotics and their mechanisms of action are actively investigated. AMPs are molecules with sizes between 2 and 10 kDa and usually contain between 8-50 different amino acids. The sources of AMP isolation are varied (bacteria, protozoa, fungi, plants, insects, animals) and their role in living organisms is the ability to act as effectors of the natural defense mechanisms. Due to their positive net charge and amphipathic properties, their three-dimensional structures favor interactions with bacterial membranes [7, 8].

1.1. Catelicidins.

Catelicidins are small molecules mainly stored in neutrophils and macrophages [9]. The first studies that were completed with the identification and isolation of catelicidins were initiated following the analysis of the immune system of insects in 1980 [10]. These peptides possess a broad spectrum of activity against bacteria, fungi and viruses. The role of catelicidins in anti-infective protection has been demonstrated by suppressing the synthesis of these peptides in mice and thus inducing the sensitivity of the organism to infections [11].

Many studies have demonstrated the importance and usefulness of the synthesis of peptides of similar structures and derived from catelicidins in the treatment of *Pseudomonas aeruginosa* and *Staphylococcus aureus* resistant infections but also of burn-associated infections [12, 13]. The potential to increase susceptibility to aminoglycosides and to bind microbial DNA has been investigated[14, 15, 16]. Gene therapy to potentiate the synthesis of catelicidins it is a strategy that can be considered in the future to kill or inhibit resistant pathogenic microorganisms [5, 17].

1.2. Defensins.

Defensins have been described since 1985 as cysteine-rich cationic antimicrobial peptides, similar to catechidins. In addition to powerful microbicidal activity, the defensins also interfere with adaptive immune system by inducing T-cell and monocytes chemotaxis, and dendritic cells maturation. Furthermore, they can induce the release of various mediators such as histamine and prostaglandin D2 and modulate host defense by activating certain receptors [18].Depending on preserved disulfide bridges, the defensins can be divided into α and β defensins that have a linear structure and are found in all vertebrates and defensins θ that have a cyclic structure and are found only in certain primates [19].

The mode of action of defensins consists in the direct lysis of microorganisms through increasing the permeability of the cell membrane resulting from the formation of electrostatic bonds between defensin arginine groups and membrane phospholipids. Defensins in low concentrations may inhibit the synthesis of peptidoglycans in the bacterial cell wall, by sequestering lipids II. In addition, they can bind to polyanionic molecules such as DNA after penetration into the bacterial cell [20].

Various defensin analogs have been synthesized and tested which possess improved properties such as resistance to proteolysis, reduced cytotoxicity and efficacy against a broad spectrum of major clinical microorganisms, including mycobacteria [21, 22, 23, 24]. Due to studies that have examined *in vitro* the potential of defensins as a treatment for cure-free infections, it has been found that these peptides could represent a new method of prophylaxis through direct effects on pathogens but also of treatment, when associated with currently used chemotherapeutic agents. However, applying these peptides as alternative treatment methods requires more research in order to investigate the probability of selecting resistance.

1.3. Lactoferrins.

Lactoferrin belongs to transferrins family and has the ability to bind iron. This glycoprotein is found in milk, as well as a constituent of the innate immune system, in lacrimal, salivary, nasal and genital secretions [25]. The antibacterial and antifungal effects are due to iron binding capacity. In addition to iron binding capacity, lactoferrin has been shown to be effective in binding endotoxins, an event that could prevent endotoxins from triggering a strong immune response that would cause septic shock [26]. Lactoferrin in combination with other peptides such as lysozyme and lactoferrin homologues have been shown to have improved microbicidal or microbiostatic effects. Derivatives such as lactoferampin and lactoferricin are described as peptides with antifungal, antiviral, antimicrobial properties, but also antibiofilm formed by one of the most dangerous nosocomial bacteria, namely *Pseudomonas aeruginosa* [27, 28].

Starting from the baseline studies of experimental infections that have the effect of prophylactic and therapeutic activity of lactoferrin as an antimicrobial agent and as modulator of the anti-inflammatory immune response, it was concluded that this glycoprotein is a promising lead for the biotechnological development of antimicrobial compounds [29, 30]. In-depth studies are required to support the practical application of native lactoferrin or homologues as unique agents for prophylaxis or in combination with antibiotics or probiotics in infection control.

The main advantages of using antimicrobial peptides are fast action, broad spectrum activity and possibly a low level of resistance. Among the disadvantages would be reduced activity in physiological liquids, sensitivity to pH variations and proteolytic enzymes, and a high cost of synthesis.

3. METAL BASED ANTIMICROBIALS

Although the mechanism of the antimicrobial action of metals is incompletely elucidated, they have been used before antibiotics due to their antimicrobial properties. Nowadays, many pharmaceutical preparations contain metals and are used in the treatment of various diseases, including infections.

2.1. Silver.

Medical uses of silver date back to the time of Hippocrates ("father of modern medicine"), who in his writings discusses the role of silver in wound care [31]. During the First World War, the soldiers used silver leaves to limit and treat the wounds. Silver has disappeared around World War II due to the emergence of antibiotics. Interest in the antimicrobial properties of silver was revived in 1960 by Moyer, who published a paper on antibacterial effects of silver nitrate [32]. Today, silver has re-emerged as a clinical treatment for the prevention and treatment of infections encountered in wounds, ulcers and burns [33].

It is believed that the release of silver ions is the basis for antimicrobial activity. Silver in its ionized form reacts with thiol groups of enzymes, inhibiting vital processes for the survival of microorganisms. Silver can prevent cell division and may induce membrane lesions by adhering to the plasma membrane and perturbing the natural electrical potential [34]. Silver ions are known to have toxic effects on bacteria, viruses, fungi and some aquatic organisms [35]. In the human body, it is known that silver has a low level of toxicity due to the fact that human body fluids contain large amounts of chlorine and sulfur ions, which form insoluble salts with silver ions and thus reduce silver toxicity. However, after long-term ingestion of colloidal silver, cases of arrhythmia and irreversible neurological toxicity followed by death have been reported [36].

2.1.1. Colloidal silver preparations. Colloidal silver solutions contain positively charged silver particles suspended in liquid media. These have traditionally been used up to Moyer's studies, which have led to the replacement of solutions with silver salts due to an improved formulation that has enhanced stability, an easier ionization capacity, and a reduction in precipitation formation [32]. Despite these drawbacks, today, silver ions are used to control bacterial growth in a variety of medical applications, including dental medicine, catheters and burn healing. Silver, metal or salt molecules can be incorporated into gels, creams and ointments for topical applications, and have biocidal effects at the wound site. These preparations may also include other antimicrobial products or wound healing products in a mixture or complex with silver metal [37].

2.1.2 Silver salts. Silver nitrate is the most commonly used silver salt, exerting an antibacterial action (*in vitro* and *in vivo*) at a concentration of 0.5% and a significant toxic effect at a concentration greater than 1%. Silver nitrate has traditionally been administered to neonates for the prevention of neonatal conjunctivitis [38].

2.1.3 Silver nanoparticles. Nanoparticles (NP) are a wide class of materials that include particulate matter with at least a size less than 100 nm. Their importance has been highlighted when researchers have found that size can influence the physico-chemical properties of a substance, such as optical properties [39].

Nanoparticles have completely new properties based on specific features such as shape, size and distribution [40]. Metals have physical and chemical properties different from the metals they come from, such as lower melting points, larger specific surfaces, specific optical properties, mechanical strength and specific magnetizations, properties that might become attractive in various industrial applications [41]. Many commercially available consumer products contain NP, especially cosmetic and sunscreen products. NPs have also been used in various fields of biology and medicine, including tissue engineering, pharmacology, and so on [42]. The positive electrical charge of silver nanoparticles gives them the possibility of binding them to the negatively charged surface of bacteria, which explains and amplifies the bactericidal effect [43, 45].

These characteristics have led to different approaches (e.g. incorporation of nanoparticles into solids or their diffusion into drugs) and numerous hypotheses on the mechanism of action have been proposed:

a) Release of toxic ions (Cd^{2+}, $Zn^{2+,}$ Ag^{+}) that bind to the sulfur-containing proteins of the cell membrane and interfere with cell permeability;

b) Toxic ions that can cause DNA damage;

c) Interruption of electron transport, oxidation of proteins and collapse of membrane potential due to contact with CeO_2 or nC60;

d) Generation of reactive oxygen species that may cause cell membrane disruption.

These mechanisms may act simultaneously [44, 45].

The antimicrobial mechanism of nanoparticles is most likely linked to their surface properties. In other words, the smallest nanoparticles possess the most powerful antimicrobial effect. So there is no doubt that size matters and this is one of the reasons why nanoparticles are considered a real improvement in antimicrobial strategy. Although antibiotic molecules are smaller than any nanoparticles, it should be borne in mind that the size of small proteins is 5-10 nm in the range of nanoparticles that may vary from 1 to 10 nm. Silver nanoparticles possess low toxicity for human cells, broad spectrum activity against bacteria, and a much lower probability of microorganisms to develop resistance than conventional antibiotics [46].

The field of nanotechnology is developing on a day-by-day basis, having an important impact on people's lives and creating enthusiasm for growth in life science, especially biotechnology and biomedical science. Nanocrystalline particles are a breakthrough in the field of high sensitivity bimolecular detection and diagnostics, therapeutic and antimicrobial catalysis, and microelectronics [40]. The use of nanotechnology in various therapeutic areas has revolutionized the field of medicine, where nanoparticles are designed and used for therapy, diagnosis and biomedical research tools. With nanotechnology, it is now possible to offer therapy at a molecular level that can further help in the treatment and pathogenesis of the disease [47, 48].

The bactericidal activity of silver is well documented. Its benefit in reducing or preventing infection can be used in many applications, from topical treatments for burns and chronic wounds to temporary and permanent medical devices incorporating silver. An ongoing study would be needed to define areas where silver provides benefits. Silver remains a reasonable addition to count against infection and relatively few side effects. However, the benefits of silver-containing products and known side effects and the other options available in choosing the most appropriate therapy should be weighed.

2.2. Copper.

Metals have had medical uses since the ancient age. The earliest dates appeared around 2400 B.C. in an Egyptian manuscript where copper was used to disinfecting wounds and decontaminate drinking water, even though bacteria were not found [49]. The copper antimicrobial mechanism involves redox reactions and the production of hydroxyl radicals through the reactions of Fenton and Haber-Weiss. The oxygen intermediates are highly reactive and cause lipid peroxidation and protein oxidation. Copper ions can inactivate proteins by destroying Fe-S groups and induce mutations in bacterial DNA. Studies have been conducted that have tested the antimicrobial activities of copper and copper nanoparticle objects and materials in hospitals and have been shown to be highly effective in reducing nosocomial pathogens [50, 51, 52].

4. NATURAL PRODUCTS

Over time, plants have been indispensable sources of treatment in traditional medicine in disease management and can provide a multitude of possibilities to combat resistance [53, 54]. Traditional remedies, which consist mainly of plant/extracts components, are natural, cheap and easily accessible, are readily accepted by the community.

The plants possess a wide range of biological activities, primarily linked to the presence of bioactive secondary metabolites or varied phytocompounds, i.e., glycosides and alkaloids, anthocyanins, coumarins, flavonoids, saponins and terpenoids [55, 56]. Therefore, any potent phytocenteric substance exhibiting broad spectrum antimicrobial activity may serve as an ideal alternative to conventional antibiotics. Bacterial resistance to antibiotics usually involves a single mechanism by which bacteria can occur, for example: inactivating or modifying the drug, modifying the target and decreasing drug accumulation by decreasing permeability and / or increasing efflux; instead, microbial cells can be affected by secondary metabolites of plants by various and multiple mechanisms [57]. The mechanisms that underlie the antibacterial properties of phytocompounds, include disruption of cell membrane function and structure [58, 59], interruption of DNA synthesis, interference with intermediate metabolism, induction of coagulation of cytoplasmic constituents and inhibition of the quorum sensing (QS) process [60, 61].

In view of their effectiveness, efforts are being made to develop standardized treatment protocols by which traditional medicine could be integrated inmodern medical practice.

Essential oils.

The emergence of microorganism resistance to chemical and conventional drugs has led to the search for new sources of broad spectrum biocides. Since ancient times, plants and their derivatives, such as essential oils (EO), have been used in traditional medicine. In nature, EOs play an important role in mediating interactions of plants with the environment by attracting insects that disperse pollen and seeds or to take away other unwanted insects [62]. EOs are secondary metabolites with strong odors produced by aromatic plants, which are often an important part of traditional pharmacopoeia [63]. Essential oils are composed of variable mixtures of terpenoids, especially monoterpenes (C10) and sesquiterpenes (C15), although diterpenes (C20) may also exist [64].

EOs are liquid, volatile and are soluble in lipids and organic solvents having a lower density than water [65]. They may be present in all plant organs including buds, flowers, leaves, seeds, branches, stems, flowers, fruits, roots, wood or bark, but are generally stored by the plant in secretory cells, caries, glands, or epidermal cells [66]. EOs can inhibit or slow the growth of bacteria, yeasts and fungi by acting on a cascade of reactions involving complete alteration of cell morphology [67]. The antimicrobial activity of EO, similar to all natural extracts, is influenced by the composition and amount of specific compounds. Identifying the mechanism of action of EO requires the study of each component [68]. For example, the potentiation of antimicrobial activity occurs in the presence of increased concentrations of cinnamic aldehyde, eugenol or citral of EO [69, 61, 70]. Monoterpenes and phenols present in EOs of thyme, sage and rosemary possess antimicrobial, antifungal and antiviral properties [71, 57, 72]. EO from basil, sage, rosemary and oregano are active against strains of *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* and *Salmonella spp*., but are less effective against *Pseudomonas spp.* [73, 74, 75, 76]. Most EOs have a stronger effect on Gram-positive bacteria than on Gram-negative species, and this effect is most likely due to differences in cell membrane compositions [77].

5. PASSIVE IMMUNOTHERAPY

Immunotherapy is a promising treatment option for bacterial or viral infectious diseases. Laboratory animal studies and clinical studies have provided evidence of the effectiveness of passive immunization with monoclonal antibodies for the prophylaxis or treatment of infectious diseases [78, 79, 80, 81]. Treatment with monoclonal antibodies specific for F protein of the respiratory syncytial virus was approved by the US Food and Drug Administration and used in clinical cases [82]. Currently, the only treatments available to prevent infections with resistant strains acquired in immunocompromised patients are "last resort" antibiotics. However, excessive use of these still effective antibiotics results in the appearance of multi-resistant strains. For this reason, monoclonal antibody therapy would be a solution in treating infections and lethal diseases.

5.1. Anti-QS antibodies.

It is vital to develop innovative approaches for the prevention and treatment of multiple or pan-drug resistant infections. Immunological strategies work through mechanisms that differ from those of antibiotics and provide attractive goals for a new method of antibacterial treatment. The QS ensures the activation of certain sets of genes as a result of the rapid and coordinated response of bacteria to an increase in population density, a phenomenon that contributes to the pathogenesis and resistance of microorganisms [83]. Pathogenic microbial behavior is governed primarily by the QS system, which includes self-inducing, downstream receptors and proteins. Bacterial autoinducers can be classified into three major chemical groups: i) *N*-acyl homoserine lactones (AHLs) that have been shown to be produced by over 70 species of Gram-negative bacteria, ii) oligopeptides, which are generally employed by Gram-positive bacteria, and iii) the ribose-like *S*-4,5-dihydroxy-2,3-pentanedione (DPD)/autoinducer-2 (AI-2), which is utilized by both Gram-negative and -positive bacteria and, thus, can be regarded as an interspecies QS signaling molecule [84].

In various studies, there have been developed methods to treat and monitor bacterial infections with *Pseudomonas aeruginosa* by administering immunoglobulins having a high affinity to HSL-derived signaling molecules. In addition to reducing the amount of extracellular signaling molecules and therefore in bacterial virulence, therapy may facilitate monitoring of bacterial load and assessment of the progress of the infection. Antibodies could be monoclonal or polyclonal which should be obtained from an animal host stimulated by antigen injection [85, 86, 87].

The importance of anti-QS antibodies is reflected in an increase in the number of extensive research in this area over the last few years. More concerted efforts are needed to understand the mechanism and possibilities of widespread application of anti-QS antibodies against infectious and multidrug resistant strains.

6. CONCLUSIONS

Antimicrobial resistance is a priority research area, and mitigation strategies are further planned and explored; however, its impact and spectrum are expanding at a much faster pace. Infection treatment is currently challenging for both microbiologists and clinicians. The alternative strategies suggested in this review cannot yet completely replace antibiotics as treatment agents, but can be successfully implemented for a preventive or adjuvant therapy, in combination with antibiotics. Another absolutely pivotal strategy is the cautious use of antibiotics. At the same time, it is necessary to strengthen the strict surveillance and enforcement of laws, together with the policies related to their use.Overall, the dual strategy, namely alternative measures suggested along with the moderate use of antibiotics, will diminish the current magnitude of the antimicrobial resistance phenomenon. Moreover, a global, multidisciplinary, long-term approach is needed to develop the new diagnosis and identify critical control points. Implementing and sustaining educational programs targeting both clinicians and consumers, as well as international collaboration between researchers and policy-makers could reduce the spread of antimicrobial resistance. Control of antimicrobial resistance should be considered a global priority before it becomes too late.

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8. ACKNOWLEDGEMENTS

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