ANTIMICROBIAL PEPTIDES CAN BE AN ALTERNATIVE TO ANTIBIOTICS

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Abstract: For the rapid growth of the pathogenic bacteria to the conventional antibiotics leads to determine the urgent need for a search of fundamentally new anti-infective drugs. Antimicrobial peptides (AMPs) of the innate immune system are promising conditions for a role of such novel antibiotics. However, some cytotoxicity of AMPs towards host cells limits their active implementationin medicine and forces attempts to design numerous structural analogues of the peptides with optimized properties 1. Antimicrobial peptides are widely distributed throughout the animal and plant kingdoms. These peptides are involved in the direct destruction of various microorganisms like bacteria, fungi, parasites, viruses etc. and also can destruct tumor cells. Antimicrobial activities of AMPs, primarily disrupts membranes, so they have a lower likelihood of inducing drug resistance.

Extensive studies show that net charge, hydrophobicity and amphipathicity are the most important physicochemical and structural determinants endowing AMPs with antimicrobial potency and cell selectivity. Designing AMPs for therapy will need to focus on such factors like their susceptibility to proteolytic degradation and reduction of toxicity to mammalian cells. Strict guidelines pertaining to their use should also be established to prevent or hinder future development of bacterial resistance to such peptides 2. This review summarizes the recent advances in the development of AMPs with respect to characteristics, structure-activity relationship, functions, their usage against microbes, expression, regulation and their applications.

Keywords: *Antimicrobial peptide, antimicrobial resistance, therapeutic agents, antibiotics, antibacterial activity, antifungal activity, antiviral peptides, antiparasitic peptides, antitumor activity.*

I. INTRODUCTION

Bacterial resistance to various antimicrobial agents has developed as a result of theresponse to their direct exposure. This can be happened, if target protein is altered by mutation or enzymatic activity, or by passing of target protein, or the extrusion of the antimicrobial from the cell

.These adaptations can arise in susceptible bacteria as a result of mutations or through horizontal genetransfer, either within or between genera , primarily employing mobile genetic elements such as plasmids, transposons or integrons 3.Alarmingly, antibiotic-resistant bacteria have been increasingly isolated from patients and animals treated with antibiotics, and this resistance to the most conventional antibiotics has become one of the most pressing global public health concerns

worldwide .Therefore we need to find an alternative antimicrobial strategy 4. Antimicrobial peptides have been described as evolutionary ancient weapons 5. Found in both animal and plant kingdoms, they play a fundamental rolein the innate non-specific defence system which confers resistance against infections without prior exposure to foreign pathogens 6 . Antimicrobial peptides are the alternative participants for design of a new antimicrobial agents for their specificity, they have natural antimicrobial properties and a low propensity for the development of bacterial resistance. In addition to rapid and broad-spectrum activities against Gram negative and Gram positive bacteria, fungi, parasites, viruses and tumor cells, AMPs also mediate chemotaxis, apoptosis, immunomoduleatory effects and wound healing 7. The antimicrobial mechanism of AMPs varies from membrane permeabilization to interaction with an array of intracellular target molecules, and differes from that of antibiotics that target specific molecular receptors of pathogens.

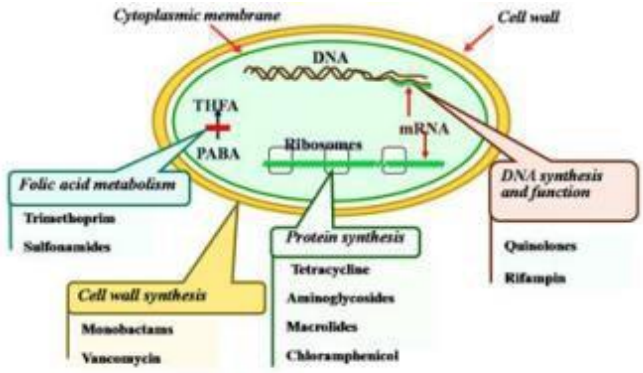


FIGURE 1 Targets of antibiotics [Color figure can be viewed at wileyonlinelibrary.com]

The membrane interactions between AMPs and bacteria to acquire resistance against them, whichsupports the wide application of AMPs in food, medicine and animals for avoiding the overuse of antibiotics. These peptides are stored in granules of phagocytic cells and exert their effects in phage lysosomes or being secreted extracellularly; they are also widely expressed and released at epithelial surfaces and in a site of inflammation 8. They have different amino acid sequences and structures, but most of them are cationic and they can adopt an amphipathic conformation, for this reason they are able to easily interact with the negatively charged components onthe surface of the bacterial cells and integrate into the lipid bilayers. The main mechanism of antibacterial action ofAMPs is related with their ability to alter membrane permeability and damage its structure 9. Some AMPs are non-membranolytic and penetrate bacterial membranes without disturbing their integrity. They have intracellular targets and interfere with the metabolic processes, including synthesis of the vitally important cell components 10 . For this wild-scale multitargeted action it is believed to be one ofthe reasons for the effectiveness of AMPs towards multidrug -resistance bacterial strains. For this widebeneficial features of AMPs such as broad- spectrum activity, swift and effective bacterial hilling that also complicates the resistance development, would healing promotion, it is thought to be used as a replacement of antibiotics and also in food, animals etc.

1. Discovery of AMPs: Most of the natural antimicrobial peptides are isolated chromatographically from bacteria, plants, fungi and animals. During the first wave ,prior to 1980s,several non-gene encoded antimicrobial peptides were discovered. From 1980s, the second wave started, during this time researchers started their studies over the innate immunity and mechanisms of action of gene-encoded AMPs as potential antimicrobials. Since around 2000, immune modulation properties of AMPs have been reported. In 1922, Sir Alexander Fleming discovered lysozyme, is now recognized as the first AMP. The discovery of lysozyme did not stir up much interest at that time. Lysozyme inhibits bacteria by cleaving saccharides on the cell wall 11. The small protein may be used topically as its size makes it unstable for systemic use 12 . Rogeres(1928)1st noticed the ability of nisin to inhibit bacteria, it was the 1st antibiotics. It contains multiple thioether rings. It is the only bacteriocin, which is approved by the US Food and Drug Administration (FDA) as a food preservative. It is used as preservatives of meat and dairy products (<12.5mg/kg food) in over 50 countries. Nisin attacks the cell wall of Gram-positive bacteria like Listeria monocytogens. Nisin also inhibits Gram-negative pathogens such as Escherichia coli and Salmonella spp. When used in combination with chelators or heat treatment 13 .Dubos discovered gramicidin from a soil bacterium Bacillus brevis. Gramicidin A consists of alternating L and D amino acids. The N-terminus of this peptide is formylated 14. It consists of hydrophobic amino acids. Such sequence is viral for the formation of a head-to-head dimer as a membrane channel. It is the 1st peptide-antibiotic which was used clinically as a topical treatment. Gramicidin S isolated from bacteria, used to treat infectious wounds. This small peptide is cyclic, forms peptide bond between the termini. It works both against Gram positive and Gram negative bacteria. Gramicidin S is still used in topical oinments and eye drops 15. Polymixin E (Colistin) is also a bacteriocine is still used clinically to treat infection caused by Gram negative pathogens . It has a cyclic peptide structure followed by a lipid tail 16. Daptomycin has a similar structure, with a negative charge. It requires the presence of Ca2+ to show its full activity. In 2003 Daptomycin was approved by FDA to treat Gram positive bacteria infections. Alamethicin isolated from the fungus Trichoderma viride is also an AMP. It consists of seven alpha-aminoisobutyric acids forming a helical structure. It prevents Gram positive bacteria and fungi. This is perhaps the only AMP with evidence to support to barrel-stone pore in membranes 17. Boman et al. discovered ceeropins(1980s) from the moth Hyalophora Ceeropia. Lehrer and his colleagues identified the first alpha defensins from human neutrophils 18. Subsequently the first beta defensin was discovered from cattle 19. Cyclic theta defensin was discovered by Tang et al.(1999). All of these defensins contain three pairs of disulfide bonds 20. Due to their small size and stability, there is substantial interest in developing therapeutic uses for theta defensin miniproteins 21.Lucifesin is a defensin, discovered in 2010 from insects. This is probably a key antimicrobial element for maggot therapy.

In summary, all of these peptides mentioned above, is used as antimicrobial agents, also some are under development. In addution, the induction of AMP expression, at a needed sitre and tine, provides a new avenue for antimicrobial development 22.

II NOMENCLATURE OF ANTIMICROBIAL PEPTIDES:

Although various methods are employed for naming the newly identified peptides, there are three mostcommon methods present

i)Source base methods

ii)Peptide base method

iii)Source and peptide combined methods

i) SOURCE BASED METHOD: It is the most common method. In this method either species or genus name is taken.Example : Sesquin is derived from *vigna sesquipedalis* ,here the name comes from the species name. Palicourein is derived from *Palicourea condensala* , here the name derived from the genus name.Sometimes peptide name is derived from the common name of the organism eg, termicin from termites. Also abbreviations of animal names are utilized such that bBD-1(bovine beta defensing -1). Other animal abbreviations include PMAP-36 where P indicates pigs. e-CATH-1, e indicates equine. Sometimes, names of organs or tissues are also utilized . eg, Human neutrophil peptide-1(HNP-1) Liver expressed antimicrobial peptide-2 (LEAP-2).

ii) PEPTIDE BASED METHOD: Same AMPs are named according to their peptides' properties. The name of defensin is derived from the word 'defence', implying the functional role of this family of peptides. On the other hand many AMPs are named after their amino acid sequences.Example : Humanhistatins are named like this as they are rich in histidine residues. PR-39 consists of 39 residues which is rich in proline and arginine residues.

iii) SOURCE AND PEPTIDE COMBINED METHOD : In some cases, source of organisms and peptide features are combined to assign a unique name of AMPs.For instance,Ib-AMP is abbreviated from Impatiens balsamina antimicrobial peptide. When there are multiple similar peptides, they are namedby giving numbers such as Ib-AMP1,Ib-AMP2,Ib-AMP3, Ib-AMP4 etc.

III MAJOR PARAMETERS THAT DETERMINE THE ACTIVITY OF ANTIMICROBIAL PEPTIDES

i) **LENGTH :** Length of the antimicrobial peptides play a major role to determine their activity as at least 7-8 amino acids are required to form amphipathic structures with hydrophilic andhydrophobic faces on opposite sides of a peptide molecule. In the barrel- stave model at least 22 amino acids should be present for alpha-helical AMPs and at least 8 amino acids areneeded for beta-sheet AMPs. Besides the effect of length on the mode of action, it also affect its cytotoxicity. For example: a shortened melttin and a shorter derivative of HP(2-20)exhibited at least 300 times less toxicity to rat erythrocytes, respectively, compared to their original length. Therefore the length of AMP should be taken into consideration when designing a new synthetic peptides with low toxicity.

ii) **CHARGE:** The net charge of the AMPs is the sum of all charges of ionizable groups of thepeptide. Charge of AMPs varies from positive to negative, and it is one of the most important factor for it's antimicrobial activity. Most of them are positively charged; ranging from +2 to +9, the interaction between AMPs and cell membrane mainly relies an electrostatic attraction. It is generally assumed that cationic AMPs initially interact with negatively charged lipid head groups on the outer surface of the cytoplasmic membrane. The peptide than penetrates the outer leaflet of the cytoplasmic membrane lipid bilayer in an approximately parallel orientation to the bilayer, which leads to the displacement of lipids23. But an excessive charge can affect it's antimicrobial activity. Continuously increasing the number of positive charges may not increases it's activity. Same anionic AMPs are also present, they are rich in Glu and Asp, theyparticipate in the eukaryotic innate immune response. These peptides have not charges ranging from-1 to -2, and they generally require cations for .zn^{2+}, as compactors for biological activity. By changing the net charge of an AMP , its antimicrobial and hemolytic activities can be altered to achieve selective killing of microbes with no or minimized effects on host cells. For eg:, increasing positive net charge of V13 K from +8 to +9 resulted in higher hemolytic activity, while decreasing the net charge to lower than +4 abolished its activity against P. aeroginase 24.

iii) **HYDROPHOBICITY:** It is the another important factor which influence the activity and selectivity of APMs . Almost 50% of amino acids in primary sequence of natural AMPs are hydrophobic residues 25. In most of the cases increase in hydrophobicity on the positively charged side below a threshold value can increase its antimicrobial activity. On the other hand, decreasing the hydrophobicity can reduce antimicrobial activity. Each and every AMPhas an optimal hydrophobicity, beyond which its activity decreases rapidly. Therefore while designing new synthetic peptides, the hydrophobicity should be selected at around optimalvalue. Some studies shown that hydrophobicity is also a critical component for selecting therange of target cells of an AMP. By changing the hydrophobicity of an AMP it's target range can be changed. For eg.,magainin is an AMP that is only effective against Gram-positive bacteria. However, some synthetic analogs with higher hydrophobicity can also kill some Gram-positive bacteria and eukaryotic cells 26. AMPs with high hydrophobicity can damage the membranestructure, which results in cell lysis or the formation of transient pores and the transport of peptidesinside the cell, this property enables them to interact with intracellular targets 27.

IV) **Amphipathicity:** It is the another important property of AMPs which ensure the activity andinteraction with microbial membranes.

Fermandes -Vidal et al. showed that amphipathicity is moreimportant than hydrophobicity for binding to microbial membranes.

Because the amphipathicity of AMPs is required for a strongpartition into the membrane interface, priority should be given to the amphipathic structure when designing synthetic AMPs for specific target cells.

It is the most important physicochemical and structural parameters for AMPs antimicrobial activity. It results from the segregation of hydrophobic and polar residues on the opposite face of the molecular framework. Some studies suggest that perfect amphipathicity often results in a simultaneous increase in both bactericidal and cytotoxic activity. Wanget al found that imperfectly amphiphilic peptides should better antimicrobial activity than the corresponding perfectly amphipathic peptides.

V) **Covalent Bonds:** Covalent bonds have profound effect on AMPs. The modification the covalent bonds should have positive or negative effect on AMPs work means it can increase or reduce it's antimicrobial activity. For eg.,Protegrin missing a disulphide bond becomes inactive against HSV; while adding disulphide bond in Sakacin P resulted in higher antimicrobial activities 28. Another studies shows that addition of a disulphide bond and a trp-trp crosslink to indolicin, increase its stability against protease with no change in antimicrobial activity.

However increase in stability does not always lead to better AMPs. For eg., Houston et al. introduced a covalent bond to form a lactam bridge between Gln and Lysresidues in two alpha-helical AMPs, eg., Cecropin and mellitin. This modification helped AMPs to form more stable alpha-helix structures but decreased the antimicrobial activity of both.

IV STRUCTURE OF AMPS

Primary structure:

Sequence Length: AMPs have variable sequence length ranging from 10-60 amino acid residues shorter AMPs are preferred for their low producing cost, many of them show similar antimicrobial activities to those of longer AMPs, eg., the hexapeptide MP196 (RWRWRW-NH_2) shows robust activity against E.coli and S. aureus with an minimum inhibitory concentration(MIC) value of 5microgram/liter
29. Moreover, long chain linear peptides are often more hemolytic and cytotoxic but their N or C- terminal- truncated sequences usually have a lower cytotoxicity but retain robust activity 30. However, peptide with very short lengths shows a decreased tendency to form amphipathic secondary structures,which leads to low antimicrobial potency. So, it can be said that AMPs have a certain threshold length for binding with membranes with a high efficiency and form helical structures.

Amino acid composition: AMPs have mainly two types of amino acid residues, cationic and hydrophobic residues. Naturally occurring AMPs contain Arg; Lys and His amino acid residues and hydrophobic residues are mainly alipathic and aromatic amino acids. The positively charged amino acidresidues of AMPs directly interact with the negatively charged component of bacterial cells. Then, the hydrophobic residues get incorporated into lipid bilayers to mediate membrane permeabilization and disruption, which lead to rapid cell death.

Characteristics of secondary structures:Alpha-helix:

According to updated database, the proportion of the natural alpha-helical peptides is the highest among AMPs (fig 2), and they are mainly derived from different species, including insects, fish, amphibians, mammalians and plants. Many studies have found that most of these peptides, interactingwith membranes are converted to alpha-helical structures. This transformation induces the segregationof hydrophilic/ charged amino acid in space from the hydrophilic residues, which results in an amphipathic structure that is recognized as a prerequisite for AMPs to act on membranes 31.

Sequence analysis of PMAP-36 showed that it has the highest proportion of cataionic amino acids (36%),most frequently at the N- terminus. Structural analysis of PMAP-36 further demonstrate

that this highly cationic sequence adopts a typical amphipathic alpha-helical conformation and random hydrophobic tail. Recent studies have indicated that N-terminal region (alpha-helical domain) of PMAP-36 is the active region 32. Researchers have found that the disruption of amphipathicity increased antimicrobialactivity with very little hemolysis.

According to the helical wheel projection, Ma et al designed a small combinatorial library of Val/ Arg - rich peptides, and the peptide G6 was found to have optimal cell selectivity. G6 has been further demonstrated to reduce peritoneal bacterial counts and increase survival after *Salmonella typhimurium*infection 33.

It was found that the net increase in charge increase antimicrobial activity and decrease hemolysis andthe substituition of Gly with Pro significantly reduce toxicity. Sources of some AMPs along with their chemical and biochemical properties are summarized in Table 1.

Beta-sheets: Besides alpha-helixes, beta-sheets are another principal secondary structures of AMPs. Inaqueous solution most of the peptides change their conformation from unstructured forms to beta- sheet structures in a membrane mimetic environment.

Generally beta-sheet peptides consist of two to ten cys residues, they form disulfide bridges for stabilizing their bioactive conformation. Additionally, peptides that contain disulfide bonds often adopt a cyclic beta-hairpin conformation, such as tachyplesin and protegrin 1 (PG 1) 34.The turn region of PG-1contains three positively charged Arg residues, and this is considered to be active center of PG-1.
Natural beta- sheet AMPs play important role in innate immune system by killing invading pathogens orregulating immune responses. Defensins have six to eight Cys residues in defined positions, conserved across most multicellular organisms.

Peptides that typically adopt alpha-helix and beta-sheet structures have been designed to improve the prospects for applying AMPs therapeutically and the relationship between structures and bio-activities has been investigated 35.The beta-sheet structure posses greater cell selectivity than that of alpha- helical peptides with equal hydrophobicity and charge. But it is difficult to form robus beta-structure forshort peptides. Studies have shown that several rational designs of synthetic beta-sheet folding peptideamplifies broad-spectrum and highly selective antimicrobial activities. Therefore, the protein folding theory and the common features of natural AMPs provide a basis for the design of beta-sheet AMPs, including:(1)the intrinsic beta-sheet propensities of amino acids in strands and cross-strand interactionsacross strands, (2) a net positive charge mediating peptide interactions with negatively charged membranes of bacteria; (3) hydrophobic residues providing lipophilic anchors and ultimately including membrane disruption, and (4) the reasonable arrangement of amino acid residues to form a structure with amphipathic characteristics, which segregates cationic and hydrophobic residues to opposite facesof the folded molecule 36. Researchers have found that synthetic hairpin structure of a *S.typhimurium* strain provided resistance to bacterial infection. Subsequently, a series of symmetric end beta-sheet peptides showed the effect of the type of amino acid on AMP activity.

Extended structures: Most of the extended properties with a high proportion of Pro and Gly residuesusually exhibit a linear structure, rather than typical secondary structures. Therefore, extended properties can be divided into Pro and Gly-rich peptides 37. Indolicidin has Pro-rich extended property and it is isolated from mammals, on the other hands apidaecins are isolated from insects. Pro-residues varies from 15-39 residues. The pro-residue is often associated in doublets and triplets with basic residues (Arg and Lys). Generally short Pro-rich peptides exhibit

effective activity against Gram negative bacteria while maintain low antimicrobial activity against Gram positive bacteria. Several Gly-rich AMPshave been isolated from various insects. The size of Gly-rich peptides varies from 8kDa (holotricin) to 30kDa (sarcotoxin II) 38.

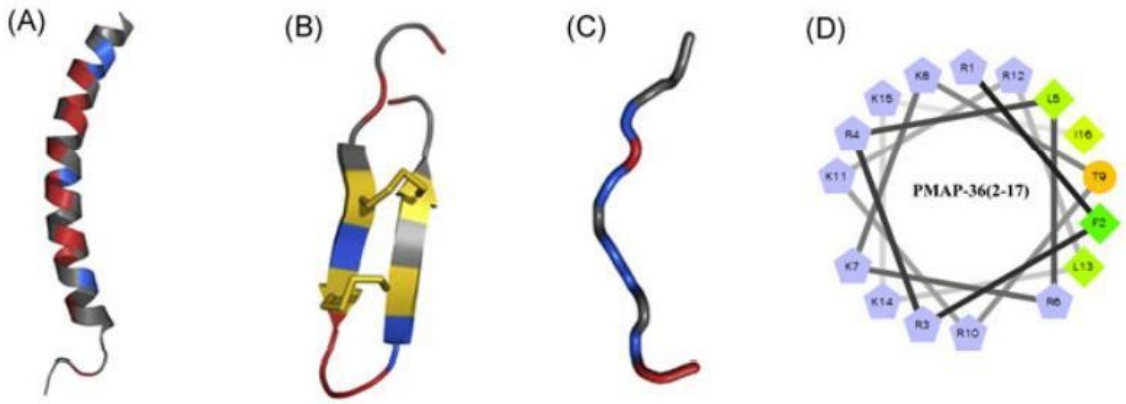


FIGURE 2 Common structural classes of antimicrobial peptides (AMPs): A, α-helix; B, β-sheet; and C, extended structures. Reproduced with permission from Takahashi et al,[9] Copyright (2010) Elsevier Masson SAS. Helical-wheel projections of the peptides: D, PMAP-36. By default, the output presents the hydrophilic residues as circles, hydrophobic residues as diamonds, potentially negatively charged as triangles, and potentially positively charged as pentagons. Hydrophobicity is color coded as well: the most hydrophobic residue is green, and the amount of green decreases proportionally to the hydrophobicity, with zero hydrophobicity coded as yellow. Hydrophilic residues are coded red with pure red being the most hydrophilic (uncharged) residue, and the amount of red decreases proportionally to the hydrophilicity. The potentially charged residues are light blue. Reproduced with permission from Zhu et al,[30] Copyright (2014) Elsevier Ltd [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Classification of antimicrobial peptides (AMPs)

Classes	Representatives	Sequences	Hosts
α-Helix	Melittin	GIGAVLKVLTTGLPALISWIKRKRQQ	Honey bee
	Magainin-1	GIGKFLHSAGKFGKAFVGEIMKS	Frog
	LL-37	LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES	Human
β-Sheet	HNP-1	AC[1]YC[2]RIPAC[3]IAGGRRYGTC[2]IYGGRKWAFC[3]C[1]	Human
	HBD-1	DHYNC[1]VSSGGQC[2]LYSAC[3]PIFTKIQGTC[2]YRGKAKC[1]C[3]K	Human
	Protegrin 1	RGGRLC[1]YC[2]RRRFC[2]VC[1]VGR	Pig
Extended	Indolicidin	ILPWKWPWWPWRR	Cow
	Tritrpticin	VRRFPWWWPFLRR	Pig
	PR-39	RRRPRPPYLPRPRPPPFFPPRLPPRIPPGFPPRFPPRFP	Pig

Abbreviations: HBD-1, human α-defensin 1; HNP-1, human α-defensin 1.
[1], [2], and [3] are disulfide bonds intramolecularly formed by Cys residues in one peptide.

V MODE OF ACTION

Different AMPs have different modes of action, some kill cells by disrupting membrane integrity, some by inhibiting proteins, DNA, RNA synthesis or several by interacting with

certain intracellular targets. All AMPs known by late-90s are cationic. However, the concept that AMPsneed to be cation was changed later with the discovery of negatively charged AMPs in 1997 39. Eg.
Maximin-H5 is an anionic peptide, isolated from frog skin.

Generally an particular AMPs is active against only one specific class of microorganisms (eg. Bacteria orfungi) . But, there are some exceptions, some AMPs have several mechanisms of action. For eg.
Indolicidin can kill bacteria, fungi and HIV 40. However, it kills *E.coli* by penetrating into the cells and inhibiting DNA synthesis, and it shows anti-HIV activities by inhibiting HIV-integrase 41. Also there are some AMPs which kill different types of cells by the same mode. For eg. PMAP-23 can kill both fungi andparasites by forming pores in their cell membranes 42.

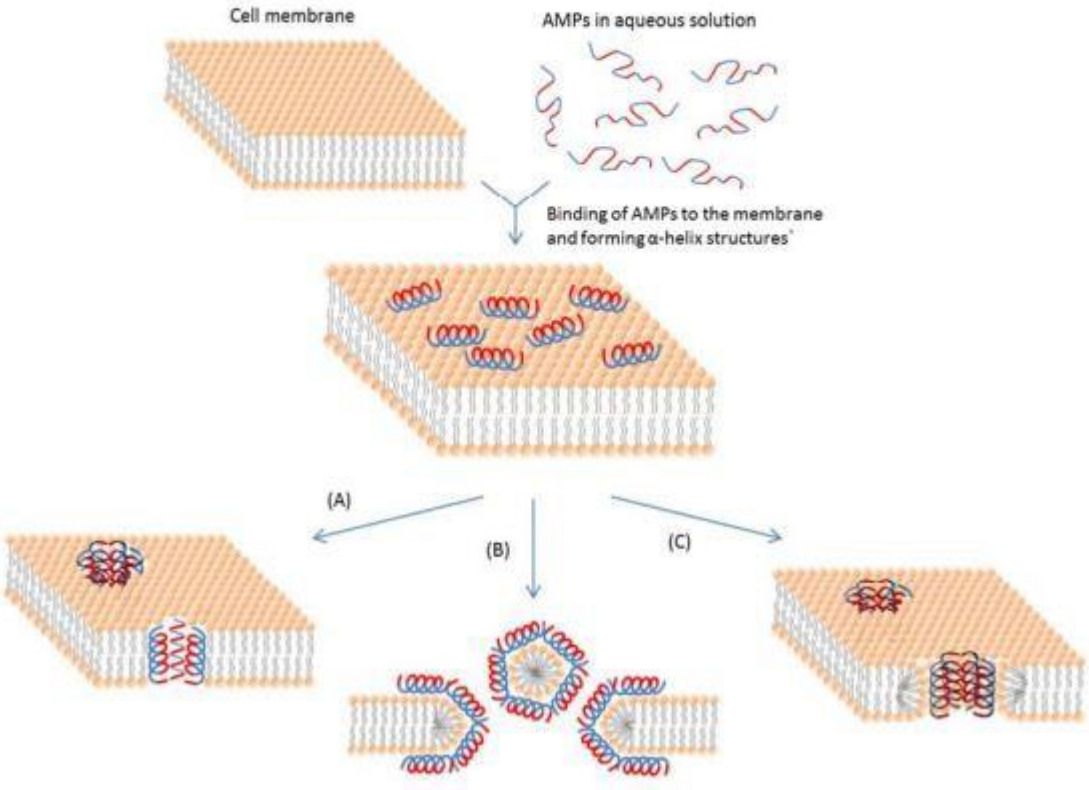
Approximately one third of the proteins of a bacterial cell are associated with the membrane and these proteins have many functions that are critical to the cell including active transport of nutrients, respiration, proton motive force, ATP generation and intercellular communication 43. Without completecell lysis, with AMP treatment, the function of these proteins can be altered. Therefore, AMPs does not only can disrupt cell membrane but can also inhibit some functional proteins.

Membrane-active AMPs: Most membrane-active AMPs are amphipathic, i.e, they both have hydrophobic and cationic faces. This feature ensures the initial electrostatic interaction with the negatively charged cell membrane and the hydrophobic part helps the AMP molecule to insert into thecell membrane. So, the interaction mainly depends on cationic state and hydrophobicity of the AMP molecule. The major types of membrane-active AMPs and the mechanisms of their actions are summarized in Table 2 and fig. 3.

Table 2 The action mechanisms of membrane -active AMPs

Interaction model	Mechanism	References
Carpet like (Detergent-like)	The peptide micelle touches the membrane first and coats a small area of the membrane. Then AMP molecules penetrate the lipid bilayer to let pore formation occur leaving holes behind.	[115–117]
Membrane thinning	AMPs insert themselves into only one side of the lipid bilayer. It can form a gap between lipid molecules at the chain region. This gap creates a force and pulls the neighboring lipid molecules to fill it.	[118–120]
Aggregate	AMPs stick to the membrane parallel to the surface. Then reorientation of AMPs occurs and they insert themselves into the membrane vertically to form sphere-like structures.	[115,121–123]
Toroidal pore	AMPs align perpendicularly into the bilayer structure with their hydrophobic regions associated with the center part of the lipid bilayer and their hydrophilic regions facing the pore.	[83,123]
Barrel-stave	Staves are formed first parallel to the cell membrane. Then barrels are formed and AMPs are inserted perpendicularly to the plane of the membrane bilayer.	[82,124,125]

Figure 3 Schematic representation of some action mechanisms of membrane-active AMPs. (**A**) Barrel-Stave model. AMP molecules insert themselves into the membrane perpendicularly. (**B**) Carpet model. Small areas of the membrane are coated with AMP molecules with hydrophobic sides facing inward leaving pores behind in the membrane. (**C**) Toroidal pore model. This model resembles the Barrel-stave model, but AMPs are always in contact with phospholipid head groups of the membrane. The blue color represents the hydrophobic portions of AMPs, while the red color represents the hydrophilic parts of the AMPs.



Intracellularly Active AMPs: Intracellularly active AMPs have been shown to interact with targets insidethe cells 44. For example indolicin was shown to bind to DNA with a preferred sequence 45. Some AMPscan inhibit DNA and protein synthesis 46. One example of this is PR-39, an AMP from pig intestines, which kills bacteria in a non-lytic process by acting like a proteolytic agent and stopping protein and DNAsynthesis 47. Similar to PR-39, indolicin does not lyse cells directly. It enters the cytoplasm and kills bacterial cells by targeting DNA synthesis 48.

VI BROAD SPECTRUM ACTIVITY:

Antifungal activity: AMPs have strong antifungal activity and it is also useful in addressing fungal infections. AMPs generally lyse the cell and they interfare with fungal cell wall synthesis. Cathelicidin peptides destroyed Candida albicans and Cryptococcus neoformans cells via membrane permeabilization and damage the microorganism.

Vylkova et al. found that human beta-defensin 2 (Hbd-2)(Synthetic) and Hbd-3(synthetic) could destroy *Candida albicans* in an energy – dependent and salt-sensitive manner without causing gross membrane disturbance or lysis 49. Research has shown that LL-37 could reduce the *C.albicans* attachment to abiotic surfaces, oral epidermis and murin urinary bladders by interacting with Yeast carbohydrate and protein cell- wall components, which is of critical importance inprevention *C.albicans* colonization and infection by AMPs 50.

Antiviral peptides: Antiviral AMPs neutralize viruses by interacting in either the viral envelope or the host cell membrane. AMPs can integrate into viral envelopes causing membrane instability, this render the viruses unable to infect host cells. For eg., defensins bind to the viral glycoproteins making herpes simplex viruses (HSV) unable to bind to the surface of the host cells 51. There are some antiviral AMPs which can prevent viral particles from entering host cells by occupying specific receptors on mammalian cells. For eg., heparan sulfate is important for the attachment of HSV viral particles to the host cell surface. The heparan sulfate molecules are negatively charged glycosaminoglycan molecules. Thus, some alpha-helical cationic peptides, eg., lactoferrin, can prevent HSV infections by binding to heparan molecules and blocking virus-receptor interactions 52. Some AMPs can cross the cell membrane and localize in the cytoplasm and changes in the gene expression profile of host cells, which can help host defense system to fight against viruses or can block viral gene expression. For eg., NP-1, prevents Vero and CaSki cell lines from infection by herpes simplex viruses type 2 (HSV-2). This AMPs stops the viruses by preventing the migration of a major viral protein, VP16, into the nucleus. This viral protein is requiredto form complexes with the host transcriptional factors to induce the expression of immediate early viral genes, which are required for the virus to defeat the first stage cellular response 53. Thus, this AMP does not compete with viral particles to bind to the receptor on cell surface but it prevents cell-to-cell spread of viral particles.

Antibacterial activity: Antibacterial AMPs are the most studied AMPs. Most of them are cationic AMPs. These AMPs target bacterial cell membranes and cause disintegration of the lipid bilayers. Themajority of these AMPs are also amphipathic with both hydrophilic and hydrophobic domains. Such structures provide AMPs the capability to bind to lipid components (hydrophobic region) and phospholipid groups (hydrophilic region) 54.

Researchers have demonstrated that some AMPs at low concentration can kill bacteria by inhibiting some important pathways inside the cell. For eg., buforin II can diffuse into cells and bind to DNA and RNA without damaging the cell membrane 55. Drosocin, pyrrhocoricin, and apidaecin are other examples of such AMPs. It is generally hypothesized that three main mechanisms could account for peptide permeation of the membrane of the target cell, including “barrel-stave model”, “carpet model” and “toroidal-pore model” 56. In the “barrel-stave model”, the attached peptides aggregate to form a bundle with a central lumen and insert into the hydrophobic coreof the membrane forming a trans-membrane pore. The “carpet model” suggests that AMPs bind onto the phospholipid head covering the surface of membranes in a carpet-like manner and disrupt the bilayer curvature like a detergent beyond a threshold concentration of membrane-bound peptide 57.

The “toroidal-pore model” involves aggregation of peptide helices into the membrane, inducing the lipidmonolayers to bend continuously through the pore so that both the inserted peptides and the lipid headgroups line the water core 58.

Antiparasitic peptides: The first antiparasitic AMP is magainin. It can kill Paramecium caudatum. Later, a synthetic peptide was developed against Leishmania parasite 59. Cathelicidin is an antiparasitic AMP, which is able to kill *Caernohabditis elegans* by forming pores in the cell membrane, the mode of action of antiparasitic peptides is the same as other AMPs. They kill cells by directly interacting with cell membrane.

Antitumor activity: Cancer was the second leading cause of death, after heart disease, in the United States in 2019. Therefore, development of antitumor drug is needed. Some cationic AMPs exhibit cytotoxic activity against tumor cells. AMPs mainly act on target cell membranes via a non-receptor- mediated pathway, for which it is more difficult for tumor cells to develop resistance compared to conventional chemotherapeutic agents 60. For example, the antitumor mechanism of B1 and its analogs involves three steps: cell membrane disruption resulting from changes in membrane permeability; penetration of the cytoplasm after membrane disruption; disruption of mitochondrial membranes and release of cytochrome C 61.

AMPs with ideal antitumor activity should meet at least three conditions: (1) high net positive charge. The existence of the high net positive charge of AMPs contributes to electrostatic attraction between the negatively charged components (such as phosphatidylserine) of tumor cells and the positively charged AMPs 62. (2) High structural flexibility. The flexibly of AMPs allows for changes in conformation in different environments (aqueous and membrane-mimic environments), which allows them to traverse the phospholipid layer of tumour cells. (3) High oligomerization. AMPs should be easily clustered on the membrane surface of a tumour cell so that they can form a pore on the tumour cell membrane.

VII APPLICATION OF ANTIMICROBIAL PEPTIDES:

In Food: In food industries natural AMPs are needed without toxicity, to preserve foods. In recent years, much attention has been focused on application of AMPs as an alternative to control undesirable microbial growth on foodstuffs. For eg, Nisin , a polycyclic peptide with 34 amino acids residues derived from Lactococcus lactis , is usually used in processed cheese, meats and beverages.

Ple/ PVA fiber, an AMP Ple (pleurocidin, a novel AMP with 25 amino acid, derived from the skin-secreted mucous of the winter flounder) incorporated into ultrafine PVA fiber mats via electrospinning technology, was demonstrated to be successfully applied in apple cider , with efficient inhibition activity against *E.coli*.

In medicine: AMPs also use in drug therapy in the clinics. Eg, Lucifensin and Lucifensin II, two insect defensins , can heal wound, especially in patients with impaired healing due to underlying disorders(eg. Diabetes). This procedure is known as debridment therapy. Pexiganan, a 22-amino-acid membrane disruptor analog of the Xenopus peptide magainin, has been clinically proven to replace ofloxacin in the treatment of diabetic foot ulcers as early as 1996, and may avoid the selection of resistant bacteria that can develop after oral systemic antibiotic therapy; however, the peptide was not approved by the Food and Drug Administration in 1999 even after completion of a phase III trial. Currently the phase III clinical trials of this peptide are being conducted again to treat mildly diabetic foot infection by Dipexium Pharmaceuticals 63. Omiganan is an indolicidin analog. It has a broad-spectrum antibacterial activity. It

has completed phase III clinical trials for catheter infections and rosacea, completed phase II for the application of omiganan in patients with vulvar intraepithelial neoplasia atopic dermatitis and acne vulgaris. POL7080 as an antimicrobial peptidomimetic specifically targets P. aeruginosa at the nanomolar level via non-membrane- disrupting activity. In addition to antibacterial activity, some AMPshave also been shown to have immune-promoting effects, such as PXL01, which is derived from humanlactoferricin and been assessed for its efficacy, safety and handling in patients with flexor tendon injuries in phase II trials 64.

In animals: It has been reported that several AMPs added in the diet have beneficial effects, including body weight, the average daily gain, nutrient digestibility and intestinal morphology as well as effects onintestinal and fecal microflora. AMPs can also improve animal performance, nutrient digestibility and support normal intestinal morphology and function. Some reports also demonstrated that AMPs can protect piglets from challenge with the mycotoxin deoxynivalenol (DON)201 and repair the intestinal injury induced by DON 65. Moreover, AMPs enhance intestinal barrier function and improve microbiota composition in the intestines of weaned piglets and reduce rates of diarrhea in them.

TABLE 3 Selected antimicrobial peptides (AMPs) in clinical phase of development

AMPs	Description	Condition or disease	Administration	Phase	Status company	Clinical trial identifier if available
Pexiganan (MSI-78)	Analog of magainin	Diabetic foot infection	Topical	Phase 3-C	MacroChem Corporation	NCT00563433 NCT00563394
					Dipexium Pharmaceuticals, Inc.	NCT01594762 NCT01590758
Omiganan	Derived from indolicidin	Catheter infections	Topical	Phase 3-C	Mallinckrodt	NCT00231153
		Atopic dermatitis		Phase 2-C	Cutanea Life Sciences, Inc.	NCT03091426
		Rosacea		Phase 3-C		NCT02576847
		Vulvar intraepithelial neoplasia		Phase 2-C		NCT02596074
		Acne vulgaris		Phase 2-C		NCT02571998
Lytixar (LTX-109)	Synthetic antimicrobial peptidomimetic	Gram-positive, skin infections Mild Eczema/Dermatoses Atopic Dermatitis	Topical	Phase 2-C	Lytix Biopharma AS	NCT01223222
		Nasal carriers MRSA		Phase 1/2-C		NCT01158235
		Non-bullous Impetigo		Phase 2-C		NCT01803035
Surotomycin	Cyclic lipopeptide	Clostridium difficile-associated diarrhea (CDAD)	oral	Phase 1-C	Merck Sharp & Dohme Corp.	NCT02835118 NCT02835105
Novexatin (NP-213)	Cyclic peptide	Onychomycosis	Topical	Phase 2-C	NovaBiotics	[197]
LL-37	Host-defense peptide	Melanoma	Intratumorally in cutaneous or subcutaneous tumors	Phase 1/2-A	M.D. Anderson Cancer Center	NCT02225366
PXL01	Derived from lactoferricin	Surgical adhesions	Hyaluronic acid-based hydrogel	Phase 2-C	Pergamum AB	NCT01022242
Iseganan (IB-367)	Derived from protegrin 1	Oral mucositis in head and neck cancer.	Oral rinse	Phase 3-U	National Cancer Institute (NCI)	NCT00022373
PAC-113	Derived from histatin 3	Oral candidiasis	Mouth rinse	Phase 2-C	Pacgen Biopharmaceuticals Corporation	NCT00659971

(Continues)

Table 3 continued

AMPs	Description	Condition or disease	Administration	Phase	Status company	Clinical trial identifier if available
Dalbavancin	Lipoglycopeptide	Bone infection Osteomyelitis Septic arthritis Joint infection Prosthetic joint infection	Intravenously	Phase 4-R	Infectious Diseases Physicians, Inc	NCT03426761
		Infectious Peritonitis	Intravenously	Phase 4-A	University of Colorado, Denver	NCT02940730
		Methicillin-resistant *Staphylococcus Aureus* skin infections	Intravenous administration in children	Phase 3-R	Durata Therapeutics Inc	NCT02814916
SGX942	5-amino acid peptide	Oral mucositis in head and neck cancer	Intravenously	Phase 3-R	Soligenix	NCT03237325
OP-145	Derived from LL-37	Chronic otitis media	Eardrops	Phase 2-C	Leiden University, The Netherlands	ISRCTN84220089
Brilacidin (PMX-30063)	Defensin mimetic	Bacterial skin infection	Intravenously	Phase 2-C	Cellceutix Corporation	NCT02052388
		Mucositis in head and neck Neoplasms	Oral Rinse	Phase 2-C	Innovation Pharmaceuticals, Inc	NCT02324335
POL7080	Peptidomimetic	Renal impairment	Intravenously	Phase 1-C	Polyphor Ltd	NCT02110459
		Ventilator-associated pneumonia aeruginosa		Phase 2-C		NCT02096328
		Healthy; Synergism with amikacin		Phase 1-C		NCT02897869
AP-214	Derivative from HDP	Prevention of (acute) kidney injury after cardiac surgery	Intravenously	Phase 2-C	Action Pharma A/S	NCT01256372
		Prevention of kidney injury after thoracic aortic aneurysm repair		Phase 2-C		NCT00903604
CD-NP	Chimeric 37-mer derived from combination of two natriuretic peptides	Acute decompensated heart failure	Infusions	Phase 2-C	Nile Therapeutics	NCT00839007
Ghrelin	Endogenous host-defense peptide	Chronic respiratory infection	Intravenously	Phase 2-C	University of Miyazaki, Japan;	JPRN-UMIN000002599
		Airway inflammation		Phase 2-C		JPRN-UMIN000001598

Abbreviations: A, active, not recruiting; C, completed; HDP, host-defense peptide; R, recruiting; U, unknown.

Table 4 Report on application of antimicrobial peptides (AMPs) in animal

Animals	AMPs	Treatments/Doses	Effects
Weanling piglets	CAP[a]	Basal diet with 4 ppm deoxynivalenol and 4% CAP	Attenuating the metabolic disturbances in amino acid, lipid, and energy metabolism induced by DON; Improving intestinal morphology, intestinal epithelial cell proliferation and protein synthesis; Improving feed efficiency, immune function, and antioxidation capacity, alleviating organ damage
Weanling piglets	SyntheticAMP-A3[b]and AMP-P5[c]	Basal diet with 60 mg/kg AMP-A3 and basal diet with 60 mg/kg AMP-P5 Basal diet with 40 and 60 mg AMP-P5/kg diet Basal diet with 0, 60 and 90 mg AMP-A3/kg diet	Improving the performance, nutrient digestibility, intestinal morphology and reducing pathogenic bacteria
Weaned piglets	Cecropin AD[d]	Basal diet with 400 mg/kg cecropin AD and piglets were orally challenged with E. coli K88	Increasing immune status and nitrogen and energy retention as well as reducing intestinal pathogens
Weanling piglets	Recombinant Lactoferrampin-lactoferricin	Basal diet with 0.1 g Lactoferrampin-lactoferricin and 0.1 g chlortetracycline/kg diet	Improving performance and affecting serum parameters
Weanling piglets	cipB-LFC-LFA	Basal diet with no addition, 100 mg cipB, 100 mg cipB-LFC-LFA/ kg diet	Improving performance, the regulation of immune function and the absorption of Fe, reducing the incidence of diarrhea
Weanling piglets	Colicin E1	Basal diets with0, 11, or 16.5 mg Colicin E1/kg and piglets were orally inoculated with E. coli	Improving the performance and reducing the incidence of postweaning diarrhea
Weaned female piglets	Lactoferrin	Basal diet with 1.0 g/kg lactoferrin	Increasing ADG, efficiency of gain, intestinal villus height and relative abundance of mRNA for PR-39 and protegrin 1
Indigenous male chickens	Cecropin AD-Asn (CADN)	Basal diets with a CADN liquid sample at 0, 2, 4, 6, and 8 ml/kg	Increasing nutrient utilization, enhancing intestinal villus heights, decreasing aerobic bacterial counts

TABLE 4 (Continued)

Animals	AMPs	Treatments/Doses	Effects	References
Arbor Acre male broiler chickens	Pig AMP (PMAP)[e]	Basal diet with PAMP at 150 and 200 mg/kg	Improving the performance, the intestinal mucosal immunity, and increasing the intestinal ability to absorb nutrients	Bao et al[212]

Abbreviations: AD, atopic dermatitis; CAP, composite antimicrobial peptides; cipB-LFC-LFA, cipB-lactoferricin-lactoferrampin; DON, deoxynivalenol.
[a]CAP consists mainly of antibacterial lactoferrin peptides, along with plant defensins and active yeast.
[b]AMP-A3 (amino acid sequence: AKKVFKRLEKLFSKIWNWK-NH_2) is an analog of antimicrobial peptide HP 2-20 (amino acid sequence: AKKVFKRLEKLFSKIQNDK-NH_2).
[c]AMP-P5 (amino acid sequence: KWKKLLKKPLLKKLLKKL-NH_2) is an analog of the hybrid antimicrobial peptide CA-MA [Cecropin A (1–8)-Magainin 2 (1–12); KWKLFKK IGIGKFLHSAKKF-NH_2].
[d]Cecropin AD is expressed in *Bacillus subtilis* and the amino acid sequence of Cecropin AD is KWKLFKKIEKVGQRVR-DAVISAGPAVAT-VAQATALAK-NH_2.
[e]PAMP is isolated from pig small intestine.

VIII PERSPECTIVE AND CONCLUSIONS

Antifungal, antibacterial, antiviral, antiparasitic properties of AMPs promising alternatives to conventional antibiotics. But, there is a limiting factor, that is it has low in vivostability. To extent the half-life of AMPs, AMP mimics with an amphiphilic studies have made great advances in recent days, but further studies are needed. Though many researches have been done on AMPs, infection control by AMP is still hindered by some factors including low specificity, high manufacture cost, potential toxicity to animal cells etc.

In this review we have discussed about various applications of
AMPs, it's mechanism of action, it's various types and it's broad- spectrum activity etc.

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