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Fish antimicrobial peptides: at a glance

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ABSTRACT

Generally, antimicrobial peptides (AMPs) are considered as an important part of innate immunity, due to which they provide the first line of defence against various pathogens. Additionally, they also kill pathogens that show resistance towards many antibiotics. Fishes are regularly challenged by various pathogens which not only affect their health but the risk of becoming resistant to conventional antibiotics is also increasing. As fishes shows more dependence on innate immunity, AMPs can aid as important defensive weapon in fishes. In general, AMPs exhibit various multidimensional characteristics such as neutralization of pathogens (viral, fungal & bacterial), rapidly diffuse to the infection site, and other immune cells recruitment to the infected tissues. AMPs also show various biological effects such as immunomodulation, neutralization of endotoxin and angiogenesis induction. There are numerous AMPs that have been isolated from fishes but not fully characterized at molecular level. In this review we basically focus on approaches used to design new AMP, machine learning approach, current objectives of AMPs and future prospects.

Introduction

Various organisms develop antimicrobial peptide (AMP) as an important component of their innate immune response. Due to dependence of fishes on their innate immune system, antimicrobial peptides are considered major component as it forms the first line of defence (Hancock, 1997; Hancock & Scott, 2000). The AMP as part of innate immunity gives the advantage that they can function even without memory or high specificity. AMP helps in defencing the host by employing cytotoxicity on the attacking pathogenic microorganisms. In higher organisms they act as immune modulators (Zanetti, 2004). There are numerous pathogens in aquatic environment. Adaptive immune system is poorly developed in fishes either due to restricted classes of immunoglobulin or their functional diversion (Magnadottir, 2010). Antimicrobial peptide is also regarded as host defence peptides that constitutes innate immune system. By pore-forming "ionophoric" or disruptive "lytic" actions, it gives protection against viral, bacterial, fungal and other pathogenic infections (Smith *et al.,* 2010; Ageitos *et al.,* 2017). The site of secretion of antimicrobial peptides are mucus, saliva, circulatory system and those areas which are at high-risk pathogen targets (Noga *et al.,* 2010). Fish are considered as "gold mines" of AMPs whose immunomodulatory and antimicrobial activities have been extensively studied (Valero *et al.,* 2013; Shabir *et al.,* 2018). For the healthy growth of fishes, it is very important to

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give them suitable conditions especially under stressful environment in aquaculture. In stress conditions immunological conditions of fish reduces making it vulnerable to pathogens. The immune capacity of fish can be enhanced by many ways. First way is to examine the immune suppression of fish and take suitable measures when required. Another method to examine elevation in the expression of antimicrobial peptide earlier to stress incident leads to immune suppression of fish (Noga *et al.,* 2011b). PGLa, magainins and maganin 2 are the most studied AMPs that were structurally linear peptide and were extracted from the skin of an African frog (*Xenopus laevis*). Numerous functions showed by them are healing of wound, immunomodulation (Chakchouk *et al.,* 2014), function as chemokines and cause their production, lipopolysaccharide inhibition and initiate response of acquired immune system by recruiting antigen presenting cells (Niggemann *et al.,* 2014). There are AMPs which act against viral infections such as cecropin and mellitin blocks the production of cell associated HIV-1 by suppressing the expression of HIV-1 gene (Weisshoff *et al.,* 2014). There are 8164 entries of peptides in database (CAMP^{R3}) Collection of Anti-Microbial Peptides, mostly entries (74%) are from animals (Figure 1) (Waghu *et al.,* 2016). A database (DADP) Database of Anuran defence Peptides contributes the sequences of at least two thousand peptides (Novkovic *et al.,* 2012).

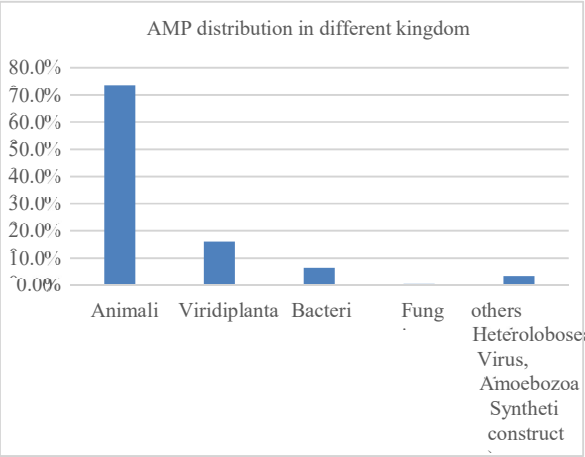

Figure 1: AMP distribution in different kingdoms on the basis of sequences in CAMP^{R3} database. Source: (Rončević *et al.,* 2019).

Features on which the activity of AMPs depends

The major physiochemical properties on which the structure-function relationship of AMP depends are: hydrophobicity (Bahar & Ren 2013; Rončević *et al.,* 2017), charge (Walkenhorst *et al.,* 2013; López *et al.,* 2018), size (Hou *et al.,* 2011; Bahar & Ren 2013), amphipathicity (Edwards *et al.,* 2016; Mahlapuu *et al.,* 2016), solubility (Chen *et al.,* 2005), helicity (Huang *et al.,* 2010), sequence, and secondary structure (Tossi *et al.,* 2000). Although some amino acid that are highly conserved at their position between many antimicrobial properties. The AMPs activity is dependent on combination of various properties. Some properties of AMPs depend upon its interaction with the lipid bilayer membrane of target cells. For the better understanding between structure-function relationship, hence, need to recognize properties that are responsible for specificity and activity of AMPs. All these properties require to observed together since to get desired modification if one parameter change may change the other parameter.

Structural properties of AMPs

On the basis of secondary structure most AMPs can be characterized as following: alpha-helix, beta-sheet, loop and extended. Among them alpha-helix and beta-sheet are most usual (Powers & Hancock 2003). Till date the most studied AMPs are alpha-helical. Structurally in alpha-helix the two adjoining amino acids are at a distance of 0.15nm and the angle between them is 100 degrees. The residues that are not present in the alpha-helical AMP sequence are cysteine (Lewies *et al.,* 2015). (Brogden 2005). Examples- melittin, dermaseptin, and cercopins. In the formation of beta-strand the two beta-strand are linked with disulphide bond. (Bahar & Ren 2013; Pasupuleti *et al.,* 2012) It is cysteine residue that helps in the formation of disulphide bond and provide stability to the structure (Brogden 2005). (Lewies *et al.,* 2015 Due to disulphide the peptide acquires cyclic configuration and which is important for antimicrobial activity (Matsuzaki *et al.,* 1999). Examples- protegrin, defensins and droscomycin. Linear extended antimicrobial peptides are linear in shape without secondary structure (Seo *et al.,* 2012). There are some amino acids which are over expressed in them. The peptides are rich in arginine, proline, or histidine. Examples- apidaecin and indolicidin (Seo *et al.,* 2012). In loop antimicrobial

peptide single disulphide bond is sufficient to acquire loop confirmation structure (Seo *et al.,* 2012). Structural modification and immobilization on the surface can be done easily in AMPs as they are made up of amino acids (Costa *et al.,* 2011). Synthetic peptides can be prepared with the help of recombination expression system (Ramos *et al.,* 2013) or by chemical synthesis (Wade *et al.,* 2012).

Synthesis of AMPs

Generally, AMPs are ribosomally synthesized and encoded by gene (in case of eukaryotes) or may accumulated by versatile enzyme named non-ribosomal peptide synthetase (NRPS) (Papagianni, 2003). Fungi and bacteria used the latter process **(**Finking & Marahiel, 2004**)** and integration of non-proteinogenic amino acid are permitted into the peptides and peptides are modified additionally with ring formation, hydroxylation, acylation and glycosylation (Walsh *et al.,* 2013; Hancock & Sahl, 2006). The known amino acids that are non-proteinogenic are ~ 500 at least, having added functional and structural properties that may help the activity of peptide significantly. Actually, in this manner the antibiotics of cyclic peptide vancomycin, gramicidin S and polymyxin B are prepared (Hancock & Sahl, 2006) and in their sequences all contain some non-proteinogenic amino acids (Walsh *et al.,* 2013). Mostly all forms of life, including bacteria produces peptides that are synthesized ribosomally, encoded by gene (Mahlapuu *et al.,* 2016; Waghu *et al.,* 2016). Often, the genes of various antimicrobial peptides are aggregated at an individual locus of chromosome, like in alpha and beta-defensins (Lai & Gallo, 2009) and may co-expressed. Moreover, they are generally expressed as an inactive precursor, having a region of signal peptide and pro-piece that function to inactive the mature peptide until it is transported to the infection site, where it is released proteolytically. That is the reason, propiece is generally anionic and mature peptide is cationic to complement each other. Mostly, N-terminal of AMP sequence is pro region, but in some instances C-terminal like for some plant and fish peptides (Patrzykat *et al.,* 2003). Thus, the potential of AMPs regulated by the level of expression as well as presence and abundance of suitable proteases at the right time to right place for peptide cleavage, majorly at dibasic cleavage sites (Lai & Gallo, 2009). The most common property of eukaryotic and prokaryotic proteins is signal peptide and require to enter secretory pathways (Von 1990). An important feature of AMPs is for a given class signal regions may be highly conserved than mature peptide (Patrzykat *et al.,* 2003). Majority of AMPs that are encoded by gene undergoes post-translation modifications, recently classified into more than 15 types like capping of C-terminal and N-terminal (amidation, pyroglutamic acid formation, acetylation), formation of disulphide bridge, glycosylation, phosphorylation, hydroxylation, halogenation etc.

Mode of action

The outer surface of eukaryotic cell is made up of zwitter ionic phospholipids namely sphingomyelin and phosphatidylcholine while the prokaryotic cell surface is made up of negatively charged teichoic acid or lipopolysaccharides (Dolis *et al.,* 1997). The primary mechanism for antimicrobial activity appears to be the electrostatic interaction of peptides with negatively charged molecules on the membrane. In some cases, the mode of action of AMPs in target cell by cell membrane translocation and inhibition of crucial cellular processes like cell wall synthesis, nucleic acid synthesis, protein synthesis, and enzymatic activities. The other factors were also important for the transportation of peptide through membrane like fluidity of the outer membrane, molecular architecture, negative charge concentration, charge and magnitude of the outside membrane (Kondejewski *et al.,* 1999). The membrane adsorption and insertion of antimicrobial peptides was regulated by membrane fluidity. On the basis of action mechanism AMPs mainly classified into membrane acting and non-membrane acting peptides. MPP (membrane permeabilizing peptides) are generally indicated by cationic peptides that are able to form transient pore on the membrane. The non-membrane permeabilizing peptides having potential to cross through the membrane without membrane permeabilization. There are some antibacterial peptides that form transmembrane pores on the cell membrane of target cells such as LL-37 (Henzler *et al.,* 2003), magainins (Hallock *et al.,* 2003), melittin (Yang *et al.,* 2001), and defensin. Some antimicrobial peptides are able to translocate through cell membrane and by inhibiting crucial cellular processes leads the cell to death such as, mersacidin (Brötz *et al.,* 1997), pyrrhocidin (Kragol

et al., 2001), indolicidin (Friedrich *et al.,* 2001), pleurocidin (Patrzykat *et al.,* 2002), dermaseptin (Patrzykat *et al.,* 2002), and buforin II (Park *et al.,* 2000). There are some antifungal peptides that shows their antimicrobial action through the production of reactive oxygen species such as, lactoferrin (Patrzykat *et al.,* 2002), histatin (Kavanagh & Dowd 2004), melittin (Park & Lee 2010), and papiliocin (Hwang *et al.,* 2011). AMPs encourage membrane damage by disruption of lipid bilayer, by formation of pores or by membrane thinning in target cells (Lohner & Prenner 1999). The mode of action of antimicrobial peptides was described through several models. The mechanism of cellular uptake of AMPs are classified into energy independent and energy dependent uptake mechanisms. The mechanisms of energy independent uptake involve carpet model, toroidal model, or barrel-stave model and mechanisms of energy dependent uptake involves micropinocytosis (Figure 2).

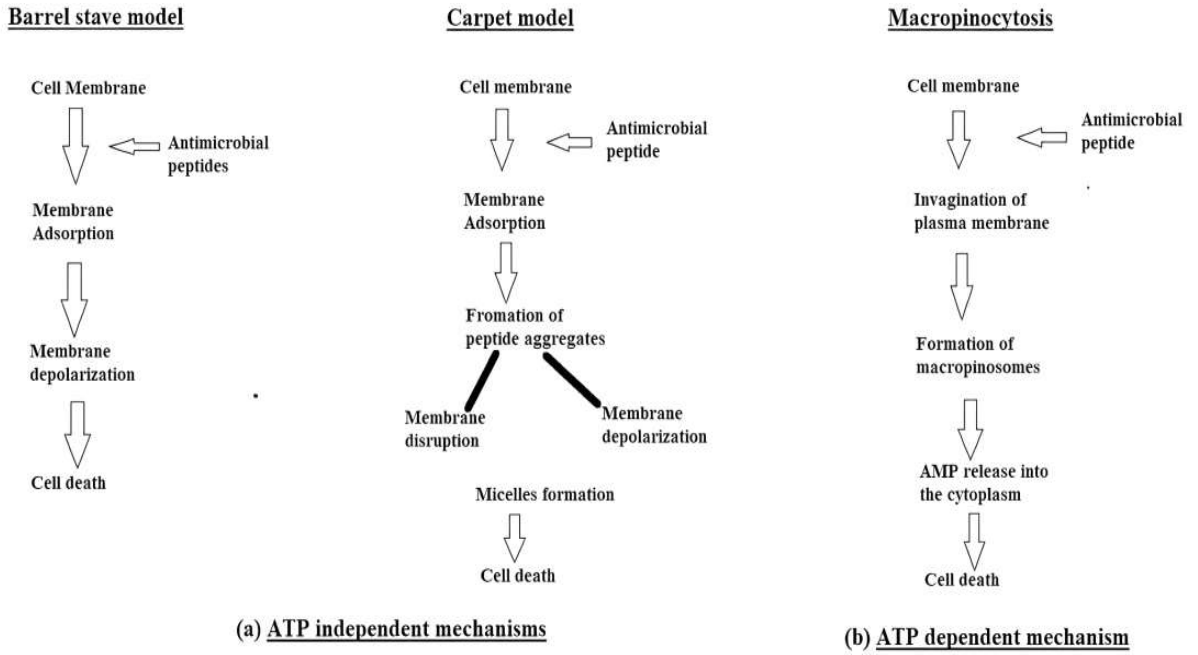

Figure 2: AMPs mode of action. (a) The mechanism that are independent of energy. (b) The mechanism that are dependent of energy (Source: Pushpanathan *et al.,* 2013).

Barrel-Stave model

The peptide monomer of AMPs gets accumulated on the surface of membrane in a perpendicular direction and acquires the structure of barrel-stave followed by membrane insertion (Yang *et al.,* 2001). Alpha-helical and beta-sheet peptides with hydrophobic surfaces are outward facing the barrel and interact with acyl chains of membrane, while hydrophobic surfaces are inward facing the barrel (Giuliani *et al.,* 2007) formed water filled pore in the transmembrane so that intracellular content released and resulting cell death. Examples, AMPs that follows this model are gramicidins and alamethicin (He *et al.,* 1996; Zhang *et al.,* 2001).

Carpet model

On the membrane surface the peptides get initially associated and establish a local carpet. After reaching threshold concentration, permeation of membrane was induced by the peptide that results in cell membrane destruction leads to microbial cells lysis (Oren & Shai 1998).

Toroidal pore model

The adsorption of AMPs takes place on the membrane in carpet form with perpendicular orientation they inserted into the membrane causing the membrane disruption (Matsuzaki *et al.,* 1997). Unlike two models, during insertion the peptides

remain bound constantly to lipopolysaccharides of the membranes of bacteria. The peptides that are aggregated either after or prior binding with the surface of membrane induced depolarization of membrane and forms transmembrane pores of toroidal shape with the formation of micelle that leads to death of cell (Sengupta *et al.,* 2008).

Macropinocytosis

Macropinocytosis is the energy independent intake way of antimicrobial peptides, in which the formation of vesicle, macropinosomes, takes place by the inward folding of the membrane of target cell with peptide. Therefore, vesicle containing antimicrobial peptides gets discharge in cytoplasm and employ its antimicrobial potency (Madani *et al.,* 2011).

Post-translational modifications of AMPs

Several AMPs are synthesizing directly in their active forms but in some AMPs posttranslational modification is must for their functions. Various post-translational modifications are: proteolytic cleavage (Shinnar *et al.,* 2003), formation of disulphide linkage (Mangoni *et al.,* 1996), glycosylation (Oman *et al.,* 2011), amidation (Rifflet *et al.,* 2012), methylation (Hancock & Chapple 1999), addition of D-amino acids (Kreil 1997; Kamatani *et al.,* 1991), and phosphorylation (Goumon *et al.,* 1996).

Expressional regulation of AMPs: In animal

There are plenty of microbial infections are faced by living organism on regular basis and hence for recognition of pathogen as well as to defend attack of pathogen have developed a complex immune response. The recognition mechanism of microbes occurs within animals that aid them to discriminate between attacking pathogens (Lemaitre *et al.,* 1997). The recognition of pathogen takes place with the interaction between pattern-recognition receptors present on of host cell surface and molecular structures present on the pathogen (Medzhitov *et al.,* 1997).In non-chordates and chordates, the recognition of pathogen was done by many proteins having c-type lectin domains (Vasta *et al.,* 1999). PGRP (Peptidoglycan recognition proteins) are also takes part in identification of pathogen and are mostly conserved from insects to mammals (Kang *et al.,* 1998). There are several pathways that have been identified and mediate the gene expression of AMP (Imler & Hoffmann 2000). The signalling pathways of antimicrobial defence is highly conserved among fishes, insects and mammals (Beutler, 2000) form *Drosophila* a perfect design for understanding innate immune responses of animal. NF-kB like transcription factors mediates the AMP gene induction in *Drosophila* that comprises of three Re1 proteins: Relish, Dorsal and DIF (dorsalrelated immunity factor) and includes two pathways namely imd (immune deficiency) and Toll pathway (Figure 3) (Levashina *et al.,* 1998), which are homologous to mammalian TNFR (Tumour necrosis factor receptor) and TLR (Toll-like receptor).

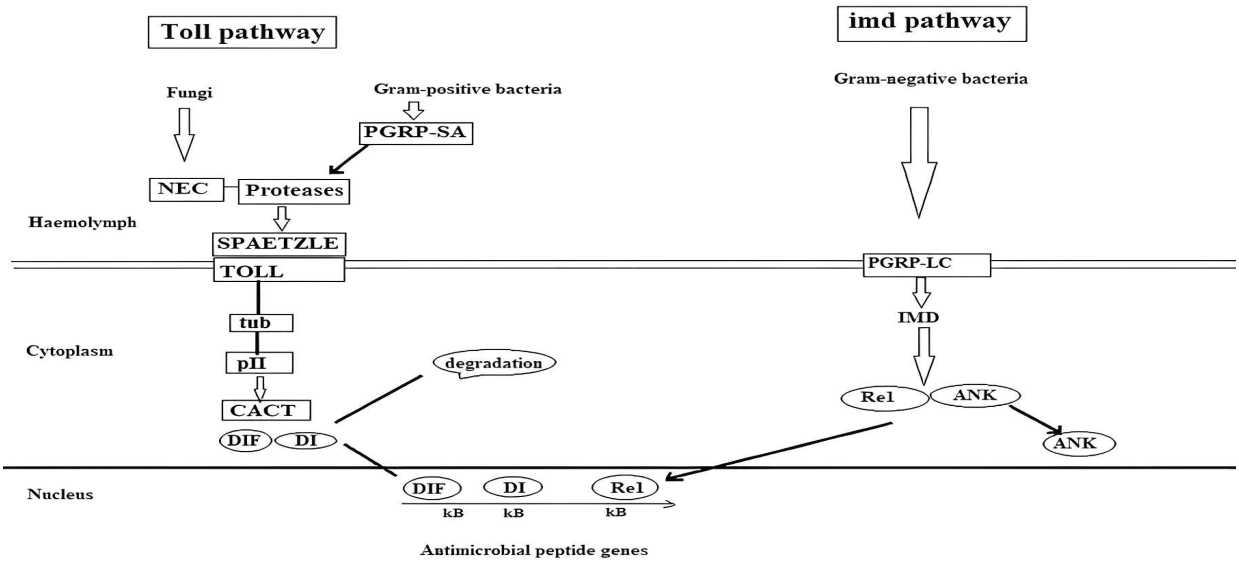

Figure 3: AMPs regulation in *Drosophila* by the pathway of Toll and imd (Source: Shabir *et al.*, 2018)

The dorsal and dorsal related immunity factor are controlled by Toll transmembrane receptor protein linked pathway (Rutschmann *et al.,* 2000), while the Relish is regulated by IMD (Immune deficiency gene). The gene expression of AMP is suppressed on mutations of these two pathways (Lemaitre *et al.,* 1996). The AMP encoding gene expression differs regarding distinct pathogens and is mostly depending on stimulation of Re1 and Toll pathways. Normally, IMD/Relish route is stimulated by the bacteria that are gram negative whereas Toll pathway is activated by the bacteria that are gram positive and fungi. For instance, the Drosomycin gene is induced by the bacteria that are gram positive and fungi which is IMD/Relish dependent signalling pathway whereas Drosomycin is regulated by Toll-DIF-Dorsal. On contrary, Diptericin is induced by the bacteria that are gram negative which depends upon Toll-DIF-Dorsal signalling pathway while Diptericin is regulated by IMD/Relish (Imler & Hoffmann 2000; Lemaitre *et al.,* 1996). The Toll pathway is very important for the survival after fungal infection, while the IMD pathway is necessary after the infection of gram-negative bacteria (Lemaitre *et al.,* 1995). NF-kB activity is also regulated by TNF signalling in mammals. In *Drosophila* and mammals, MAPK (mitogen activated protein kinase) pathway have been involved in gene regulation of AMP (Han *et al.,* 1998).

In fishes

The bacterial antigen of a typical *Aeromonas salmonicida* induces the piscidine gene in Atlantic cod (*Gadus morhua*) (Browne *et al.,* 2011). In the same way, numerous PAMPs like peptidoglycan and LPS induces the expression of beta-defensin genes in fishes (Casadei *et al.,* 2013). The cell walls of bacteria like peptidoglycan & LPS and poly I:C the viral synthetic analog might be manage as immunostimulant in fishes to activate pattern recognition pathway causing AMP expression and finally given immunity against pathogens in fish. Under stress conditions DAMPs (Damage-associated molecular patterns) releases that induces histone derived AMPs (Terova *et al.,* 2011). Imbalance iron levels or anaemia in biological system induces hepcidin in fish and in response to transferrin its expression changes (Chen *et al.,* 2008; Fraenkel *et al.,* 2009). Additionally, AMPs promotor region having binding site for many sequence-specific DNA-binding factor that establish their control by certain stimulation pathways and aiding their essential role in immunity and other biological functions (Shewring *et al.,* 2011; Katzenback 2015; Chaturvedi *et al.,* 2018)**.**

Approach used to design new AMP Extraction and assay –guided isolation

Earlier, novel AMPs recognition required to operate many samples from similar species to acquire little amount of functional peptides. Homogenization of primary tissue was succeeded by the removal of peptide and many steps was involved to isolate crude peptide, especially by chromatographic techniques. There are cases, where AMP production stimulation was done with animals treated initially by noradrenaline or bacterial infection or electric shock (Giuliani *et al.,* 2010). The antimicrobial peptides were segregated by assay-guided fractionation and by the help of various techniques like mass spectrometry & Edman degradation sequence was determined. Magainin was isolated in this manner (Destoumieux *et al.,* 1997). Although, the approach is successful but time consuming and produces low yield (Figure 4).

Sequencing approach

The fast progress with reducing amount of sequencing techniques (high throughput sequencing), associated with effective and comparatively economic solid phase synthesis techniques, has unlock the hidden sequence data in genome and their functional testing, without the need of polypeptide isolation. For instances, the peptides of frog have been recognized by extracting the complete RNA and reverse transcription of mRNA on the basis of 3' poly-A tail. With the help of suitable vectors cDNA library was constructed and selection of positive clones and finally analysed with the help of nucleotide sequencing (Figure 4). QSAR approaches can involve the studies of virtual screening where construction of molecular descriptors of known active peptides is used based on their biophysical properties that are combined with various functional aspects. With the help of these descriptors the biological activity of a novel sequence is linked (Wang *et al.,* 2012). The important inference is that a mathematical function can be made that precisely relates physio-chemical characteristics with an observable outcome.

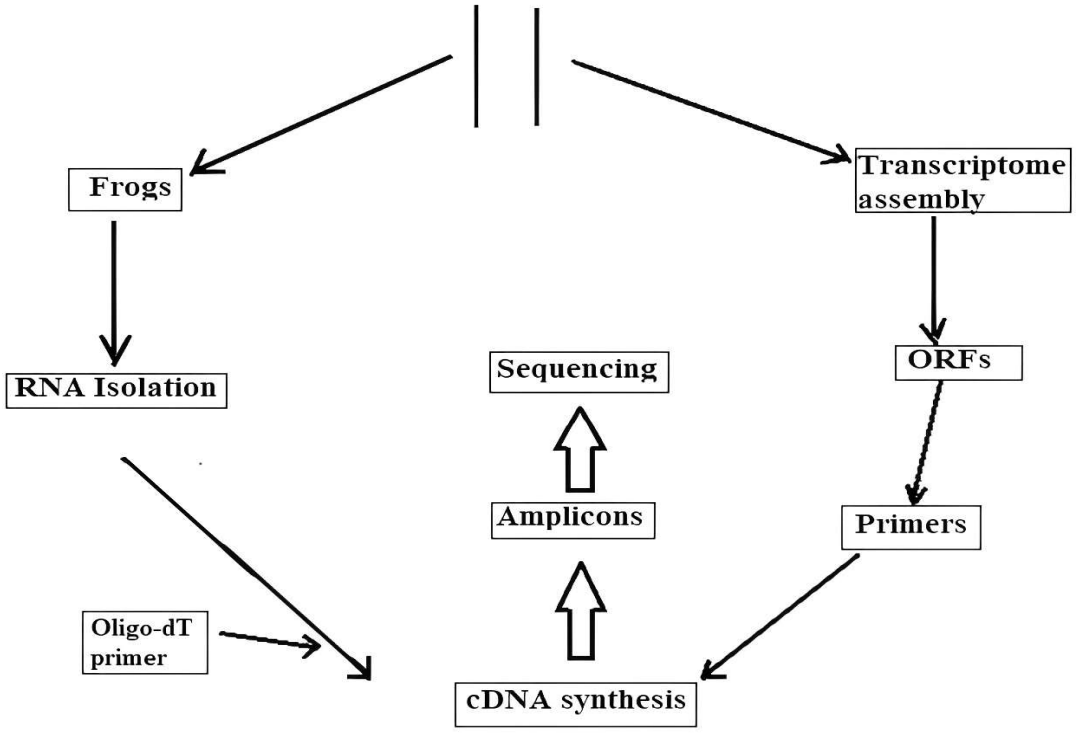

Figure 4: Diagram showing targeted DNA sequencing method (Source: Rončević *et al.*, 2019).

Quantitative Structure-Activity Relationship (QSAR) approach

After this statistical analysis is done to decide which combination of parameters or descriptor give an estimated functional value that relates to practically-determined values. Then QSAR design is validated on an external set of peptides (Figure 5) (Taboureau *et al.,* 2010; Veerasamy *et al.,* 2011). **Databases and tools of AMPs**

The steadily raising resistance of microbes against drugs force the researcher to develop antimicrobial agents. Past decade, numerous tools of AMP production and databases have been confirmed and are accessible online. There are databases that were developed to cover the sequences of AMP from many origins such as APD, ANTIMIC, AMSDb, YADAMP and CAMP. Some emphasize on particular families of AMP such as, EnzyBase (large lytic proteins), CyBase (cyclotides), Defensins (defensins), and THIOBASE (bacterial thiopeptides). There are some databases that assemble AMPs originated by shrimp (PenBase), fungi (peptaibols), bacteria (BACTIBASE and BAGEL), amphibians (DADP), and plants (PhytAMP). Swiss Prot database and AMPer are other tools of AMPs. Various computational techniques were established to hasten the process of classification and prediction of AMPs (Lin *et al.,* 2018). Quantitative structure-active relationship (QSAR) models were the earliest machine learning models that provide optimization and systematic screening of a peptide for experimental evaluation. These models operate on physico-chemical descriptors to find out the biological activity of a molecule and which is highly expensive and time consuming. Newly, machine learning approach is adopted because of their high speed, high efficiency and low cost. Following methods are involved for prediction power in a condition of supervised classification: hidden markov models (HMMs) (Fjell *et al.,* 2013), decision tree model (Lira *et al.,* 2013), neural network model (Veltri *et al.,* 2018), random forests (RFs) (Joseph *et al.,* 2012), nearest neighbor (Wang *et al.,* 2011) or k-nearest neighbour algorithm (Xiao *et al.,* 2013) and support vector machine (SVM) (Meher *et al.,* 2017).

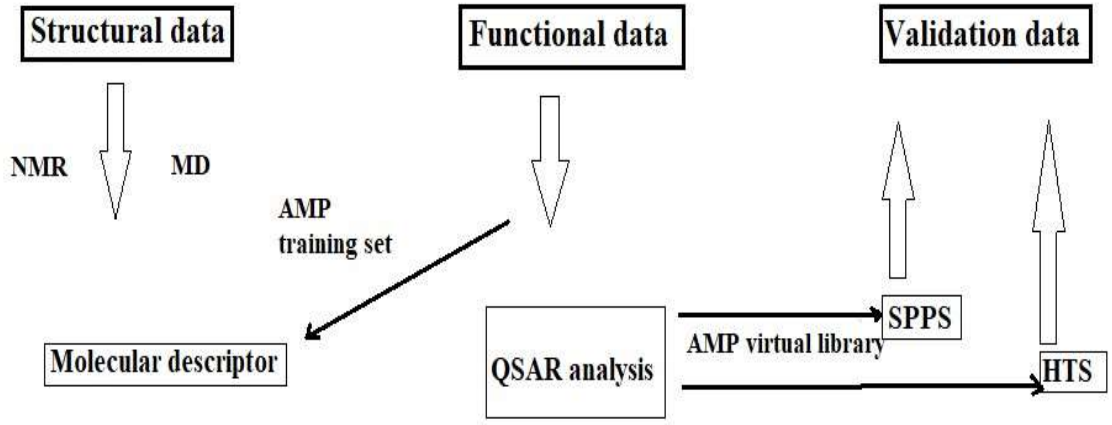

Figure 5: Outline of QSAR method (Source: Rončević *et al.*, 2019).

Machine Learning approach

Recently, "deep" network architecture for classification and chemical data analysis. To find out if the unknown sequence is AMP or not some predictors only use binary classifiers (Meher *et al.,* 2017; Wang *et al.,* 2011). For more detailed quantitative analysis, multiclass classifier is used. Four classes were identified to classify the antimicrobial activity of synthetic peptide with the help of decision tree model (Lira *et al.,* 2013). When we compare the sequences in database a common phenomenon observed was the occurrence of same sequence in different subclasses. Therefore, it is very crucial to establish a mechanism for quickly and precisely learning from multi-label datasets, to design novel and highly effective antimicrobial agents.

Current objectives of AMPs: Biofilms, Persister cells and drug resistant bacteria. As bacterial cells are directly target by the AMPs, they have potency to check antibiotic tolerant cells. Biofilms are the collective population of microorganisms that are immovable and able to grow on surfaces like medical implants and human tissues. Biofilms is responsible for causing almost 80 % bacterial infections in human (Harro *et al.,* 2010). Additionally, antibiotic resistance related to biofilm is also contributed stagnant biofilm cells (Mah & O'Toole 2001).However, there are some antibiotics that have been demonstrated to invade the matrix of biofilm (Dunne *et al.,* 1993), but their effectiveness is not shown against stagnant cells, mainly persister cells (Stewart & Costerton 2001).

Biofilm control

The electrostatic interaction between negatively charged matrix of biofilm and cationic peptides is the main difficulty of using AMPs against biofilms (Otto 2006). The other problem is the treatment of mature biofilms is highly challenging (Stewart & Costerton 2001). The coating of surface with AMPs has also preferred including free antimicrobial peptides as alteration of surface with antimicrobial peptides may help to lower the device related infections (Gao *et al.,* 2011). The biofilm matrix is supposed to create a diffusion barrier against some antimicrobial peptides (Lewis 2001). This barrier having negative charge and preserve the cells from antimicrobial agents that are positively charge and the diffusion of antimicrobial agents is reduced by the alginate in the matrix of biofilm (Shigeta *et al.,* 1997). Thus, AMPs must diffuse into biofilms and kill the cells of biofilms.

Persister control

Persister cells are dormant cells that found in any microbial populations and show tolerance to antibiotics (Lewis 2010). Though, for the survival of bacteria the integrity of membrane is must not the metabolic stages of cell and AMPs target mainly the cell membrane. Hence, they have great potency to kill persister cells.

Resistance to antimicrobial peptides

The mechanisms of resistance are basically of two types: inducible and constitutive resistance (Yeaman & Yount 2003). The constitutive resistance mechanisms include, formation of biofilm (Yeaman & Yount 2003), electrostatic shielding (Friedrich *et al.,* 1999), and alter the potential of membrane during various cell growth stages (Yeaman *et al.,* 1998). The inducible resistance mechanisms involve acylation (Guo *et al.,* 1998), substitution (Lewis *et al.,* 2009), modification (Gunn 2001) of molecules of the membrane, some proteolytic enzyme activation and efflux pumps and intracellular target alterations (Figure 6).

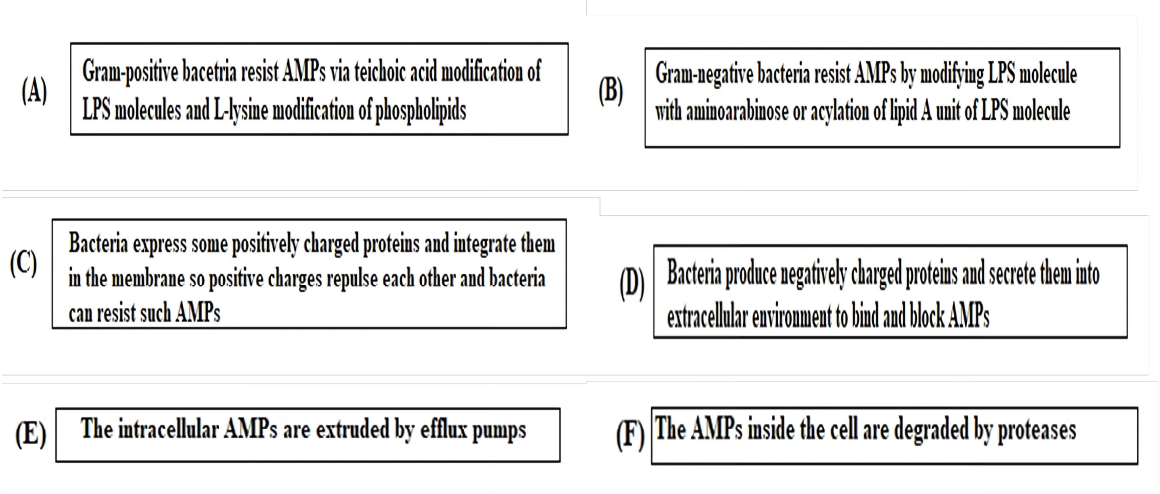

Figure 6: Diagram A to F showing the resistance mechanism of AMPs (Source: Bahar & Ren 2013).

AMPs categorization

Generally, in antimicrobial potency of AMPs enzymatic mechanisms are not involved (Phoenix *et al.,* 2013). For example, lysozyme is not considered as an antimicrobial peptide due of its large size (148 aa). It destroys the bacteria by enzymatic mechanism through dissociation of 1,4β-linkage in peptidoglycan chain (Kirby 2001).

Anticancer activity

There are certain amphiphilic alpha-helical antimicrobial peptides that reveal anti-cancerous characteristics as they have similar mode of action towards bacteria and cancer cell (Sang *et al.,* 2017). Their affinity to specific cell membrane linked glycoproteins describes the selectiveness towards cancerous cells. The AMP having cationic nature named Sapecin containing KLK motifs, interacting through negatively charged residues on the surface of host cell (Bednarska *et al.,* 2017). Fast and selectively cytotoxic activity (12µg/ml) was demonstrated by magainin-2 and their analogues against haematopoietic and rigid tumour cells (Deslouches & Di 2017). In contrary, against normal lymphocytes their cytotoxic activity was not understood even up to 200µg/ml.

Antiviral peptides

The neutralization of viruses by antiviral AMPs takes place with integration in the membrane of host cell or either in the viral envelop. It is shown in studies that antiviral AMPs target the enveloped RNA and DNA viruses (Horne *et al.,* 2005). AMPs causes instability of membrane by integrating into viral envelopes, so that viruses become unable to infect host cells (Sitaram & Nagaraj, 1999).

Antiviral antimicrobial peptides may inhibit the virus entry into the host cell capturing particular receptors on mammalian cells (Song *et al.,* 2001). For instance, for the attachment of Herpes Simplex virus (HSV) to the membrane of host cell heparin sulphate is very crucial (Wu & Spear 1989) and these are negatively charged molecules of glycosaminoglycan (Laquerre *et al.,* 1998). Lactoferrin is α-helical cationic peptide (Andersson *et al.,* 2004) may check the infection of HSV by linking to heparin molecules and checking virus-receptor associations (Jenssen *et al.,* 2004). There

are certain antiviral AMPs that confined in the cytoplasm and organelle by crossing the membrane of cell, leads to changes in the expression profile of gene in host cell, make it easier for host defence system to fight against viruses or check the gene expression of virus. The antiviral functions of beta-defensin (BD)-1 peptide was shown against VHSV (Viral hemorrhagic septicaemia virus) infection in rainbow trout (Falco *et al.,* 2008) and against NNV (Nervous necrosis virus) the antiviral functions are shown by epinecidin-1 from grouper and TH-5 from tilapia (Chia *et al.,* 2010).

Antiparasitic activity

The group of antiparasitic peptides is smaller in contrast with remaining groups of AMPs (Bahar & Ren, 2013). However, parasitic organism is multicellular, their mode of action includes killing of cells by compromising the integrity of cell membrane like other AMPs. *Trypanosoma brucei* and *leishmania* are the parasites against which antiparasitic peptides have been recognized (Jenssen *et al.,* 2006). The anti-parasitic functions in channel catfish was showed by a beta-hemoglobin peptide family AMP against *Ichthyophthirius multifiliis* (ich) that causes ichthyophthiriosis (Ullal & Noga 2010).

Antifungal activity

Despite differences in the cell wall of bacteria and fungus their mode of action in killing cells are same. Their mode of action of antifungal peptides (AFPs) involves disintegration of fungal cell causes lysis, osmotic stress, and finally cell death (Pushpanathan *et al.,* 2012). According to some research they may also intervene with intracellular machinery (Bahar & Ren 2013). Fungal cell wall is made up of chitin which binds selectively to antifungal peptides (Pushpanathan *et al.,* 2012). Striped bass secretes a peptide, piscidine-2, that act as fungicide by disrupting fungal membrane. Many AFPs that are derived from plant have been studied to acquire lectin like activity such as Ac-AFP1/2, Tu-AMP1/2, and Pp-AMP1/2. They alter the intracellular actin skeleton through binding fungal chitin. This disorganization of chitin has been found to compromise the morphology of fungal cell and integrity of membrane (Rautenbach *et al.,* 2016).

Antibacterial peptides

Mostly antibacterial AMPs are cationic AMPs that target the cell membrane of bacteria and causing the breakdown of lipid bilayer (Zhang *et al.,* 2001). As these AMPs have both hydrophobic and hydrophilic domains so they are amphipathic in nature. This property help AMPs to phospholipid group (hydrophilic region) as well as lipid component (hydrophobic region) (Jenssen *et al.,* 2006). In some research it was studied that certain AMPs kills the bacteria at low concentration without altering the integrity of membrane. These AMPs do not directly interact with membrane instead they block some pathways such as replication of DNA and protein synthesis to kill bacteria (Brogden 2005). For example, an AMP that dispersed into cells and attach to RNA & DNA without causing any damage to the membrane of cell is buforin II (Park *et al.,* 1998) other examples of such AMPs are apidaecin, pyrrhocoricin and drosocin. There is some example in which AMP have been demonstrate to kill the bacteria that are antibiotic resistant. For example, blocking the synthesis of cell wall can be done by an AMP (nisin) and an antibiotic (vancomycin). Although, a strain MRSA (Methicillin resistant *Staphylococcus aureus*) was found to be sensitive tom nisin, while resistant to vancomycin (Brumfitt *et al.,* 2002). There are two most common properties of AMPs are amphipathicity and cationicity (Bahar & Ren 2013; Mahlapuu *et al.,* 2016). There are numerous AMPs that are derived from food and have been found to act on the cell membrane of bacteria. For instance, the membrane of inner *Escherichia coli* changes rapidly by lactoferricin B have been showed by electron microscopy resulting its depolarization, disintegration of the transmembrane potential, outer membrane permeabilization (Théolier *et al.,* 2014). Majorly these peptides show salt sensitive antibacterial activity. For example, the activity of lactoferricin B is repressed if the addition of divalent cations (Mg^{2+}, Ca^{2+}, Fe^{2+}) in adequate amount takes place (Théolier *et al.,* 2013). Nearly all AMPs reveal antibacterial or bacterio-static functions against many strains of gram positive and gram-negative bacteria. The amino acids which are positively charged of these peptides binds to negatively charged molecule of the membrane of pathogens leads to the formation of pore causes degradation of membrane.

Multidisciplinary properties of AMPs

The various properties of antimicrobial peptides that are explained below (Figure 7).

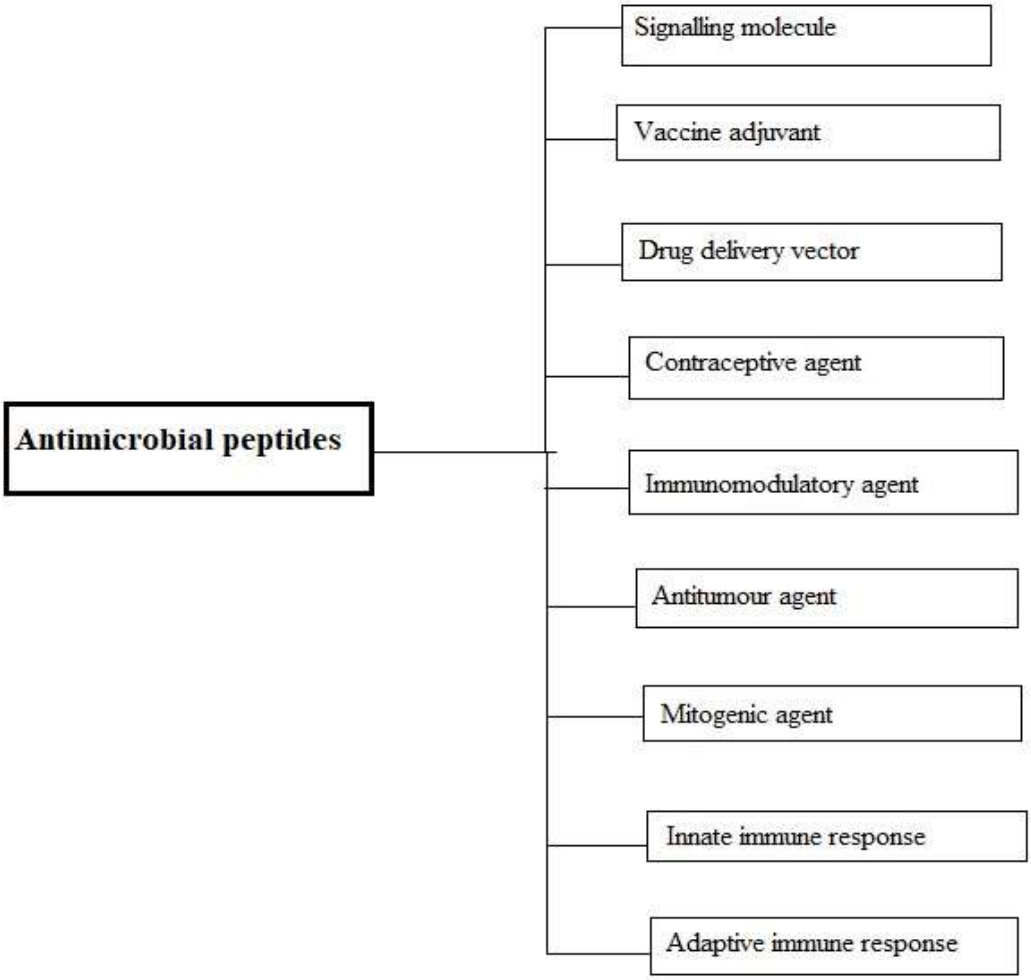

Figure 7: Layout of multidisciplinary properties of AMPs (Source: Pushpanathan *et al.*, 2013).

AMPs as signalling molecule

The immune system of many organisms produces short cationic AMPs known as Host Defence Peptide (HDP) that plays a crucial role in innate immunity (Steinstraesser *et al.,* 2011). As host defence many HDPs are involved in immune response modulation. They function as modulators of signal transduction pathways by affecting the activity of intracellular signalling targets like protein kinase. HDPs namely defensins are created by various cell types like small intestinal epithelial cells, lymphocytes, keratinocytes, neutrophils, cardiomyocytes, & tissue macrophages and are categorized into two groups such as alpha-defensins and beta-defensins. Defensins act as chemoattractant of immune cells, host cell receptor interaction, recruitment of neutrophils, activation of classical complement pathways, mobilization of immunocompetent T-cells as well as enhancer of cell adhesion (Hata *et al.,* 2008). The host defence AMP named LL-37 is synthesized by various cell types like mast cell, neutrophils, macrophages and monocytes that act as chemoattractant of mast cells and neutrophils, inhibit apoptosis of keratinocytes and neutrophils, promote induction of chemokine, angiogenesis, and stimulate differentiation of monocytes and proliferation of vascular endothelium. Moreover, it also shows anti endotoxin and anti-inflammatory effects (Steinstraesser *et al.,* 2011).

AMPs as vaccine adjuvants

Adjuvants helps to enhance the less-immunogenic potency of protein antigens or subunit vaccines (Kovacs *et al.,* 2009). There are some AMPs that function as inducers of proinflammatory cytokines such as IFN, TNF and COX-2. By encouraging differentiation of specific cell lineages, like dendritic cells, antigen-specific immunity is achieved, also cytokine expressions modulation changes the switch between Th1 and humoral Th2 polarization of adaptive immune responses (Kindrachuk & Napper 2010). Adjuvants utilized this property, and research focused with fish AMPs may promote the search toward alternative harmless vaccine adjuvants. According to some research, in the cell line of trout macrophage RTS11, upregulation of COX-2 and IL-1β with fish cecropin and pleurocidin analog peptide, CF17 (Chiou *et al.,* 2006). This explains that these compounds reveal the properties of adjuvants, and AMPs optimization with adjuvants will bring diversity in structure and choices while determining the adjuvants (Kindrachuk *et al.,* 2010).

AMPs in the development of inactivated vaccines

By inactivating pathogens, AMPs can be used as vaccines against particular pathogens. There are some problems like allergic reaction related with inactivated vaccines that are formalin based (Solomon 2008). Therefore, to reduce the aftereffects of formalin an alternative compound needs to be found to produce inactivated vaccines (Solomon 2008). As AMPs are developed from biological sources, they can be a bio alternative to formalin.

AMPs as drug delivery vector

Cell penetrating AMPs that are non-lytic were used as vector for drug delivery to manage and treat many diseases. There are some drugs that are large and hydrophilic in nature are unable to cross through the barriers of cell membrane. In such instances, the translocation property of AMPs helps them to enter into cells without causing any damage to the cell membranes were used as vectors for drug delivery (Henriques *et al.,* 2006). The most important property of AMPs to serve as delivery vector is that they must have the ability to cross the membrane of cell at very low concentration (micro molar) without any particular receptors and able to deliver the cargo such as drug into the interior of cell (J¨arver & Langel 2006). Antibacterial peptides such as pVEC, TP10, and LL-37 were involved in damage of bacterial membrane and function as CPP (cell penetrating peptides) without causing toxicity to the host cells of eukaryotes (Zhang *et al.,* 2010). There were analogues of AMP like buforin 2 and magainin, by membrane translocating mechanisms they enter into the carcinoma cells of human. The translocation of buforin2 analogue over the membrane takes place by passive mechanism which is less concentration dependent and with causing notable toxicity to carcinoma cells whereas for translocation of magainin 2 analogue the formation of transient pore is required as an intermediary step and leads to higher toxicity to carcinoma cells (Takeshima *et al.,* 2003).

AMPs as an active compounds of drug

Some study revealed that, for immunogenic drugs the fish AMPs can utilize as crucial compound. For example, on infection of *Vibrio vulnificus* the hybrid tilapia was tested for the defensive effects of pre-treating, co-treating and post-treating fish with TP3 and TP4 (Pan *et al.,* 2017). There is increased in survivability up to 95.3 % and 88.9 % after co-treatment with the pathogen and TP3 and TP4 while higher mortalities were observed after pre and post treatment (Pan *et al.,* 2017). However, the expression of immune related genes like *il1b, il6, il8, mcp8* was inhibited in all the treatment, and some AMPs, in co-treatment more pronounced effects were found, helps in the survival of fish, emphasizing the prospects of TP3 and TP4 to be utilize as antibacterial drug (Pan *et al.,* 2017).

AMPs as contraceptive agents for vaginal prophylaxis

In the reproductive tract of mammals there are many AMPs that are identified and they serve dual role in preventing sexually transmitted diseases as well as regulating fertility (Rana *et al.,* 2006). In the mucosal plug and vaginal fluid Lactoferrin was found, which checks fusion of virus and under acidic conditions its entry by binding and microbial membrane disruption. In seminal plasma, vaginal secretions and mucosal secretions Cathelicidin was found, that prevent the infection of microbes by neutralizing the polysaccharides of microbial cell. Magainins and dermaseptins are amphipathic, cationic alpha-helical peptides found in the skin extract of frogs *Xenopus laevis* and *Phyllomedusa sauvagei* that shows contraceptive activities against

numerous sexually transmitted infections causing pathogen and HIV infections (Zairi *et al.,* 2009). Nisin having contraceptive effect by stopping the mobility of sperm without causing damage to the epithelial cells of vagina. Thus, might be used as unusual contraceptive microbicides (Gupta *et al.,* 2009).

Immunomodulatory effects

Immunomodulators are also regarded as immunosuppressant, immunoadjuvants, and immunostimulants. In immunotherapy the disease is treated by modulating the immune system of host. There are various targets on which immunomodulatory peptides or proteins acts like macrophages, T and B lymphocytes, NK cells and monocytes. The mode of action chiefly affects by the activation of macrophage, immunoglobulins and cytokines, phagocytosis stimulation, stimulation of NK cells and MAPK-dependent and NK-kB pathways activation (Figure 8).

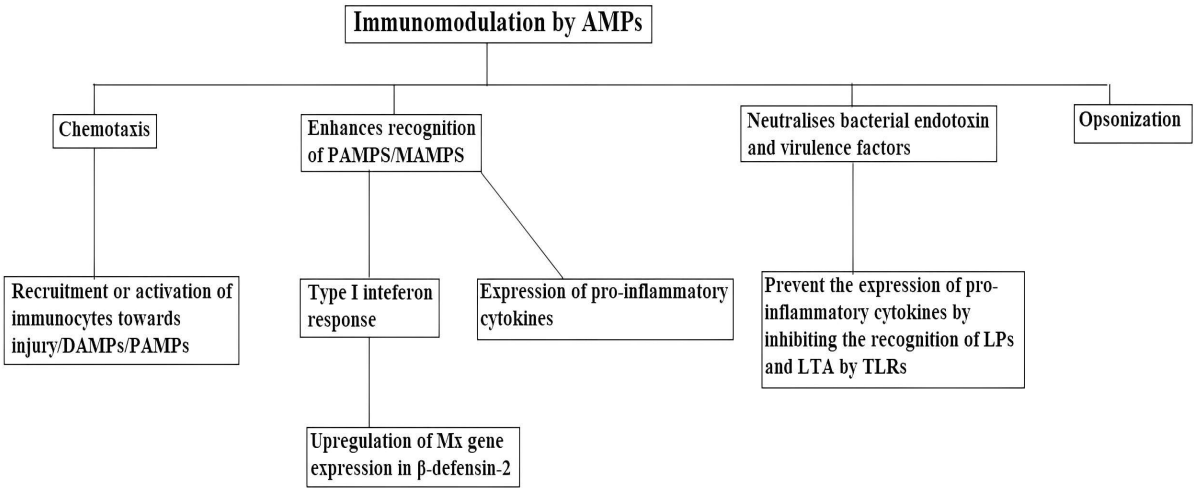

Figure 8: Immunomodulation by AMPs (Source: Chaturvedi *et al.,* 2018).

Future prospects: CRISPR-Cas and nutra ceutics

CRISPR-Cas system

Due to advancement in recent technologies the quality of synthesis of synthetic or recombinant peptides have increased and their expression levels also improved. Although, there are still challenging and important barriers. To overcome these problems, the improvement of target genome is an innovative technology. CRISPR (clustered regularly interspaced short palindromic repeat) system is the novel approach for pathogen-correcting genome and alters the target regions of pathogens (Gupta *et al.,* 2014). Cas (CRISPR associated proteins) are the mechanism that is adapted by the archaeal and bacterial immune system, which particularly identify genetic material of different pathogens that seek to infect them. By this approach they recognize earlier infections and hence resistance is developed against those infections by RNA-guided method (Pursey *et al.,* 2018). The two main objectives of producing antimicrobial peptides are: (1) to target pathogens by AMPs action. (2) the development of novel approaches to challenge against silent pathogens or avoid their progress and evading antimicrobial resistances (AMRs) (Greene 2017). The bacterial CRISPR-Cas system can be redesigned to attack rather than protect bacteria. Thus, guide RNAs may be able to target chromosomes that are essential for the survival of bacteria or responsible for virulence. Certainly, CRISPR-Cas approach has been modified and used with phagotherapy by encoding CRISPR-Cas9 targeting chromosomal genes of bacteria within the capsid of phage to treat resistances against *Staphylococcus aureus* and *Escherichia coli* (Citorik *et al.,* 2014). As bacterial adaptation got triggered due to the extensive use of antibiotics, hence antimicrobial resistance is the major issue to be solved. Therefore, priority should be given for combating AMR in aquaculture (Santos & Ramos 2018). CRISPR-Cas can be redesigned to target

AMR genes transported by a phage to resensitize bacteria to β-lactam antibiotics (Yosef *et al.,* 2015). These all innovations are based on the CRISPR-Cas tool emphasize higher probabilities to come in gene editing.

Nutraceutics

Food or parts of food, give health or medical aids, including the treatment and prevention of diseases is known as nutraceutics. Fishes are the biggest sources of nutraceutics (Chiesa *et al.,* 2016). Generally, AMPs observed as bioactive are firstly appear as precursors, after digestion a section is detached, indicating their useful effects on human health (Mohanty *et al.,* 2016). Many bioactive peptides of fish have been functioning as nutraceutics, though, they are hydrolysate of fish meal and as they are actually commercial products their impacts on human health have been determine, shows that they should cleared the clinical trials (Cheung *et al.,* 2015). Many AMPs of fish were earlier proposed as nutraceuticals, like epinecidin-1, tilapia piscidine 4 or tongue sole NKL 27, and grouper epinecidin-1 as they show an antibacterial function against known human pathogens (Cheung *et al.,* 2015).

Conclusion

AMPs are present as host defence molecules in prokaryotes as well as in eukaryotes. Fishes are regularly faced various pathogens which not only affects their health but also there is higher chances of becoming resistant to conventional antibiotics. Hence, in aquaculture AMPs may be a potential candidate for evolving therapeutic agents. Additionally, to the activity of microbicidal, AMPs also bears many other applications like immune modulators, signaling molecule, drug delivery vehicles, antitumor agents etc. The control of infection is still hindered by many challenges like high cost of manufacturing, low specificity, and animal cell toxicity. Even a small alteration can change the properties of AMPs but the results of these changes are still a challenging task. With the involvement of computational approaches, there is a better understanding of mode of action of AMPs and their activity. By combining machine learning molecular dynamics simulation and experiments it has been possible to design antimicrobial peptides from scratch. Machine learning not to directly discover and design AMPs with enhanced potency and antimicrobial efficacy, but rather to help glean understanding about the relationship between AMP sequences and function.

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Conflict of interest

The authors declare that they have no conflict of interest.

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