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Antimicrobial peptides as new antibiotics: A comprehensive review

Amanpreet Thakur, Puja Gupta*
Department of Biotechnology, School of Biosciences, RIMT University, Mandi Gobindgarh, India..

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ABSTRACT

Antibiotics have long been the foundation stone of combating infectious diseases, but the widespread and often indiscriminate use of these drugs has given rise to drug-resistant pathogens, presenting a global health crisis. There is an urgent need to explore alternative therapeutic strategies that are less susceptible to resistance mechanisms as traditional antibiotics are losing their efficiency. Antimicrobial peptides (AMPs), small bioactive proteins naturally produced by a wide range of organisms, have emerged as promising candidates in the search for new antibiotics. AMPs serve as the first line of defense against a broad spectrum of pathogens, including bacteria, viruses, and fungi. This review article looks into the wide potential of AMPs, not only as antibacterial agents but also in their roles as antifungal, antiviral, and anticancer therapies. The present review article provides an in-depth exploration of the structural diversity of AMPs, examining how their unique properties contribute to their broad-spectrum activity. It further discusses the mechanisms and modes of action that differentiate AMPs from conventional antibiotics. Despite their immense potential, several challenges such as toxicity, stability, and high production costs hinder the clinical application of AMPs. This article not only outlines these challenges but also discusses emerging strategies aimed at overcoming these barriers. Overall the review presents AMPs as a critical focus in the development of future antimicrobial therapies..

1. INTRODUCTION

Multidrug resistance amongst pathogenic bacteria in recent decades has been alarming, largely due to the overuse of antibiotics. The development of novel antibiotics with unique modes of action is desperately needed in light of this circumstance to eradicate these resistant microorganisms. Antimicrobial peptides (AMPs) are promising substitute options for antibiotic treatment, offering several benefits above existing drugs. AMPs are naturally existing antibiotics found in a variety of organisms, including bacteria, plants, and animals. The term "AMPs" refers to peptides that can kill microbes, excluding enzymes that destroy microbes through hydrolytic actions such as chitinases, glucanases, and lysozymes. These tiny molecular peptides shield host organisms from various bacteria, viruses, fungi, and parasites and are essential for their innate immunity [1]. They fight with pathogenic microbes through natural mechanisms, targeting essential structures like bacterial membranes and, in many cases, molecules within the cells. It is challenging to develop resistance in the bacteria for such peptides due to their wide range of targets. The general features of AMPs include helical polypeptides with short amino acid sequences (less than 100 amino acid residues) including excessive amounts of the positively charged amino acids lysine and arginine [2] (Fig. 1).

AMPs and their derivatives have the potential to create new categories of antimicrobial drugs. AMPs have various biological functions that include immunoregulation, wound healing, angiogenesis, anti-cancer activity, treatment of inflammatory disorders, antiviral, and antitumor effects [3,4]. The development of AMPs in biomedicine as wound-healing agents is due to their ability to enhance cell proliferation and tissue repair. Their immunomodulatory properties could be beneficial for treating autoimmune disorders. AMPs are being studied in the cosmeceutical industry for inclusion in skincare products because of their antioxidant properties (which offer anti-aging benefits) and the antibacterial activity that helps eliminate bacteria causing acne and many other skin issues. While some AMPs have a narrow range of activity, others exhibit a very broad spectrum of action against a variety of microbes, including Gram-negative and Gram-positive bacteria, fungi, parasites, and viruses.

This article gives a detailed overview of AMPs, including their structure, classification, mechanisms of action, production methods, and potential applications for AMPs. It also discusses the probable uses of AMPs and the challenges in applying them.

2. HISTORICAL PERSPECTIVE

In 1939, microbiologist René Dubos made the initial discovery of AMPs. He discovered that the soil bacterium *Bacillus brevis* produced an antibiotic chemical known as gramicidin. Mice exposed to

*Corresponding Author
Puja Gupta, Department of Biotechnology, School of Biosciences, RIMT University, Mandi Gobindgarh, India.
Email: rzepedab @ ipn.mx

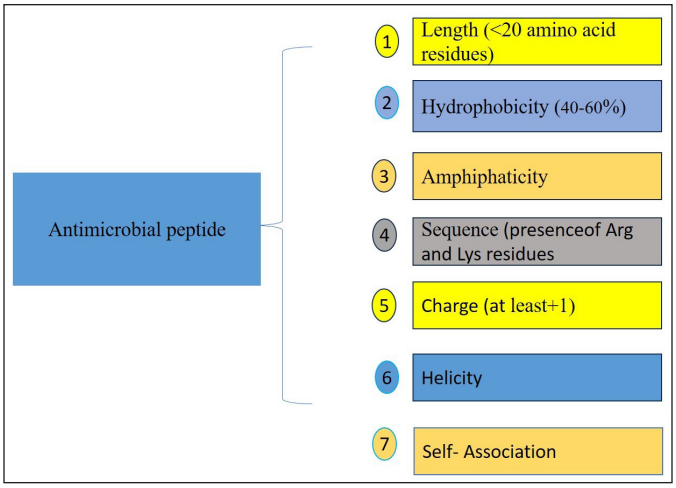

Figure 1. Characteristic features of antimicrobial peptides (AMP).

pneumococcal infections were demonstrated to benefit from this drug [5,6]. Gramicidin demonstrated bactericidal or bacteriostatic effects against a broad spectrum of Gram-positive bacteria both *in vitro* and *in vivo* [5]. Since then, many AMPs have been discovered in both prokaryotic and eukaryotic organisms, e.g., *B. brevis* also produced tyrocidine, which is effective against Gram-positive bacteria, *Triticum aestivum* produces purothionin, which is active against Gram-positive bacteria and fungi [7]. The first animal-based AMP known as defensin was extracted from the white blood cells of rabbits in 1956 [8]. Later on, bombinin was extracted from epithelia [9]; lactoferrin from cow milk; small AMPs from lysosomes of human white blood cells [10]; and the female human reproductive tract [11].

3. SOURCES OF AMPS

3.1 Microbes as Source of AMPs

Bacteria and fungi act as sources of AMPs, with bacteria being the first to be discovered and studied [12]. Bacterial AMPs, known as bacteriocins, are produced not to defend against infections but as a competitive tactic to eliminate other microbes rivalling for the same nutrients [13]. These small molecules, produced by both Gram-negative and Gram-positive bacteria, often have stronger antimicrobial effects than those from eukaryotic organisms, e.g., AMPs from *Pseudomonas* and *Bacillus* species have broad antibacterial effectiveness. The human microbiota also produce AMPs, helping to maintain balance in different body areas, e.g., lactocillin produced by *Lactobacillus gasseri* residing in the vaginal is effective against Gram-positive bacteria, e.g., *Gardnerella vaginalis* and *Staphylococcus aureus* [14]. Filamentous fungi, like *Aspergillus giganteus* and *Penicillium chrysogenum,* produce defensin-like AMPs effective against fungal pathogens [15]. Clinically, gramicidin, a bacterially derived AMP from *B. brevis*, is used in Neosporin® against Gram-positive bacteria, Daptomycin, extracted from *Streptomyces roseosporus*, is an approved AMP for treating skin infections that are caused by Gram-positive bacteria [16].

3.2 Plants as Source of AMPs

AMPs are bioactive peptides found in plants that are essential to their defense against bacterial and fungal infections [17,18]. These peptides are divided into groups according to the formation pattern of disulfide bridges and their amino acid composition. Three prominent families include the thionin, defensin, and snakin families. The biological activity of snails is dependent on six disulfide bridges formed by their 12 cysteine residues. Snakin-Z obtained from *Ziziphus jujube* composed of 31 amino acids is more poisonous to fungus than to bacteria [19].

The earliest plant-based AMP, i.e., purothionin obtained from wheat flour (*T. aestivum*) demonstrates efficacy against *Corynebacterium fascians*, *Corynebacterium poinsettia,* and *Pseudomonas solanacearum* [20]. The efficiency and broad-spectrum activity of plant AMPs underscore their significance in combating microbial threats.

3.3 Animals as Source of AMPs

Animal antimicrobial peptides are produced in exposed areas like skin and mucosal barriers that are more accessible to microorganisms [17]. Several vertebrates, such as fish, amphibians, and mammals, as well as invertebrates have been found to comprehend these peptides. Invertebrates primarily rely on their effective innate immune system in the lack of an adaptive immune system, with AMPs being essential for protecting against microbial threats. According to Jenssen *et al.* [13], invertebrates are capable of producing a wide variety of proteins and peptides found in phagocytes, hemolymph, and epithelial cells [13]. The b-hairpin-like peptides polyphemusin and tachyplesin from horseshoe crabs as well as melittin from bee venom are notable examples of invertebrate AMPs [21,22]. Qi and coworkers 2019 showed that pre-treating mice with Tachyplesin III offers protection against infections caused by *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. Invertebrates can make a diverse array of proteins and peptides located in phagocytes, hemolymph, and epithelial cells [23]. However, these findings still need confirmation through human clinical trials.

More than 200 AMPs are produced by insects depending on the species. Certain species, such *Acyrthosiphon pisum*, do not produce any AMPs; however, *Hermetia illucens* and *Harmonia axyridis* are capable of producing up to 50 AMPs [24]. These AMPs are mostly produced in the hemocytes and fat body of insects and then released into the hemolymph. Based on their amino acid compositions and antibacterial properties, insect AMPs are categorized into groups including, cecropins, defensins, glycine-rich and proline-rich peptides. The first insect AMP discovered, cecropin, is present in both Diptera and Lepidoptera and consists of linear peptides. It is effective against both Gram-positive and Gram-negative bacteria [25]. Peptides known as insect defensins, which range in length from 29 to 34, exhibit robust activity against Gram-positive bacteria and less activity against Gram-negative bacteria [26].

Gram-negative bacteria such as *Escherichia coli* are effectively inhibited by attacins, a glycine-rich AMP type. Although insect-derived antimicrobial medications (AMPs) like diptericin, coleoptericin, and sarcotoxin IIA exhibit promising substitutes for traditional antibiotics, their practical use is restricted, as the majority have only been examined *in vitro*. An exception is the peptide melittin, which is extracted from honeybee venom and employed in medicine due to its wide range of antibacterial and anti-inflammatory effects.

Amphibians, particularly frogs, are rich sources of AMPs, mostly isolated from frog skin. Magainin is a well-known AMP from frogs, that exhibits activity against yeasts, fungi, bacteria, and viruses. Frogs of the genus *Rana* produce peptides like esculentins, nigrocins, brevinins, and temporins, e.g., esculentin-1 which is composed of 46 amino acids demonstrates strong activity against human pathogens. Peptides like brevinin-2Ta show promise in reducing bacterial loads and promoting angiogenesis in pre-clinical studies. Amino acid

substitutions may be explored to decrease hemolytic activity while enhancing antimicrobial effects [27].

Anionic AMPs, such as temporin-1Ja from the Japanese Frog *Rana japonica*, exhibit moderate activity against *S. aureus* and *E. coli*. Some AMPs protect amphibians from ingested pathogens in the stomach mucosa, such as the peptides buforin and buforin II of the Asian toad *Bufo bufo gargarizans*. Synthetic peptides like Pexiganan (MSI-78), an analogue of magainin-2, have been developed for bacterial infection treatment but were rejected by the FDA due to no significant advantage over conventional antibiotics [28].

Mammalian AMPs have been discovered in various species, including humans, cattle, and sheep [29,30]. Some of these peptides, found in mammals, serve a dual role by not only exhibiting antimicrobial activity but also inducing chemoattraction and activating host cells for innate defense [31]. AMPs could be stored in cells like epithelial cells and phagocytes and released in response to stimuli, aiding in infection defense [32]. Defensins and cathelicidins are prominent AMPs in mammals, displaying structural diversity and various functions. The cathelicidin family includes peptides with distinct antibacterial structures, such as a-helical, b-hairpin, and arginine and proline-rich peptides [34]. The BMAP-28, a-helical peptide that belongs to the cathelicidin family, demonstrates antimicrobial effects on bacteria and fungi [35]. Defensins, another group of AMPs, require proteolytic processing for activation and have been identified in various mammalian species, with some being constitutively produced and others inducible [36]. Research on mice infected with *Salmonella typhimurium* revealed that the administration of certain defensins increased mortality and reduced bacterial loads in different organs [37]. Dermcidin, an anionic peptide found in humans, undergoes proteolytic processing in sweat, generating truncated peptides with antimicrobial activity [38]. Many mammalian AMPs, including lactoferricin derived from bovine lactoferrin, show potential clinical applications. Bovine lactoferricin exhibits strong antimicrobial activity, immunological properties, and antitumor effects. It has been successfully used to treat infections, enhance antibiotic effects against ocular isolates, improve diabetic wound healing, and address osteo-articular diseases [39,40]. Additionally, human saliva contains AMPs like histatins, with histatin 5 being particularly effective against various yeasts [41]. Histatins are tested in topical gels for treating oral fungal infections, and efforts to identify fragments with pharmaceutical applications. Peptide P113 has shown promising results in clinical studies [42].

3.4 Synthetic and Engineered AMPs

3.4.1 Synthetic AMPs

Natural AMPs have several drawbacks, e.g., limited availability, frequent folding issues, a short half-life due to rapid degradation, potential toxicity to the entire body, and challenging delivery to the target site [43,44]. Synthetic AMPs have been developed to address the failings of natural AMPs. These synthetic versions exhibit superior efficacy, reduced cytotoxicity, and increased resistance to enzymatic destruction. An example of a synthetic antimicrobial is Novarifyn (NP432). It mainly targets *A. baumannii, C. difficile, E. coli,* and *P. aeruginosa* [12]. The natural peptide AamAP1 from the scorpion *Androctonus amoreuxi* effectively combat infections caused by *Candida albicans, E. coli,* and *S. aureus* at doses between 20 and 150 μM. A synthetic version of this peptide, called AamAP1-Lysine, is 4 to 20 times more effective and can fight these infections at much lower doses, between 5 and 7.5 μM. Although synthetic and natural peptides vary in effectiveness, they work the same way to fight pathogens and have similar traits. These traits can be used to design synthetic peptides to increase the efficacy.

New AMPs can be developed and tested by modifying certain amino acids through the use of *in silico* technology [45]. The mentioned technology aims to speed up biosynthesis and reduce manufacturing costs by improving biological activities and increasing production efficiency. These specially designed peptides are a new type of medication that can both increase the killing of microbes and overcome disease resistance. Hydrophobic and cationic residues are added to increase antibacterial action. Several techniques used to increase stability in the body include acetylation, cyclization, D-amino acids, and peptidomimetics [46]. Yang *et al.* [47] designed a peptide called Sushi-replacement peptide (SRP)-2, which is rich in arginine and has a strong α-helical structure. This peptide effectively kills a wide range of bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA) and multidrug-resistant *A. baumannii*, while being safe for mammalian cells [47]. SRP-2 works by directly interacting with bacterial cell membranes, leading to their death. It also reduces inflammation caused by bacteria. SRP-2 reduced bacterial infections and inflammation when tested on mice. It was found that arginine works better than lysine for making AMPs that target bacteria. Some AMPs have sugar molecules, called glycans, attached to them, which are important for their function. These glycans are usually added to the Golgi apparatus of cells. Since plant and mammalian glycans are different, it is important to avoid allergenic plant glycans in AMPs for humans. Efforts are focusing on changing the glycosylation process to avoid allergenic glycans. Although progress has been made for other proteins, it is yet to be fully applied to AMPs [48].

3.4.2 Engineered AMPs

Biological systems like *E. coli, Pichia pastoris,* and *Saccharomyces cerevisiae* are used to make large amounts of peptides and proteins at a low cost [49]. The most common biological systems for making these AMPs are *E. coli* and *S. cerevisiae*, which account for more than 95%. Bacteria are often used more than yeast to get recombinant antibacterial peptides as many AMPs produced in yeast are either in limited amounts or are inactive [50,51]. One of the main challenges in producing AMPs in microbes is that the peptides can be toxic to the host cells. However, this is not usually a big problem because many AMPs are effective at very low and non-toxic doses. Another issue is that the quality of the peptides can be low due to changes after they are made in the cells. Because of these problems, plants are being considered as a promising alternative for producing recombinant AMPs [52].

Gleba and collaborators 2013 developed a new technology known as "Magnifection" at the German biotech company from Icon Genetics. This technology enables to make of large amounts of proteins and peptides from plants in a shorter duration. It uses *Nicotinia tabacum* or *Nicotinia benthamiana* and involves injecting them with special bacteria carrying viral instructions. These bacteria spread through the plants, delivering the instructions to produce the desired proteins. The process, which combines elements from viruses, bacteria, and plants, results in high yields of AMPs within a few days, making production faster and cheaper [53].

Sampaio de Oliveira *et al.* [50] developed a new method to produce AMPs using a special vector. This vector works in both plants and bacteria, allowing for large-scale AMP production by transforming chloroplasts. Producing AMPs in plants has extra advantages, such as avoiding endotoxin contamination and enabling oral delivery of medicine from grown fruits, which is not possible with bacteria. There

are some problems like the toxicity of AMPs when they are produced in other host cells other than plant-based production systems. Each plant cell can have up to 10,000 copies of the modified chloroplasts, resulting in higher AMP production [50].

4. STRUCTURAL CLASSIFICATION OF AMPS

The vast open-access Database of Antimicrobial Activity and Structure of Peptides (DBAASP) (https://dbaasp.org/) contains data on amino acid compositions, biological impacts, chemical modifications, three-dimensional arrangements, and possible toxicity of peptides with antimicrobial properties. Version 3.0 (DBAASP v3), the most recent version, has more than 22,119 entries [54].

AMPs are categorized into various sub-groups based on amino acid sequences, peptide net charge, and protein structure. The AMP database [DRAMP Data Repository of AMPs, http://dramp.cpu-bioinfor.org/] lists 3,791 AMPs from six kingdoms: 824 from plants, 4 from archaea, 431 from bacteria, 7 from protozoa, 6 from fungi, and 2,519 from animals [55]. A different database, the Antimicrobial Peptide Database (https://aps.unmc.edu), has over 3940 AMPs from the six kingdoms of life (including 383 bacteriocins/peptide antibiotics from bacteria, 5 from archaea, 8 from protists, 29 from fungi, 250 from plants, and 2463 from animals), as well as 190 predicted and 314 synthetic AMPs (as of July 8, 2024) [29]. These peptides are generally classified into four types according to their secondary structures: α-helix, β-sheet, and extended loop, Among the various structural configurations α-helix and β-sheet formations stand out as the most prevalent, with α-helical peptides particularly reaping widespread research attention in the area of AMPs [56]. A few instances of α-helical peptides are melittin, which was taken from the venom of the honey bee *Apis mellifera*, and human cathelicidin, which was produced from LL-37. Magainin was obtained from the African clawed frog by *X. laevis*. It is commonly recognized that these peptides, when exposed to membrane mimic conditions, adopt an amphiphilic α-helix secondary structure [57]. LL-37 is the C-terminal segment of human cationic antimicrobial protein (hCAP-18), the only human cathelicidin known to date that is primarily expressed by epithelial and neutrophil cells.

4.1 Classification of AMPs Based on Charge

A classification based on their net charge is one of the most common ways of classifying the AMPS which significantly influences their mode of interaction with microbial membranes. This charge-based classification is essential for understanding the varying mechanisms through which AMPs target and eliminate pathogenic microorganisms. AMPs can be categorized as anionic, cationic, cationic alpha-helical, cationic β-sheet AMPs, extended cationic AMPs, and fragments from antimicrobial proteins. The various types are explained below in detail:

a) Anionic AMPs: Anionic AMPs, ranges from 5 to 70 amino acids and exhibit a net charge spanning from –1 to –8 (Dennison 2018). They are acknowledged as essential components of natural immune systems across various organisms such as vertebrates, molluscs, and plants. These peptides demonstrate antimicrobial, fungicidal, and anti-infective properties. The major anionic AMPs are peptide fragments resulting from proteolysis, although few are small molecules that are encoded genetically. According to Torres *et al.* [58], they possess a lot of residues of aspartic acid or glutamic acid, which can help bind the metal ions needed for their antibacterial action. Furthermore, tryptophan and other aromatic residues may play a significant role in securing the AMPs to membranes [58]. By constructing salt bridges out of the negatively charged elements and metal ions of the microbial membrane, their method of interacting with microbes seems to be comparable to that of larger proenzymes. The first anionic AMP, i.e., ovine pulmonary surfactant-associated anion peptide (SAAP) with 5-7 aspartate residues made the ovine pathogen *Mannheimia haemolytica* susceptible by constructing salt bridges using zinc ions of the microbial membrane and negatively charged elements [59,60]. The detailed mode of action is mentioned elsewhere in the section. Maximin H5 from amphibians, Dermcidin produced through human excretions [60], Xlasp-p1 [61], and AMP AP2 [62] are a few examples of anionic antibacterial peptides.

b) Cationic AMPs: Cationic AMPs, or CAMPs, are essential elements of the innate immune systems found in many different animals. The majority of AMPS are cationic. They are effective against a broad range of pathogens, such as bacteria, viruses, fungi, and insect pests. They are therefore suitable candidates for antibacterial medication development because their various modes of action mostly comprise interactions with microbial membranes. Their capacity to combat a wide range of species, including fungi, viruses, parasites, and antibiotic-resistant strains of both Gram-positive and Gram-negative bacteria, has been demonstrated by extensive research. These peptides exhibit diverse structural configurations, ranging from alpha-helical and beta-helical to extended forms, further highlighting their versatility and potential in therapeutic applications [63,64].

c) Cationic alpha-helical: Cationic alpha-helical AMPs, typically with an amidated C-terminus, are usually below 40 amino acids and hold a net charge ranging from +2 to +9 [65]. These AMPs, characterized by linear cationic α-helical structures, do not contain cysteine. Cathelicidins comprise a group of cationic AMPs and exhibit amphiphilic α-helical structures. These have N-terminal structural domain that is highly conserved, cathelin is connected to a c-terminal peptide and possesses antimicrobial properties. There are about thirty different types of cathelicidins found in mammals, however, humans only have one, termed as human cationic protein 18 kDa (hCAP18)/LL-37, and mice have one, named cathelin-related antimicrobial peptide (CRAMP). Magainin, cecropins, and LL-37 have all been thoroughly explored [3]. The human hCAP18/LL-37 C-terminal section is the source of LL-37, which has antibacterial potential. It successfully eradicates a wide range of microorganisms, including Gram-positive and Gram-negative bacteria, protozoa, and fungi. The physiological roles of human and mouse cathelicidin peptides have been extensively explored compared to other AMPs apart from their antimicrobial efficacy as demonstrated in the laboratories. Other examples of cationic alpha-helical peptides include andropin, cecropins, moricin, melittin, and ceratotoxin, found in insects; magainin, bombinin, dermaseptin, buforin II from amphibians brevinin-1 and esculentins as well as CAP18 from rabbits [66].

d) Cationic β-sheet AMPs: Peptides typically have 2–8 cysteine residues, forming 1–4 pairs of intramolecular disulfide bonds [67,68]. These disulfide bonds are crucial for the biological functions and structural stability of these peptides. Defensins are the primary component of β-sheet AMPs. Mammalian defensins are categorized into two groups: α-defensins and β-defensins [36]. The tertiary structures of mammalian defensins are strikingly similar, though there is a difference in their covalent structures. In the case of α-defensins, they form a cyclic structure by combining cysteine and disulfide bonds near the amino terminus, and a three-stranded chain through hydrogen bonding with the β-hairpin. The ability of amphipathic α-defensins to disrupt bacterial membranes by interacting with phosphatidyl chains depends on their hydrophobic amino acids and positive charge. The primary mechanism for membrane degradation and bacterial killing may involve the interaction between hydrophobic residues and the bacterium, or between negatively charged molecules and the cationic

α-defensin residues on the bacterial surface. Some β-defensins consist of both an α-helix and a β-sheet, e.g., the middle region of insect defensin A (residues 14–24) has an 11-amino acid α-helix, with the N-terminal β-hairpin parallel to the α-helix, and the first 13 amino acid residues forming a cyclic structure. New research also shows that the cyclic backbone of defensins is essential to their antibacterial and membrane-binding abilities, while the quantity and location of disulfide bonds determine the shape and stability of the protein [2,69].

e) Extended cationic AMPs: The fifth category consists of extended cationic AMPs, which lack typical secondary structures but contain precise amino acids such as tryptophan, glycine, arginine, proline, and histidine. These peptides rely on hydrogen bonds and vanderWals forces interacting with lipids in the membrane for structural stability, e.g., PR-39 is a composed ofarginine (24%), proline (49%), prophenin-1 contains phenylalanine (19%), andproline (53.2%), indolicidin has proline (23%), tryptophan (38%), and histatin-8 includes histidine (33.3%) [57].

f) Fragments from antimicrobial proteins: The sixth category comprises fragments derived from antimicrobial proteins. These proteins, along with their fragments, possess broad-spectrum bactericidal properties. The innate immune system's fight against infections is greatly aided by lysozyme, the earliest antimicrobial protein discovered, which targets invasive microorganisms. Its extracellular segment, consisting of 130 amino acids, adopts a structure comprising α-helix and β-sheet. Similar to lysozyme found in humans and chickens, other proteins with membrane-active and DNA-binding functions exhibit a helix-loop-helix (HLH) motif. This HLH peptide demonstrates potent bactericidal effects against both Gram-negative bacteria and Gram-positive, as well as *C. albicans* fungus. Toda *et al.* [70] identified a gene in fruit flies that amplifies the requirement for sleep. This gene, called nemuri, the Japanese word for "sleep" was determined to be responsible for this increased sleep [70]. The NEMURI protein, which is produced by this gene has immunomodulatory qualities and is located in an arginine-rich area [71]. Interestingly, NEMURI shows strong bactericidal action that is comparable to kanamycin. Interestingly, the amino-terminal copper and nickel (ATCUN) binding motif, which is represented by the N-terminal sequence H2N-XXH and in which XX stands for any amino acid other than proline, is present in certain AMPs. This motif exhibits a high attraction towards Cu^{2+} and Ni^{2+}. It is known that reactive oxygen species (ROS) can damage proteins, lipids, and nucleic acids. The Cu^{2+}-ATCUN combination is capable of producing ROS [70].

5. MODE OF ACTION OF AMPS

AMPs attack the lipopolysaccharide (LPS) layer of the cell membrane, which is present in all microorganisms, in contrast to antibiotics that focus on particular biological processes like DNA or protein production. In addition to this specificity, they possess various characteristics such as hydrophobicity, amphipathic, stereotic geometry, size, charge, and their ability to interact with biological membranes, all of which contribute to their wide-ranging antimicrobial effects. AMPs can easily diffuse due to their small size and quickly release outside of cells, facilitating a swift response against harmful microbes.

AMPs have a strong cell-specificity. They effectively eliminate prokaryotic microbes while posing no harm to mammalian cells. This characteristic is explained by the differences in lipid content between prokaryotic and eukaryotic cell membranes [26]. Positively charged AMPs and negatively charged microbial surfaces interact through electrostatic forces that enhance the contacts between membranes. Microbial surfaces become negatively charged due to teichoic acids in Gram-positive bacteria and LPS cell walls in Gram-negative bacteria which improves the interaction with AMPs [7]. Conversely, sphingomyelin and zwitterionic phosphatidyl choline, which constitute the outermost covering of eukaryotic membranes, do not promote interactions with AMPs as they have a neutral charge at physiological pH.

The capability of AMPs to engage and function against their target cells is largely dependent on both the cell surface and the amino acid makeup of the peptides. The high retention of positive amino acid residues in peptide sequences from different organisms is conducive to this idea. Furthermore, it is essential that the peptide adhere to negatively charged particles like anion phospholipids within the target membrane by means of its dual structure. Depending on the peptide/lipid ratios and affinities, these peptide molecules may be oriented perpendicularly, enabling them to come into contact with the lipid bilayer and form transmembrane holes. Not all AMPs have the same mode of action to break down bacteria membranes.

AMPs are classified into two groups based on how they work: "membrane-acting peptides," which disrupt bacterial membranes by destabilizing them, and "non-membrane-acting peptides," which can cross membranes without harming them but interfere with normal cell activities [7]. In this review article, we have focused only on membrane-acting models.

Membrane Model: AMPs execute their antibacterial activity by triggering bacterial membrane lysis, increasing membrane permeability, and releasing cell content through their interaction with the negatively charged bacterial membrane. As AMPs move toward the cytoplasmic membrane via electrostatic interaction with the microbial membrane, they latch to and interact with the anionic elements of the plasma membrane. AMPs cannot pass through the bacterial cell wall until they've crossed the capsular polysaccharide and other elements like the peptidoglycan and lipoteichoic acid of Gram-positive bacteria and the LPS of Gram-negative bacteria.

The two primary factors influencing the interaction at this step are the peptide-lipid ratio and the conformational shift. Research indicates that α-helical AMPs attach themselves to the anionic lipid membrane to enhance the contact, changing its disordered structure in the aqueous solution into an amphiphilic α-helical structure. Unlike α-helical peptides, β-sheet peptides interact with membranes without undergoing a significant conformational change because of their stable disulfide bond bridge. One important aspect influencing the way AMP interacts with the cell membrane is the peptide-lipid ratio. AMPs are parallel on the plasma membrane surface at low peptide-lipid ratios. As the peptide-lipid ratio rises, AMPs are vertically inserted into the hydrophobic core of the membrane. Membrane penetration ultimately results in the leakage of intracellular ions, metabolites, and biosynthesis, which finally causes cell death.

Four models have been put out to describe how AMPs cause bacterial membranes to permeabilize (Fig. 2).

a) The Barrel-Stave Mechanism

The barrel-stave model illustrates how AMP implantation explains how peptide bundles form transmembrane pores or channels. The hydrophobic residues of α-helical and β-sheet peptides face outward after binding, whereas their hydrophilic surfaces form pore linings [72]. These peptides undergo a conformational phase shift upon engagement, which compels the polar phospholipid head groups to

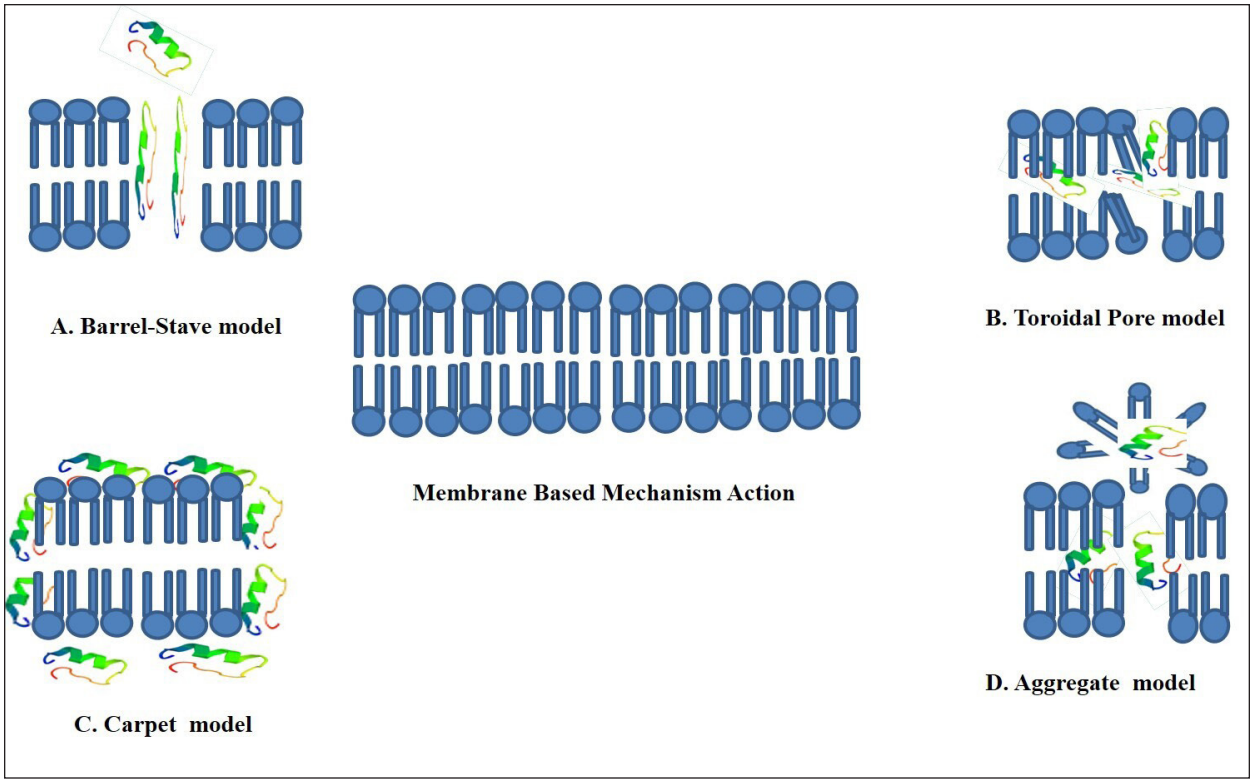

Figure 2. Models of antibacterial mechanisms of antimicrobial peptides (AMP).

align and causes the membrane to taper. This mode of action has been suggested on alamethicin, a peptide formed by the fungus *Trichoderma viride* [73]. In the barrel-stave model, AMPs come together to form aggregates, break through the cell membrane's bilayer as multimers, also, allow the cytoplasm to escape thereby creating channels. AMPs can potentially cause cell death by inducing cell membrane collapse in harsh situations [74]. Furthermore, simulations demonstrate that in explicit and implicit membranes, hairpin AMP protegrin-1 can form stable octameric β-barrels and tetrameric arcs (half barrels) [72].

b) The Torroidal Pore Mechanism

The toroidal concept refers to a membrane deflection that can result from AMP insertion that is perpendicular to the lipid head groups [75]. It is known as the supramolecular complex or wormhole model. This well-studied peptide-membrane interaction forms a membrane-spanning pore that is lined with head groups of phospholipids and polar peptide surfaces. These peptides' hydrophobic residues push aside the polar head groups, rupturing the hydrophobic portion of the membrane and causing a strain in the direction of positive curvature [76]. The peptides align perpendicular to the membrane when they reach a crucial peptide-to-lipid ratio (about 1:30 for magainin). The Macagainin 2, lacticin Q, and arenicin are common examples of this model [77]. After that, the helices self-associate, moving their polar residues away from the membrane's hydrocarbon chains to create a dynamic peptide-lipid supramolecular structure. Omarien *et al.* [78] claim that cationic peptides that form fluid domains, such as TC19, TC84, and BP2, also reduce the membrane barrier [78].

c) The Carpet Mechanism

Gazit *et al.* [79] proposed that AMPs can also be arranged parallel to the membrane, covering it entirely and forming micelles simultaneously with the breaking membranes [79,80]. Electrostatic binding is the cause of the initial interaction. The peptides then induce membrane penetration, which disintegrates the microbial cell, when this contact reaches a threshold concentration. Because they aid in reducing the repulsive electrostatic forces between positively charged peptides, negatively charged lipids are necessary for the formation of a peptide carpet. In this model, AMPs are bound parallel to the membrane surface by the interaction of positively charged cationic peptides with negatively charged polar phospholipid heads. The peptides realign inside the membranes after reaching a particular concentration, creating micelles with a hydrophobic center that eventually causes membrane disintegration [80].

d) In the aggregate model

AMPs force the peptides and lipids to combine into a peptide-lipid complex micelle by binding to the anionic cytoplasmic membrane. AMP-formed channels, together with lipid and water channels, facilitate the leakage of intracellular contents and ions, which ultimately results in cell death, in contrast to the carpet model. Additionally, these channels might facilitate the entry of AMPs into the cytoplasm where they can operate. This process elucidates why AMPs can operate on intracellular molecules not just on the cytoplasmic membrane but also across it. The mechanisms underlying the activity of anionic AMPs are yet unknown, in contrast to cationic AMPs. The antibacterial activity of Maximin H5 against *S. aureus* has been associated in multiple investigations with membrane disruption. Aspartic acid residues play a minor structural role in maximin H5 due to their distance from the microbial membrane, limiting their direct involvement in membrane disruption. The antimicrobial activity of the peptide primarily arises from its N-terminal α-helical region, which binds and destabilizes the microbial membrane. Aspartic acid residues mainly help maintain the peptide's structure, indirectly supporting its function. Stabilizing the α-helix structure of the peptide requires hydrogen bonds created by

amidation of the C- and N-terminal. Additionally, it seems that low pH enhances the degree of α-helix of maximin H5 and encourages the "Carpet"-like mechanism of killing *S. aureus*. The anionic AMP Xlasp-p1 exhibits significant broad-spectrum antibacterial action against both Gram-positive and Gram-negative bacteria via the disruption of cell membranes and intracellular material efflux.

6. FUNCTION OF AMPS

6.1 Antibacterial Activity of AMPs

The antibacterial effect of AMPs may or may not involve the cell membrane. As previously mentioned, cationic AMPs are strongly attracted to microbial pathogens due to the presence of specific anionic components in the plasma membranes of bacteria and fungi. These components include lipoteichoic acid in Gram-positive bacteria, LPS in Gram-negative bacteria, and mannan in fungi. AMPs can cause membrane perforation or permeation, leading to either penetration into the membrane or leakage of intracellular contents, thus exerting their intracellular effects.

6.2 Antiviral Activity

Antimicrobial peptides not only have antibacterial properties but also exhibit a wide range of antiviral activities against enveloped viruses, e.g., Bovine antibiotic peptide-13 inhibits the transmissible gastroenteritis virus by disrupting virus protein synthesis and gene expression [81]. AMPs such as protegrin and indolicidin block herpes simplex virus (HSV) by targeting viral membrane glycoproteins, preventing virus adhesion and entry. LL-37 inhibits viruses like HIV, influenza A virus, and others by destroying their membranes and inhibiting DNA replication [82]. LL-37 and CRAMP in mice significantly inhibit non-enveloped enterovirus 71 by regulating antiviral responses and preventing viral binding [83].

Le Messurier *et al*. [84] 2016 indicated that AMPs can enhance immune responses to influenza A virus thereby boosting the protection of host [85]. Peptides such as pa-MAP and temporin B inhibit HSV1 by preventing viral attachment, with temporin B also damaging the virus envelope. Temporin G blocks the fusion of the influenza virus envelope with host cells by interacting with viral hemagglutinin protein [86]. Parainfluenza respiratory virus, temporin G impairs viral replication by blocking late replication steps, inhibiting viral release. The peptide HD5, which is derived from human defensin, inhibits the adhesion and penetration of viruses, hence preventing viral infection [87]. The amino acids GF-17 and BMAP-18, derived from cathelicidin, work against the Zika virus by directly rendering it inactive and disrupting the interferon pathway. Other AMPs show antiviral activities against dengue and pseudo-rabies viruses. AMPs can also fight non-enveloped viruses. LL-37, for example, is effective against adenovirus, rhinovirus, and Aichi virus. Besides directly inhibiting viral particles and replication, AMPs modulate the host immune system to indirectly inhibit virus growth.

6.3 Antiparasitic Activity

The antiparasitic efficacy of AMPs is not well documented, particularly *in vivo* and in clinical contexts, despite extensive research on their antibacterial and antiviral roles. Parasites, including protozoa and worms, significantly contribute to the global disease burden, posing major health problems worldwide. WHO has classified eleven types of parasites as Neglected tropical diseases due to their impact on millions of people, particularly the poor. Major parasitic diseases include malaria, leishmaniasis, trypanosomiasis, and schistosomiasis. Recently, there has been growing interest in using antibacterial peptides for antiparasitic therapy [88]. Leishmanicidal AMPs are notably present in a variety of organisms, e.g., the venom of honeybees contains halictine-2, which exhibits strong anti-leishmanial activity without endangering mouse macrophages or human red blood cells; the cyanobacteria *Lyngbyamajuscula* contains the linear lipopeptides Attacin, cecropin, and defensin 2, which react to *Leishmania infantum* chagasi infection via the Toll and Imd pathways; and finally, the cyanobacteria *Lyngbya majuscula* contains the linear lipopeptide Dragomide E, which is active against the promastigotes of *Leishmania donovani*. Furthermore, a peptide from snake venom called LZ1 dramatically lowers the blood stage of *Plasmodium falciparum* and selectively inhibits ATP activity in red blood cells infected with malaria. Because of its distinct chemical structure, Phylloseptin-1, which is secreted by *Phyllomedusa azurea*, has strong antiparasitic activity and inhibits the emergence of cross-resistance.

6.4 Anticancer Activity

New anticancer treatments are being explored due to cancer cells' resistance to existing therapies and the toxicity of chemotherapy drugs. Antibacterial peptides, which might help prevent cancer growth, have become a research focus. Zhao *et al.* [89] reported that the HPRPA1 peptide from *Helicobacter pylori* has anticancer properties. Additionally, combining the homing peptide iRGD with HPRPA1, was found to enhance its anticancer effects, with iRGD improving HPRPA1's penetration into A549 MCS184 cells. Moreover, it has been demonstrated that Lk6 can kill M7CF breast cancer cells by causing nuclear disruption without damaging the cell surface [90].

6.5 Immune Modulation Activity

Antimicrobial peptides may be able to stimulate and suppress immune cells: which results in better control of inflammation, and increased cell killing [91]. The AMPs can also trigger various immune responses, including: activation, attraction, and differentiation of lymphocytes; stimulation of angiogenesis; reducing inflammation by lowering the production of pro-inflammatory chemokines; and repressing expression of reactive oxygen nucleic acids [92]. In addition, AMPs such as those of neutrophils and macrophages are also produced by many immune cells that can provide the first defense against invading microbes.

6.6 Anti-Biofilm Activity

Biofilms have a high level of resistance to traditional antibiotics and play a significant role in spreading germs in the environment. They have been linked to as much as one-third of human infections [93]. Studies have found that some aminoglycosides have anti-biofilm activity, separate from their ability to target free-swimming planktonic cells. The discovery that LL-37, at one-sixteenth of its minimum inhibitory concentration (MIC), can hinder the establishment of *Pseudomonas aeruginosa* biofilms and disperse existing biofilms [94] led to the idea of using cationic peptides as anti-biofilm therapies. Consequently, there has been a surge in research to identify natural and synthetic agents with anti-biofilm potential. These agents work differently from antibiotics, with many effective anti-biofilm peptides acting at concentrations well below their MICs for planktonic biofilm cells.

Mechanistic investigations have demonstrated that synthetic bactenecin derivatives block biofilm activity by targeting and destroying guanosine tetraphosphate (ppGpp), a chemical that signals a rigorous

stress response. Bacterial production of ppGpp is essential for biofilm formation and maintenance under nutrient-restricted conditions. By targeting ppGpp, AMPs prevent biofilm formation, disperse existing biofilms, and increase bacterial susceptibility to conventional antibiotics. The significance of anti-biofilm compounds has grown due to their association with numerous clinical infections. Several techniques have been developed to evaluate the anti-biofilm impact of AMPs on various harmful bacteria. The most basic technique involves staining polymers with crystal violet dye to quantify the amount of biofilm and determine the minimum biofilm inhibitory concentration. Other assays include the Biofilm Ring Test and the Calgary Biofilm Device, which involve static biofilms and the use of crystal violet to quantify biofilm mass. Nevertheless, these static techniques have drawbacks, including limited availability of new growth medium, a high background of dislodged bacterial cells, and comparatively fresh biofilms. The colony biofilm assay measures anti-biofilm activity on peptide-infused agar but has questions regarding its validity as a biofilm model. The enhanced observation of biofilm adhesion and development in the presence of AMPs can be achieved by flow cell equipment and confocal imaging, although with inferior throughput.

7. CLINICAL APPLICATIONS

Antimicrobial peptides are currently being evaluated for their effectiveness in treating local infections. Indolicidin is found to be effective against *Aspergillus* fungal infections. The α-helical peptide SMAP29 is effective against *P. aeruginosa* in peritoneal infections; β-sheet protegrin works against vancomycin-resistant *Enterococcus faecalis* (VRE); MRSA and *P. aeruginosa* [95]. Magainin Pharmaceuticals, Inc. (Plymouth Meeting, PA) has advanced the α-helical magainin variant MSI-78 to a phase III clinical trial to test its efficacy against polymicrobial foot-ulcer infections in diabetics. The trial results indicated that MSI-78 is as effective as orally administered ofloxacin, with fewer side effects. Applied Microbiology has started trials to test the lantibiotic peptide nisin's effectiveness against *Helicobacter pylori* in stomach cancer (http://www.businesswire.com/cnn/ambi.htm). Additionally, some peptides have shown efficacy in treating systemic infections. Human lactoferricin has also demonstrated effectiveness in treating systemic infections.

8. CHALLENGES AND LIMITATIONS

The toxicity, instability, and high manufacturing costs of AMPs are some of the obstacles to their use as therapeutic candidates [96]. This can be worked around by synthesizing shorter, more digestible AMPs, or short antimicrobial peptides, with 2–10 amino acid residues. While it's widely acknowledged that there are obstacles in turning nonclinical candidate AMPs into profitable clinical products. Combining peptides with other drugs can improve bioavailability, address multi-drug resistance, and increase efficacy, especially during pandemics. Developing rapid, cost-effective, and eco-friendly synthesis techniques is vital. Advances in gene editing, AI, and CRISPR-Cas9 support peptide drug development, but any modifications must maintain the peptides' biological functions and avoid toxicity.

9. FUTURE PERSPECTIVES

The traditional discovery of AMPs involves screening peptide libraries from specific organisms, a process marked by trial and error. However, newer computational methods predict AMPs from proteomic or genomic data. Although selecting suitable organisms is challenging, the microbiota is a rich source of unexplored AMPs. Combining experimental and bioinformatics tools for metagenomic data will aid AMP discovery. Analyzing omic data from diverse microbiomes and developing new AMP discovery tools are crucial. AMPs are essential for limiting pathogenic microbiota and shaping the microbiome, potentially leading to therapies for diseases related to microbiota imbalances, including infections of various body systems. Using mobile elements like bacteriophages or plasmids to deliver AMPs can combat resistant pathogens without encouraging antibiotic resistance. The future of AMP research is promising, with many microbiota-produced AMPs yet to be discovered, posing an excellent research opportunity. There is much to learn about their discovery, characterization, and mechanisms. AMP research will progress significantly, with applications in food preservation, agriculture, healthcare, cosmetics, and industry.

10. CONCLUSION

Traditional antibiotics can be replaced with AMPs to treat bacterial infections. The search for novel AMPs with increased potency, selectivity, or affordability is gaining momentum. The combination approach, which uses AMPs in addition to traditional antibiotics, can increase the efficiency of the former while lowering the latter's resistance. The two main ways to produce AMPs are by chemical synthesis and genetically modified bacteria; nevertheless, for practical application, it is essential to improve biological preparation techniques, lower costs, and increase yields. As more natural AMPs are found, it will be vital to comprehend how AMPs are expressed in organisms and to find better expression vectors to produce AMPs in large quantities in the future. The structure-function correlations of reported AMPs also require more investigation. Apart from their microbicidal properties, AMPs also exhibit other biological characteristics, which could make them useful as immunological modulators, signaling molecules, antitumor agents, and drug delivery vehicles. Therefore, comprehending the diverse biological characteristics of AMPs and their mechanism of action can be crucial for the clinical advancement of peptide-based treatments.

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13. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

14. DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

15. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

16. PUBLISHER'S NOTE

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The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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