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Bacterial Biofilms in Diabetic Foot Ulcers: Potential Alternative Therapeutics

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Abstract

Diabetes *mellitus* is a major health problem that affects approximately 171 million people globally. One of its most severe complications is the development of diabetic foot ulcers (DFU). Ischemic and neurophatic lesions are of major importance for DFU onset; however, it is the infection by multidrug-resistant and biofilm-producing microorganisms, along with local microenvironmental conditions unfavorable to antibiotics action that ultimately cause infection chronicity and lower limbs amputation. Novel therapeutic protocols for DFU management are extremely urgent. Bacteriophages, probiotics and antimicrobial peptides (AMP) have recently been proposed as alternatives to currently available antibiotics. Bacteriophages are viruses that specifically infect and multiply within bacterial cells. Their ability to diffuse through polymeric matrixes makes them particularly efficient to eradicate biofilm-based bacteria. Promising results were also observed with probiotic therapy. Probiotics are well-characterized strains with the ability to compete with pathogenic microorganisms and modulate the host immune response. AMP are molecules produced by living organisms as part of their innate immune response. Unlike conventional antibiotics, AMP also act as immunomodulators and resistance to AMP was rarely observed, supporting their potential as therapeutic agents. These innovative therapeutic strategies may in the future substitute or complement antibiotherapy, ultimately contributing for the decrease in multidrug-resistant bacteria dissemination.

Keywords: antimicrobial peptides, antimicrobial resistance, bacteriophages, biofilm, diabetic foot ulcer, probiotics

1. Introduction

Diabetes *mellitus* is a serious health problem in rapid expansion worldwide. It is estimated that there are 171 million diabetic patients worldwide and this number is expected to double by the year 2030 [1]. Diabetic foot ulcers (DFU) are one of the most frequent complications of diabetes, resulting from a complex interaction of factors, namely ischemia and neuropathy [2].

Neuropathy, which is characterized by modifications in sensitive and autonomic functions, causes ulceration due to trauma or excessive pressure in a deformed foot without protective sensibility. Autonomic neuropathy causes dryness of the skin by decreasing sweating, and therefore the vulnerability of the skin to break down increases. Once the protective layer of skin is damaged, deep tissues are exposed to bacterial colonization [3].

Diabetes-associated ischemia is caused by peripheral arterial disease. Poor arterial inflow decreases blood supply to ulcer area and is associated with reduced oxygenation, nutrition, and ulcer healing [3].

These ulcers are frequently colonized by pathogenic bacteria and infection is facilitated by immunological deficits related to diabetes [4], rapidly progressing to deeper tissues, increasing the presence of necrotic tissue, rendering amputation inevitable [5]. In fact, diabetic patients frequently require minor or major amputations of the lower limbs (15-27%) [2], which not only contribute dramatically to high morbidity among diabetic patients, but is also associated with severe clinical depression and increased mortality rates [6].

Although ischemic and neuropathic changes have the initial role in DFU pathophysiology, in the majority of cases it is the infection by multidrug-resistant microorganisms and the unfavorable microenvironmental conditions to the action of antibiotics that leads to amputation [5].

Diabetes-associated foot ulcer infections are predominantly polymicrobial and several bacterial genera can be part of the DFU microbiota, namely *Staphylococcus*, *Pseudomonas*, *Streptococcus*, *Enterococcus*, *Corynebacterium*, *Acinetobacter*, *Prevotella*, *Porphyromonas*, and members of the family *Enterobacteriaceae*. The predominant Gram-positive and Gram-negative species present in DFU are *Staphylococcus aureus* and *Pseudomonas aeruginosa*, respectively [7–9].

There is, to date, little understanding of the ecology of such chronic infections, but bacterial biofilms seem to play a major role [10]. These are ubiquitous and complex structures consisting of an interactive community of polymicrobial cells embedded in a self-produced extracellular matrix of hydrated polymeric substances, such as proteins, polysaccharides, nucleic acids and others, irreversibly attached to the biological surface of the ulcer. These characteristics make them recalcitrant to the action of most antibiotics and also resistant to the innate immune system [11].

administration of biofilm-based infections generally requires local surgical procedures as well as antibiotic administration. However, in infected DFU, because of deficient vascularization, antibiotics frequently reach the local ulcer microenvironment only at subtherapeutic concen-

trations [5]. Even when topically applied, antibiotics rarely reach bacteria that reside within mature biofilms at therapeutic concentrations [12].

Biofilm formation is a major mechanism of adaptation that is able to protect bacteria from antibiotics, due to several physiological traits. Firstly, biofilm spatial structure provides a protective coat against antimicrobial compounds. Secondly, in most cases, biofilms are polymicrobial, formed by complex mixtures of different species. It was proposed that, in such biofilms, the chemical interactions that occur between polymeric substances produced by different bacterial strains might lead to a more viscous matrix, impairing the contact between the bacterial cell wall and the antibiotic. Lastly, the production of degradative enzymes by different pathogenic species can act synergistically against antimicrobial compounds. These biofilm features are responsible for a reduced diffusion of the antibiotic within the biofilm matrix [13, 14].

In addition, patients suffering from DFU face the emergence and dissemination of antibiotic resistant bacteria, which is not a recent biological phenomenon. Seventy years ago, after the discovery of penicillin and the beginning of the antibiotic era, Alexander Fleming noticed the emergence of bacterial strains resistant to penicillin. Indeed, resistance began to appear in target microorganisms, including *S. aureus* isolates from hospitals, a few years after the introduction of penicillin into medical practice [15]. Fleming described the occurrence of antibiotic resistance and warned the scientific and medical community of this phenomenon in his Nobel Prize lecture in 1945 [16].

Several causes can explain the emergence and dissemination of antibiotic resistance. Firstly, the overuse and, most importantly, the misuse of antibiotics in different but interconnected areas, like human and veterinary medicine, agriculture and animal production. Secondly, the effects of antibiotic compounds in the environment are not yet completely described and understood. Finally, antibiotic compounds are stable and static chemical substances that are used to fight living and evolving bacterial cells [17]. Microorganisms, namely bacteria, are ubiquitous and interact with all other living beings. Considering that nature is a highly complex system supported by extremely dynamic interactions and exchanges between all its elements, the emergence and evolution of bacterial populations able to resist against antibiotic substances is not surprising. In fact, over the last decades, microbiologists have demonstrated the influence that antibiotics exert upon bacterial populations. Previously seen as miracle drugs, capable of virtually eradicating all species of bacteria, antibiotics are now seen as substances with limited antimicrobial capacity and multifaceted proprieties. These compounds have the ability to induce or inhibit different bacterial responses and to influence bacterial virulence and survival strategies [18, 19].

As mentioned above, biofilm formation is a well-known virulence factor of some bacterial strains that, along with many other advantages, confers them a protective layer against adverse elements. Recently, it was demonstrated that some antibiotics are able to induce this adaptive strategy. In 2005, when Hoffman *et al*. [18] were testing the efficacy of aminoglycosides, a widely exploited antibacterial therapeutic agent, against biofilm-forming bacteria, they observed an unexpected bacterial response. Aminoglycosides not only did not eliminate the *P. aeruginosa* strain used in the study, but also stimulated their ability to form biofilm. In fact,

they demonstrated that aminoglycosides interact with the *P. aeruginosa* aminoglycoside response regulator gene, *arr*, which encodes for an inner-membrane phosphodiesterase essential to the regulation of cyclic di-guanosine monophosphate levels, which represents a bacterial second messenger that regulates cell surface adherence [18]. Later on, Kaplan *et al*. [19] also reported that in *Escherichia coli*, not only sub inhibitory antibiotic concentrations but also disinfectants such as chlorhexidine are responsible for the induction of biofilm formation. From their work, one can conclude that, for some bacterial strains, biofilm formation can be a specific defensive reaction to the presence of antibiotics.

Despite all the evidences showing that biofilms provide advantages to microorganisms, namely enhanced resistance towards environmental stresses including the presence of antimicrobial compounds, many antibiotics that are currently in use were developed, tested, and regulated using *in vitro* tests against planktonic bacteria.

It is known that microbial cells growing within a biofilm are physiologically distinct from planktonic cells of the same strain. The overall resistance level in biofilms is distinct from the one observed at a cellular level [20]. As a consequence, the antimicrobial concentration required to inhibit biofilms can be up to hundreds or even a thousand times higher than the corresponding concentration necessary to eliminate free-living bacterial cells [21]. Such phenomena cannot be overlooked in the development of novel strategies to combat infectious diseases.

Taking into account that biofilm formation is a threatening characteristic of the microbiome that colonizes diabetic foot wounds, it is not unexpected that in the past few decades a major problem in treating DFU infections has been the increasing rate of colonisation by antibiotic resistant pathogens. This is the case of methicillin-resistant *S. aureus* (MRSA), and to a lesser degree, glycopeptide-intermediate *S. aureus*, vancomycin-resistant enterococci, extended-spectrum β-lactamase- or carbapenamase–producing gram-negative bacilli, and highly resistant strains of *P. aeruginosa*. In fact, the infection by polymicrobial communities of multidrug-resistant bacteria is an important cause of DFU healing impediment [7, 22–27].

The rates of isolation of these multidrug-resistant pathogens vary widely among geographical area and treatment center. However, the increasing incidence of multidrug-resistant microorganisms together with the incapacity of antibiotics to act on resistant and biofilm-producing bacteria at therapeutical concentrations emphasizes the importance of developing new treatment strategies to effectively eradicate these infections.

Considering that biofilms were only described by the scientific community by the end of the twentieth century, it is comprehensible that research on biofilms is still an expanding area [28]. The lack of understanding of the mechanisms behind the biofilm mode of life has impaired the development of antimicrobial compounds that specifically operate on biofilm polymicrobial communities [28]. However, in recent years, the increased failure in infectious diseases therapeutic protocols and the dissemination of antibiotic resistance has demonstrated the importance of developing such substances and several novel therapeutic strategies, namely bacteriophages, probiotics and antimicrobial peptides (AMP), are recently been explored and proposed as potential alternatives to eradicate bacterial biofilms in DFU.

2. Bacteriophages

Bacteriophages were discovered almost a century ago by two independent microbiologists, Twork in 1915 in the United Kingdom and D'Herelle in 1917 in France. D'Herelle named these bacteria-eating entities as bacteriophages and explored them as antibacterial agents [29, 30].

Bacteriophages are bacteria-specific viruses that infect and multiply within bacterial cells. In contrast to lysogenic bacteriophages, the replication of lytic bacteriophages and release of the newly formed virus particles always involves lysis of the host bacterial cell. Bacteriophage therapy is the use of lytic bacteriophages to reduce or eliminate pathogenic bacteria [31].

Lytic bacteriophages seem to be efficient therapeutical agents in biofilm microenvironment due to several particular characteristics: specificity and efficiency in lysing pathogenic bacteria; absence of pathogenicity to man and animals; efficiency over bacteria organized in polymeric matrixes, namely biofilms; action in microaerophilic environments with high bacterial load; and rapid and economical accessible production capability [32, 33].

Bacteriophage therapy has become a broadly relevant technology for veterinary, agricultural and food microbiological applications; however, the treatment of human infections with bacteriophage-based protocols attracts the greatest interest [34].

Bacteriophages are viruses that specifically infect prokaryotic bacterial cells. In fact, the prokaryotic biochemical machinery that enables the interaction between bacteriophages and bacterial cells has particular characteristics that are not present in eukaryotic cells. For instance, the outer membrane receptors of bacterial cells, with which bacteriophage capsid coat or molecular appendages first connect with the purpose of being anchored on the bacterial cell wall, as well as the polymerases required for the bacteriophage genome replication, are specific of prokaryotic bacterial cells and are structurally and functionally different from those presented by eukaryotic cells [31]. For that reason, bacteriophages can only directly interact and infect bacterial cells, and not eukaryotic cells. The bacterio-specificity features allow classifying bacteriophages as 'safe' for use in eukaryotic organisms, namely plants and animals, including humans.

The use of bacteriophages as antibacterial agents for suppurative infections began shortly after their discovery, with Bruynoghe's and Maisin's application for treating *S. aureus* skin infections [35]. However, following the discovery and general application of antibiotics, interest in the therapeutic uses of bacteriophages waned. Recently, the increase in antibiotic-resistant bacterial strains has reinvigorated enthusiasm about these bacteria-specific viruses [36]. This interest is particularly true in cases in which bacteriophages can be applied topically, as is the case of DFU.

Recently, a topically delivered bacteriophage suspension was tested for its antimicrobial activity and wound healing capability against ulcers chronically infected with *S. aureus*, *P. aeruginosa* and *Acinetobacter baumannii*. In this study, conducted by Mendes *et al.* in 2013 [37], the bacteriophage suspension was applied in debrided infected cutaneous wounds and microbiologic, histological and planimetric parameters were evaluated. It was shown that the

bacteriophage treatment successfully decreased bacterial colony counts and improved wound healing, as indicated by smaller epithelial and dermal gaps. The bacteriophage therapy protocol developed was proven to be an effective methodology in the treatment of two animal models of Diabetes *mellitus*, rodents and porcines [37].

The same bacteriophage suspension also demonstrated *in vitro* activity against both planktonic cells and established biofilms. Using metabolic activity as a measure of cell viability, it was observed that bacteriophage treatment significantly increased cell impairment within biofilms. Moreover, bacteriophage exposure repeated every four hours caused a further decrease in cell activity [9].

There is still much to unravel regarding bacteriophage therapy. For instance, not all phages are suitable for clinical application. More information is required, namely detailed studies of potentially useful phages with respect to their interaction with target bacteria and their genetic content.

Nonetheless, despite the paucity of experimental data regarding bacteriophage therapy in DFU, a consensus appears to have emerged on the feasibility of this potential alternative to treat biofilm-infected DFU.

3. Probiotics

The increasing global antimicrobial drug resistance problem led to an urge in researching alternatives to drug therapies, making the concept of bacteriotherapy more interesting and pertinent than ever. Bacteriotherapy is a promising alternative approach to fight infections by employing harmless bacteria to displace pathogenic microorganisms [38].

The concept of 'probiotic' arose in 1907 from a hypothesis proposed by Noble Prize-winning Ilya Mechnikov. At the turn of the twentieth century, Mechnikov noticed that peasant populations in Bulgaria had increased average life spans in comparison with wealthier European populations [39]. He also observed that yogurt and other fermented milk products were a substantial part of their diets and described the beneficial effects of the 'Bulgarian *bacillus*' present in those foods [40, 41]. These healthy bacteria, later classified *Lactobacillus bulgaricus*, helped digestion, impaired the putrefactive effects of gastrointestinal metabolism, and contributed to the improvement of the immune system [41].

Mechnikov was not the only one to notice the health benefits of lactic acid bacteria. A few years before, in 1899, another important discovery was made at the Pasteur Institute in Paris. Henri Tissier demonstrated that children suffering from diarrhea had a low number of bacteria characterized by a peculiar Y-shaped morphology. On the other hand, these "bifid" bacteria were abundant in the gut flora of healthy breast-fed infants. Moreover, Tissier demonstrated that the administration of these Y-shaped bacteria, later classified *Bifidobacterium*, to patients with diarrhea allowed them to re-establish a healthy intestinal microbiome [42].

The definition of probiotic as well as their characteristics have evolved in the last century and nowadays probiotics are defined By the Food and Agriculture Organization and the World

Health Organization as: 'live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" [43]. Probiotics are either a single strain or a mixture of commensal microorganisms with the ability to outcompete pathogenic bacteria through several mechanisms of action. The two most common are direct modification of the microbial populations and modulation of host immune system [43].

Direct modification of the microbiome includes competition with pathogenic bacteria for adhesion to epithelial receptor, production of antimicrobial substances like acids, hydrogen peroxide and bacteriocins, and inhibition of toxic substances produced by pathogens. Immunomodulation includes strengthening of host immune response, promotion of anti-inflammatory action, and enhancement of the wound healing process by stimulating the accumulation of inflammatory cells like lymphocytes, macrophages and polymorphonuclear cells in the site of wound [44].

As one would expect, not all commensal bacteria are suitable to be used as a probiotic. The screening and selection of probiotics includes a rigorous evaluation of the probiotic candidate strain in order to determine whether it fulfills all the required criteria.

Firstly, it is important to assess its safety. An evaluation that includes strain identification and typing, antimicrobial resistance profiling, and determination of virulence and pathogenic properties, including metabolic activities associated with toxic compounds production, is mandatory [45]. Secondly, it is relevant to determine its technological potential. It is essential for a probiotic strain to be genetically stable and bacteriophage-resistant. In addition, it must present viability during processing and storage and be adequate for large-scale production [46]. Thirdly, it is required to establish its physiological properties. To survive the host inner environment, which is rather complex and hostile, a probiotic strain must possess specific characteristics such as gastric acid and bile tolerance and mucosal surface adhesion stability [47]. Lastly, the functional properties must be evaluated. Validated and documented health effects are mandatory, namely antagonistic activity towards pathogens, immunomodulatory activity, and anticarcinogenic properties. Some probiotic strains are also able to interfere with the host cholesterol and lactose metabolism, preventing damages by its metabolites [48].

Probiotics have already been exploited for prevention as well as treatment of a number of health disorders including irritable bowel syndrome, hypersensitivity such as food allergies, hypercholesterolemia, renal failure, gastritis and gut infection, parasitic infections, urogenital infections, colorectal cancer, and dental disorders [49, 50]. Since the putative probiotic mechanisms of action should be the same in the peripheral wounds as they are in other parts of the body, these can be considered as a potential DFU treatment alternative.

Lactic acid bacteria (LAB), in particular *Lactobacillus* and *Bifidobacterium* species, have been extensively used as probiotic strains. The genus *Lactobacillus* is formed by ubiquitous and usually harmless bacteria. In animals, including humans, they are present in the gastrointestinal and genitourinary tracts where they act as health promoters [51]. The genus *Bifidobacterium* includes anaerobic bacteria that produce acetic and lactic acid without release of carbon dioxide. *Bifidobacterium* is the third most abundant genus in the complex microbiome of the human intestinal tract where it exerts beneficial functions of paramount importance [52].

However, other species of bacteria, and even some fungi, also present probiotic properties, such as *Enterococcus faecium, Bacillus cereus, E. coli* strain Nissle, *Propionibacterium freudenreichii, Propionibacterium acnes* and the yeasts *Saccharomyces cerevisae* and *Saccharomyces boulardii* [53–55].

LAB commonly produce antimicrobial substances with effect against gastric and intestinal pathogens and compete for cell surface and mucin binding sites [56]. Recent studies have demonstrated the efficacy of LAB-based therapy for DFU infections control. A study on effectiveness of bacteriotherapy using *Lactobacillus plantarum* on infected chronic DFU demonstrated that topical application of this bacterial culture induced debridement, granulation tissue formation and total healing in half of the diabetic patients treated [57, 58]. *Lactobacillus fermentum* also showed promising applications in treating DFU infections. When co-incubated *in vitro* with *S. aureus* and *P. aeruginosa, L. fermentum* reduced the cytotoxicity and biofilm formation ability of several pathogenic strains [59].

Additional studies have suggested that *Lactobacillus reuteri, Lactobacillus rhamnosus, Lactobacillus casei, Lactobacillus acidophilus* and *Lactococcus lactis* are also promising probiotics with the ability to naturally eliminate pathogenic microorganisms, including MRSA clinical isolates [60].

In the last years, probiotics have been widely studied and all these recent data point out the beneficial effects of probiotics to human and animal health. Naturally, no probiotic strain will provide all the proposed benefits. However, one can no longer ignore the emergence of probiotics as a novel approach to fight multidrug-resistant and biofilm-producing bacteria commonly present in DFU.

4. Antimicrobial peptides

Antimicrobial peptides are major components of the host innate immune system that act as endogenous antibiotics [61, 62]. These multifunctional molecules are produced by living organisms from all kingdoms, including bacteria, fungi, plants, insects and vertebrates, as part of their defense strategy against pathogens. Most AMP act as the first defense barrier against dissemination of a wide spectrum of microorganisms, such as bacteria, fungi, viruses and protozoan parasites [62].

In addition to their antimicrobial activity, AMP serve as modulators of the immune system and even show antitoxic activity, since they neutralize bacterial toxins, including lipopolysaccharide lipid A [63, 64]. Some AMP are also able to prevent biofilm formation and act on pre-formed biofilms [65].

The majority of AMP are polypeptides with ten to forty amino acid residues; however, some can have up to a hundred. AMP are amphipathic molecules, with two regions in their structure, a polar or hydrophilic region and a nonpolar or hydrophobic region. Due to the presence of multiple lysine, arginine, and histidine residues, the polar region of AMP is cationically

charged. On the other hand, hydrophobicity derives from the abundant presence of hydrophobic amino acids, such as tryptophan, tyrosine and phenylalanine [66, 67].

The distinctive physical-chemical properties of AMP are what confers them their potential as antimicrobial compounds. It has been generally accepted that AMP exert their bactericidal activity through electrostatic interactions with the negatively charged bacterial cytoplasmic membrane, followed by permeabilization of the membrane, which causes cell lysis. Membrane permeabilization can occur through pore formation in the lipid membrane, membrane dissolution, narrowing of the membrane bilayer or lipid-peptide domain formation [68]. The AMP amphipathic structure, namely their cationic and hydrophobic regions, interacts with the negatively charged phospholipids present in the surface of the microorganisms' cytoplasmic membranes. Bacterial membranes are rich in lipids such as phosphatidilglycerol and cardiolipin, whereas host cells have eukaryotic membranes that are rich in phosphatidylcholine, cholesterol, and sphingomyelin [69].

It is the difference in the lipids that constitute the membranes of bacteria and host cells that allows AMP to selectively target the microbial cells over mammal cells and confers them the criterion of safety to be use in eukaryotic organisms, including humans.

Additionally to their role as membrane disruptors, several studies have also suggested alternative targets for AMP. In fact, it was proven that some AMP are able to translocate into the cytoplasm of pathogens and attack intracellular targets. This way, AMP impair essential bacterial metabolic processes, including nucleic acids synthesis and cell wall assembly [70–72]. AMP can present multiple and simultaneous mechanisms of action, including both membrane permeabilization and intracellular effects. This property is probably the reason why they present antimicrobial activity against such a wide range of pathogens.

Regarding their immunological functions, AMP are also known as host-defense peptides [73–76]. By interacting with a variety of host cell receptors, AMP promote the recruitment of leukocytes to the site of infection through direct chemotactic activity and stimulation of chemokine production by leukocytes, epithelial cells, and other cell types [77, 78]. Finally, some AMP also play a role in angiogenesis and wound healing [79, 80].

The production of AMP is not limited to multicellular organisms; bacteria can also synthesize AMP that are active against other bacteria. These AMP of bacterial origin include nonribosomally synthesized peptides such as gramicidins, and ribosomally synthesized peptides such as bacteriocins, and have been used for years [81, 82]. Gramicidin S is a cyclic decapeptide produced by *Bacillus aneurinolyticus* and has been used as a topical antimicrobial agent against Gram-positive bacteria since 1946 [83]. Nisin is a bacteriocin produced by *L. lactis* that acts primarily against Gram-positive bacteria and has been used safely as a food preservative for over 50 years [84].

Several studies have analyzed the *in vitro* activity of different AMP against DFU clinical isolates. In 2013, Okuda *et al*. [85] evaluated the antimicrobial activity and mode of action of three bacteriocins, nisin A, lacticin Q, and nukacin ISK-1, against a clinically isolated and biofilm-producing MRSA strain. Nukacin ISK-1, produced by *Staphylococcus warneri*, presented only bacteriostatic effects. However, both nisin A and lacticin Q, produced by *L. lactis*,

showed bactericidal efficacy against planktonic and biofilm cells [85]. Synthetic cationic antimicrobial peptides, namely NP101 and NP108, also showed *in vitro* activity against bacterial species commonly associated with DFU infections, such as *S. aureus* and *P. aeruginosa*, as demonstrated by O'Driscoll *et al.* [86] in 2013. These results suggest that bacteriocins that act on biofilm-producer cells are highly suitable for the treatment of DFU infections.

However, there are some limitations in the use of AMP as a clinical alternative for Antibiotics, in spite of the fact that bacteria resistance to AMP is rare, in opposition to what is observed towards classic antibiotics [87]. This characteristic of AMP is likely to be related to the ionic interaction between the positively charged AMP and the negatively charged bacteria membrane. Since these interactions are not dependent of specific protein binding sites, in order to develop resistance to AMP, bacteria would have to change the basic structure, namely the lipid bilayer, of its cytoplasmic membrane [88]. Moreover, attachment of the AMP with the bacterial membrane and consequent cell lysis happens in such a short period of time, rendering the possibility to develop AMP resistance quite scarce [89]. However, there are reports of distinct species of bacteria, which present resistance towards AMP. The mechanisms of resistance include degradation of AMP through secretion of proteases; removal of AMP from their site of action via efflux pumps; production of inhibitors that bind to AMP and prevent them from reaching their target; and modulation of AMP gene expression [90–92].

Another obstacle to the successful implementation of AMP as an alternative to conventional antibiotics is the production costs. AMP discovery and development is time consuming, reaching up to 10 years, and can cost millions of euros or dollars. In fact, production costs are estimated to be approximately 50-400 American dollars per gram of amino acid [93].

Even so, AMP are still a promising alternative to antibiotics. A possible solution to reduce costs associated with AMP production is the reduction of the peptide size, maintaining its antimicrobial activity [94]. Moreover, AMP exhibit physiological and functional advantages over other molecules that make them so attractive to be used in clinical practice. For instance, physiological concentrations of AMP *in vivo* are much lower than the minimal inhibitory concentrations required for its antimicrobial activity *in vitro* [95]. In fact, AMP are antimicrobial agents with a broad-spectrum activity displayed at micromolar concentrations, usually in the 1-50 μg/ml range [96]. A plausible justification for this fact may be the synergistic effect that some AMP possess, which enhances their antimicrobial activity *in vivo* [97].

For all these reasons, the development of AMP-based therapies to eliminate microbial pathogens, such as those present in DFU infections, is extremely promising and deserves further exploration.

5. Conclusive remarks

The severity of diabetic foot infections and the economic burden associated with its prevention, treatment and control have compelled scientists and clinicians to invest substantial time and effort in not only understanding how these mechanisms work, but also how they can interfere with them.

As mentioned before, a major factor responsible for healing impediment of DFU are infections by multidrug-resistant or biofilm-producing bacteria. Dissemination of these strains, coupled with disinvestment in new antibiotics development, calls for increasing research to find new approaches to prevent and control these pathogens. In this chapter, the potentialities of bacteriophage viruses, probiotic strains and antimicrobial peptides as novel strategies for management of DFU, were reviewed. Several studies, conducted by independent research teams, have demonstrated promising results, both *in vitro* as *in vivo*, regarding their competence to eradicate the pathogenic microorganisms present in DFU. However, further investigation is required so that in the future, these strategies could be applied in clinical practice alongside with conventional therapeutics.

Acknowledgements

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References

[1] Hadaegh F, Zabetian A, Tohidi M, Ghasemi A, Sheikholeslami F, Azizi F. Prevalence of metabolic syndrome by the Adult Treatment Panel III, International Diabetes Federation, and World Health Organization definitions and their association with

coronary heart disease in an elderly Iranian population. Ann Acad Med Singapore. 2009; 38(2):142–149.

[2] Jeffcoate W, Harding K. Diabetic foot ulcers. Lancet. 2003; 361(9368):1545–1551. DOI: 10.1016/S0140-6736(3)13169–8

[3] Vuorisalo S, Venermo M, Lepäntalo M. Treatment of diabetic foot ulcers. J Cardiovasc Surg (Torino). 2009; 50(3):275–291.

[4] Geerlings SE, Hoepelman AI. Immune dysfunction in patients with diabetes mellitus (DM). FEMS Immunol Med Microbiol. 1999; 26(3–4):259–265. DOI: 10.1111/j.1574-695X.1999.tb01397.x

[5] Lipsky B, Berendt A, Deery H, Embil J, Joseph W, Karchmer A, et al. Diagnosis and treatment of diabetic foot infections. Clin Infect Dis. 2004; 39(7):885–910. DOI: 10.1086/424846

[6] Ismail K, Winkley K, Stahl D, Chalder T, Edmonds M. A cohort study of people with diabetes and their first foot ulcer: the role of depression on mortality. Diabetes Care. 2007; 30(6):1473–1479. DOI: 10.2337/dc06-2313

[7] Spichler A, Hurwitz B, Armstrong D, Lipsky B. Microbiology of diabetic foot infections: from Louis Pasteur to 'crime scene investigation'. BMC Med. 2015; 7:2–13. DOI: 10.1186/s12916-014-0232-0

[8] Banu A, Noorul M, Rajkumar J, Srinivasa S. Spectrum of bacteria associated with diabetic foot ulcer and biofilm formation: a prospective study. Australas Med J. 2015;8(9):280–285. DOI: 10.4066/AMJ.2015.2422

[9] Mendes J, Leandro C, Mottola C, Barbosa R, Silva F, Oliveira M, Vilela C, et al. *In vitro* design of a novel lytic bacteriophage cocktail with therapeutic potential against organisms causing diabetic foot infections. J Med Microbiol. 2014;63(Pt 8):1055–1065. DOI: 10.1099/jmm.0.071753-0

[10] James G, Swogger E, Wolcott R, Pulcini E, Secor P, Sestrich J, et al. Biofilms in chronic wounds. Wound Repair Regen. 2008; 16(1):37–44. DOI: 10.1111/j.1524-475X.2007.00321.x

[11] Dickschat JS. Quorum sensing and bacterial biofilms. Nat Prod Rep. 2010; 27(3):343–369. DOI: 10.1039/b804469b

[12] Lipsky B, Holroyd K, Zasloff M. Topical versus systemic antimicrobial therapy for treating mildly infected diabetic foot ulcers: a randomized, controlled, double-blinded, multicenter trial of pexiganan cream. Clin Infect Dis. 2008; 47(12):1537–1545. DOI: 10.1086/593185

[13] Bridier A, Dubois-Brissonnet F, Greub G, Thomas V, Briandet R. Dynamics of the action of biocides in *Pseudomonas aeruginosa* biofilms. Antimicrob Agents Chemother. 2011; 55(6):2648–2654. DOI: 10.1128/AAC.01760-10

[14] Burmølle M, Webb JS, Rao D, Hansen LH, Sorensen SJ, Kjelleberg S. Enhanced biofilm formation and increased resistance to antimicrobial agents and bacterial invasion are caused by synergistic interactions in multispecies biofilms. Appl Environ Microbiol. 2006; 72(6):3916–3923. DOI: 10.1128/AEM.03022-05

[15] Wenzel RP. The antibiotic pipeline – Challenges, costs and values. N Engl J Med. 2004; 351:523–526. DOI: 10.1056/NEJMp048093

[16] Fleming A. Penicillin: Nobel prize lecture [Internet]. 1945. Available from: http://www.nobelprize.org/nobel_prizes/medicine/laureates/1945/fleming-lecture.pdf [Accessed: 2016/02/05].

[17] Levy SB, Marshall B. Antibacterial resistance worldwide: causes, challenges and responses. Nat Med. 2004; 10(12):122–129. DOI: 10.1038/nm1145

[18] Hoffman LR, D'Argenio DA, MacCoss MJ, Zhang Z, Jones RA, Miller SI. Aminoglycoside antibiotics induce bacterial biofilm formation. Nature. 2005;436(7054):1171–1175. DOI: 10.1038/nature03912

[19] Kaplan JB. Antibiotic-induced biofilm formation. Int J Artif Organs. 2011; 34(9):737–751. DOI: 10.5301/ijao.5000027

[20] Stewart P, Costerton W. Antibiotic resistance of bacteria in biofilms. Lancet. 2001;358:135–138. DOI: 10.1016/S0140-6736(01)05321-1

[21] Ceri H, Olson ME, Stremick C, Read RR, Morck D, Buret A. The Calgary biofilm device: new technology for rapid determination of antibiotic susceptibilities of bacterial biofilms. J Clin Microbiol. 1999;37(6):1771–1776.

[22] Lipsky B, Berendt A, Cornia P, Pile J, Peters E, Armstrong D, et al. 2012 Infectious Diseases Society of America clinical practice guideline for the diagnosis and treatment of diabetic foot infections. Clin Infect Dis. 2012;54(12):132–173. DOI: 10.1093/cid/cis346

[23] Dang C, Prasad Y, Boulton A, Jude E. Methicillin-resistant *Staphylococcus aureus* in the diabetic foot clinic: a worsening problem. Diabet Med. 2003;20:159–161. DOI: 10.1046/j.1464-5491.2003.00860.x

[24] Stanaway S, Johnson D, Moulik P, Gill G. Methicillin-resistant *Staphylococcus aureus* (MRSA) isolation from diabetic foot ulcers correlates with nasal MRSA carriage. Diabetes Res Clin Pract. 2007;75:47–50. DOI: 10.1016/j.diabres.2006.05.021

[25] Tascini C, Gemignani G, Palumbo F, Leonildi A, Tedeschi A, Lambelet P, et al. Clinical and microbiological efficacy of colistin therapy alone or in combination as treatment for multidrug resistant *Pseudomonas aeruginosa* diabetic foot infections with or without osteomyelitis. J Chemother. 2006;18:648–651. DOI: 10.1179/joc.2006.18.6.648

[26]   Kandemir O, Akbay E, Sahin E, Milcan A, Gen R. Risk factors for infection of the diabetic foot with multi-antibiotic resistant microorganisms. J Infect. 2007;54:439–445. DOI: 10.1016/j.jinf.2006.08.013

[27]   Richard J, Sotto A, Jourdan N, Combescure C, Vannereau D, Rodier M, et al. Risk factors and healing impact of multidrug-resistant bacteria in diabetic foot ulcers. Diabetes Metab. 2008;34:363–369. DOI: 10.1016/j.diabet.2008.02.005

[28]   Costerton JW, Lewandowski Z, Caldwell DE, Korber DR, Lappin-Scott HM. Microbial biofilms. Annu Rev Microbiol. 1995;49:711–745. DOI: 10.1146/annurev.mi.49.100195.003431

[29]   Twort FW. Investigation on the nature of the ultramicroscopic viruses. Lancet. 1915;186:1241–1243. DOI: 10.1016/S0140-6736(01)20383-3

[30]   d'Herelle F. Sur le rôle du microbe bactériophage dans la typhose aviaire. C R Acad Sci. 1919;169:932–934.

[31]   Sulakvelidze A, Kutter E. Bacteriophage therapy in humans. In: Kutter E, Sulakvelidze A, editors. Bacteriophages: Biology and Application. 1st ed. Florida: CRC Press; 2004. p. 381–436. DOI: 10.1201/9780203491751.ch14

[32]   Njoroge J, Sperandio V. Jamming bacterial communication: new approaches for the treatment of infectious diseases. EMBO Mol Med. 2009;1(4):201–210. DOI: 10.1002/emmm.200900032

[33]   Sillankorva S, Oliveira R, Vieira M, Sutherland I, Azeredo J. Bacteriophage Phi S1 infection of *Pseudomonas fluorescens* planktonic cells versus biofilms. Biofouling. 2004;20(3):133–138. DOI: 10.1080/08927010410001723834

[34]   Kutter E, De Vos D, Gvasalia G, Alavidze Z, Gogokhia L, Kuhl S, Abedon S. Phage therapy in clinical practice: treatment of human infections. Curr Pharm Biotechnol. 2010;11: 69–86. DOI: 10.2174/138920110790725401

[35]   Bruynoghe R, Maisin J. Essais de thérapeutique au moyen du bacteriophage. C R Soc Biol. 1921;85:1120–1121.

[36]   Chopra I, Hodgson J, Metcalf B, Poste G. The search for antimicrobial agents effective against bacteria resistant to multiple antibiotics. Antimicrob Agents Chemother. 1997;41:497–503.

[37]   Mendes J, Leandro C, Corte-Real S, Barbosa R, Cavaco-Silva P, Melo-Cristino, et al. Wound healing potential of topical bacteriophage therapy on diabetic cutaneous wounds. Wound Repair Regen. 2013; 21: 595–603. DOI: 10.1111/wrr.12056

[38]   Leone S, Pascale R, Vitale M, Esposito S. Epidemiology of diabetic foot. Infez Med. 2012; 20 (Suppl. 1): 8–13.

[39]   Metchnikoff E. The prolongation of life: Optimistic studies. 1st ed. New York and London: G. P. Putman's Sons; 1908. p. 161–183.

[40] Azizpour K, Bahrambeygi S, Mahmoodpour S, Azizpour A. History and basic of probiotics. Res J Biological Sci. 2009;4(4):409–426.

[41] Kingsley CA, Gregor R. Probiotics: 100 years (1907–2007) after Elie Metchnikoff's observation. In: Méndez-Vilas A, editor. Communicating Current Research and Educational Topics and Trends in Applied Microbiology. 1st ed. Spain: Formatex.org; 2007. p. 466–474.

[42] Tissier H. The treatment of intestinal infections by the method of transformation of bacterial intestinal flora. C R Soc Biol. 1906;60:359–361.

[43] FAO/WHO Working Group. Guidelines for the evaluation of probiotics in food [Internet]. 2002. Available from http://www.who.int/foodsafety/fs_management/en/probiotic_guidelines.pdf [Accessed: 2016/02/05]

[44] Oelschlaeger T. Mechanisms of probiotic actions – a review. Int J Med Microbiol. 2010;300(1):57–62. DOI: 10.1016/j.ijmm.2009.08.005

[45] Sanders ME, Akkermans LM, Haller D, Hammerman C, Heimbach J, Hörmannsperger G, et al. Safety assessment of probiotics for human use. Gut Microbes. 2010:1(3):164–185. DOI: 10.4161/gmic.1.3.12127

[46] Conway P. Selection criteria for probiotic microorganisms. Asia Pacific J Clin Nutr. 1996;5:10–14.

[47] Tuomola E, Crittenden R, Playne M, Isolauri E, Salminen S. Quality assurance criteria for probiotic bacteria. Am J Clin Nutr. 2001;73:393–398.

[48] Donovan SM, Schneeman B, Gibson GR, Sanders ME. Establishing and evaluating health claims for probiotics. Adv Nutr. 2012;3(5):723–725. DOI: 10.3945/an.112.002592

[49] Hickson M. Examining the evidence for the use of probiotics in clinical practice. Nurs Stand. 2013;27(29):35–41. DOI: 10.7748/ns2013.03.27.29.35.e6363

[50] Singh Y, Ahmad J, Musarrat J, Ehtesham N, Hasnain S. Emerging importance of holobionts in evolution and in probiotics. Gut Pathog. 2013;5(1):12. DOI: 10.1186/1757-4749-5-12

[51] Salminen S, Isolauri E, Salminen E. Clinical uses of probiotics for stabilizing the gut mucosal barrier: successful strains and future challenges. Antonie van Leeuwenhoek. 1996;70(2–4):347–358. DOI: 10.1007/BF00395941

[52] Finegold SM, Sutter VL, Mathisen GE. Normal indigenous intestinal flora. In: Hentges DJ, editors. Human intestinal microflora in health and disease. 2nd ed. New York: Academic Press; 1983. p. 3–31. DOI: 10.1016/B978-0-12-341280-5.50007-0

[53] Endres JR, Qureshi I, Farber T, Hauswirth J, Hirka G, Pasics I, et al. One-year chronic oral toxicity with combined reproduction toxicity study of a novel probiotic, *Bacillus coagulans*, as a food ingredient. Food Chem Toxicol. 2011;49(5):1174–1182. DOI: 10.1016/j.fct.2011.02.012

[54] Franz CM, Huch M, Abriouel H, Holzapfel W, Gálvez A. Enterococci as probiotics and their implications in food safety. Int J Food Microbiol. 2011;151(2):125–140. DOI: 10.1016/j.ijfoodmicro.2011.08.014

[55] Psomas E, Andrighetto C, Litopoulou-Tzanetaki E, Lombardi A, Tzanetakis N. Some probiotic properties of yeast isolates from infant faeces and Feta cheese. Int J Food Microbiol. 2001;69(1–2):125–133. DOI: 10.1016/S0168-1605(01)00580-3

[56] Ljungh A, Wadström T. Lactic acid bacteria as probiotics. Curr Issues Intest Microbiol. 2006;7(2):73–89.

[57] Peral M, Rachid M, Gobbato N, Huaman M, Valdéz J. Interleukin-8 production by polymorphonuclear leukocytes from patients with chronic infected leg ulcers treated with *Lactobacillus plantarum*. Clin Microbiol Infect. 2010;16(3):281–286. DOI: 10.1111/j.1469-0691.2009.02793.x

[58] Valdéz J, Peral M, Rachid M, Santana M, Perdigón G. Interference of *Lactobacillus plantarum* with *Pseudomonas aeruginosa in vitro* and in infected burns: the potential use of probiotics in wound treatment. Clin Microbiol Infect. 2005;11(6):472–429. DOI: 10.1111/j.1469-0691.2005.01142.x

[59] Varma P, Nisha N, Dinesh K, Kumar A, Biswas R. Anti-infective properties of *Lactobacillus fermentum* against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. J Mol Microbiol Biotechnol. 2011;20(3):137–143. DOI: 10.1159/000328512

[60] Sikorska H, Smoragiewicz W. Role of probiotics in the prevention and treatment of methicillin-resistant *Staphylococcus aureus* infections. Int J Antimicrob Agents. 2013;42(6):475–481. DOI: 10.1016/j.ijantimicag.2013.08.003

[61] Zasloff M. Antimicrobial peptides of multicellular organisms. Nature. 2002;415:389–395. DOI: 10.1038/415389a

[62] Hancock R, Sahl H. Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. Nat Biotechnol. 2006;24:1551–1557. DOI: 10.1038/nbt1267

[63] Rosenfeld Y, Papo N, Shai Y. Endotoxin (lipopolysaccharide) neutralization by innate immunity host-defense peptides: Peptide properties and plausible modes of action. J Biol Chem. 2006;281:1636–1643. DOI: 10.1074/jbc.M504327200

[64] Kirikae T, Hirata M, Yamasu H, Kirikae F, Tamura H, Kayama F, et al. Protective effects of a human 18-kilodalton cationic antimicrobial protein (CAP18)-derived peptide against murine endotoxemia. Infect Immun. 1998;66:1861–1868.

[65] Overhage J, Campisano A, Bains M, Torfs E, Rehm B, Hancock R. Human host defense peptide LL-37 prevents bacterial biofilm formation. Infect Immun. 2008;76:4176–4182. DOI: 10.1128/IAI.00318-08

[66] Baltzer SA, Brown MH. Antimicrobial peptides: promising alternatives to conventional antibiotics. J Mol Microbiol Biotechnol. 2011;20(4): 228–235. DOI: 10.1159/000331009

[67] Hou S, Liu Z, Young AW, Mark SL, Kallenbach NR, Ren D. Effects of Trp- and Arg-containing antimicrobial-peptide structure on inhibition of Escherichia coli planktonic growth and biofilm formation. Appl Environ Microbiol. 2010;76(6):1967–1974. DOI: 10.1128/AEM.02321-09

[68] Gaspar D, Veiga AS, Castanho MA. From antimicrobial to anticancer peptides. A review. Front Microbiol. 2013;4:294. DOI: 10.3389/fmicb.2013.00294

[69] Wimley WC. Describing the mechanism of antimicrobial peptide action with the interfacial activity model. ACS Chem Biol. 2010;5(10):905–917. DOI: 10.1021/cb1001558

[70] Schneider T, Kruse T, Wimmer R, Wiedemann I, Sass V, Pag U, et al. Plectasin, a fungal defensin, targets the bacterial cell wall precursor Lipid II. Science. 2010;328:1168–1172. DOI: 10.1126/science.1185723

[71] Brogden K. Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? Nat Rev Microbiol. 2005;3:238–250. DOI: 10.1038/nrmicro1098

[72] Subbalakshmi C, Sitaram N. Mechanism of antimicrobial action of indolicidin. FEMS Microbiol Lett. 1998;160(1):91–96. DOI: 10.1111/j.1574-6968.1998.tb12896.x

[73] Nijnik A, Hancock R. The roles of cathelicidin LL-37 in immune defences and novel clinical applications. Curr Opin Hematol. 2009;16:41–47. DOI: 10.1097/MOH.0b013e32831ac517

[74] Lai Y, Gallo R. AMPed up immunity: how antimicrobial peptides have multiple roles in immune defense. Trends Immunol. 2009;30:131–141. DOI: 10.1016/j.it.2008.12.003

[75] Bowdish D, Davidson D, Hancock R. A re-evaluation of the role of host defence peptides in mammalian immunity. Curr Protein Pept Sci. 2005;6:35–51. DOI: 10.2174/1389203053027494

[76] Bowdish D, Davidson D, Hancock R. Immunomodulatory properties of defensins and cathelicidins. In: Shafer W, editors. Antimicrobial peptides and human disease. 1st ed. Berlin: Springer; 2006. p. 27–66. DOI: 10.1007/3-540-29916-5_2

[77] Davidson D, Currie A, Reid G, Bowdish D, MacDonald K, Ma R, et al. The cationic antimicrobial peptide LL-37 modulates dendritic cell differentiation and dendritic cell-induced T cell polarization. J Immunol. 2004;172(2):1146–1156. DOI: 10.4049/jimmunol.172.2.1146

[78] Nijnik A, Pistolic J, Wyatt A, Tam S, Hancock R. Human cathelicidin peptide LL-37 modulates the effects of IFN-gamma on APCs. J Immunol. 2009;183:5788–5798. DOI: 10.4049/jimmunol.0901491

[79] Heilborn J, Nilsson M, Kratz G, Weber G, Sørensen O, Stähle-Bäckdahl M, et al. The cathelicidin anti-microbial peptide LL-37 is involved in re-epithelialization of human skin wounds and is lacking in chronic ulcer epithelium. J Invest Dermatol. 2003;120:379–389. DOI: 10.1046/j.1523-1747.2003.12069.x

[80] Koczulla R, von Degenfeld G, Kupatt C, Krötz F, Zahler S, Gloe T, et al. An angiogenic role for the human peptide antibiotic LL-37/hCAP-18. J Clin Invest. 2003;111:1665–1672. DOI: 10.1172/JCI17545

[81] Hancock R, Chapple D. Peptide antibiotics. Antimicrob Agents Chemother. 1999;43:1317–1323. DOI: 10.1016/S0140-6736(97)80051-7

[82] Cotter D, Hill C, Ross P. Bacteriocins: developing innate immunity for food. Nat Rev Microbiol. 2005;3:777–788. DOI: 10.1038/nrmicro1273

[83] Gause G. Gramicidin S. Lancet. 1946;2:46.

[84] Cleveland J, Montville T, Nes I, Chikindas M. Bacteriocins: safe, natural antimicrobials for food preservation. Int J Food Microbiol. 2001;71:1–20. DOI: 10.1016/S0168-1605(01)00560-8

[85] Okuda K, Zendo T, Sugimoto S, Iwase T, Tajima A, Yamada S, et al. Effects of bacteriocins on methicillin-resistant *Staphylococcus aureus* biofilm. Antimicrob Agents Chemother. 2013;57(11):5572–5579. DOI: 10.1128/AAC.00888-13

[86] O'Driscoll N, Labovitiadi O, Cushnie TP, Matthews K, Mercer D, Lamb A. Production and evaluation of an antimicrobial peptide-containing wafer formulation for topical application. Curr Microbiol. 2013;66(3):271–278. DOI: 10.1007/s00284-012-0268-3

[87] Yeaman MR, Yount NY. Mechanisms of antimicrobial peptide action and resistance. Pharmacol Rev. 2003;55(1):27–55. DOI: 10.1124/pr.55.1.2

[88] Wimley WC, Hristova K. Antimicrobial peptides: successes, challenges and unanswered questions. J Membr Biol. 2011;239(1–2):27–34. DOI: 10.1007/s00232-011-9343-0

[89] Fernebro J. Fighting bacterial infections-future treatment options. Drug Resist Updat. 2011;14(2):125–139. DOI: 10.1016/j.drup.2011.02.001

[90] Otto M. Bacterial sensing of antimicrobial peptides. Contrib Microbiol. 2009;16:136–149. DOI: 10.1159/000219377

[91] Guilhelmelli F, Vilela N, Albuquerque P, Derengowski LdaS, Silva-Pereira I, Kyaw CM. Antibiotic development challenges: the various mechanisms of action of antimicrobial peptides and of bacterial resistance. Front Microbiol. 2013;4:353. DOI: 10.3389/fmicb.2013.00353

[92] Nawrocki KL, Crispell EK, McBride SM. Antimicrobial peptide resistance mechanisms of grampositive bacteria. Antibiotics (Basel). 2014;3(4):461–492. DOI: 10.3390/antibiotics3040461

[93] Marr AK, Gooderham WJ, Hancock RE. Antibacterial peptides for therapeutic use: obstacles and realistic outlook. Curr Opin Pharmacol. 2006;6(5):468–472. DOI: 10.1016/j.coph.2006.04.006

[94] Seo MD, Won HS, Kim JH, Mishig-Ochir T, Lee BJ. Antimicrobial peptides for therapeutic applications: a review. Molecules. 2012;17(10):12276–12286. DOI: 10.3390/molecules171012276

[95] Lai Y, Gallo RL. AMPed up immunity: how antimicrobial peptides have multiple roles in immune defense. Trends Immunol. 2009;30(3):131–141. DOI: 10.1016/j.it.2008.12.003

[96] Diamond G, Beckloff N, Weinberg A, Kisich KO. The roles of antimicrobial peptides in innate host defense. Curr Pharm Des. 2009;15(21):2377–2392. DOI: 10.2174/138161209788682325

[97] Cassone M, Otvos L Jr. Synergy among antibacterial peptides and between peptides and small molecule antibiotics. Expert Rev Anti Infect Ther. 2010;8(6):703–716. DOI: 10.1586/eri.10.38