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The development of antimicrobial peptides as an approach to prevention of antibiotic resistance

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In recent years, the widespread use of conventional antibiotics has led to many microbial pathogens becoming resistant to these antibiotics. Therefore, the development of novel and alternative therapeutic strategies for controlling and reducing the effects of these pathogens is urgently needed. Studies have shown that antimicrobial peptides (AMPs) and proteins are important members of the host defense system in eukaryotes. These peptides are potent agents with broad-spectrum activity against many Gram-positive and Gram-negative bacteria. In this review, we discuss the diversity, the broad spectrum of antimicrobial activity and related properties of AMPs that could be exploited for their application as potential drug candidates in therapeutic strategies against multiresistant pathogens.

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Introduction

Resistance to antibiotics is a type of drug resistance in which microorganisms can survive in the presence of one or more antibiotics. Many of the resistance genes are located on plasmids that some of them can be transferred through conjugation, transduction and transformation from a bacterium to other and by which antibiotic-resistant strains are created (Table 1) [1]. One of the major causes of antibiotic resistance is the indiscriminate and inappropriate use of antibiotics in medicine and veterinary medicine, and the excessive use of antibiotics in food and farmed animals; accordingly, prolonged exposure of microorganisms to antibiotics increases the risk of resistance [2,3]. Bacterial resistance, in addition to reducing the impact of one or more drugs, may increase colonization in the presence of a specific group of antibiotics. For example, in the case of methicillin-resistant *Staphylococcus aureus*, the use of glycopeptides, cephalosporins and especially quinolones increases the colonization and disease severity [4]; resistant *Clostridium difficile* shows enhanced colonization in the presence of specific cephalosporins, quinolones and clindamycin [5].

Antibiotic resistance mechanisms

Antibiotic resistance may result in the transfer of genes from one bacterium to another. Point mutations may also occur in the pathogen genome with an approximate ratio of one in 100 million chromosome replications. The effects of the mutations are transferred to the next generation with the proliferation of mutant bacteria, and quite resistant clones may emerge. Four main mechanisms of microbial resistance to antimicrobial agents include [6–16]:

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Table 1. Genetic elements involved in the transmission of antibiotic resistance [1].

Genetic element	General characteristic	Resistance determinants specified and examples
Plasmid	Variable size (1 to >100 kb), conjugative, and mobilizable	R factor: multiple resistance
Insertion sequence	Small (<2.5 kb), contains terminal inverted repeats, and specifies a transposase	IS1, IS3, IS4
Integron	Facilitates acquisition and dissemination of gene cassettes; specifies an integrase, attachment sites, and transcriptional elements to drive expression of multiple resistance genes	Class 1: multiple single determinants and MDR Efflux pump (Qac) Class 2: Tmp, Strp, Str, and Spc (Tn7) Class 3: carbapenems Class 4: *Vibrio* spp. superintegron
Transposable bacteriophage	A bacterial virus that can insert into the chromosome	Mu
Composite (compound) transposon	Flanked by insertion sequences and/or inverted repeats	Tn5: Kan, Bleo, Str
Complex transposon	Large (>5 kb), flanked by short terminal inverted repeats, and specifies a transposase and recombinase	Tn1 and Tn3: β-lactamase Tn7: Tmp, Str, Spc Tn1546: glycopeptides
Conjugative transposon	Promotes self-transfer	Avoids early activation of QS
Other transposable elements	Other than composite, complex, and conjugative transposons	Tn4: Amp, Str, Sul, and Hg Tn1691: Gen, Str, Sul, Cm, and Hg

(1) Inactivation of the agent or alteration in its structure: for example, the inactivation of penicillin G has been seen in some resistant strains due to the beta-lactamase production.
(2) Alteration in the target of the agent: for example, a change in penicillin binding protein in methicillin-resistant *S. aureus* has been seen.
(3) Alteration of metabolic pathways: for example, some sulfonamide-resistant bacteria that do not require para amino benzoic acid (PABA) use the folic acid available in the environment directly with some changes in their metabolic pathway (PABA is an important precursor for the synthesis of folic acid and nucleic acid in bacteria).
(4) Prevention of the entry of adequate amounts of the agent into the cytoplasm: some bacteria reduce the permeability to antibiotics by making some changes in their membrane or remove the drug by activating and increasing the number of efflux pumps. Different ways of creating antibiotic resistance are described in Fig. 1.

Possible methods to deal with the problem of antibiotic resistance

The use of resistance changing materials

One way to suppress the microbial resistance is to develop some compounds that cause bacteria to revert from their resistant status. Examples of these compounds are phenylalanine, arginine and phenylamide, which prevent the drug to be excreted by efflux pumps [17]. Another example is clavulanic acid and sulbactam that act as beta-lactamase inhibitors.

Phage therapy

Phage therapy is based upon the use of a special group of viruses, which are able to attack specific bacteria [18,19]. Phages are usually a part of the ecology surrounding bacteria, which control the bacteria population in the gut, oceans, soil and other environments. Phage therapy in humans was used between 1920 and 1940 in America and Europe. The success of phage therapy has been controversial and various unsubstantiated studies have been conducted to show its usefulness [20]. After the discovery of penicillin in 1940, Europe and America abandoned phage therapy in favor of antibiotics, but it was continued in the Soviet Union [21]. With the rise of antibiotic-resistant bacteria, attention has returned toward phage therapy. Recently, companies like Intralytix, Novolytics and Gangagen and some universities and research centers of North America and Europe have restarted to research in this area [22,23].

Removal of nutrients

Removal of nutrients is a potential approach to replace the need for antibiotics. Limiting the iron available for bacteria is a way by which the human body prevents bacterial proliferation. Researchers are developing chelators to absorb the iron available for pathogens, thereby inhibiting their growth and subsequent infection [24,25].

The use of probiotics

Probiotics are now being used to suppress infections due to antibiotic-resistant pathogens. Probiotics are microorganisms which, as a coexistent competitor, prevent pathogen colonization. Probiotics need to be not harmful to health, resistant to acid and bile, have long-term survival in the gastrointestinal environment, adherence to

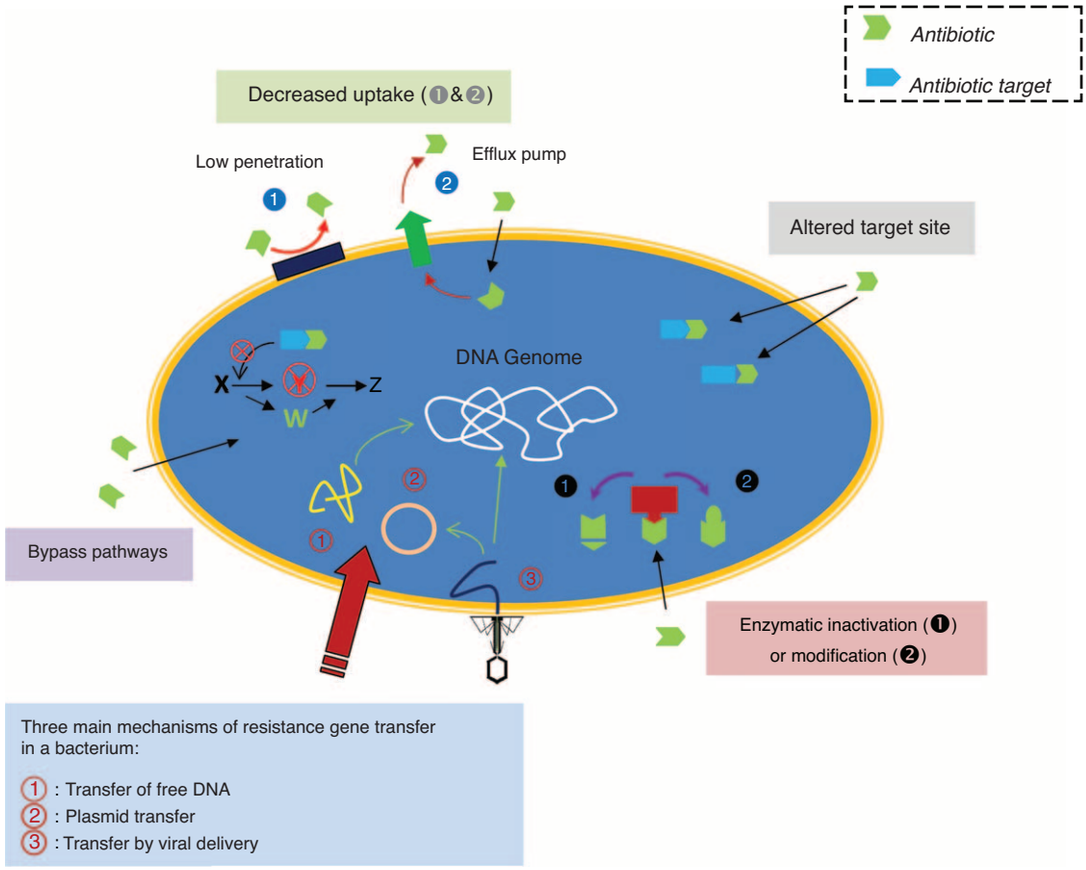

Fig. 1. Mechanisms of resistance to antibiotics in bacteria and major ways for uptake of resistance genes.

intestinal epithelial cells, the ability to produce antimicrobial agents, regulation and modulation of immune responses. Probiotic microorganisms consist mostly of strains of *Lactobacillus*, *Bifidobacterium* and *Streptococcus;* these bacteria have been used for centuries in the production of fermented dairy products [26,27].

The use of bacteriocins

Bacteriocins are small antibiotic molecules, which are produced by bacteria to inhibit the growth of similar or closely related bacterial strains [28]. These antibiotics were found among Gram-negative bacteria, Gram-positive bacteria and Archaea, for example, the bacteriocins from *Escherichia coli* are called colicins and bacteriocins produced by *Staphylococcus warneri* are called warnerins. Also many lactic acid bacteria produce a high diversity of different bacteriocins called lantibiotics [29]. Generally, because these antibiotics are made by nonpathogenic bacteria that normally colonize the human body, they are of interest in medicine. These molecules exhibit significant potency against antibiotic-resistant bacteria, are stable and can have narrow or broad activity spectra. Bacteriocins can even be produced *in situ* in the gut by probiotic bacteria to combat intestinal infections [30]. Generally, bacteriocins are often referred to as bacterial antimicrobial peptides (AMPs) that are small in size (20–50 amino acids) but some important differences are between recognized bacteriocins and other AMPs (eukaryotic), including bacteriocins that are often very potent, acting at pico- to nanomolar concentrations, whereas other AMPs need micromolar concentrations for activity. Most bacteriocins have a very narrow target spectrum with activity against only a few species/genera closely related to the producer, but other AMPs are generally less specific and target a large diversity of bacteria [31].

The use of antimicrobial peptides

AMPs are of the important components of the body's natural defense against pathogens. They are produced by granulocytes, macrophages and most epithelial cells in humans and have antimicrobial, antifungal, antiviral and even antiprotozoal activity [32]. AMPs, as new antibiotics having strong bactericidal properties, are used against antibiotic-resistant bacteria; some have entered clinical trials [11,12].

AMPs are found in almost all life forms, from bacteria to plants, vertebrates and invertebrates. They are part of the adaptive immune response, which acts as a major defense

system against severe infections [33,34]. Hundreds of natural AMPs have been isolated so far such that a list of these peptides has been drawn together [35]. Also, thousands of AMPs have been designed and synthetically produced by scientists. These peptides act on a wide range of organisms including bacteria, fungi, protozoa, enveloped viruses and even on cancer cells. In addition, AMPs have immunomodulatory activity that is essential in adaptive immune regulation and inflammatory response (Table 2) [36–38]. During the last decade, AMPs are widely studied as a secondary treatment apart from commonly used antibiotics, especially for the treatment of infections caused by drug-resistant bacteria (Table 3) [32,39].

Sources of antimicrobial peptides

The main sources of AMPs have been classified and include microorganisms, plants, invertebrates and vertebrates [40,41].

Antimicrobial peptides in microorganisms

As Alexander Fleming discovered the antimicrobial potential of the peptide derivatives of penicillin produced by the fungus *Penicillium notatum*, microorganisms were more considered as a source of bioactive compounds. Microorganisms produce a wide range of AMPs, such as bacteriocins, fungal defensins, peptaibols, cyclopeptides and pseudopeptides [42,43]. These peptides were recently divided into two main groups: peptides with lanthionine (Lantibiotics) and peptides without lanthionine, which differ in the presence or absence of the unusual amino acid of lanthionine [43]. Nisin is the most widely recognized of the lantibiotics, which is produced by *Lactococcus lactis* and used as a food preservative for nearly 50 years and no resistance has emerged since. Mersacidin is another lantibiotic, which is produced by *Bacillus* species and shows the same antibacterial activity as vancomycin against methicillin-resistant *S. aureus*, but resistance to mersacidin does not develop [44].

Table 2. General characteristics of antimicrobial peptides.

Induction by	Bacterial components (LPS, CpG, PGN)
	Viral components
	Fungal components
	Cytokines/chemokines (interleukin-1β, interleukin-6, TNFα)
Functions	Antimicrobial effects
	Clearance endotoxins
	Chemotaxis
	Modification of proinflammatory and anti-inflammatory cell response
	Angiogenesis and vasculogenesis
	Apoptosis including anticancer activity
	Wound healing and tissue repair/remodeling
Advantages	Broad-spectrum activity (antibacterial, antiviral and antifungal)
	Rapid onset of killing
	Cidal activity
	Potentially low levels of induced resistance
	Concomitant broad anti-inflammatory activities
Disadvantages	Discovery costs of synthesis and screening
	Patent exclusivity for economic viability
	Systemic and local toxicity
	Reduced activity based on salt, serum and pH sensitivity
	Susceptibility to proteolysis
	Pharmacokinetic and pharmacodynamic issues
	Sensitization and allergy after repeated application
	Natural resistance (e.g., *Serratia marcescens*)
	Confounding biological functions (e.g., angiogenesis)
	High manufacturing costs

Antimicrobial peptides in plants

Many studies showed that AMPs play a major role in plant adaptive immune [45]. Given the defensive role of AMPs, they are found at the part of a plant in which it is invaded by pathogens, such as leaves, flowers, seeds and warty protuberances. Eight different families of AMPs have been identified in plants. They all have a beta-sheet spherical structure stabilized by two to six disulfide bonds. Defensins and thionins are two important families of them on which many studies have been done. Mature thionins are typically composed of 45–47 amino acids and are divided into at least five classes (I–V). Thionins have antimicrobial and antifungal properties in in-vitro conditions and their greater expression in transgenic plants leads to their resistance against plant pathogens [46]. Plant defensins usually consist of 45–54 amino acids and are divided into four different groups or subfamilies based on the type of their sequence, these peptides have antibacterial and antifungal properties [45].

Antimicrobial peptides in invertebrates

AMPs are essential components of adaptive immune in invertebrates [47]; their protected adaptive immune mechanism (also known as an ancestral mechanism) is considered as the most effective adaptive immune systems among all living organisms [47,48]. In the early 1980s, AMPs in invertebrates were studied by Bomen *et al.* [49] leading to the isolation and identification of cecropins from insects. From that time onwards, many AMPs were identified in hemolymph cells as well as special epithelial cells of invertebrates. These peptides can be expressed continuously or by induction in response to invasion by microbes [41,50]. The study of how AMPs are regulated and expressed in *Drosophila melanogaster*, as a model led to the successful discovery of a pathogen recognition receptor. AMPs in invertebrates show widespread antimicrobial activity against invading pathogens. Defensins are the most widespread group of AMPs in invertebrates. They are circular peptides with a base terminal with three to four disulfide bonds and were first isolated from the carnivorous fly *Sarcophaga peregrine* [51]. However, alpha-helix AMPs such as cecropins isolated from *Hyalophora cecropia* hemolymph and melittin found in bee venom [52] have been described. It is noteworthy

Table 3. A number of antimicrobial peptides are being investigated for clinical use [39].

Name	Source and description	Indication	Phase	Company
Magainin peptide/ pexiganan acetate	22-amino-acid linear antimicrobial peptide, isolated from the skin of the African clawed frog (*Xenopus laevis*)	Diabetic foot ulcers	3	Dipexium Pharma (White Plains, New York)/Macro Chem/Genaera
Omiganan	Synthetic cationic peptide derived from indolicidin	Rosacea	2	BioWest Therapeutics/ Maruho (Vancouver)
OP-145	Synthetic 24-mer peptide derived from LL-37 for binding to lipopolysaccharides or lipoteichoic acid	Chronic bacterial middle-ear infection	2	OctoPlus (Leiden, the Netherlands)
Novexatin	Cyclic cationic peptide, 1093 daltons	Fungal infections of the toenail	1/2	NovaBiotics (Aberdeen, UK)
Lytixar (LTX-109)	Synthetic, membrane-degrading peptide	Nasally colonized MRSA	1/2	Lytix Biopharma (Oslo, Norway)
NVB302	Class B lantibiotic	*Clostridium difficile*	1	Novacta (Welwyn Garden City, UK)
MU1140	Lantibiotic	Gram-positive bacteria (MRSA, *C. difficile*)	Preclinical	Oragenics (Tampa, Florida)
Arenicin	21 amino acids; rich in arginine and hydrophobic amino acids	Multiresistant Gram-positive bacteria	Preclinical	Adenium Biotech Copenhagen, (Denmark)
Avidocin and purocin	Modified R-type bacteriocins from *Pseudomonas aeruginosa*	Narrow spectrum antibiotic for human health and food safety	Preclinical	AvidBiotics (S. San Francisco, California)
IMX924	Synthetic five-amino-acid peptide innate defense regulator	Gram-negative and Gram-positive bacteria (improves survival and reduces tissue damage)	Preclinical	Iminex (Coquitlam, British Columbia, Canada)

that beta-hairpin peptides called tachyplesin and polyphemusin, isolated from horseshoe crab blood cells, have shown high antibacterial and antifungal activities [53].

Antimicrobial peptides in vertebrates

So far, abundant AMPs have been isolated from a wide range of vertebrates, including fish, amphibians and mammals, indicating that, despite the presence of an adaptive immune system, AMPs play an important role in host defense [54]. These peptides are found on the surface of mucous membranes and skin, inside the immune cell granules, as well as within the small intestine pores. A large number of AMPs (about 500) have been isolated from glands under the skin and stomach mucous membrane of amphibians such as frogs and toads [41]. Among them, magainin, isolated from frog skin secretions, is typical with an alpha-helix structure, which is common among amphibian AMPs [55]. In addition to magainin, many AMPs with an alpha-helix structure have also been isolated from other amphibians: bombinins, dermaseptins and temporins are examples. Defensins and cathelecidins are two large and diverse groups of vertebrate AMPs. Cathelecidins contain a protected section at their amino end known as the cathelin domain comprising about 100 amino acids. Cathelecidins have been isolated from different mammalian species, such as pig, horse, sheep, cow, mouse, rabbit, and even human. Humans and mice produce only one type of cathelecidin, which is called hCAT-18/LL-37 in humans and cathelin-related antimicrobial peptide in mice [41]. Vertebrate defensins are a part of cyclic peptide family that are subclassified in three groups (alpha-defensin, beta-defensin, theta-defensin). Both alpha and beta subgroups exist almost in all vertebrate species, whereas theta-defensin is seen only in the neutrophils and monocytes of European and Asian monkeys [54,56].

Diversity in antimicrobial peptides

AMPs have a large diversity, which is caused by their high performance against various pathogens in various organisms [47]. Despite this diversity, AMPs have common features. For example, they are relatively short (usually shorter than 100 amino acids) and most of them have a positive charge about +2 to +9 (cationic peptides), which is because of the presence of arginine, lysine and histidine amino acids in them. They have also adaptability and can acquire an amphipathic structure with separate hydrophobic and hydrophilic domains. These peptides are remembered as cationic AMPs due to the general net positive charge of AMPs. It is hard to categorize AMPs because of their numerous varieties. One way to categorize them is based on their synthetic pathways, either with or without ribosomal help. One of the most accepted ways to classify them is based on their secondary structure, by which they are divided into four categories (Tables 4 and 5) [57,58].

Group I: linear peptides with an alpha-helix structure

This group is mostly composed of linear AMPs such as magainin, temporins, interleukin 37 and several designed

Table 4. Antimicrobial peptide structures and the relationship between peptide structure and antibacterial activity.

AMP structure	Example	Activity type	Disruption model
α-Helical peptides	Magainin	Most α-helical	Barrel stave
	Cecropin	AMPs disrupt bacterial membranes by forming amphipathic helices in membranes	Carpet
	Pexiganan		Toroidal
β-Sheet peptides	α-Defensins	Many of β-sheet AMPs exert their antimicrobial activities by disrupting bacterial membranes	Toroidal
	β-Defensins		
	Protegrin		
Extended peptides[a]	Indolicidin	Most extended AMPs are not active against the membranes of pathogens and penetrating across the membranes	Interacting with intracellular proteins[b]
	Bac5		
	Bac7		
Loop peptides	Thanatin	Disrupting bacterial membranes	–
	Lactoferricin B		
	Bactenecin-1		

AMP, antimicrobial peptide.
[a]Predominantly rich in specific amino acids and have no regular secondary structure elements.
[b]But some extended peptides, such as indolicidin, are membrane active and induce membrane leakage.

peptides. This class of AMPs is highly irregular and flexible in the aquatic environment and all parts of it possess an amphipathic alpha-helix structure interacting with membrane and membrane-like environments.

Group II: peptides with beta-sheet structure stabilized with disulfide bonds

Unlike alpha-helix peptides, the structure of the beta-sheet peptides is less flexible and that is because of the rotational structure caused by disulfide bonds (such as tachyplesin, protegrin and defensins like HBD-1) or by the rotation of the peptide structure itself (such as polymyxin B and gramicidin). These peptides adopt beta-sheet structure in aquatic environment, which stabilize in contact with lipid surface.

Group III: linear peptides with an elongated structure in which one or more amino acids are predominant

The group is made up of elongated peptides rich in one or more amino acids, such as glycine, tryptophan, arginine or histidine. This group, like alpha-helix peptides, has a flexible structure in the aquatic environment, although it adopts an amphipatic structure in the interaction with the membrane and membrane-like structures. Indolicidin and Bactenecin-5, which are, respectively, rich in tryptophan and proline/arginine, both are members of the group.

Group IV: peptides with loop-shaped structure

The last group consists of peptides with a loop structure is such as lantibiotics and peptides with a disulfide bond. The circular shape of lantibiotics is formed by a thioether linkage: the linkage is formed with the reaction of cysteine with threonine side chains dehydrated after the translation and result in creating unusual amino acids of lanthionine and methyllanthionin. Nisin and mersacidin are the most prominent members of the lantibiotics. Thanatin, lactoferricin B and bactenecin-1 are also among loop peptides created by a disulfide bond.

Antimicrobial peptides: mode of action

Biological activity of AMPs often results from their interaction with phospholipids in cell membranes. Most researchers believed that the first antimicrobial mechanism of these peptides is to disrupt the cell membrane [33,59]. But some evidence has demonstrated that the AMPs inhibit some intracellular reactions, such as protein synthesis, nucleic acid synthesis, enzyme activity and cell wall synthesis (Fig. 2 and Table 6) [54,60,61]. According to these studies, the large AMPs with more than 100 amino acids are often lytic enzymes, nutrient-binding proteins or contain sites that target specific microbial macromolecules, whereas small AMPs act mainly through disrupting the structure or the function of microbial-cell membranes or interact with ATP and directly inhibit the action of certain ATP-dependent enzymes [11,62,63].

The general structural characteristics of AMPs are essential to their mode of action. For example, their positive charge causes electrostatic interactions and also their accumulation on the poly anionic surface of the bacterial cell while their amphopathic properties enable them to penetrate into the hydrophobic region of the cell membrane [54,59,62].

In bacteria, before reaching the cytoplasmic membrane AMPs must pass the bacterial envelope. Antimicrobial activity was a primary consequence of the capacity of peptides to interact and disrupt biological membranes as a result of having two main factors, including cationic and amphipathic properties, which leads directly to the death of cells. Studies have shown that cationic peptides, as the most important group of AMPs that play a significant role

Table 5. Classification of antimicrobial peptides based on the content of amino acid.

Classification of antibacterial peptides	
Anionic peptides (rich in glutamic and aspartic amino acids)	Antimicrobial activity
Neuropeptide derived	
Enkelytin (bovine/human)	Bacteria
Peptide B (bovine/human)	Bacteria
Aspartic acid rich	
Dermcidin (human)	Bacteria
Linear cationic α-helical peptides	
Cecropins (insects/pig)	Bacteria, fungi, virus, protozoa, metazoa
Buforins	Bacteria, fungi
Cationic peptides enriched with specific amino acids	
Proline-rich	
Drosocin (fruit fly)	Bacteria
Metchnikowins (fruit fly)	Bacteria
Pyrrhocoricin (hemipteran)	Bacteria, fungi
Glycine-rich	
Diptericins (dipterans)	Bacteria
Attacins (dipterans)	Bacteria
Histidine-rich	
Histatin (human)	Bacteria, fungi
Tyrosine-rich	
Indolicidin (cattle)	Bacteria
Anionic and cationic peptides that contain cysteine and form disulfide bonds	
Single disulfide bridge	
Thanatin (hemipteran)	Bacteria
Brevinins (frog)	Bacteria, fungi
Two disulfide bridges	
Androctonin (scorpion)	Bacteria, fungi
Protegrin I (pig)	Bacteria, fungi, virus
Three disulfide bridges	
Defensins (insects)	Bacteria, fungi, protozoa
Penaeidins (shrimp)	Bacteria, fungi
More than three disulfide bridges	
Defensins (plant)	Fungi
Gambicin (mosquito)	Bacteria, fungi, protozoa
Drosomycin (fruit fly)	Fungi
Aromatic dipeptides	
p-Hydroxycinnamaldehyde (saw fly)	Bacteria, fungi
Peptides derived from oxygen-binding proteins	
Lactoferrin	Bacteria, fungi

in host defenses, cross from the outer membrane by the self-promoted uptake pathway. In this pathway, the divalent cations, magnesium (Mg^{2+}) and calcium (Ca^{2+}) that form stabilizing cross bridges between adjacent lipopolysaccharide (LPS) molecules, are displaced by cationic peptides. Because stabilization of LPS as the major portion of the outer membrane in Gram-negative bacteria occurs by these positive charged cataions and in comparison with these agents the cationic peptides have more tend to LPS, so during exposure to bacteria cell, these peptides are replaced with Mg^{2+} and Ca^{2+} causing a local disruption in the outer membrane. This process that facilitates the entry of the peptide into the outer membrane is called self-promoted uptake. Following this process, disruption of the cytoplasmic membrane occurs leading to bacterial killing. Studies have shown that this process does not have a specific receptor and, as noted, the distortion of the bilayer occurs by disruption or pore formation via direct interaction with the cell membrane (Figs 3 and 4) [11,12,59].

However, so far the accurate detail of mechanisms of AMP action has not been identified. Several models have been suggested as to how interactions between the peptide and membrane are formed and cause membrane disruption. The most relevant models include the barrel stave model, the carpet model and the toroidal model. These models represent the morphological changes and membrane disruption due to peptide–membrane interactions, such as pore formation, cell lysis and peptide transfer into the cytoplasm. Generally, in all these models the interaction of the peptide with the negatively charged lipid heads in the bacterial cytoplasmic membrane is the major step. As this continues, the AMPs accumulate facing parallel to the lipid bilayer until a critical threshold concentration is reached, after which they self-organize to form a permeation pathway. Studies have shown that changes in the structure of cell membrane and pore formation are dependent on the amount of positive charge, peptide size and distribution of hydrophilic/hydrophobic regions and combinations of amino acids comprising the peptide [62,64].

Barrel–stave model

In this model constant pores are formed by certain number of peptides. Peptides as monomers or a group

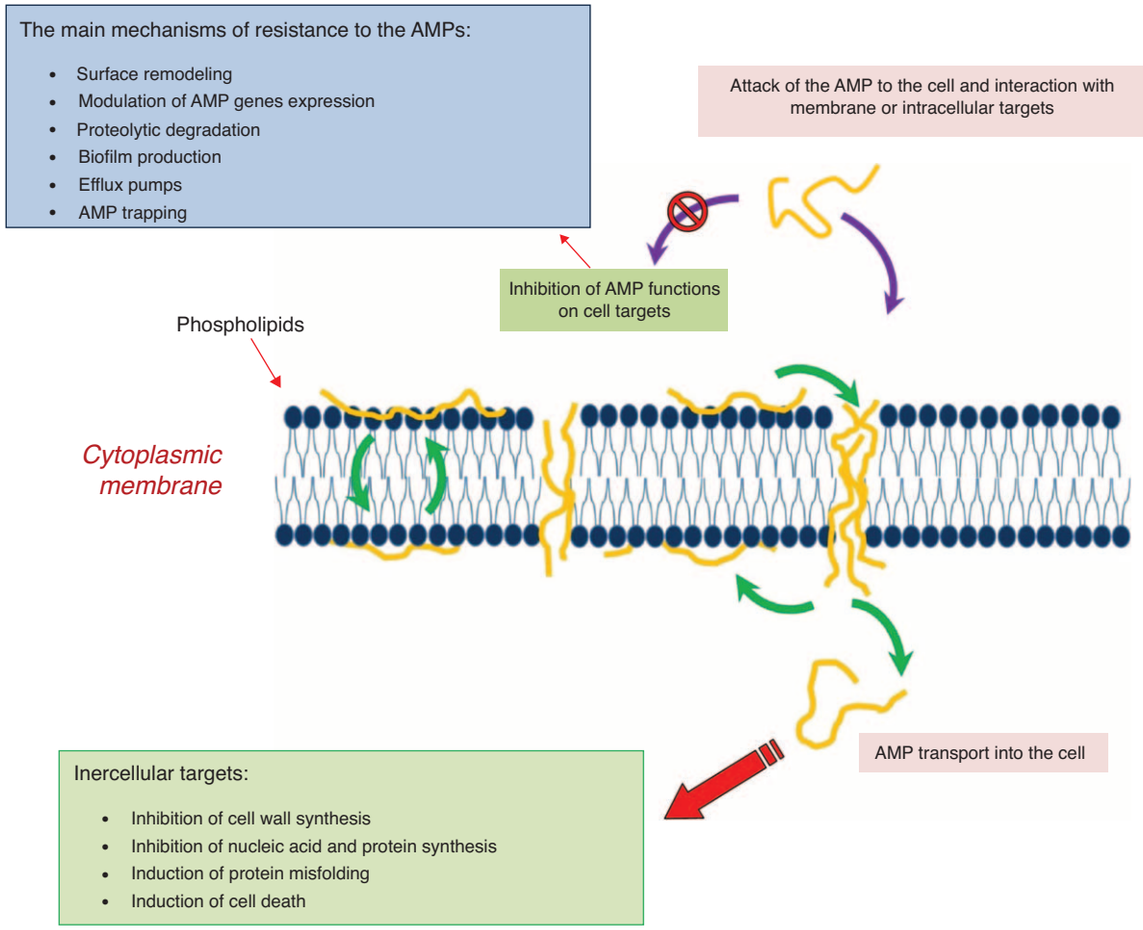

Fig. 2. The main mechanisms of cell killing by antimicrobial peptides (AMPs) through targeting of the intracellular.

arrive at the cell membrane and are bonded to its surface. Then, they are inserted vertically into the middle of the membrane and results in a number of other peptides accumulating in this area and forming a pore in proportion with their size.

Amphipatic AMPs that form pores have a regular structure, so that hydrophilic regions are located in the interior side of the pore and hydrophobic regions are in the exterior side and in contact with fatty acid tails. The mode of action of gramicidin S, a cycle peptide, is consistent with this model [65].

Carpet model

In the carpet model, the peptide's action on the membrane is similar to that of a detergent. In this model, interactions between negatively charged phospholipid and cationic AMPs lead to a carpeting and thinning of the membrane, respectively. According to this mechanism, at a critical threshold concentration, the peptides form

Table 6. Examples of nonlytic antimicrobial peptide mechanisms.

Peptide	Target organisms	Interaction	Mechanism
Nisin	Bacteria	Lipid II	Inhibition of peptidoglycan synthesis
Indolicidin2	Bacteria	Lipopolysaccharide	Binding to nucleic acids/inhibition of proteins and nucleic acid synthesis
Lactoferricin B	Bacteria	Lipopolysaccharide	Binding to nucleic acids/inhibition of macromolecular synthesis
Pyrrhocoricin	Bacteria	?	HSP (DnaK) binding/prevention of chaperone protein folding
Psd1	Fungi	?	Binding to Cyclin F/cell cycle impairment
Osmotin PR5	Yeast	Membrane receptors/ phosphomanno proteins	Apoptosis
Dermaseptin S3	Yeast	?	Apoptosis, ROS and DNA damage
BM0	Yeast	?	Inhibition of plasma membrane H^{+}-ATPase

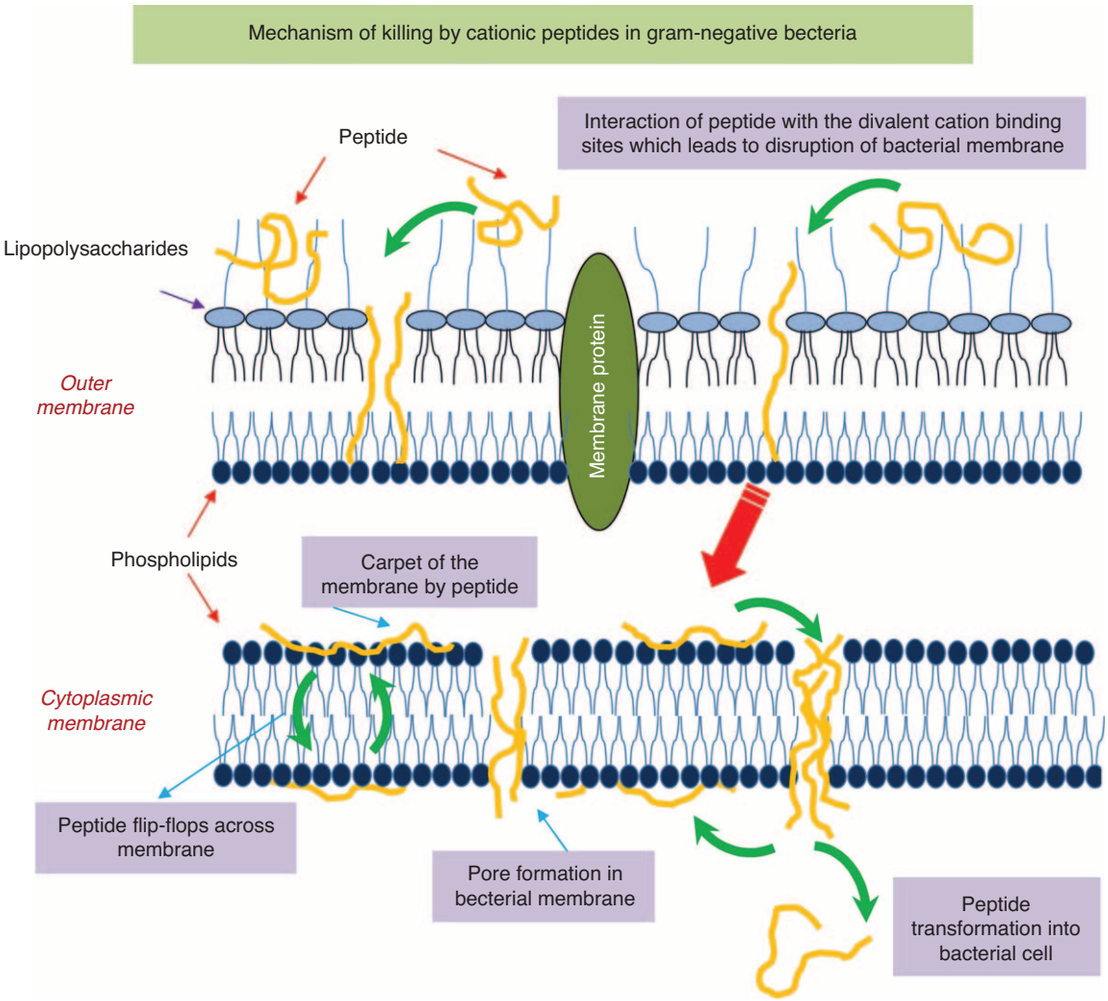

Fig. 3. A model of the cell membrane – antibacterial peptide interaction in Gram-negative bacteria.

toroidal transient holes in the membrane and above this concentration; the membrane disintegrates and forms micelles after disruption of the bilayer curvature. The action of dermaceptin S, cecropin and ovisporin is consistent with this model [62,66].

Toroidal pore model

In toroidal model, AMPs form unstable and temporary pores. At the first stage, the peptides are placed in the vicinity of the upper leaflet of the membrane and then penetrate the cell membrane through the hydrophobic regions and binding to the membrane lipid heads. This interaction leads to the introduction of mechanical stress in the lipid bilayer and its position is irregular and thinner. When the peptide concentration reaches their threshold their positions are switched from a parallel to a perpendicular position forming a hydrophobic cylindrical pore. These pores are unstable and are created in the membrane and are destroyed again, finally, the peptides are distributed on both sides of the membrane. This model draws the phospholipids in a flip-flop reaction and peptides shift into the cytoplasm that is not seen in the other models. Magainin and LL-37 follow this model [62,67].

Molecular basis of antimicrobial peptide cell selectivity

Two important factors related to structure of the peptides that are involved in cell selection including hydrophobic amino acids and total positive charge of the peptide. These can cause the strong interaction between AMPs and anionic phospholipids in the bacterial membrane, other anionic components in the bacterial outer membranes increase the intensity of antimicrobial peptide

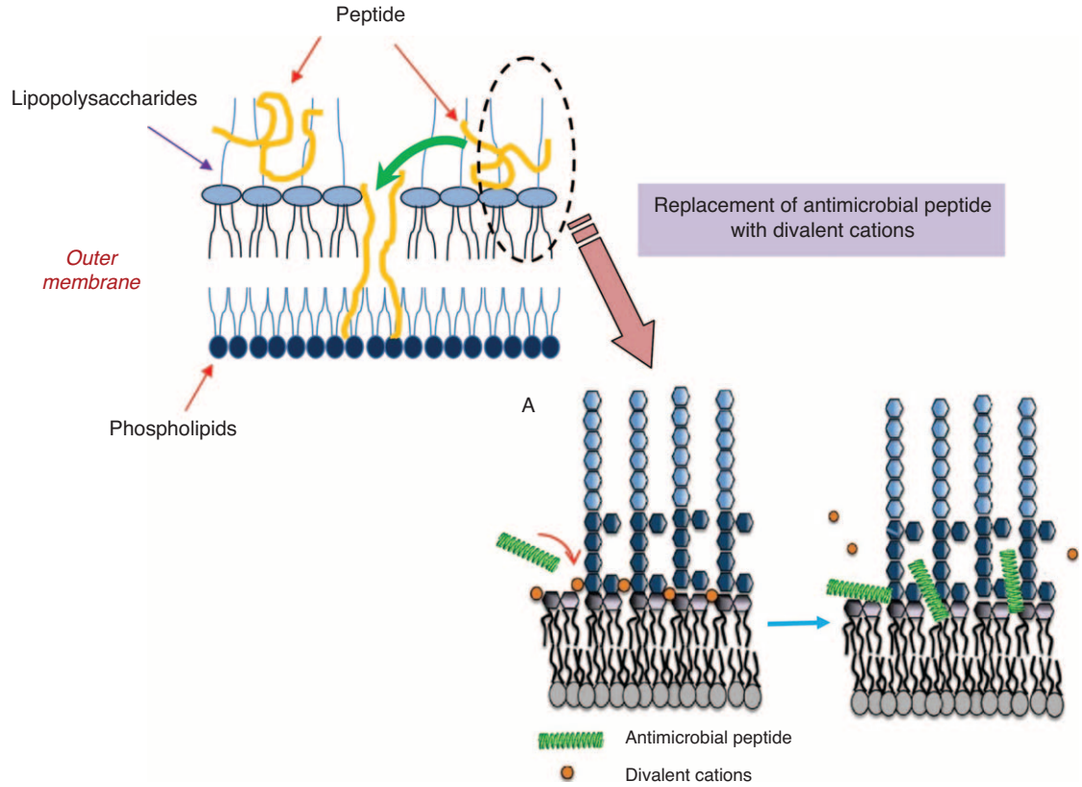

Fig. 4. Binding competition between the antimicrobial peptide and LPS stabilizing ions which leads to local disruption of outer membrane in Gram-negative bacteria.

binding. In the mammalian cell membrane, the outer leaflet is composed of neutral phospholipids and acidic phospholipids located in the inner leaflet. On the other hand, the presence of hydrophobic amino acids is essential for activity and entry of AMPs to the bacterial-cell membrane. The high hydrophobicity of these peptides causes a strong affinity for a neutral mammalian membrane: this is the cause of toxicity of certain peptides. Melittin, temporin-L and mastoparan are examples of these peptides, which are a powerful toxin. There are two properties about the bacterial and mammalian membranes that influence in the cellular selectivity of peptides, including the presence of sterols in the mammalian membrane that protect cells against AMPs, so mammalian membranes that have higher level of sterols have a lower sensitivity to AMPs. In addition, the potential of the bacterial inner membrane is more negative in comparison with the mammalian membrane and acts as an additional force in direction entry and replacement of cationic peptides into bacterial cells [68,69].

In general, cell selectivity, cell cytotoxicity and the damaging effects of AMPs are dependent to the charge, hydrophobicity, amphipathicity, stereochemistry and propensity of peptides to form barrels. Sensitivity to peptides and differences in viability of eukaryotic cells are also dependent on variations in membrane lipid composition, hydrophobicity and the metabolic activity of the cells [11,70].

Bacterial resistance to antimicrobial peptides

Depending on the mode of action of AMPs, it seems unlikely that bacterial resistance to these peptides can be attributed to a dramatic change in the structure or organization of the phospholipids [33]. However, several views of the ability of bacteria resistant to the AMPs have been presented (Fig. 2) [62,71].

Enzymatic digestion of proteins

Bacterial resistance to AMPs may be the result of peptidase and protease production that results in fragmentation of the peptides. There are different ways to escape peptidase and protease digestion, including presence of proline within the peptide sequence, carboxyl terminal amidation of peptide and formation of cyclic peptides.

Capturing and extruding

Identification of extracellular binding agents, attraction and repulsion of AMPs to them is another way to obtain

resistance against AMPs. Bacteria are able to produce extracellular proteins that bind to the AMPs and inactivate them. Bacteria are able to produce macromolecules that facilitate excretion of AMPs from bacterial cells. Although the mechanism of this process is not entirely clear, it is clear that the identification of specific sequences or structural motifs is required by these macromolecules.

Changes in surface charge

Bacterial resistance to AMPs could be due to a partial reduction in the negative surface charge of the bacterial cell membrane. Despite the change in the bacterial cell envelope macromolecules, it seems unlikely they can neutralize the bacterial membrane's anionic components using molecules with positive charges. For example, the incorporation of D-alanine in the teichoic acid component of the cell wall in Gram-positive bacteria, L-lysin in phospholipids or aminoarabinose in the LPS in Gram-negative bacteria are possible. This mechanism relative to the capacity of bacteria to reduce the negative charge is limited. Other mechanisms that cause bacterial resistance to AMPs include biofilm formation and changes in the fluidity of the outer membrane in Gram-negative bacteria that reduce permeability and increase bacterial resistance. However, the high diversity of AMPs decreases the risk for resistance development in microorganisms [71].

Synthetic and modified antimicrobial peptides for pharmaceutical applications

AMPs with beneficial properties, such as broad-spectrum activity, selected functional properties against bacteria, low risk of developing bacterial resistance have led to the development of appropriate drug candidates (Table 2) [42,72]. Their practical use has limitations such as sensitivity to proteases and low biological activity. To overcome these limitations, there are a number of methods including the design and synthesis of short AMPs with high potency and no toxicity. Many new AMPs based on changes to natural AMPs and proteins, such as melittin, cecropin magainin, indolicidin and temporin, or by de-novo methods have been produced. These changes included adding, removing, replacing one or more amino acids and modification to the amino or carboxyl termini, which may lead to a suitable peptide for clinical application [11,12,73,74].

In comparison with natural AMPs the synthetic peptides have less toxicity and higher potency. Chimeric AMPs, which inherit traits from their parents, such as those derived from cecropin, melittin and magainin have found success; most studies have been done with the cecropin-mellitin hybrids [11,12,72,75]. Although the design of hybrid AMPs using a combination of hydrophobic positive and negative charged amino acids and regions is easy but optimization of other features, such as peptide stability, toxicity rate and sensitivity to salts has identified problems in relation to their clinical applications. Recent studies demonstrated that a change of basic amino acids affects the activity and antimicrobial properties of peptides. For example, the electrostatic interaction property of lysine residues within the bacterial membrane phospholipids is weaker in comparison with arginine, so substitution of these amino acids together not only increases their attraction to the bacterial membrane phospholipids but also reduced peptide affinity to erythrocyte cells [76,77].

In summary, the antimicrobial activity of AMPs is due to the combination of hydrophobicity, cationic units and sequence of peptides. However, because of the complexity of the target cell (LPS and outer membrane) quantitative structure activity relationship studies failed to demonstrate a clear relationship between the activity and sequence of the peptides. On the contrary, based on related studies, the amphipathic structure of AMP is more important than its secondary structure. Changes in hydrophobicity without manipulation of sequence peptides can be useful in the development of peptides, which are more active and are resistant to protease. Also, Otvos *et al.* [78] synthesized chimeric dimers, in which pyrrhocoricin was connected to drosocin and generated a new class of AMPs with potent inhibition effect on DnaK and degradation activity on cell membrane. Eckert *et al.* [79] reported the first target-specific AMPs, which contained a killing domain (novispirin G10) and targeting domain (K homology domain) that was designed specific against *Pseudomonas*.

Restrictions on the use of antimicrobial peptides as antibiotics

The main reasons for nonuse of AMPs as antibiotic are cell toxicity and excessive cost. The cost of these peptides is 5–20 times more expensive than the traditional antibiotics. For example, treatment of an infection with AMPs costs about 400–500$ for 1 mg/1 kg body weight [32]. One suitable method to reduce production costs is to produce the recombinant peptides in resistant bacterial strains as host. Based on this subject, Mygind *et al.* [80] reported plectasin as a useful peptide for treatment of Gram-positive bacterial infection especially that caused by *Streptococcus pneumoniae*, including strains resistant to conventional antibiotics. This peptide (consisting of 40 amino acids) that was isolated from a fungus (*Pseudoplectania nigrella*) is the first therapeutic (clinical trial) fungal defensin produced in very high yields in *Aspergillus* as a recombinant peptide. At the end of 2008, Novozymes Company signed a global licensing agreement with Sanofi-Aventis Company for the further development and marketing of NZ2114, a derivative of plectasin, as a treatment for Gram-positive bacterial infections [81].

Conclusion

In recent decades, with growing microbial resistance to conventional antimicrobial agents, unconventional therapeutic options are urgently needed. Based on antibiotic resistance studies in the United States, only in 1998, 80 million prescriptions of antibiotics for human use were filled that is equal to 12 500 tons in 1 year. On the contrary, animal and agricultural uses of antibiotics are added to human use. Agricultural practices account for over 60% of antibiotic usage in the United States, so this adds an additional 18 000 tons per year to the antibiotic burden in the environment [82]. Thus, development of microbial resistance, as well as economic incentives, has resulted in research and development in the search for new antibiotics in order to maintain a pool of effective drugs at all times. According to the characteristics of AMPs especially cationic peptides, these molecules are one of the best new alternative antibiotic agents as an innovative response to the increasing problem of multidrug resistance. The main advantage of these peptides, over antibiotics, is their broad spectrum of activity with rapid onset of killing and low levels of induced resistance compared with conventional antibiotics. But the size of some peptides and side-effects such as toxicity are a major problem. Hence, to overcome the high production costs of long peptides and to improve their biological properties and reduce toxicity, short synthetic peptides can be designed and synthesized, because AMPs can be readily modified through substitutions, chain elongation or deletions of amino acid sequence to improve their efficiency in specific host–pathogen interactions [11,12].

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Conflicts of interests

There are no conflicts of interest.

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