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A review on antimicrobial peptides (AMPs)

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Abstract
In nature, antimicrobial peptides (AMPs) are widely involved in the innate host defense. Fish are considered as an great source of antimicrobial peptides because they represent all the major AMP classes, including cathelicidins, hepcidins, defensins, histone-derived peptides, and piscidins. Antimicrobial peptides (AMPs) are said to be highly promising as natural alternative to chemical antibiotics. The fish peptides exhibit broad-spectrum antimicrobial action, killing both fish and human pathogens. Moreover, they are immunomodulatory, and their genes are highly reactive against microbes and innate immuno-stimulatory molecules. Later research has illustrated that some of the special properties of fish peptides, including their ability to act even in very high salt concentrations, make them good potential targets for development as therapeutic antimicrobials. AMPs have demonstrated diverse biologic effects, like endotoxin neutralization, immunomodulating activity and induction of angiogenesis.

Keywords: Antimicrobial peptides, defensin, cathelicidin, hepcidin, piscidin

Introduction
Antimicrobial peptides or proteins (AMPs) are the first line of defence molecules occurring naturally in all multicellular species. The proteins constitute a broad family embracing ability to destroy yeasts, bacteria, fungi, viruses and even cancer cells, with a broad area of action. AMPs are also referred to as 'host defence peptides' in higher eukaryotic cells. The main groups of AMPs, including cathelicidins, hepcidins, defensins, histone-derived peptides, and a fish-specific class of the cecropin family, called piscidins, fish are an excellent source of these peptides (Masso and Diamond, 2014) [39]. AMPs are low molecular weight proteins of about 12–100 amino acid residues, usually positively charged. The structure of AMPs gets folded into an amphipathic structure which makes it soluble in aqueous and lipid because about 40-50% of AMPs residues are hydrophobic.

AMPs are distinct molecules with the ability to kill targeted microbes using a mechanism in which some cationic peptides create electrostatic attraction for negatively charged phospholipids of microbial membranes and integrate into cell membrane of microbes resulting in membrane disintegration. AMPs are broad ranged molecules which have the ability to act against the gram-positive and gram-negative bacteria, viruses and fungi. They have other functions like mediators of inflammation, cell proliferation, angiogenesis, wound healing, chemotaxis, immune induction and protease–antiprotease balance. Antimicrobial peptides (AMPs) have been considered as the good natural alternative to the chemical antibiotics. Fish being a eukaryotic organism, very much relies on their innate immunity, also the fish is a good source of AMPs having their application in a wider range (A. Gautam *et al.*, 2016) [71]

Fish antimicrobial peptides (AMPs) are small peptides whose activities significantly affect the teleost fish's innate immune response, is considered the first line of protection against a broad spectrum of pathogens. Their antitumor effects make them possible medications to be used in human subjects for oncological therapies (Valero *et al.*, 2013) [60]. AMPs properties as antimicrobial agents and other concern behaviours, such as cardioprotective (anticoagulant, antihypertensive and antiatherosclerotic) antioxidant, anxiolytic, analgesic, appetite suppressant, antidiabetic, and neuroprotective, have a tremendous potential to be used in many industrial sectors (Shabir *et al.*, 2018) [55]. AMPs have enormous potential for mass production for further use, and cloning seems to be uncomplicated because the genes that encode for those peptides are small and highly conserved regions are usually present (Valero *et al.*, 2020) [61]

A large number of AMPs have been isolated from a wide number of fish species during last years, among which pleurocidin from winter flounder (*Pleuronectes americanus*) cathelicidins from rainbow trout (*Oncorhynchus mykiss*), defensins from zebrafish (*Danio rerio*), piscidins

from hybrid striped bass (white bass, *Morone chrysops*, female, x striped bass, *Morone saxatilis*, male), dicentracin from sea bass (*Dicentrarchus labrax*) and hepcidin from channel catfish (*Ictalurus punctatus*), epinecidin from the grouper (*Epinephelus coiodes*) (F. Buonocore 2011)

History of AMPs

Lysozyme, the first recorded human antimicrobial protein, was described by Alexander Fleming in 1922 from nasal mucus (Zhang and Gallo, 2016) [68]. Nisin, the oldest yet most common antimicrobial peptide, was first described in 1928. A strain of lactic acid bacteria, *Lactococcus lactis*, produces this 34-residue peptide. It belongs to lantibiotics, a family of bacteriocins that are strongly modified post-translationally. In 1971, the structure was explained (Gross and Morell, 1971) [20]. The first major report of alpha-helical AMPs was detecting 'cecropins' (Zhang and Gallo, 2016) [68]. It is reported that a total of 3,240 AMPs have been recored in the antimicrobial peptide database (APD3[1]) updated on August 24, 2020 (Y. Huan *et al.*, 2020) [25]. Teleost fish species express a significant number of AMPs: to date, roughly 117 out of 2,927 AMPs belonging to two prominent families have been isolated, piscidins and hepcidins.

It was reported that for the another ten years the antimicrobial activity of fish peptides was not described. Intially n 1980, toxic peptide, named pardaxin, was characterized fom the Moses sole fish (*Pardachirus marmoratus*), (N. Primor and AT Tu, 1980) [49], but until 1996 its antimicrobial activity was not discovered (Z Oren, Y Shai, 1996) [45]. Afterwards, a peptide was isolated from the skin secretions of the fish winter flounder (*Pleuronectes americanus*), (Cole *et al*., 1997) [13]. Thereafter, the field advanced as with other vertebrate species also, with the identification of homologous peptides in the piscidin family along with defensin, cathelicidin, and hepcidin families, which are found in many other species (JA Masso-Silva and G Diamond, 2014) [39].

Classification of antimicrobial peptides

AMPs can be classified in two ways based on

(i) Secondary structure
(ii) Composition in amino acids.

In the first form of classification, AMPs are divided into (a) Linear peptides with an amphipathic α helical conformation, (b) Linear peptides with a significant proportion of one residue such as P, R or W, (c) Peptides containing loop structures, (d) β-strand structures containing disulfide bridges (Jegou *et al.*, 2013) [28].

Their amino acid composition can be divided into two main clusters: cationic antimicrobial peptides and non-cationic antimicrobial peptides (Zasloff, 2002 and Narayana and Chen, 2015) [67, 30].

Cationic peptides

Linear α-helix peptides: α-helical AMPs are composed of piscidins, gaduscidins, mononucleosis, grammistins, pardaxin, epinecidin and chemokine.

Disulphide bonded peptides: Peptides with disulphide brides (one, three or more) as their primary structure are composed of different families. The single disulphide is divided into three groups: linear, distinctive disulphide bond in their constitution and no homology with the previous ones (Masso and Diamond, 2011) [38].

Non-cationic antimicrobial peptides

Their amino acid structure is high in aspartic and glutamic acids, varies significantly from cationic peptides. In fish, most non-cationic AMPs have been isolated during the last decade. They can be derived from oxygen-binding proteins, linear α-helical peptides, those with disulphide bridges or various other structures (Buonocore *et al.*, 2012) [7].

General Properties of AMPs

Generally, the AMPs are synthesized in exposed tissues, such as skin, intestine, lungs and red blood cells. As compared to the immunoglobulin these peptides are synthesized 100 times at more rapid rate and at low metabolic cost, but they can also be stored as a reserve into cells and be released when cells are stimulated by the contact with pathogens. These peptides are a fast non-specific way to fight against microorganisms. (J Huerta-Cantillo and F Navarro-García, 2016) [26]

Bacteriocins are labeled Ribosomally synthetized AMPs from prokaryotes (Jegou *et al.* 2013) [28]. It has been shown that they are effective tools to protect or secure an ecological niche (Riley and Wertz 2002) [50]. It is now well known that the plasmatic membrane is the primary target of these peptides. By disrupting the plasmatic membrane, they operate on the responsive cells (Epand and Vogel 1999) [18].

In order to account for anti-viral activity, three methods have been proposed:

1) By disrupting the lipid bilayers of viral envelopes, immediate inactivation of viral particles (Daher *et al.* 1986) [15].
2) By inhibiting viral-cellular membrane fusion, prevention of viral penetration into the host cell (Baghian *et al.* 1997) [2].
3) Inhibition of viral replication in infected cells by suppressing viral gene expression (Wachinger *et al.* 1998) [62].

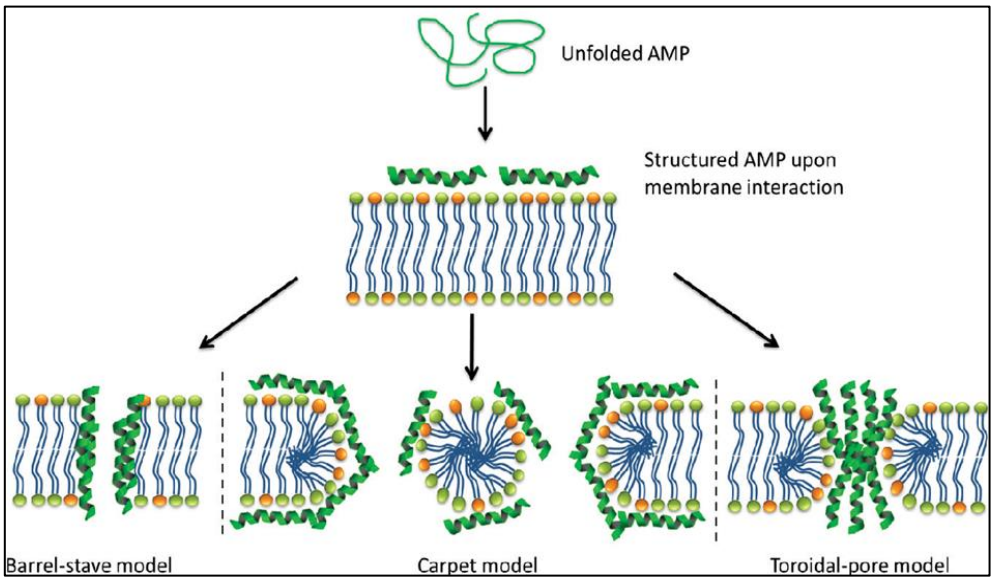

Fig 1: Models commonly used to describe the mechanism of killing bacteria by AMPs. Several mechanistic models (barrel-stave model, carpet-like model, toroidal pores model) have been proposed

Antimicrobial peptides isolated from fish

By forming aggregates on the cell's surface at the proper position, AMPs may modify membranes to form transmembrane pores that induce the release of intracellular material. As a consequence of peptide-induced permeation, these peptides bind with each other until they achieve a sufficient concentration to disrupt the cell membrane and lyse the cell. On the other side, other AMPs enter the macropinosomes-contained microbial cell and are eventually released into the host cytoplasm, ready to develop their antimicrobial function (Shabir *et al.*, 2018) [55].

Table 1: Classification and function of most known fish AMPs

AMP name	Fish species	Function
Piscidin-1	*Gadus morhua* (Atlantic cod)	Antibacterial
Piscidin-2	*Gadus morhua* (Atlantic cod)	Antibacterial
Piscidin-1	*Morone saxatilis* (Striped bass)	Antibacterial
Piscidin-2	*Morone saxatilis* (Striped bass)	Antiparasitic, Antibacterial, Antifungal
Piscidin-1, -2, -4, -5)	*Morone saxatilis* x *M chrysops* (Hybrid Striped bass)	Antibacterial
Dicentracin	*Dicentrarchus labrax* (European sea bass)	Unknown
Moronecidin	*Morone chrysops* (White bass)	Antibacterial, Antifungal
GAD-1, -2	*Gadus morhua* (Atlantic cod)	Antiviral, Antibacterial
Epinecidin-1	*Epinephelus coioides* (Orange spotted-grouper)	Antibacterial, Antiviral, Immune modulator
Pleurocidin	*Pseudopleuronectes americanus* (Winter flounder)	Antibacterial
Chrysophsin-1, -2, -3	*Chrysophrys major* (Red seabream)	Antibacterial, Hemolytic
asCath-1 asCath-2	*Salmo salar* (Atlantic salmon)	Antibacterial Immune modulator
CATH1-SALTR	*Salmo trutta fario* (Brown trout)	Unknown
CATH1-SASLFO CATH2-SASLFO	*Salvelinus fontinalis* (Brook trout)	Unknown
aCath	*Plecoglossus altivelis* (Ayu)	Antibacterial
Grammistins Pp1, Pp2a, Pp2b, Pp3, Pp4a, Pp4b	*Pogonoperca punctata* (Soapfish)	Antibacterial Hemolytic Ichthyotoxic
Grammistins GsA, GsB, GsC, GsD, GsD	*Grammistes sexllineatus* (Golden-striped grouper)	Unknown
Astacidin-2	*Pacifastacus leniusculus* (crayfish)	Antibacterial
HLP1	*Oncorhynchus mykiss* (Rainbow trout)	Antibacterial
H1	*Morone saxatilis x M. chrysops* (Hybrid striped bass)	Antibacterial
Parasin 1	*Parasilurus asotus* (Catfish)	Antibacterial Antifungal
Hbβ P1, P2, P3	*Ictalurus punctatus* (Channel catfish)	Antiparasitic Antibacterial
Astacin-1	*Pacifastacus leniusculus* (Crayfish)	Antibacterial
omBD-1	*Oncorhynchus mykiss* (Rainbow trout)	Antibacterial Antiviral
zfBD1, 2, 3	*Danio rerio* (Zebrafish)	Unknown
Defensin	*Epinephelus coioides* (Orange-spotted grouper)	Antibacterial Antiviral
Hepcidin	*Sparus aurata* (Gilthead seabream)	Antibacterial
Hepcidin	*Morone saxatilis x M. chrysops* (Hybrid striped bass)	Antibacterial
Sal -1, Sal - 2	*Salmo salar* (Atlantic salmon)	Unknown
LEAP - 2	*Ictalurus furcatus* (Blue catfish)	Unknown

AMP name	Fish species	Function
apoA - I apoA -II	*Cyprinus carpio* (Common carp)	Antibacterial
NK -lysin	*Ictalurum punctatus* (Channel catfish)	Unknwon
JF -NK - 2	*Paralichthys olivaceus* (Japanese flounder)	Antibacterial
NK -lysin	*Danio rerio* (Zebrafish)	Unknown
MAPP	*Misgurnus anguillicaudatus* (Loach)	Antibacterial
Myxinidin	*Myxine glutinous* (Hagfish)	Antibacterial
Misgurin	*Misgurnus anguillicaudatus* (Loach)	Antibacterial Antifungal
From pepsin hydrolysate	*Setipinna taty* (Scaly hairfin anchovy)	Antibacterial

Piscidins

A 25-residue peptide isolated and characterized from winter flounder skin mucous secretions, *Pleuronectes americanus*, named pleurocidin, was the first member of the family (Cole *et al*., 1997) [13]. Piscidins against several microorganisms exhibits potent antimicrobial activity. They are widely active against Gram-positive and gram-negative bacteria species, with the highest antibacterial values obtained against *Streptococcus* species, *Pseudomonas*, *Bacillus* and *Vibrio*. Chrysophsin-3 was observed to kill the three stages of *Bacillus anthracis* (sporulated, germinated and vegetative), penetrate and kill the spores without full germination (Pinzon-Arango *et al.*, 2013) [48]. Piscidins have also shown to possess anti-fungal activity (Niu *et al.*, 2013) [42], anti-parasitic activity (Pan *et al.*, 2009) [46], and anti-viral activity (Chinchar *et al.*, 2004). Browne *et al.*, (2011) [11, 6] proposed that Piscidins are primarily present in the gill, skin and intestinal region, and also can be found in the head kidney and spleen. Buonocore *et al.*, (2012) [7] stated that mast cells, rodlet cells, phagocytic granulocytes and eosinophilic granular cells are clearly among the cell types where piscidin are expressed.

Furthermore, pleurocidin (or piscidin) expression is found in winter flounder at 13 days post-hatch, which is suggested to play an essential role in protection during development (Douglas *et al.*, 2001) [17]. In addition to microorganisms, piscidin-mediated antitumor activity has been shown by the growth inhibition and killing of many different cancer-derived cell lines such as A549 (adenocarcinomic human alveolar basal epithelial cells) (Lin *et al.*, 2009) [33]. Other appealing characteristics of piscidin include its capacity to maintain antibacterial activity at high salt concentrations (Lauth *et al*., 2002) [32], thermostability, as observed in seahorse brooding pouch's piscidin maintained maximum activity after exposure for 30 minutes from 20-80 °C, and 20 per cent loss of activity observed while boiling for 30 minutes at 100 °C (Sun *et al.*, 2012) [58]. Expression of pro-inflammatory and other immune-related genes can be modulated in fish, such as IL-1β, IL-10, IL-22, IL-26 (Interleukin), TNF-α (Tumour Necrosis Factor alpha), IFN-γ(Interferon gamma), NF-κB (Nuclear factor kappa B), lysozyme, NOS2 (nitric oxide synthase 2), MyD88 (Myeloid differentiation primary response 88), TLR4a (toll like receptor 4), TLR1(toll like receptor 1), TLR3 (toll like receptor 3) (Lin *et al*., 2009) [33].

Defensins

A general term for cysteine-rich, cationic antimicrobial peptides found in plants, fungi, invertebrates and vertebrates, defensins exhibit a general conformation made by cysteine-stabilized α-helical and β–sheet folds. Fish defensins were first identified through a database mining technique in zebrafish, Fugu, and tetraodon (Zou *et al.*, 2007) [70]. Three exons and two introns are found in fish Casadei *et al.* (2009) [9] encoding a prepropeptide (including signal peptide, propeptide and mature peptide) consisting of 60 to 77 amino acids and a mature peptide of 38 to 45 cationic amino acids with a pI of approximately 8 (except for olive flounder peptides of approximately 4, indicating an anionic nature) (Nam *et al.* 2010) [40]. Fish β-defensins, albeit with very moderate activity, are active against Gram-negative, *Aeromonas hydrophila* (Zhao *et al*., 2009) [69] and Gram-positive bacteria, *Planococcus citreus* (Ruangsri *et al.*, 2013) [53] with limited MIC (minimum inhibitory concentration) values, are exceptions to these records of MICs in the large μM scale. Also, β-defensins are involved in the treatment of fish-related viruses such as Singapore grouper iridovirus (SGIV), viral nervous necrosis virus (VNNV), Haemorrhagic septicaemia virus (VHSV) and Rana grylio virus (RGV) specific to frogs (Guo *et al.*, 2012) [21]. β-defensins have been shown to show numerous immunomodulatory functions in addition to their antimicrobial activities (Semple *et al.*, 2012). Cuesta *et al.*, (2008) [54, 14] observed that a chemotactic behaviour was demonstrated by β-defensins from the gilthead seabream, indicating the ability to recruit head-kidney leukocytes. There are another evidence in zebrafish (Liu *et al*., 2009) [35] and rainbow trout (Dixon *et al.*, 2013) [16] of CCR6 (chemokine receptor 6) mammalian orthologs that can help resolve the process. Several factors, including cell wall components such as LPS (Lipopolysaccharide) (Guo *et al*., 2012) [21], β-glucans (Van der Marel *et al.*, 2012) [37] and peptidoglycan stimulate fish β-defensin genes (Casadei *et al*., 2013) [8].

Hepcidins

Hepcidins are antimicrobial peptides rich in cysteine and first found in humans (Krause *et al.*, 2000). Shike *et al.* 2002 [29, 32] first described and isolated fish hepcidin from the hybrid striped bass and since then hepcidins have been identified in at least 37 fish species. A β-sheet-composed hairpin-shaped with four disulfide bridges (formed by eight cysteines) along with an odd vicinal bridge at the hairpin turn is the general structure of Human Hepcidin (Hunter *et al.*, 2002) [27], which is also the general structure of fish hepcidin (Huang *et al*., 2007 [24], Nam *et al.*, 2011 [41] and Xu *et al.*, 2012 [58]). Two forms of Hepcidin are found in fish, HAMP1 and HAMP2. However, while HAMP1 is present in actinopterygian and non-actinopterygian fish, only actinopterygian fish have been shown to have HAMP2 (Masso-Silva *et al.*, 2011) [38]. Like other AMP genes, exposure to both Gram-positive and Gram-negative bacteria can trigger fish hepcidins (Solstad *et al*., 2008) [57], viruses can also induce hepcidin genes in fish (Yang *et al.*, 2013) [65], and poly I:C (polycytidylic acid) (Solstad *et al.*, 2008) [57], as well as mitogens (Cuesta *et al.*, 2008) [14].

The capacity to affect the viability of cancer cells has also been demonstrated by fish hepcidin. Tilapia hepcidin TH2-3, for example, has demonstrated a concentration-dependent inhibition of proliferation and migration of the human

fibrosarcoma cell line HT1080a. In addition, in HT1080 TH2-3 was able to induce cell membrane destruction and findings also show that TH2-3 down-regulates c-Jun contributing to apoptosis (Chen *et al.* 2009) [10]. However, beyond the possible antimicrobial and immunomodulatory consequences, Hepcidin is best recognized as a crucial ferroportin-controlled iron regulator capable of degrading its internalization, which decreases the transfer of iron into the blood (Ganz and Nemeth 2011) [19].

Cathelicidins

The first cathelicidins found in fish were initially isolated from the Atlantic hagfish, *Myxine glutinosa*, as antimicrobial peptides (Uzzell *et al.*, 2003) [59]. Fish cathelicidins can be subdivided into two classes; the linear peptides, and the characteristic disulphide bond. Considerable sequence homology is seen among members of the groups (up to 90%) and minute homology between the classes compared to mammalian cathelicidins. Moreover, a third-class (focused on sequence homology between themselves and a lack of homology with any of the other two classes) tends to be the newly described cathelicidins found in cod (Maier *et al.*, 2008) [36]. It also shows intense anti-fungal activity against *C. albicans* (Broekman *et al.*, 2011) [5].

On the other hand, Hagfish cathelicidins are active against Gram-positive and Gram-negative bacteria, but inactive against *Candida* sp. (Uzzell *et al.*, 2003) [59]. More precisely, rainbow trout cathelicidins are active against *Y. ruckeri*, while Atlantic salmon cathelicidins are not (Bridle *et al.*, 2011) [4]. It is studied that, in mammals, cathelicidins showed multiple activities, both as immune and non-immune, as well as in excess of their *in vitro* antimicrobial activities (KY Choi *et al.*, 2011) [12]. Although, in fish, research has not approached this level, a recent study demonstrated that two Atlantic salmon cathelicidins induced the rapid and transient expression of IL-8 in peripheral blood leukocytes. This suggests that the immunomodulatory activities seen by mammalian cathelicidins may be shared by their fish counterparts, and may thus be an evolutionarily conserved mechanism of innate immune regulation.

Histones derived peptides

In a variety of fish species, histone-derived AMPs have been identified with broad-spectrum action against both human and fish pathogens (Noga *et al.*, 2011) [44], including water moulds (Robinette *et al.*, 1998) [51] and a parasitic dinoflagellate (Noga *et al.*, 2001) [43]. In fish skin, they are expressed and secreted and found in other tissues, including the gill, spleen, and intestines. Robinette and Noga (2001) [43] further showed that they play a part in host fish defence from experiments demonstrating that histone-derived AMP gene expression is triggered in various fish species' particular tissues under stress conditions.

Therapeutics

All AMPs exhibit a common characteristics of development as therapeutic antimicrobials which include wide range of activity against variety of pathogens; potent activity under a wide range of conditions, including temperature and in secretions such as saliva; and a reduced capacity to the development of resistance by bacteria. The identification and characterization of peptides from fish has provided a unique contribution.

Since many AMPs are sensitive to high salt concentrations, while some of the fish AMPs have the ability to kill microbes even at extremely high salt concentrations, such as those found in the marine environment.

The AMPs from fishes are active against the pathogens like nervous necrosis virus. Also AMPs have dual functions for example hepcidins are involved in iron regulation as well Piscidins are present in both phagocytic granulocytes as well as in mast cells. For instance, piscidin 2 concentrations in various tissues of hybrid striped bass are lethal to different ectoparasites and the concentrations of piscidin 4 in gills are fatal to various bacteria.

Conclusion

Antimicrobial peptides are found as host defense molecules in all animals from primitive prokaryotes to most evolved eukaryotes. As examined fishes are challenged many times by a variety of pathogens which not only effect their health but also increase the risk of becoming resistant to conventional antibiotics which severely affects the aquaculture industry. Therefore, AMPs can be considered as the potential proteins for developing therapeutic agents in the field of aquaculture. AMPs are examined to have diverse biologic effects like endotoxin neutralization, immune modulating activity and induction of angiogenesis and therefore, they are considered as very vital and attractive therapeutic tools. For the future research study should be done on different human health benefits and therapeutic effects of AMPs which can be used for treating diseases like microbial as well as cancerous. The synthetic drugs are either least effective or completely ineffective and have lesser safety margin, these endogenous peptides will prove to be potent and effective, thus the health benefits of AMPs need to be evaluated.

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