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MICROBIOLOGÍA

ANTIMICROBIAL PEPTIDES, AN ALTERNATIVE TO COMBAT BACTERIAL RESISTANCE

Péptidos antimicrobianos, una alternativa para el combate de la resistencia bacteriana

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ABSTRACT

Antimicrobial peptides (AMPs) of higher organisms have been studied for the past 25 years, and their importance as components of innate immunity is now well established. The essential simplicity of their chemical structure, along with the lower likelihood of developed resistance compared to conventional antibiotics, has made them attractive candidates for development as therapeutics. The objective of this review article is to describe the current relevance, main mechanisms presented, and the uses of antimicrobial peptides as new therapies in the clinical area. The information used was mainly compiled from scientific articles based on a systematic review of scientific papers with data on human AMPs and their different applications, searching without date limits and only documents in English and Spanish. Gray literature was excluded, and no restrictions were made involving study design for a retrospective study. Although these products have not yet been commercialized, they have advantages over the currently available treatments since they are not expected to cause bacterial resistance due to their three-dimensional structure, amphipathic tendency, and cationic character; however, the technique of peptide production is still new and is in the early stages of innovation of new molecules.

Keywords: Antimicrobial potential, immune system, therapeutic agents.

RESUMEN

Los péptidos antimicrobianos (AMPs) de organismos superiores se han estudiado durante los últimos 25 años y su importancia como componentes de la inmunidad innata está ahora bien establecida. La simplicidad básica de la estructura química de los péptidos antimicrobianos, junto con la menor probabilidad de aparición de resistencia en comparación con los antibióticos convencionales, los ha convertido en candidatos atractivos para el desarrollo como terapéuticos. El objetivo de este artículo de revisión es describir su relevancia actual, los mecanismos principales que presentan y los usos que se les están dando como nuevas terapias en la clínica. La información usada se recopiló principalmente de artículos científicos con datos sobre AMPs humanos y sus diferentes aplicaciones. Aunque aún no se comercialicen estos productos, tienen ventajas sobre los tratamientos existentes, puesto que se prevé que no causen resistencia bacteriana, esto debido a su estructura tridimensional, su tendencia anfipática y su carácter catiónico. Aunque la técnica de producción de péptidos es aún nueva y está en las primeras etapas de innovación de nuevas moléculas, promete importantes logros en un futuro cercano en el diseño de péptidos más eficientes o que sean estables en diferentes ambientes.

Palabras clave: Agentes terapéuticos, potencial antimicrobiano, sistema inmunológico.

INTRODUCTION

The objective of this review article is to describe the current relevance, the main mechanisms presented, and the uses of antimicrobial peptides (AMPs), as new therapies in the clinical area are being evaluated. The information used was mainly compiled from scientific articles with data on human AMPs and their different applications, based on a systematic review of scientific papers and searching without date limits and only papers in English and Spanish. Gray literature was accessed through manual search; no restrictions were made involving study design for a retrospective study. AMPs are an essential part of innate immunity that evolved, in most living organisms, over 2.6 billion years to combat microbial challenge against various bacteria, viruses, fungi, and parasites. The treatment of bacterial infections is increasingly complicated by the ability of bacteria to develop resistance to antimicrobial agents, making it essential to find new therapeutic alternatives (WHO, 2012), and a new therapeutic alternative is antimicrobial peptides.

Antimicrobial peptides

With the increase in antibiotic resistance, the search for alternative antibiotics has become a priority for the treatment of impending antibiotic-resistant strains; therefore, the study of peptides is relevant because they comprise diverse amino acid sequences and structures and perform functions that are essential actors in biological systems, facilitating the effective management of the necessary information that provides the basis for revealing the unanswered questions on the natural behaviors of organisms. It is becoming more urgent to find alternative options for the treatment of antibiotic-resistant strains; as a result, AMPs have been proposed as possible candidates for their use as antimicrobial agents since their mode of action is assumed to be substantially different from existing antibiotics, mainly due to their extensive spectral activity and their low resistance potential (Maróti *et al., 2011).*

AMPs are a family of approximately 900 molecules that are part of the most primitive innate immune systems in vertebrates, insects, and plants (Tomasinsig *et al., 2010).* Therefore, an AMP database exists that allows efficient search, prediction, and design of peptides with antibacterial, antiviral, antifungal, antiparasitic, insecticidal, spermicidal, anticancer, chemotactic, immunological modulation or antioxidant activities. Genes encode most AMPs and synthesized by ribosomes, although others are products of secondary metabolites such as lactoferricin, a globular protein isolated mainly from bovine milk, which is synthesized by neutrophils in the blood. Lactoferricin deprives free iron from microorganisms, such as *E. coli*, that need it to grow and invade the host.

Among the range of peptide, classifications are cationic antimicrobial peptides (AMPs), which are relatively small peptides that have a net positive charge and exhibit some antimicrobial activity, often of broad-spectrum, allowing them to be a source of innate defense against microbial infections (Wang, 2015). Based on Wang *et al*. (2016), the APD3 database lists more than 2600 examples of AMPs, of which 112 are human peptides, and 100 of those have antimicrobial activities. These examples, in addition to their families and most essential characteristics, are shown in Table 1.

It is important to emphasize that AMPs differ fundamentally from antibiotics in their pharmacodynamic characteristics; the pharmacodynamic differences combine producing a much lower probability of resistance that evolves against antimicrobial peptides (Rodriguez and McDaniel, 2001). Many of the antibiotics produced by microorganisms are peptide molecules. These are produced by the synthesis of nonribosomal peptides (NRPS), a process that involves the expression of large sets of genes that encode multiple enzymes that work sequentially to catalyze the sequence of chemical reactions necessary to synthesize the antibiotic (Yu *et al., 2017),* and the manipulation of modular polyketide synthases (PKSs), which has led to the production of, for example, various erythromycin analogs (Marahiel, 2016).

Antibiotics based on peptides include β-lactamases, such as penicillin cyclic peptide antibiotics, such as polymyxins and bacitracin, glycopeptides, such as vancomycin (Yim *et al., 2014),* and the lipopeptide daptomycin, one of the most recent antibiotic classes introduced (Robbel and Marahiel, 2010).

AMPs, including those made in human cells, contrast with NRPS antibiotics because AMPs are produced by the normal process of ribosomal translation on an mRNA template. The first product is usually a preprotein that is then processed to the final length of the active AMP. The difference in the genetic origins of AMP and NRPS-antibiotics has consequences for the compositions of the final products since the ribosomally-produced AMPs contain only the usual complement of amino acids found in proteins, and NRPS, on the other hand, are not limited by ribosomal translation and usually contain a mixture of typical amino acids along with noncanonical amino acids that are not found in proteins (Walsch *et al., 2013).*

There are two common characteristics of most antimicrobial peptides regardless of their structure or size. First, AMPs have a positive charge due to the presence of a large number of basic amino acids (mostly lysine and arginine), and second, approximately 50 % of the amino acids that constitute them are hydrophobic, which enables them to interact with the membranes (Andersson *et al., 2016; Waghu et al., 2016*). Also, defensins attack the surface of the membrane that surrounds a pathogen, generating holes in the membrane, and beta-defensins such as hBD-1 and hBD-2 become active against gram-negative bacteria. With an abundant expression of hBD-2 in gram-positive infections, the inflammatory response and vasodilation increase and stimulation of cytosines or bacteria are required. hBD-3 is active against gram-positive and gram-negative bacteria and

Table 1. Families of human AMPs and their main characteristics and mechanisms.

Family	Characteristics	Example	Mechanism
Alpha (group I in humans, cathelicidins)	Helical structures, linear without cysteines. They are found mainly in blood cells and epithelial cells.	α-Helical human cathelicidin LL-37/hCAP 18 (polymorphonuclear chemoattractant). For the cathelicidin gene to be activated, it requires active vitamin D (1,25 OH2 vitamin D3)	Immunomodulatory activities: inhibition of apoptosis, cytokine stimulating, lipopolysaccharide (LPS) neutralizing, promotion of wound healing, and regulation of adaptive immune responses.
Beta (group II in humans, defensins)	Beta strands. Regularly found in epithelial cells. Rich in Arginine	Human α-defensins (6 isolated types of neutrophils HNP-1 to HNP-4 and the other 2 in Paneth cells in the small intestine and epithelial cells of the female urogenital tract) Encoded in chromosome 8p23.	Chemoattraction of keratinocytes and chemotactic for human monocytes and mast cells. Promote a strong Th1 response and induce cytotoxic T-cells, NK activity, and IL-12 and IFN-γ production
Alphabetic (group II in humans, defensins)	α-Helical structures and β-braids (cyclic molecules) in the same 3D fold, include 6 cysteine residues that stabilize the structure forming 3 intramolecular disulfide bonds. Rich in arginine.	Human α-defensins. hBD-1 in genitourinary and respiratory tract expressed in keratinocytes and serous glands, hBD-2 in external epithelium, hBD-3 bactericidal action, derived from keratinocytes, hBD-4 induced by keratinocytes. Encoded in chromosome 8p23	Chemoattractants of leukocytes and dendritic cells.
Non-alphabetic (group III in humans, histatins)	No helical alpha or β strand, high proportion of specific amino acids.	Indolicidin, histatin 5 (saliva). The genes that code for histatins 1 and 3 have been mapped to chromosome 4q13	Inhibitory activity over proteolytic enzymes.

induced by the pathogenicity of microorganisms (PAMP and by TNF-alpha, IL-1beta and IFN-gamma) (Wang, 2015).

Interaction of AMPs with the bacterial cell membrane

The barrel model suggests that peptides accumulate on membrane surfaces and insert into the membrane when a threshold amount is reached. Interactions between the peptide and lipid hydrophobic side chains are then formed, creating a hydrophilic pore, which produces an osmotic imbalance and disrupts the membrane potential. Conversely, the toroidal pore model suggests that the peptide causes the formation of pores stochastically, requiring fewer peptides for inferred pores (Rivera *et al., 2007*); however, these models are based on studies performed on lipid vesicles and cannot fully explain the interaction of AMPs with the complex bacterial cytoplasmic membrane.

On the other hand, the aggregate mechanism is like how detergents act, in which the peptide binds to the membrane, forming structures similar to the mycelia, and a channel is formed, through which ions are released, causing cell death due to loss of cytoplasmic content. Because AMPs that act on vegetative cells cause damage to the membrane, there is a loss of transmembrane potential and essential molecules (Zhang and Gallo, 2016), and it has been demonstrated that damage to the membrane disturbs cellular homeostasis, leading to an increase in cell volume and a decrease in total cell numbers (Nguyen *et al., 2011).* The formation of pores or channels in cells has been studied using fluorescent dyes such as propidium iodide (Nguyen *et al., 2011)* and Sytox® Green (Lee *et al., 2015).*

Certain studies have only examined the damage on the membrane, which does not indicate whether the membrane is the initial and only objective of the AMP or if the peptide moves through the membrane into the cytoplasm to target another essential cellular function, such as DNA and RNA synthesis. However, a study by Barns and Weisshaar (2013) suggests that at a low peptide concentration, the peptide interacted with the membrane, but the damage to the membrane was repairable; on the contrary, at a high peptide concentration, a threshold was reached that caused irreversible damage to the membrane. Therefore, it is necessary to give intervals of lethal and sublethal concentrations to obtain more information on how the peptide is directed to the cell. It is also known that rhodamine labeling (Rh-LL-37) has different effects on antimicrobial activity against *E. coli*, suggesting that this compound binds to peptidoglycan. In other studies, cecropin A (an antimicrobial peptide with leishmania activity) containing CL (anionic phospholipids) causes dissociation in cellular proteins, leading to cellular homeostasis (Wang *et al., 2013*).

It has also been shown that human α-defensin 5 translocates to the cytoplasm of *E. coli* and accumulates at the site of cell division, and buforin antimicrobial peptide analogs (Fig. 1) exert their bactericidal activity on

E. coli by binding to DNA and RNA after penetrating the cell membrane (Barns and Weisshaar, 2013).

AMP resistance mechanisms

The mechanisms of resistance to AMPs include proteolytic degradation or sequestration by secreted proteins, impedance by exopolymers and biofilm matrix molecules, elusion of cell surface attraction/membrane alteration and export by efflux pumps. Segregated bacterial proteins, such as proteases, are the first bacterial defense mechanisms that AMPs encounter when interacting with bacteria.

The proteolytic degradation of AMPs by extracellular enzymes represents a simple but effective way to provide resistance of AMPs to microorganisms. Commensal bacteria that live on the epithelial surfaces of mammals, such as staphylococci, secrete various proteases, including metalloproteases such as aureolysin and SepA, and serine endopeptidases such as V8 protease, which are known to degrade linear AMPs, such as human cathelicidin LL-37 (Omardien *et al., 2016*).

Group A *Streptococcus* produces a protease called SpeB, and this cysteine protease has been shown to fragment many host AMPs, including LL-37 and β-defensins (Sieprawska-Lupa, 2004; Hao *et al., 2013*). Interestingly, the exploitation of host proteins can enhance the proteolytic activity of SpeB; interactions between SpeB and cell wall anchored G-related linkage of alpha2M (GRAB) proteins brings SpeB complexed to the host inhibitory proteinase alpha2-macroglobulin, which shows increased activity towards LL-37, to the membrane surface (Nguyen *et al., 2011;* Lee *et al., 2015*).

In addition, as a secondary effect of SpeB proteolytic activity, proteoglycans degrade host-released dermatan sulfate, which completely neutralizes human alpha-defensin, HNP-1. Finally, the proteases of another gram-positive pathogen, *Enterococcus faecalis*, and the gram-negative *Pseudomonas aeruginosa* and *Proteus mirabilis* have also been reported to degrade LL-37 (Nelson *et al., 2011*).

For what has been considered the continued high interest in AMPs as potential therapies for bacterial infections, surprisingly few studies have tried to assess the risk of developing resistance and explore the mechanisms of how it is acquired. The methods to determine this resistance are the broth microdilution method, to obtain the minimum inhibitory concentration (MIC), and the other is the time of death test. The molecular basis of acquired resistance has been characterized in bacterial pathogens through the isolation of resistant strains of natural origin. The serial passages in the presence of AMPs and the direct placement of AMPs are shown in Table 2. (Schmidtchen *et al., 2001;* modified from Frick *et al., 2011;* Dobias *et al., 2017;* Hashemi *et al., 2017*).

Defensins are essential components of the defense mechanisms in epithelial cells and constitute a family of

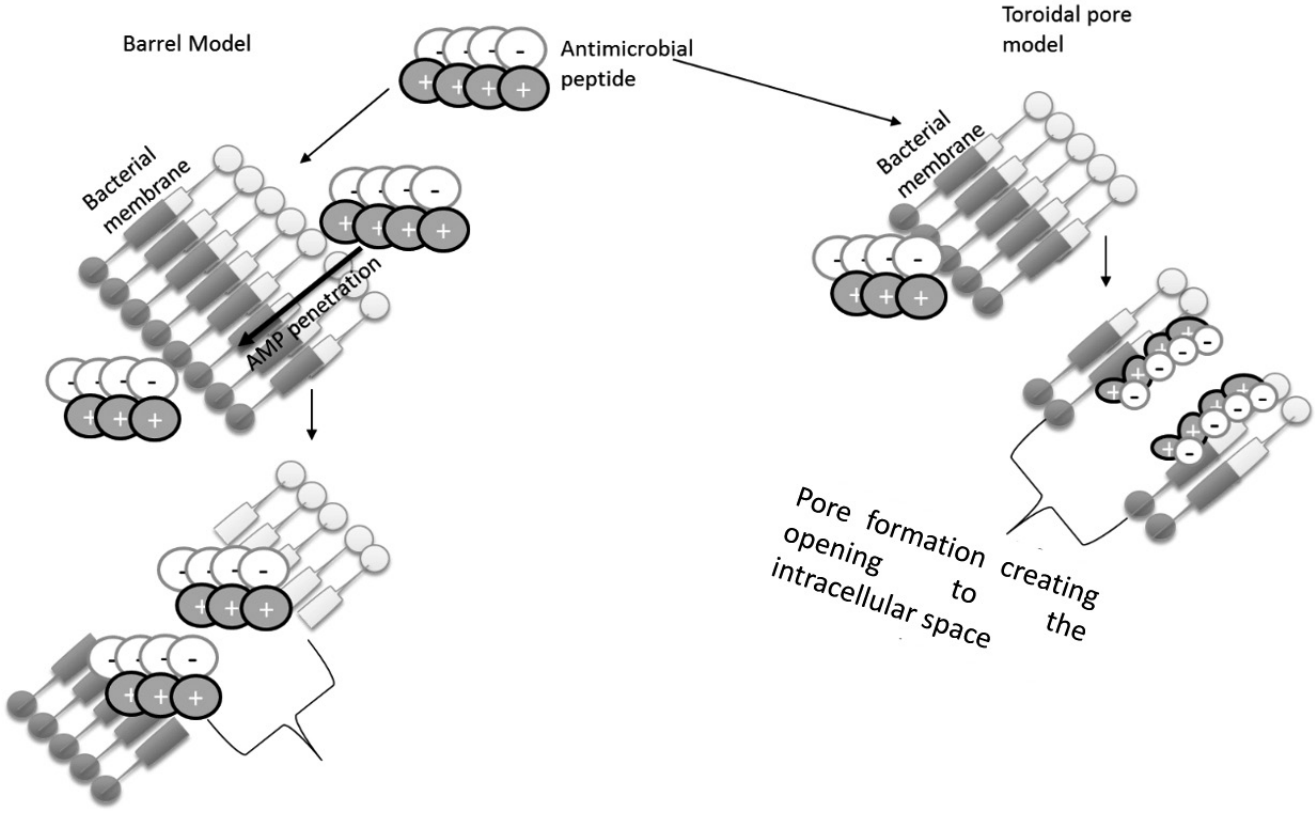

Figure 1. Mechanisms of AMP on the bacterial membrane.

Table 2: Mechanisms of AMP resistance in some microorganisms and genes involved in AMP resistance.

Organism	Isolation	AMP resistance	Genes involved AMP resistance	Proposed mechanism
S. aureus	Clinical	LL-37, β-defensin 2, β-defensin 3, lactoferricin B	*hemB*	Inactivation results in a small colony variant (SCV) phenotype with reduced AMP ligation/uptake
E. coli*, *K. pneumoniae	Clinical	Colistin, polymyxin B, defensins, CSA-131, CSA-44	*mcr-1*	Encodes a transferase of PEtN that modifies lipid A, reducing the anionic charge
L. monocytogenes	Direct leucocin A	Leucocin A	*mptACD*	Unknown
A. baumannii	Direct colistin	Colistin, polymyxin B, CSA-131	*lpxA*, *lpxD*, or *lpxC*	Inactivation results in complete loss of LPS production, reduction of AMP binding
S. epidermidis	Clinical	PSMγ, PSMδ	unknown	Unknown
S. typhimurium	DES mutagenesis	Polymyxin, CAP37, CAP57, protamine, polylysine	*phoQ*	The constitutive activation in Mg^{2+} of modifications of LPS regulated by phoP reduces the anionic charge
	Direct colistin	Colistin, polymyxin B	*pmrA*, *pmrB*	The constitutive activation of the modification of Ara4N and PEtN LPS regulated with pmrAB reduces the anionic charge
	Direct	PR-39	*sbmA*	Inactivation reduces absorption of AMP
	Direct protamine	Protamine, colistin, lactoferricin, α-defensin 1	*hemA*, *hemB*, *hemC*, *hemL*	Inactivation results in a small colony variant (SCV) phenotype with reduced AMP ligation/uptake
	LL-37 o CNY-100HL	LL-37, CNY100HL,	*pmrB*, *phoP*	Constitutive activation of several modifications of the LPS reducing the anionic charge
P. syringae	unknown	Camalexin	*CYP71B15/PAD3*	Unknown

small cationic peptides (3 kDa-6 kDa). They are classified as defensins α, β and θ. An antimicrobial agent present in bovines is the calcium-binding protein S100A7, also known as psoriasin (Chapman *et al.,* 2016). To date, little is known about its physiological function in bovines, particularly in the mammary gland. In humans, it has been observed to protect the skin from infections against *E. coli*, although it is also expressed in healthy skin. The importance of β-defensins and psoriasin in the local defense against mastitis lies in the constitutive and inducible expression of the antimicrobial peptides in bovine mammary glands and its antibacterial activity against pathogens.

AMPs for therapeutic use

Currently, there are many AMPs in clinical development for the treatment of various bacterial pathogens, but most of them are intended for topical use only (Table 3). This is probably a direct result of the toxicity observed after the systemic administration of polymyxin B and colistin. The only AMP in clinical trials for intravenous administration is lactoferrin 1-11 (hLF1-11) of human origin to treat life-threatening infections that occur in patients with stem cell transplantation. A study showed that hLF1-11 is well tolerated in single and multiple doses as high as 5 mg after intravenous administration (Tetens *et al.,* 2010).

In contrast to standard antibiotics, AMPs are effective against quiescent and actively growing bacteria; they do not require metabolic processes for antimicrobial activity; they show rapid effectiveness kinetics (seconds or minutes) and demonstrate a low propensity for bacterial resistance *in vitro*. However, AMPs have several limitations that have delayed their successful development for clinical use, including the inhibition of activity in the presence of acidic pH and in a pulmonary environment.

Over the last two decades, there has been a focus on the rationality of engineering AMP amphipathic structures to overcome these intrinsic limitations. Recently, cationic AMP engineering activity (eCAP) has been mentioned. Recent advances in the design of eCAPs and the technology used to produce them have prompted a renaissance of interest in their therapeutic potential. Numerous groups have achieved greater efficacy and reduced toxicity with eCAPs and have demonstrated their ability to kill multiresistant and selected pathogens. Perhaps the most exciting is the recent reports of eCAPs that prevent and disrupt biofilms formed by important human pathogens (Van der Velden *et al.,* 2009; Tellez and Castaño, 2010; Lashua *et al.,* 2016).

Table 3: Commercial AMPs as new molecules for clinical use.

Peptide	AMP source (host)	State	Administration	Indication	Company
OP-145	LL-37 (human)	Phase I/II	Eardrops	Chronic otic infection	OctoPlus Inc.
hLF1-11 (Lactoferrin)	Lactoferrin 1-11 (human)	Not specified	Intravenous	Patients with neutropenic stem cell transplantation	AM-Pharma B.V.
Pexiganan (MSI-78)	Magainina (frog)	Phase III	Topical cream	Diabetic foot infection	Dipexium Pharmaceuticals, Inc.
		Phase III	Topical cream	Diabetic foot ulcers	MacroChem Corporation
Iseganan (IB-367)	Protegrin-1 (porcine leucocytes)	Phase III	Mouth washing	Prevention of mucositis induced by chemotherapy	National Cancer Institute (NCI)
		Phase II/III	Mouth washing	Prevention of ventilator-associated pneumonia	IntraBiotics Pharmaceuticals
Omiganan (MBI 226, CLS001)	Indolicidin (bovine neutrophils)	Phase III	Topical cream	Topical cutaneous antisepsis, prevention of catheter infections	Mallinckrodt
		Phase III	Topical cream	Rosacea	Cutanea Life Sciences, Inc.
		Phase II	Topical cream	Usual type of vulvar intraepithelial neoplasia (uVIN)	Cutanea Life Sciences, Inc.
		Phase II	Topical cream	Moderate to severe inflammatory acne vulgaris	Cutanea Life Sciences, Inc.
		Phase II	Topical cream	Mild to moderate atopic dermatitis	Cutanea Life Sciences, Inc.
Lytixar (LTX-109)	Synthetic antimicrobial peptidomimetic	Phase II	Topical cream	Gram-positive skin infections without complications	Lytix Biopharma AS
		Phase I/IIa	Nasal	Nasal carriers of *S. aureus*	Lytix Biopharma AS
C16G2	Synthetic specifically directed antimicrobial peptide	Phase II	Mouth washing	Avoid caries caused by *S. mutans*	C3 Jian, Inc.
CP22/MX-226/ CLS001	Bactolisin analog	Phase IIIb		Avoid infections caused by catheter and dermatology	Migenix
Mersacidin	Bacteriocin	Preclinical phase		Avoid gram-positive infections	Novacta Biosystems
Plectasin	Defensin	Preclinical phase		Avoid systemic pneumococcal and streptococcal infections	Novozymes A/S
PAC113	Synthetic peptide based on histatin 5			Human candidiasis	Pacgen
PTX002, 005, 006, 007	Synthetic peptides by molecular mimicry			Antiendotoxins with broad antimicrobial spectrum	PepTx
CZEN-002	Derived from alpha melanocyte stimulating hormone	Phase II		Vulvovaginal candidiasis	Zengen

MATERIALS AND METHODS

This systematic review was carried out in July of 2018. To conduct the most complete possible compilation, literature was collected using the databases ScienceDirect, Redalyc, Web of Science, Scopus, SciELO, and Google Scholar in English and Spanish without restricting the publication year. We searched for references that contained in the title, abstract, and/or keywords the following search terms with all possible combinations: antimicrobial peptides, antimicrobial peptide products, antimicrobial peptide effects. After eliminating the duplicated documents, we selected original articles, book chapters and theoretical references (i.e., reviews, viewpoints, comments). Gray literature, such as theses, congress memories, and technical

reports, were excluded from the analysis, and no restrictions were made involving study design for a retrospective study.

DISCUSSION AND CONCLUSIONS

Since the isolation of the first peptide from frog skin in 1983, several breakthroughs have been made in the isolation, synthesis, and application of AMPs. Nevertheless, many challenges have yet to be overcome in the field of AMP peptidomics, reflecting mainly their behavior *in vivo* and their structure–function relationships. In terms of costs, producing AMPs can be several hundred times more expensive than the production of conventional antibiotics; in terms of the increase in bacterial multi resistance, many resistances are based on changes in the physico-chemical properties of surface molecules and the cytoplasmic membrane. These changes usually confer moderate levels of resistance and are relatively nonspecific. It is possible that these proteins are not exclusively involved in AMP resistance; thus, they can be an alternative for clinical use.

For drug development efforts in the field of AMPs, it is essential that resistance to AMPs, which can develop due to selective pressure, does not rely on dedicated resistance genes that are conferred by horizontal gene transfer, as in the case of many mechanisms of resistance to antibiotics (Juhas, 2015; Melvin *et al., 2016*).

Even if many details regarding bacterial resistance to AMPs are uncovered, the therapeutic potential of AMPs that lack direct antimicrobial activity but have immunostimulatory properties that enhance natural innate immunity requires further assessment. Although some early preclinical studies have been encouraging, much more data need to be obtained before the value of this intriguing proposition can be adequately evaluated. In summary, although antimicrobial peptides are generally recognized as essential components of natural host innate immunity against the microbial challenge, their promise as a new class of drugs and their value as external therapeutic agents remains to be revealed.

Another major hurdle facing commercialization of peptides is their relatively short shelf life and potential for degradation during storage (Eckert, 2011). Several solutions for this have been proposed, including chemical modification of functional groups within the peptide and various formulation strategies, including loading peptides into various nanoparticles or encapsulating peptides in lipid vessels (da Costa, 2015). Additionally, the use of recombinant fusion peptides allows higher yields of soluble proteins and is potentially a more cost-efficient alternative to solid-phase synthesis chemistry (Haney, 2013).

Several issues require further investigation, such as membrane repair mechanisms, the role of fluidity/rigidity of the membrane, the participation of host components, the mechanisms of resistance of gram-negative membrane receptors (Juhas, 2015; Lohner *et al.,* 2017), as well as continuing to innovate and be able to mass-produce and market new molecules based on the findings. Several studies using AMPs in *Drosophila in vitro* have demonstrated the potential for synergistic interactions of AMPs in the microbial killing. Hanson *et al.* (2019) found that certain combinations of AMPs have synergistic contributions to defense against *P. burhodogranariea*; synergistic loss of resistance may arise in a general fashion: cooperation of AMPs using similar mechanisms of action may breach a threshold microbicidal activity that pathogens are no longer able to resist. For instance, the action of the bumblebee AMP *Abaecin*, which binds to the molecular chaperone DnaK to inhibit bacterial DNA replication, is potentiated by the presence of the pore-forming peptide *Hymenoptaecin* (Rahnamaeian *et al*., 2016).

The approach of using multiple compound mutants, now possible with the development of new genome editing approaches, was especially useful in deciphering the logic of immune effectors. Understanding the role of AMPs in innate immunity holds great promise for the development of novel antibiotics (Chung *et al*., 2017) and will be predicting key parameters that predispose individuals or populations to certain kinds of infections (Chapman *et al*., 2018). It will be necessary to study the role of AMPs in not only systemic immunity but also local immune responses, and the various roles that AMPs may play in aging, neurodegeneration, anti-tumor activity, regulation of the microbiota, etc. An approximation at this are toxins found in scorpion venom might be useful to design drugs, tumoral markers, and adjuvants to cancer treatment taking advantage of their high affinity and specificity for certain ionic channels over expressed in many malignant cells. Should be taken into account that these same substances can have other applications, e.g., treatment of infectious diseases taking advantage of their wide spectrum and low resistance that they could generate (Rave *et al*., 2019).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

Andersson DI, Hughes D, Kubicek-Sutherland JZ. Mechanisms and consequences of bacterial resistance to antimicrobial peptides. Drug Resist Updat. 2016;26:43-57. Doi: https://doi.org/10.1016/j.drup.2016.04.002

Angel OD, Samael K, Sánchez-Evangelista G, Carmona-Navarrete I, Galicia-Sánchez MDC, Gómez-Luna A, *et al*. Péptidos antimicrobianos, una alternativa prometedora para el tratamiento de enfermedades infecciosas. Gac Med Mex. 2018;154(6),681-688. Doi: https://doi.org/10.24875/GMM.18003445

Baltz RH. Synthetic biology, genome mining, and combinatorial biosynthesis of NRPS-derived antibiotics: a perspective. J Ind Microbiol Biotechnol. 2018;45(7),635-649. Doi: https://doi.org/10.1007/s10295-017-1999-8

Barns KJ, Weisshaar JC. Real-time attack of LL-37 on single *Bacillus subtilis* cells. Biochim Biophys Acta Biomembr. 2013;1828(6):1511-1520. Doi: https://doi.org/10.1016/j.bbamem.2013.02.011

Campos OR, Crocomo WB, Labinas AM. Comparative biology of the whitefly *Trialeurodes vaporariorum* (West.) (Hemiptera - Homoptera: Aleyrodidae) on soybean and bean cultivars. Neotrop Entomol. 2003;32(1):133-138. Doi: http://dx.doi.org/10.1590/S1519-566X2003000100020

Chapman A, Lindermayr C, Glawischnig E. Expression of antimicrobial peptides under control of a camalexin-biosynthetic promoter confers enhanced resistance against *Pseudomonas syringae*. Phytochemistry. 2016;122:76-80. Doi: https://doi.org/10.1016/j.phytochem.2016.01.001

Chapman JR, Hill T, Unckless RL. Balancing selection drives maintenance of genetic variation in *Drosophila* antimicrobial peptides. bioRxiv. 2018. Doi: https://doi.org/10.1101/298893

Chung EMC, Dean SN, Propst CN, Bishop BM, van Hoek ML. Komodo dragon-inspired synthetic peptide DRGN-1 promotes wound-healing of a mixed-biofilm infected wound. Npj Biofilms and Microbiomes. 2017;3. Doi: https://doi.org/10.1038/s41522-017-0017-2

Da Costa JP, Cova M, Ferreira R, Vitorino R. Antimicrobial peptides: an alternative for innovative medicines? Appl. Microbiol. Biotechnol. 2015;99(5):2023-2040. Doi: https://doi.org/10.1007/s00253-015-6375-x

Dobias J, Poirel L, Nordmann P. Cross-resistance to human cationic antimicrobial peptides and to polymyxins mediated by the plasmid-encoded MCR-1? Clin Microbiol Infect. 2017;23(9):676-e1-e5. Doi: https://doi.org/10.1016/j.cmi.2017.03.015

Eckert R. Road to clinical efficacy: challenges and novel strategies for antimicrobial peptide development. Future Microbiol. 2011;6(6):635-651. Doi: https://doi.org/10.2217/fmb.11.27

Frick IM, Nordin SL, Baumgarten M, Mörgelin M, Sørensen OE, Olin AI *et al*. Constitutive and inflammation-dependent antimicrobial peptides produced by epithelium are differentially processed and inactivated by the commensal *Finegoldia magna* and the pathogen *Streptococcus pyogenes*. J Immunol. 2011;187:4300–4309. Doi: https://doi.org/10.4049/jimmunol.1004179

Haney EF, Hancock REW. Peptide design for antimicrobial and immunomodulatory applications. Biopolymers. 2013;100(6):572-583. Doi: https://doi.org/10.1002/bip.22250

Hanson MA, Dostalova A, Ceroni C, Poidevin M, Kondo S, Lemaitre B. Synergy and remarkable specificity of antimicrobial peptides in vivo using a systematic knockout approach. Elife. 2019;9:e44341. Doi: https://doi.org/10.7554/eLife.44341

Hao G, Shi YH, Tang YL, Le GW. The intracellular mechanism of action on *Escherichia coli* of BF2-A/C, two analogues of the antimicrobial peptide Buforin 2. J Microbiol. 2013;51(2):200-206. Doi: https://doi.org/10.1007/s12275-013-2441-1

Hashemi MM, Rovig J, Weber S, Hilton B, Forouzan MM, Savage PB. Susceptibility of colistin-resistant, Gram-negative bacteria to antimicrobial peptides and ceragenins. Antimicrob Agents Chemother. 2017;61(8):e00292-17. Doi: https://doi.org/10.1128/AAC.00292-17

Juhas M. Horizontal gene transfer in human pathogens. Crit Rev. Microbiol. 2015;41(1):101–108. Doi: https://doi.org/10.3109/1040841X.2013.804031

Lashua LP, Melvin JA, Deslouches B, Pilewski JM, Montelaro RC, Bomberger JM. Engineered cationic antimicrobial peptide (eCAP) prevents *Pseudomonas aeruginosa* biofilm growth on airway epithelial cells. Antimicrob Agents Chemother. 2016;71(8):2200-2207. Doi: https://doi.org/10.1093/jac/dkw143

Lee H, Hwang JS, Lee J, Kim JI, Lee DG. Scolopendin 2, a cationic antimicrobial peptide from centipede, and its membrane-active mechanism. Biochim Biophys Acta Biomembr. 2015;1848(2):634-642. Doi: https://doi.org/10.1016/j.bbamem.2014.11.016

Lohner K. Membrane-active antimicrobial peptides as template structures for novel antibiotic agents. Curr Top. Med Chem. 2017;17(5):508-519.

Marahiel MA. A structural model for multimodular NRPS assembly lines. Nat Prod Rep. 2016;33(2):136-140. Doi: https://doi.org/10.1039/C5NP00082C

Maróti Gergely G, Kereszt A, Kondorosi É, Mergaert P. Natural roles of antimicrobial peptides in microbes, plants and animals. Res Microbiol. 2011;162(4):363-374. Doi: https://doi.org/10.1016/j.resmic.2011.02.005

Melvin JA, Montelaro RC, Bomberger JM. Clinical potential of engineered cationic antimicrobial peptides against drug resistant biofilms. Expert Rev Anti Infect Ther. 2016;14(11):989-991. Doi: https://doi.org/10.1080/14787210.2016.1236687

Nelson DC, Garbe J, Collin M. Cysteine proteinase SpeB from *Streptococcus pyogenes* – a potent modifier of immunologically important host and bacterial proteins. Biol Chem. *2011;* 392(12)*:*1077–1088. Doi: https://doi.org/10.1515/BC.2011.208

Nguyen LT, Chau JK, Zaat SAJ, Vogel HJ. Cyclic tritrpticin analogs with distinct biological activities. Probiotics Antimicrob Proteins. 2011;3(2):132-143. Doi: https://doi.org/10.1007/s12602-011-9067-6

Omardien S, Brul S, Zaat SAJ. Antimicrobial activity of cationic antimicrobial peptides against gram-positives: current progress made in understanding the mode of action and the response of bacteria. Front Cell Dev Biol. 2016;4:111. Doi: https://doi.org/10.3389/fcell.2016.00111

Rave LJG, Bravo AXM, Castrillo JS, Marín LMR, Pereira CC. Scorpion venom: new promise in the treatment of cancer. Acta Biol Colomb. 2019;24(2):213-223. Doi: https://doi.org/10.15446/abc.v24n2.71512

Rahnamaeian M, Cytryńska M, Zdybicka-Barabas A, Vilcinskas A. The functional interaction between abaecin and pore-forming peptides indicates a general mechanism of antibacterial potentiation. Peptides. 2016;78:17–23. Doi: https://doi.org/10.1016/j.peptides.2016.01.016

Robbel L, Marahiel MA. Daptomycin, a bacterial lipopeptide synthesized by a nonribosomal machinery. J Biol Chem. 2010;285(36):27501-27508. Doi: https://doi.org/10.1074/jbc.R110.128181

Shurko JF, Galega RS, Li C, Lee GC. Evaluation of LL-37 antimicrobial peptide derivatives alone and in combination with vancomycin against *S. aureus*. Jpn J Antibiot. 2018;71(11):971-974. Doi: 10.1038/s41429-018-0090-7

Téllez GA, Castaño JC. Antimicrobial peptides. Infection. 2010;14(1):55-67.

Tetens J, Friedrich JJ, Hartmann A, Schwerin M, Kalm E, Thaller G. The spatial expression pattern of antimicrobial peptides across the healthy bovine udder. J Dairy Sci. 2010;93(2):775-783. Doi: https://doi.org/10.3168/jds.2009-2729

Tomasinsig L, De Conti G, Skerlavaj B, Piccinini R, Mazzilli M, D'Este F, *et al*. Broad-spectrum activity against bacterial mastitis pathogens and activation of mammary epithelial cells support a protective role of neutrophil cathelicidins in bovine mastitis. Infect Immun. 2010;78(4):1781-1788. Doi: https://doi.org/10.1128/IAI.01090-09

Van der Velden WJ, van Iersel TM, Blijlevens NM, Donnelly JP. Safety and tolerability of the antimicrobial peptide human lactoferrin 1-11 (hLF1-11). BMC medicine. 2009;7(1):44. Doi: https://doi.org/10.1186/1741-7015-7-44

Waghu FH, Barai RS, Gurung P, Idicula-Thomas S. CAMPR3: a database on sequences, structures and signatures of antimicrobial peptides. Nucleic Acids Res. 2016;44(D1):1094-1097. Doi: https://doi.org/10.1093/nar/gkv1051

Walsh CT, O'Brien RV, Khosla C. Nonproteinogenic amino acid building blocks for nonribosomal peptide and hybrid polyketide scaffolds. Angew Chem. 2013;52(28):7098-7124. Doi: https://doi.org/10.1002/anie.201208344

Wang G. Improved methods for classification, prediction, and design of antimicrobial peptides. In Computational Peptidology. New York, NY: Humana Press; 2015. p. 43-66.

Wang K, Dang W, Yan J, Chen R, Liu X, Yan W *et al*. Membrane perturbation action mode and structure-activity relationships of Protonectin, a novel antimicrobial peptide from the venom of the neotropical social wasp *Agelaia pallipes pallipes*. Antimicrob Agents Chemother. 2013;57(10):4632-4639. Doi: https://doi.org/10.1128/AAC.02311-12

Yim G, Thaker MN, Koteva K, Wright G. Glycopeptide antibiotic biosynthesis. Jpn J Antib. 2014;67(1):31-41. Doi: https://doi.org/10.1038/ja.2013.117

Yu G, Baeder D, Regoes R, Rolff J. Predicting drug resistance evolution: antimicrobial peptides vs. antibiotics. bioRxiv. 2017;138107. Doi: https://doi.org/10.1101/138107

Zare-Zardini H, Salehvarzi M, Ghanizadeh F, Sadri Z, Sheikhpour R, Zare Bidoki F, *et al*. Antimicrobial peptides of innate immune system as a suitable compound for cancer treatment and reduction of its related infectious disease. Iran J Ped Hematol Oncol. 2018;8(1):62-70.

Zhang LJ, Gallo RL. Antimicrobial peptides. Curr. Biol. 2016;26(1):14-19. Doi: https://doi.org/10.1016/j.cub.2015.11.017