Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(3): 2658-2663
Received: 24-03-2019
Accepted: 26-04-2019

Bhoomika
Ph. D Scholar, Division of Veterinary Public Health, ICAR-IVRI, Izatnagar, Bareilly, Uttar Pradesh, India

Anukampa Kadwalia
Ph. D Scholar, Division of Veterinary Public Health, ICAR-IVRI, Izatnagar, Bareilly, Uttar Pradesh, India

Palvi Thakur
Veterinary Officer, Veterinary Hospital Mandap, Department of Animal Husbandry, District Mandi, Himachal Pradesh, India

Diksha Gourkhede
Mv.sc. Scholar, Division of Veterinary Public Health, ICAR-IVRI, Izatnagar, Bareilly, Uttar Pradesh, India

Vivekanandhan R
PhD Scholar, Division of Veterinary Public Health, College of Veterinary Science & AH, Mannuthy, Kerala, India

Correspondence
Bhoomika
Ph. D Scholar, Division of Veterinary Public Health, ICAR-IVRI, Izatnagar, Bareilly, Uttar Pradesh, India

Antimicrobial peptides: As an emerging alternative to combat drug resistant

Bhoomika, Anukampa Kadwalia, Palvi Thakur, Diksha Gourkhede and Vivekanandhan R

Abstract
The increasing resistance toward conventional antibiotics suggests that, without urgent action, we are now in "post-antibiotic era," in which the formerly effective therapeutic strategies are no longer relevant. Due to the limited number of available antibiotics, and the similarities in their activity spectrum as well as mode of action, intensive nonclinical and clinical research is now invested into identification of new and non-conventional anti-infective therapies, including adjunctive or preventive approaches such as antibodies targeting a virulence factor, probiotics, and vaccines. Interestingly, the antimicrobial peptides (AMPs) have rapidly captured attention as novel drug candidates. Virtually AMPs have been found in all organisms and they display remarkable structural and functional diversity. Along with direct antimicrobial activity, AMPs also carry immunomodulatory properties, which make them especially interesting compounds for the development of novel therapeutics. Present review focuses on the structure, mode of action, limitations and strategies to overcome the shortcoming.

Keywords: Antimicrobial peptides; antibiotic resistance; membrane active; toxicity

Introduction
In recent times, antimicrobial peptides (AMPs) have interestingly captured attention as novel drug candidates. The existence of humans and mammals in the environment is constantly threatened by exposure to a myriad of diverse pathogenic species, for which the bodies are well equipped with evolutionarily conserved innate immune defense system that enables to tackle the pathogens. Antimicrobial peptides (AMPs) are unique and assorted class of molecules produced by living organisms of all types, considered to be a part of the host innate immunity (Peters *et al*., 2010) [41]. Generally, antimicrobial peptides (synonymously termed as, host defense peptides or HDPs) are considered to be small polycationic peptides comprising of 7–100 amino acids and are shared by all forms of life. Currently, these compounds are being tested as alternatives to classical antibiotic therapies or, at least, as complementary to antibiotics to treat infectious diseases (Ageitos *et al*., 2017) [1]. More significantly, the ability of these natural molecules to kill multi-drug resistant microorganisms has gained them considerable attention and clinical interest in the near future (Giuliani *et al*., 2007; Peters *et al*., 2010) [17, 41]. Besides direct antimicrobial activity, some AMPs carry immuno-modulatory properties (Mahlapuu *et al*., 2016) [26] and thereby indirectly promote pathogen clearance (Yeung *et al*., 2011) [55]. Natural AMPs can be found in both prokaryotes and eukaryotes (Radek and Gallo, 2007; Peters *et al*., 2010) [44, 41]. In animals, AMPs are mostly found in the tissues and organs that are exposed to airborne pathogens and are believed to be the first line of the innate immune defense against viruses, bacteria and fungi (Peters *et al*., 2010) [41]. Several types of eukaryotic cells are involved in AMP production such as lymphs, epithelial cells in gastrointestinal and genitourinary systems, phagocytes and lymphocytes of the immune system. Of late, more than 2800 experimentally reported AMPs, including both synthetically synthesized and compounds produced by living organisms are documented in specialized AMP databases such as Antimicrobial peptide database (Wang *et al*., 2016) [52], Biofilm active AMPs database (Di Luca *et al*., 2015) [11], Collection of AMPs (Waghu *et al*., 2015) [51], Yet Another Database of Antimicrobial Peptides (Piotto *et al*., 2012) [42].

History of AMP's
Earlier lysozyme was identified as an AMP by Alexander Fleming during 1922, however, the historical discovery of penicillin, dissembled its advent to biological sciences. Later, in the year 1939, Dubos extracted an antimicrobial agent from a soil *Bacillus* strain and demonstrated to protect mice from pneumococci infection (Dubos, 1938, 1939) [13, 14]. Hotchkiss and

Dubos (1940) fractionated this extract and identified an AMP which was named gramicidin. Despite some reported toxicity associated with intra-peritoneal application, gramicidin was found effective for topical treatment of wounds and ulcers. In 1941, another AMP, tyrocidine, was discovered and found to be effective against both Gram-negative and Gram-positive bacteria. However, tyrocidine exhibited toxicity to human blood cells (Rammelkamp and Weinstein, 1942) [45]. In the same year, yet another AMP was isolated from a plant *Triticum aestivum*, which was later named purothionin, that was found effective against fungi and some pathogenic bacteria (Balls *et al.*, 1942) [3]. The first reported animal-originated AMP is defensin (Hirsch, 1956) [20], which was isolated from rabbit leukocytes. In the following years, bombinin from epithelia (Kiss and Michl, 1962) [23] and lactoferrin from cow milk were both described (Groves *et al*., 1965) [18]. At the same time, it was also proven that human leukocytes contain AMPs in their lysosomes (Zeya and Spitznagel, 1963) [56]. More than 5000 AMPs have been discovered and synthesized, so far. Since the initial discovery of antimicrobial peptides (AMPs) in insects and animals was in the 1980s, they have now been acclaimed as a promising alternative to today's antibiotics.

Classification of AMP's

Of the AMPs identified so far**,** approximately 10% are anionic in nature, while 90% of the remaining peptides are cationic in nature; as such, the later are the principal focus of current research. On the basis of their amino-acid composition, size and conformational structures, AMPs can be classified as (i) linear; (ii) cysteine-rich peptides and (iii) peptides rich in specific amino acids, such as glycine, proline, arginine or histidine based on amino acid composition. However, on the basis of their secondary structure, they are classified as (i) α-helical, (ii) β-sheet, (iii) mixed and (iv) random coil (Narayana and Chen, 2015) [34].

Anionic antimicrobial peptides (AAMPs)

AAMPs have been increasingly identified in invertebrates, vertebrates and plants over the last decade and it is progressively apparent that these peptides form an integral part of the innate immune system. AAMPs constitute part of various vital organs of the body including respiratory tract, brain, epidermis, epididymis, blood and gastrointestinal tract. AAMPs act through a wide range of antimicrobial mechanisms. Respiratory tract surfactant- associated anionic peptides undergo translocation across the membrane and target internal components to exert their activity whereas, the other AAMPs act by rupturing the membrane (Narayana and Chen, 2015) [34]. The bovine anionic peptide, Kappacin, isolated from milk and cheese is the cleavage product of caseino macropeptide (CMP) with no post-translational modification (Malkoski *et al*., 2001). Bovine AAMP, Peptide B/enkelytin was first identified in the secretory granules of bovine chromaffin cells (Salzet *et al*., 2000) [47]. Yet another AAMP, chromacin tend to play an important role in inflammatory protective barrier responses against the infection and thereby having a potential host defense role (Strub *et al*., 1996) [50]. Dermcidin (DCD) is one of the well-studied human AAMPs and its gene sequence was identified in malignant melanoma (Schittek *et al*., 2001) [49]. Amphibian AAMP, temporin, isolated from the skin secretions of European red frog synergises with other temporins to aid in endotoxin neutralisation and could be used as a potent candidate for antisepsis (Rosenfeld *et al*., 2006) [46].

Cationic antimicrobial peptides (CAMPs)

Most AMPs are relatively short, commonly consisting of 10–50 amino acids, displaying an overall positive charge ranging from +2 to +11, and contain a substantial proportion (typically, 50%) of hydrophobic residues (Pasupuleti *et al*., 2012) [40]. An essential requirement for any antimicrobial agent is that it has selective toxicity for the microbial target, which is an important feature of AMPs, as their preferential interaction with microbial cells makes them non-toxic to mammalian cells. The antimicrobial activity of a given AMP is specifically related to its amino acid composition and physico-chemical properties such as net positive charge, flexibility, size, hydrophobicity and amphipathicity (Peters *et al*., 2010) [41]. The cationicity of AMPs promotes interactions with negatively charged moieties on other biomolecules such as outer membrane lipids, nucleic acids and phosphorylated proteins that promotes selectivity for negatively charged microbial cytoplasmic membranes over zwitter ionic mammalian membranes whereas hydrophobic interaction facilitates interactions with the fatty acyl chains (Nguyen *et al*., 2011) [35]. During an infection, a host animal may release multiple isoforms or structurally similar AMPs that act by distinct mechanisms to achieve an overall synergistic effect (Mangoni and Shai, 2009) [28].

Mechanisms of action

Antimicrobial peptides kill cells by disrupting membrane integrity, inhibiting protein, DNA and RNA synthesis or by interacting with certain intracellular targets (Bahar and Ren, 2013) [2]. Even if intracellular targets are involved, an initial cell membrane interaction with peptides is required for the antimicrobial activities of AMPs; this interaction determines the spectrum of target cells. Most membrane active AMPs are amphipathic in nature, which means that they have both cationic and hydrophobic faces. This feature ensures the initial electrostatic interaction with the negatively charged cell membrane and the insertion into inner membrane. This initial membrane interaction will not hinder the actions of AMPs. The hydrophobic part of AMP helps in inserting the AMP molecule into the cell membrane. Hence, the interaction mainly includes ionic and hydrophobic interactions. These interactions mostly depend on two properties, the cationic and hydrophobic property of the peptide (Nguyen *et al*., 2011) [35]

Membrane active AMPs

Membrane permeabilization by AMPs is suggested to initially lead to leakage of ions and metabolites, depolarization of the trans-membrane potential with subsequent membrane dysfunction (e.g. impaired osmotic regulation and inhibition of respiration) which leads to membrane rupture and rapid lysis of microbial cells (Brogden, 2005; Eckert, 2011) [5, 16]. Besides leading to membrane dysfunction and disruption, membrane permeabilisation is important for translocation of certain AMPs into the cytoplasm, where they target key cellular processes including DNA/RNA and protein synthesis, protein folding, enzymatic activity and/or cell wall synthesis (Nguyen *et al*., 2011) [36].

The cytoplasmic membranes of both Gram-positive and Gram-negative bacteria are rich in the phospholipids phosphatidylglycerol, cardiolipin and phosphatidylserine, which have negatively charged head groups, highly attractive for positively charged AMPs (Ebenhan *et al*., 2014) [15]. The presence of teichoic acids in the cell wall of Gram-positive bacteria and lipopolysaccharides (LPS) in the outer membrane of Gram negative bacteria provide additional electronegative

charge to the bacterial surface (Ebenhan *et al*., 2014) [15]. In order to reach the cytoplasmic membrane of Gram negative bacteria, AMPs have to first translocate through the outer membrane. This outer membrane constitutes a permeability barrier for many macromolecules, partly due to the divalent cations Ca^{2+} and Mg^{2+} that binds to the phosphate groups of the inner core of LPS and thereby provide stabilization of the outer leaflet. AMPs are proposed to be trans located through this outer membrane via so called self- promoted uptake (Giuliani *et al*., 2007) [17]. By being bulky, the AMPs then cause transient cracks and permeabilize the outer membrane, thereby permitting passage of the peptide itself across the membrane.

Various hypotheses have been postulated to describe the mechanism of action of AMPs after translocation into the cell membrane: three models are important *viz*., toroidal pore, barrel- stave and carpet model (Table 1).

Table 1: Different membrane interaction models

Interaction model	Mechanism	References
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.	(Mecke, 2005) [30]
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.	(Pouny *et al*., 1992) [43]
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.	**(**Wu *et al*., 1999) [53]
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.	**(**Zhang, 2001) [57]
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.	**(**Brogden, 2005) [5]

These membrane interactions will ultimately result in the formation of a transient channel, dissolution of the membrane and micellarization or translocation across the membranes causing an increase in the membrane permeability. Finally, there is efflux of essential ions and nutrients, leading to rapid cell death (Datta *et al*., 2016) [10].

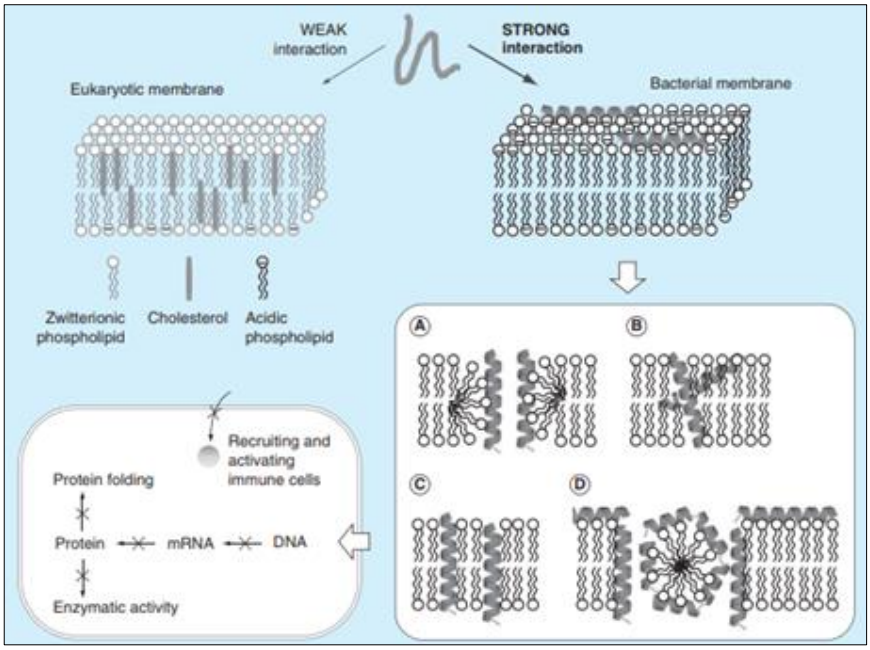

Fig 1: Mechanisms of action of antimicrobial peptides. Because the composition of the eukaryotic cell membrane and the bacterial membrane is different, cationic antimicrobial peptides strongly interact with the bacterial plasma membrane. (A) Toroidal pore model, (B) aggregate model, (C) barrel-stave model and (D) carpet model.

Intracellular Active AMPs

In early AMP studies it was thought that the permeabilization of bacterial cell membrane by AMP as the primary mechanism of killing. The finding that some AMPs can kill their target cells without causing membrane permeabilization suggests that there may be other mechanisms of killing. Recently, intracellular active AMPs have been shown to interact with targets inside the cells (Otvos, 2005; Chen and Harrison, 2007; Mookherjee *et al.*, 2009) [39, 7, 32]. Some AMPs can inhibit DNA and protein synthesis (Nicolas 2009; Hilpert *et al*., 2010) [37, 19]. For example, Indolicin and PR-39 follow non-lytic process by acting like proteolytic agents (Boman *et al*., 1993) [4], does not lyse cell directly, it enters the cytoplasm and kills the bacteria by targeting DNA synthesis (Subbalakshmi and Sitaram, 1998; Nicolas, 2009) [37]. Some AMPs can also inhibit proteases of microbes for example Histatin 5 (Nishikata *et al*., 1993) [38]. Among these intracelluler active AMPs, some of them have multiple targets for example, seminal plasmin inhibits RNA polymerase and can top RNA synthesis (Scheit *et al.*, 1979) [48], while some AMP activate an autolysin protein inside the target cells and causes autolysis (Chitnis *et al*., 1987; Chitnis *et al*., 1990) [8, 9]. Intracellular pathway of killing the bacterial cells proposed two mechanism of the cellular uptake: direct penetration and endocytosis (Madani *et al*., 2011) [25]. Endocytosis includes macropinocytosis and receptor mediated endocytosis.

Macropinocytosis is a vesicle like structure forms due to the folding of the inner membrane of the bacterial cell (Madani *et al*., 2011) [25]. In receptor mediated endocytosis, a part of the membrane is coated with clathrin and caveolin proteins followed by pit formation (Jones, 2007; Mayor and Pagano, 2007) [21, 29].

AMPs in drug development

The rapid bactericidal activity of AMPs makes them promising candidates for therapeutic anti-infectives. Furthermore, several AMPs have a broad range of action, which is an advantage in certain therapeutic areas, such as complicated skin and soft tissue infections, where a rapidly increasing incidence of polymicrobial infections involving both Gram-positive and Gram-negative organisms has been reported over the last decade (Dryden, 2010) [12]. Several AMPs have been successfully developed for pharmaceutical and commercial applications (Moberg and Cohn, 1990) [31]. Representative AMPs in clinical trials are summarized in Table 2, for detail information refers to Mahlapuu *et al.*, 2016. [27]

Table 2: Selected AMPs in clinical phase of development

AMP	Description	Phase	Indication	Administration	Clinical trial identifier if available
Pexiganan (MSI-78)	Analog of magainin (skin of African clawed frog)	Phase III	Infected diabetic foot ulcers	Topical cream	NCT00563394, NCT00563433
Omiganan	Derived from indolicidin (bovine)	Phase II/III	Catheter infections and rosacea	Topical gel	NCT00231153, NCT01784133
Lytixar (LTX-109)	Syntheticantimicrobial peptidomimetic	PhaseI/II	Uncomplicated Gram-positive skinin fections, impetigo, and nasal colonization with *S. aureus*	Topicalhydrogel	NCT01223222, NCT01803035, NCT01158235
hLF1-11	Derived from lactoferricin (human)	PhaseI/II	Bacteraemi aandfungalinfectionsin immunocompromized haematopoetic stem cell transplant recipients	Intravenoustreatment (insaline)	NCT00509938
Novexatin (NP-213)	Derived from defensins (human)	PhaseII	Onychomycosis (fungalnailinfection)	Topical brush-on-treatment	
CZEN- 002 Vaginalgel	Dimeric octamer derived from a-MSH (human)	Phase IIb	Vaginal candidiasis		
LL-37	LL-37 (human)	Phase I/II	Hard-to-heal venous leg ulcers	Polyvinyl alcohol-based solution for administration in the wound bed	
PXL01	Derived from lactoferricin (human)	Phase II	Prevention of post-surgical adhesion formation in hand surgery	Hyaluronic acid-based hydrogel for administration at the surgical site	NCT01022242
Seganan (IB-367	Derived from protegrin 1 (porcine leukocytes)	Phase III	Oral mucositis in patients receiving radiotherapy for head and neck malignancy	Oral solution	NCT00022373
PAC-113	Derived from histatin 3 (human saliva)	Phase II Oral	candidiasis in HIV seropositive patients	Mouthrinse	NCT00659971

Limitations into the market

Despite the great potential of AMPs including their wide spectrum bactericidal activity (antibacterial, antiviral, antifungal), rapid onset of action, potentially low levels of induced resistance to the physical action of AMPs and concomitant broad anti-inflammatory activities they still have several problems with application to clinical cases discovery costs of synthesis and screening, patent exclusivity for economic viability, reduced activity based on salt, serum, and pH sensitivity, systemic and local toxicity, High manufacturing costs, susceptibility to proteolysis, pharmacokinetic (PK) and pharmacodynamic (PD) issues, sensitization and allergy after repeated application, natural resistance (e.g., *Serratia marcescens*), Confounding biological functions (e.g., angiogenesis) (Koczulla and Bals, 2003; Bradshaw, 2003; Yeaman and Yount, 2003; Kang *et al*., 2014) [24, 54, 22]

Strategies for new therapeutic drug development-

To develop new antibiotics from AMPs, the fundamental issues of stability, toxicity and cost are now being targeted by a number of distinct strategies which is summarize in Table 3 (Kang *et al*., 2014) [22].

Table 3: Strategies for new Therapeutic drug development

Limitations	Strategies
Stability	Cyclization, D- or nonnatural amino acids, Acetylation, amidation Modification of amphipathic balance, Reducing cationic residue content
Toxicity	Control of hydrophobicity, Molecular targeted AM, Polymeric nanoencapsulation, Liposomal formulations, PEGylation, Drug delivery systems
Cost	Size reduction, De novo synthesis, New expression system Cost–effective purification

Conclusions

The serious problems caused by drug resistant bacteria have created an urgent need for the development of alternative therapeutics. In this respect, AMPs offer promising alternatives to standard therapies as anti-infectives and immunomodulatory agents with mechanisms of action which are less prone to resistance induction compared to conventional antibiotics. Although challenges in translating nonclinical candidate AMPs into successful clinical products are well recognized, the discovery and commercial development of next-generation therapeutic peptides and peptide mimetics is predicted to be accelerated by recent

advances in overall understanding of their mechanism of action, resistance patterns, and smart formulation strategies.

References

1. Ageitos JM, Sánchez-Pérez A, Calo-Mata P, Villa TG. Antimicrobial peptides (AMPs): Ancient compounds that represent novel weapons in the fight against bacteria. Biochem. Pharmacol. 2017; 133:117-138.
2. Bahar AA, Ren D. Antimicrobial peptides. Phamaceuticals. 2013; 6(12):1543-1575.
3. Balls AK, Hale WS, Harris TH. A crystalline protein obtained from a lipoprotein of wheat flour. Cereal Chem. 1942; 19(19):279-288.
4. Boman HG, Agerberth B, Boman A. Mechanism of action on *E. coli* of cercopin *p* 1 and pr-39, two antimicrobial peptides from pig intestine, Infect. Immune. 1993; 61:2978-2984.
5. Brogden KA. Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? Nat. Rev. Microbiol. 2005; 3(3):238-250.
6. Brogden KA. Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? Nat. Rev. Microbiol. 2005; 3(3):238-250.
7. Chen L, Harrison SD. Cell-penetrating peptides in drug development: Enabling intracellular targets. Biochem. Soc. Trans. 2007; 35:821-825.
8. Chitnis SN, Prasad KN, Bhargava PM. Bacteriolytic activity of seminalplasmin. Microbiology. 1987; 133(5):1265-1271.
9. Chitnis SN, Prasad KS, Bhargava PM. Isolation and characterization of autolysis-defective mutants of Escherichia coli that are resistant to the lytic activity of seminalplasmin. Microbiology. 1990; 136(3):463-469.
10. Datta A, Bhattacharyya D, Singh S, Ghosh A, Schmidtchen A, Malmsten M *et al*. Role of aromatic amino acids in lipopolysaccharide and membrane interactions of antimicrobial peptides for use in plant disease control. J Bio. Chem. -M116, 2016.
11. Di Luca, M, Maccari G, Maisetta G, Batoni G. BaAMPs: the database of biofilm-active antimicrobial peptides. Biofouling. 2015; 31(2):193-199.
12. Dryden MS. Complicated skin and soft tissue infection. J Antimicrob. Chemother. 2010; 65(3):35-44.
13. Dubos RJ. Studies on a bactericidal agent extracted from a soil bacillus: I. Preparation of the agent. Its activity *In vitro*. J Exper. Med. 1938; 70(1):1.
14. Dubos RJ. Studies on a bactericidal agent extracted from a soil bacillus: II. Protective effect of the bactericidal agent against experimental Pneumococcus infections in mice. J Exper. Med. 1939; 70(1):11.
15. Ebenhan T, Gheysens O, Kruger HG, Zeevaart JR, Sathekge MM. Antimicrobial peptides: their role as infection-selective tracers for molecular imaging. Bio Med Res. Int. 867381, 2014.
16. Eckert R. Road to clinical efficacy: challenges and novel strategies for antimicrobial peptide development. Fut. Microbiol. 2011; 6(6): 635-651.
17. Giuliani A, Pirri G, Nicoletto S. Antimicrobial peptides: an overview of a promising class of therapeutics. Open Life Sci. 2007; 2(1):1-33.
18. Groves ML, Peterson RF, Kiddy CA. Polymorphism in the red protein isolated from milk of individual cows. Nature. 1965; 207(5000):1007-1008.
19. Hilpert K, McLeod B, Yu J, Elliott MR, Rautenbach M, Ruden S, *et al*. Short cationic antimicrobial peptides interact with ATP. Antimicrob. Agents Ch. 2010; 54(10):4480-4483.
20. Hirsch JG. Phagocytin: a bactericidal substance from polymorphonuclear leucocytes. J Exper. Med. 1956; 103(5):589-611.
21. Jones AT. Macropinocytosis: searching for an endocytic identity and role in the uptake of cell penetrating peptides. J Cell Mol. Med. 2007; 11(4):670-684.
22. Kang SJ, Park SJ, Mishig-Ochir T, Lee BJ. Antimicrobial peptides: therapeutic potentials. Expert review of anti-infective therapy. 2014; 12(12):1477-1486.
23. Kiss G, Michl H. Uber das Giftsekret der Gelbbauchunke, Bombina Variegata L. Toxicon. 1962; 1(1):33-34.
24. Koczulla AR, Bals R. Antimicrobial peptides: current status and therapeutic potential. Drugs. 2003; 63:389-406.
25. Madani F, Lindberg S, Langel U, Futaki S, Graslund A. Mechanisms of cellular uptake of cell-penetrating peptides. J Biophys, 2011.
26. Mahlapuu M, Håkansson J, Ringstad L, Björn C. Antimicrobial peptides: an emerging category of therapeutic agents. Front. Cell. Infect. Microbiol. 2016; 6:194.
27. Mahlapuu M, Hakansson J, Ringstad L, Bjorn C. Antimicrobial peptides: an emerging category of therapeutic agents. Front. in cell. and infect. microbiol, 2016; 6:194.
28. Mangoni ML, Shai Y. Temporins and their synergism against Gram-negative bacteria and in lipopolysaccharide detoxification. Biochim. et Biophys. Acta (BBA)-Biomem. 2009; 1788(8):1610-1619.
29. Mayor S, Pagano RE. Pathways of clathrin-independent endocytosis. Nat. Rev. Mol. Cell Biol. 2007; 8:603-613.
30. Mecke A, Lee DK, Ramamoorthy A, Orr BG, Holl, MMB. Membrane thinning due to antimicrobial peptide binding: an atomic force microscopy study of MSI-78 in lipid bilayers. Biophys. J. 2005; 89(6):4043-4050.
31. Moberg CL, Cohn ZA. Lauching the antibiotic era. Personal accounts of the discovery and use of the first antibiotics. Rockefeller University Press; New York, 1990.
32. Mookherjee N, Lippert DN, Hamill P, Falsafi R, Nijnik A, Kindrachuk J *et al*. Intracellular receptor for human host defense peptide ll-37 in monocytes. J Immunol. 2009; 183:2688-2696.
33. Mylonakis E, Moreno R, El Khoury JB, Idnurm A, Heitman J, Calderwood SB, *et al*. *Galleria mellonella* as a model system to study *Cryptococcus neoformans* pathogenesis. Infect. Immun. 2005; 73(7):3842-3850.
34. Narayana JL, Chen JY. Antimicrobial peptides: possible anti-infective agents. Peptides. 2015; 72:88-94.
35. Nguyen LT, Haney EF, Vogel HJ. The expanding scope of anti-microbial peptide structures and their modes of action. Tren. Biotechnol. 2011; 29(9):464-472.
36. Nguyen LT, Haney EF, Vogel HJ. The expanding scope of anti-microbial peptide structures and their modes of action. Tren. Biotechnol. 2011; 29(9):464-472.
37. Nicolas P. Multifunctional host defense peptides: intracellular-targeting antimicrobial peptides. FEBS J. 2009; 276(22):6483-6496.
38. Nishikata M, Kanehira T, Oh H, Tani H, Tazaki M, Kuboki Y. Salivary histatin as an inhibitor of a protease produced by the oral bacterium Bacteroides

gingivalis. Biochem. Bioph. Res. Co. 1991; 174(2):625-630.
39. Otvos L. Antibacterial peptides and proteins with multiple cellular targets. J Pept. Sci. 2005; 11:697-706.
40. Pasupuleti M, Schmidtchen A, Malmsten M. Antimicrobial peptides: key components of the innate immune system. Crit. Rev. Biotechnol. 2012; 32(2):143-171.
41. Peters BM, Shirtliff ME, Jabra-Rizk MA. Antimicrobial peptides: primeval molecules or future drugs? PLoS Pathog. 2010; 6(10):e1001067.
42. Piotto SP, Sessa L, Concilio S, Iannelli P. YADAMP: Yet another database of antimicrobial peptides. Int. J Antimicrob. Agents. 2012; 39(4):346-351.
43. Pouny Y, Rapaport D, Mor A, Nicolas P, Shai Y. Interaction of antimicrobial dermaseptin and its fluorescently labeled analogs with phospholipid membranes. Biochemistry. 1992; 31(49):12416-12423.
44. Radek K, Gallo R. Antimicrobial peptides: natural effectors of the innate immune system. Semin. Immunopathol. 2007; 29(1):27-43.
45. Rammelkamp CH, Weinstein L. Toxic effects of tyrothricin, gramicidin and tyrocidine. J Infect. Dis. 1942; 71(2):166-173.
46. Rosenfeld Y, Barra D, Simmaco M, Shai Y, Mangoni ML. A synergism between temporins toward Gram-negative bacteria overcomes resistance imposed by the lipopolysaccharide protective layer. J Biol. Chemist. 2006; 281(39):28565-28574.
47. Salzet M, Vieau D, Day R. Crosstalk between nervous and immune systems through the animal kingdom: focus on opioids. Tren. Neurosci. 2000; 23(11):550-555.
48. Scheit KH, Reddy ESP, Bhargava PM. Seminal plasmin is a potent inhibitor of *E. coli* RNA polymerase in vitro. Nature. 1979; 279(5715):728-731.
49. Schittek B, Hipfel R, Sauer B, Bauer J, Kalbacher H, Stevanovic S, *et al*. Dermcidin: a novel human antibiotic peptide secreted by sweat glands. Nat. Immunol. 2001; 2(12):1133-1137.
50. Strub JM, Goumon Y, Lugardon K, Capon C, Lopez M, Moniatte M, *et al*. Antibacterial activity of glycosylated and phosphorylated chromogranin A-derived peptide 173-194 from bovine adrenal medullary chromaffin granules. J Biol. Chemist. 1996; 271(45):28533-28540.
51. Waghu FH, Barai RS, Gurung P, Idicula-Thomas S. CAMPR3: a database on sequences, structures and signatures of antimicrobial peptides. Nucl. Acids Res. gkv1051, 2015.
52. Wang G, Li X, Wang Z. APD3: the antimicrobial peptide database as a tool for research and education. Nucl. Acids Res. 2016; 44(D1):D1087-D1093.
53. Wu M, Maier E, Benz R, Hancock RE. Mechanism of interaction of different classes of cationic antimicrobial peptides with planar bilayers and with the cytoplasmic membrane of *Escherichia coli*. Biochemistry. 1999; 38(22):7235-7242.
54. Yeaman MR, Yount NY. Mechanisms of antimicrobial peptide action and resistance. Pharmacol. Rev. 2003; 55:27-55.
55. Yeung AT, Gellatly SL, Hancock RE. Multifunctional cationic host defense peptides and their clinical applications. Cell. Mol. Life Sci. 2011; 68(13):2161.
56. Zeya HI, Spitznagel JK. Antibacterial and enzymic basic proteins from leukocyte lysosomes: separation and identification. Science. 1963; 142(3595):1085-1087.
57. Zhang L, Rozek A, Hancock RE. Interaction of cationic antimicrobial peptides with model membranes. J Biol. Chem. 2001; 276:35714-35722.