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Computational Tools and Designing Methodologies for Antimicrobial Peptides: Wonder Molecules in Medical Field

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ABSTRACT
Antimicrobial peptides (AMPs), found throughout the nature, are a family of tiny peptides which perform vital functions in different organisms' innate immune systems. AMPs show a broad spectrum of anti-bacterial, anti-viral, anti-fungal, and anti-parasitic properties. A recent rapid growth in the spread of antibiotic-resistant microbes and a growing concern regarding the excessive utilization of antibiotics has prompted the evolution of AMPs' that have shown to have the potential of a promising future in the medical field. However, detailed experimental studies of AMPs involve time-consuming procedures, and are difficult to conduct because of their high production cost. In order to reduce this cost and time; more recently, computational tools are becoming quite popular as they provide a sufficient discernment into the activity of AMPs, and can consequently expedite the exploitation of their prospects as next generation anti-microbials. However, natural AMPs have a short half-life, making them highly instable; hence, there is a need to modify the existing natural antimicrobial peptides, or design new peptide analogues that show antimicrobial activity over a longer period of time. The present review outlines the development of research in respect of AMPs, including the various sources and potential applications of AMPs in the medical field; and a major focus being on the various computational tools available for finding new potential antimicrobial peptides, and the several methodologies that can be used for designing novel AMPs.

KEYWORDS: AMPs, Computational tools, Designing methodologies, Medical field.

I. INTRODUCTION

In 1922, Alexander Fleming discovered lysozyme, which heralded the beginning of contemporary innate immunity, and initiated the identification of antibiotics as well as antimicrobial peptides (AMPs). But, more recently, it has been observed that an excessive use of antibiotics throughout the past century, particularly in the underdeveloped countries, has resulted into an increase in generating antimicrobial resistance in several bacterial species. Since the prevalence of such antibiotic-resistant microorganisms is increasing at a very fast pace, it has become necessary to take immediate counter-actions against them. In this direction, AMPs which have a broad range of microbe-killing, immunomodulatory and wound-healing activities[1] hold a great potential to serve as alternate effective therapeutic molecules.

AMPs are evolutionarily conserved molecules that are found in almost all living organisms, and possibly synthesized through two ways, either ribosomally or non-ribosomally[2]. Most varieties of AMPs share some common characteristics such as: amino acid residues vary from 5 to 60 in number, most of the AMPs are cationic and contain usually 50 percent of hydrophobic residues and most of the AMPs are amphipathic in nature. However, in addition to cationic AMPs, several anionic AMPs having acidic amino acids as glutamic and aspartic are also found upto some extent[3-5].

In medical research field, AMPs are constantly evolving, and different AMP databases, for instance, APD, CAMP, PhytAMP, BACTIBASE, LAMP, DRAMP etc. have amassed a large quantity of data regarding this subject. However, the mechanism of activity of several AMPs' is still unknown, and more research is required to establish a correlation between different physicochemical properties in terms of developing cost-effective and highly secure AMPs. There is also a need for further exploring and enhancing certain properties like specificity and capacity of AMPs and its synergies[6].

More recently, several computer-based tools have also been evolved to find potential candidate AMPs. These tools are providing a new

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avenue for designing of potential novel AMPs which might overcome the limitations of basic natural AMPs that hinder the progress of AMP in drug discovery. Keeping in view this importance of computational biology in AMP research, the present analysis is an attempt to shed light on the present status of the various tools and methodologies which have become almost an essential part of discovery and designing of AMPs as potential candidates for their effective use in medical biotechnology and therapeutics.

II. SOURCES OF AMPs

Many different kinds of AMPs have been identified from different forms of life. However, some main sources of AMPs are mammals (humans), amphibians, plants, microorganisms, and insects.

Antimicrobial peptides from mammals

Mammals including humans, sheeps, cattle, and other animals, all have anti-microbial peptides. The two most common AMP families in mammals are cathelicidins and defensins. On the basis of location of disulfide linkage, defensins are categorized as alpha, beta or theta defensins[7]. A well-known AMP named cathelicidin LL-37 produced from humans, is commonly found in the dermis of neonatal infants; however, human beta defensin2 (hBD-2) is commonly found in the older human beings rather than the younger ones[8]. Human host defense peptides (HDPs) can defend humans from microbial illnesses, although they have different expressions at different stages of development. HDPs can be found in thehuman body parts like eyes, ears, mouth, skin, respiratory system, gut, and urethra etc. Furthermore, human breast milk contains AMPs that havea crucial role in nourishing because they can reduce ill-health and fatality of infants[9]. Defensins and cathelicidins also influence the immunological modulation, apoptosis, and wound healing in addition to having an antimicrobial effect[10].

Antimicrobial peptides from amphibians

Amphibian antimicrobial peptides are vital in protecting amphibians from infections which are responsible for causing a global reduction in amphibian populations[11]. Cancrin, with a peptide sequence as GSAQPYKQLHKVVNWDPYG, discovered in the sea amphibian Ranacancrivorawas the first AMP to be identified in amphibians[12]. Ranacancrivora indicates animmense source of amphibian AMPs. However, the primary origin of amphibian AMPs remains the common frog, from which magainin AMP has been isolated. Frogs from different genera i.e.Xenopus, Pseudhymenochirus, Hymenochirus and Silurana belonging to the Pipidae family have also shown to have a high concentration of AMPs in their skin secretions[13].

Antimicrobial peptides from insects

Antimicrobial peptides are mostly produced in insects' haemocytes and adipocytes, which remains the core reason for their high adaptation to viability[14]. The major prominent class of insect AMPs is Cecropin, which is present in silkworm, bees, and Drosophila etc. Cecropin A has been known to have anti-cancer and anti-inflammatory properties[15]. A bee named royal jelly was found to produce a peptide, i.e. Jellein that has antibacterial and antifungal properties, a conjugated form of jellein's lauric acid impedes Leishmania parasite[16].

Antimicrobial peptides from microorganisms

Microorganisms i.e. fungi and bacteria perform a vital role in obtaining antimicrobial peptides.Some widely-known AMPs include nisin, gramicidin, enterocin etc. Nisin, a type of bacteriocin has been isolated from Lactococcus lactis that has shown inhibition activity against Gram-positive pathogens. Gramicidin A, isolated from Bacillus brevis displays antimicrobial response against both Gram-positive and Gram-negative pathogens. Enterocin produced from Enterococcus lactis are also known to be efficient against both Gram-positive and Gram-negative bacteria. A few antimicrobial peptides produced from some Bacillus strains and Pseudomonas sp. have been found to be efficient against Shigella, E.coli and Salmonella.

Antimicrobial peptides from plants

Different parts of plants including root, seed, stem, leaf and flowers are used for isolation of AMPs. The plant AMPs are categorized into thionins, snakins, defensins, glycine-rich proteins, hevein-type proteins, cyclotides and lipid transfer proteins[17]. The first plant AMP, purothionin was isolated from endosperm of Triticum aestivum that displays inhibitory effects against Pseudomonas solanacearum, Erwinia amylovora, Xanthomonas phaseoli,
Xanthomonascampestris, Corynebacterium flaccumfaciens etc.[18]. Hispidulin, an antimicrobial peptide has been extracted from the seed part of a medicinal plant Benincasa hispida. Hispidulin shows inhibition action against various bacterial and fungal pathogens that cause infectious diseases in human [19].

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III. APPLICATIONS OF AMPs IN MEDICAL FIELD

AMPs are potential candidates for antimicrobial treatments because of their broad-spectrum and quick bactericidal effects, as well as due to a low risk of resistance development[20]. However, only a very few AMPs have been validated till now, for instance polymyxins and gramicidins. Both gramicidins and polymyxins are cyclic peptides that have been studied the most. Gramicidins are usually operated for the treatment of sepsis, i.e., throat, eyes, nose infection and surface lesion[21]; however, due to the consequences of hemolytic effect of gramicidins, they cannot be employed intensively. Polymyxins are mainly used for the treatment of ocular infections, and particularly detoxification of the digestive tract; but they can also be used to cure infections which are caused by drug-resistant Gram-negative bacteria systemically[22]. Daptomycin is a cyclic AMP that was recognized by the FDA in 2003 to be used against Gram-positive pathogens, specifically Staphylococcus aureus in complicated skin and skin-structure infections (cSSSI)[23].

A wide variety of supplementary AMPs are also presently being assessed to treat infections caused by several bacteria and fungi; for example, pexiganan, omiganan and DPK-060. These AMPs are linear AMPs that have been carefully designed by incorporation of certain modifications. A 12 amino acid residue derivative of indolicidin named omiganan (CLS001 or MBI-226), has been found to show inhibitory action against infections caused by Gram- positive bacteria, Gram-negative bacteria, and fungi; and is used for the treatment of atopic dermatitis, acne vulgaris, genital warts, catheter-induced infections and rosacea. A 22-amino-acid derivative of magainin named Pexiganan has also been found to exhibit high antimicrobial activity. Pexiganan is found to have maximum potency against Gram-positive and Gram-negative bacteria, fungi and some MDR bacteria. Pexiganan has also been assessed to treat the infections of diabetic foot ulcer in clinical phase III trials, and some other clinical trials in case of crucial. Some tools involved in this are as stated below (Figure 1).

cSSSI are allegedly in the process[23]. Similar to omiganan and pexiganan, DPK-060 also exhibits high antimicrobial effect against a wide variety of microorganisms[24-26]. The effectiveness of DPK-060 ointment to treat eczema[26] and otitis externa has been evaluated in clinical studies.

Despite the challenges of bringing non-clinical candidate AMPs into clinical use, recent advances in characterization of their mechanisms of antimicrobial activity, designing methods that work to improve stability of chemical and metabolic processes, and state-of-the-art chemical synthesis procedures which lower the production costs, are expected to accelerate the progression of AMPs as therapeutic agents in the coming generation.

IV.IN VITRO STUDY OF AMPs

In vitro study of AMPs involves the extraction, purification and characterization of peptides (using chromatography/ mass spectrometry technique) from different sources of living organisms; followed by their further molecular validation by antimicrobial assay, sequencing of the validated peptide, and a final de novo synthesis of the sequenced peptides.

However, invitro studies of AMPs is a laborious process involving high production cost, and the isolated natural AMPs are many-a-times unstable, having a short half-life that hinders the progress of AMP discovery. Hence, search for alternate mechanisms for studying these different AMPs has become the need-of-hour. In this direction, the various computational tools which have come–up mainly in the last decade have become quite popular. Some of these tools which aid in speeding-up and reducing the cost involved in studies related to various AMP molecules are detailed in the subsequent sections.

V. COMPUTATIONAL TOOLS FOR PREDICTION OF POTENTIAL AMPs

Wet-lab experiments to identify AMPs are costly and laborious. As a result, developing a computationally intensive tool to find the best potential AMP preceding the in vitro studies is highly

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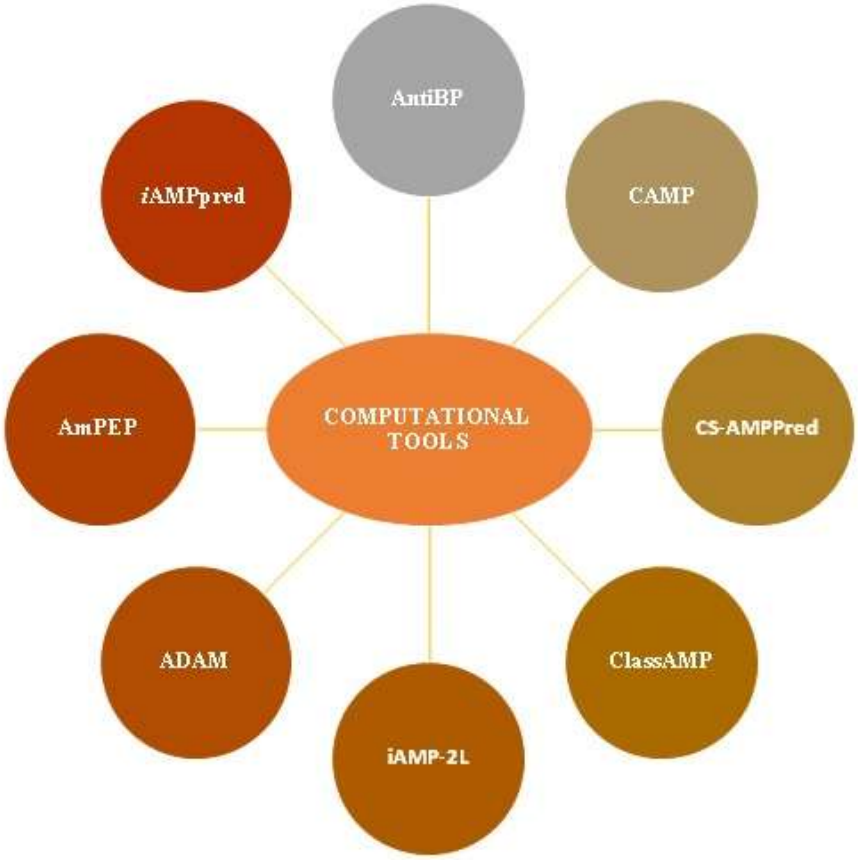

Figure 1: Different computational tools available for prediction of potential AMPs

Antibacterial Peptide Prediction Server (AntiBP)

The AntiBP tool supports "Support Vector Machine (SVM)", "Quantitative Matrices (QM)" and "Artificial Neural Network (ANN)" algorithms, and employs binary patterns and a 15-residue sliding window. In case of binary patterns, one binary pattern codes for each amino acid. This method achieves 88.17, 90.37 and 92.11 percent accuracy by combining 15 residues from N- and C-terminals using ANN, QM and SVM model, respectively. However, the N+C-terminal strategy depicts a lower accuracy (91.64 percent) when compared with blind data-sets in the second version (AntiBP2), where only SVM was implemented. Nonetheless, this version was more trustworthy than the prior version because it was trained with large number of sequences. AntiBP tool is freely accessible at http://www.imtech.res.in/raghava/antibp/[27,28].

Collection of Anti-Microbial Peptides (CAMP)

Prediction tools available in CAMP database support "Support Vector Machine(SVM), Random Forest (RF), and Discriminant Analysis (DA)"; and the method considers 257 factors, including each amino acid's composition, physicochemical characteristics, and structural traits. SVM and DA display an accuracy of 91.5% and 87.5%, respectively; but RF displayed the highest accuracy (93.2%) when tested on a blind data-set. In the newer version of this tool, a larger training data-set and a total of 64 features were employed, resulting in a little enhancement in the accuracy of SVM (92.6%) and RF (93.4%) over the blind data-set, whereas DA exhibited a little decline in accuracy (86.9%). This system is freely accessible on the web at http://www.camp.bicnirrh.res.in/[29,30].

Cysteine-Stabilized Antimicrobial Peptides Predictor (CS-AMPPred)

CS-AMPPred tool involves only SVM algorithm. The initial version of CS-AMPPred was dependent on nine physicochemical features as sequence descriptors, and could predict antimicrobial activity of sequences of various lengths. The hydrophobic moment descriptor was incorporated in the system, since an alteration of the sequences changes the hydrophobic moment.

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This method achieved an overall accuracy of 83 percent, when compared to a blind data-set. Later, this system was recreated and a modified version was developed, which was based on only five descriptors: (i) α-helix, (ii) loop formation, and averages of (iii) net charge, (iv) hydrophobicity, and (v) flexibility; these five were implemented and hydrophobic moment was eliminated. After implementation, the modified version had 90% accuracy, which was the maximun accuracy for cysteine-stabilized peptides when compared to others using the same data-set. CS-AMPPred is accessible as a stand-alone package at http://sourceforge.net/projects/csamppred/[31,32].

Classification of antimicrobial peptide prediction tool (ClassAMP)

This tool anticipates the tendency of the peptides to have antifungal, antibacterial or antiviral properties using SVM and RF algorithms. Three prediction models (antibacterial, antifungal or antiviral) were developed using these three against SVM and RF, where sequences from one class were considered positive and sequences from the remaining two classes were considered negative (i.e. for antibacterial, the negative set was composed of antiviral and antifungal peptides). These three models predict the sequences, and provide the class on the basis of higher number of votes. The accuracy of each model is greater than 90 percent. ClassAMP tool is freely accessible on the web at http://www.bicnirrh.res.in/classamp/[33].

Two-level multi-label classifier for identifying AMPs and their functional types (iAMP-2L)

This tool is developed on the basis of fuzzy K-nearest neighbour (FKNN) algorithm that has been trained by a pseudo amino acid composition (PseAAC) chou approach. Using decomposition of sequences, 40 descriptors were made. The first 20 represented the frequency of each amino acid, and the remaining 20 represented the combinations between 5 physicochemical features (pK1, pK2, pI (Isoelectric point) at 25°C, hydrophobicity and molecular weight), and 4 tiers of correlation factors (5x4=20).The contributions of the order of the sequence are added by the tiers of correlation factors. This tool is based on 2L prediction i.e. 2-Level prediction, in which prediction of sequence in the form of AMP or non-AMP with 86.32 percent accuracy in the first level. If a favourable prediction is made, multilevel prediction is used to predict the type of activity with 66 percent accuracy. This system is accessible as an online server at http://jci-bioinfo.cn/iAMP-2L[34].

ADAM

A database of antimicrobial peptide (ADAM) includes a prediction tool which is based on SVM algorithm, and the amino acid compositions (AAC) are used as feature to train the system. The established evidences related to accuracy of ADAM are not available; however, blind data-sets from different prediction tools i.e. iAMP-2L and CS-AMMPred have been used to find out the accuracy of SVM based ADAM. The accuracies of the system were found to be 88.04% and 91.33%, respectively. The system is accessible on the web at http://bioinformatics.cs.ntou.edu.tw/adam/to[35].

Improved Prediction of Antimicrobial Peptides Server (iAMPpred)

iAMPpred, a SVM-based AMP classifier, uses 66 features incorporating computational features, structural features and physicochemical features of a peptide. This system predicts different activities of the peptide i.e., antibacterial, antifungal, or antiviral. The accuracy of this tool was found to be higher than AntiBP2 and ClassAMP. It is user friendly web server which is accessible at http://cabgrid.res.in:8080/amppred[36].

AmPEP

It is a sequence-based prediction tool, based upon the Random Forest (RF) algorithm and distribution patterns of amino acid characteristics. Several physicochemical features of peptides such as solvent accessibility, hydrophobicity, secondary structure, polarity, charge, normalized van der Waals volume, and polarizability comprise around 105 distribution descriptors in the feature set. The class distribution pattern was defined on the basis of sequence as position percentage for each feature, viz. the first residue, 25% residue, 50%, 75%, and 100% residue type of distribution. This tool was found to have a higher accuracy than iAMPPred[37]. In fact, upon comparison of all the above listed tools, it can be said that AmPEP tool has the highest accuracy.

All these computational tools bring a new approach for developing a relationship between activity and structure of AMPs, which in turn facilitates the designing of novel potential AMPs having high antimicrobial activity, long half-life, and a low toxicity. Some methodologies involved in designing of novel potential AMP molecules are detailed in the subsequent section.

VI. METHODOLOGIES FOR DESIGNING OF NOVEL POTENTIAL AMPs

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Though the AMPs have a very wide and bright scope, but, they still have certain limitations such as: (i) they may destroy the animal cell membrane due to which destruction of red blood cells can occur; (ii) the stability of AMPs is restricted at a definite pH; (iii) factors like iron and some serum can decrease the activity of AMPs; (iv) AMPs are quickly hydrolyzed by proteases; and finally (v) the increasing production cost, and the short half-life of AMPs. In view of the above shortcomings, a natural AMP needs to be enriched with some of the following features, i.e., AMPs should be designed to have: (i) high antimicrobial activities; (ii) low toxicity; (iii) high restriction to proteases; (iv) good accessibility and minimum manufacturing cost; and (v) the binding capacity for serum should be lesser[38]. Consequently, for achieving these expected results, designing of AMPs has gained quite popularity in recent years. Figure 2 represents some of the methodologies which can be used for designing of novel potential AMPs.

Figure 2: Different methodologies used for designing of novel AMPs

Site directed mutagenesis- based designing of peptides

The addition, deletion, or replacement of one or more amino acid residues in natural antimicrobial peptides is referred to as site-directed mutagenesis- based designing of AMPs. A single mutation on known antimicrobial peptide sequence can have functional consequences that may be easily understood. However, a methodical mutation of AMP molecules is required to span the entire sequence space and find AMP-variants having enhanced antimicrobial activity. For instance, inspecting lysine or alanine residues is a beneficial methodology since these amino acids cover all places within a peptide sequence; allowing researchers to investigate the impact of every amino acid side chains on the peptide's structure and function[39].

De novo- based designing of peptides

De novo based designing of peptides is based on patterns or frequencies of amino acids, and the design of amphiphilic AMPs is important in de novo designing of peptides[40]. GALA (Glu-Ala-Leu-Ala), for instance, is a famous de novo- based designed AMP. GALA is an α-helical peptide which is amphipathic in nature that is formed by positioning protonatable glutamic acid residues in utmost area with a distancing of i to i+4[41]. Repeating sequences such as $(AABB)_n$ (where A1, A2 & B1, B2 are hydrophobic & cationic amino acids, respectively, and n is number of entity repeated), are designed upon the basis of hydrophobicity cycle that successfully constructs a wide range of alpha-helical AMPs which resembles the natural alpha helical peptide. $(LKKL)_3$ and$(WKKW)_{2.5}$ sequences have been shown to have maximun selectivity[42]. Besides, $L_lK_mW_2$ model peptides are also de novo-designed peptides. In this, Leu (L) and Lys(K) residues and two Trp (W) residues have been used to establish amphipathic helical characteristics. Two Trp (W) residues have been placed at the amphipathic

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interface between the hydrophilic terminating side and the hydrophobic beginning side. $L_4K_5W_2$ has been shown to exhibit the best anti-MRSA activity among the few designed model peptides[43].

Template- based designing

In this methodology, by assimilating a wide range of structurally homologous stretches of innate AMPs, and by procuring conserved motifs depending upon the kind of residue (such as charged, polar, hydrophobic, etc.), sequence templates can be acquired[44]. Factors such as tendency of helix formation, cationic nature, amphiphilicity and overall hydrophobicity can be altered in a proper order, based on the modification. For example, some natural AMPs that were employed as templates include cecropin, magainin, protegrin and lactoferrin[1].

Self-assembly of antimicrobial peptides-based designing

In the course of self-assembly of peptides, nanostructures like micelles, vesicles, nanotubes, nanoparticles, nanobelts, nanofibres, and nanotubes are created by peptides, which in turn, can acquire an ability to enhance or communicate antimicrobial activity to AMPs. For instance, a 12 amino acid self-assembling peptide, KLD-12 (KLD) (Lys-Leu-Asp), can acquire nanostructures which have been recognized for tissue engineering characteristics. However, β-sheet secondary structure of KLD and properties of self-assembly of KLD forming nanostructures reveal no notable modifications when arginine residues are added[45]. Besides, to improve the antibacterial activity and to minimise the toxicity of AMPs, dimer structures are often exploited. After formation of dimers, consequences of membrane-destabilization are diminished[46].

Chemical modification- based designing

To enhance the steadiness of peptides, many alterations in the chemical properties of AMPs have been employed. These include: phosphorylation of residues, addition of D-amino acids or synthetic amino acids, cyclization, acetylation, halogenation, and peptide-mimetics. Due to the stereospecificity property of an enzyme, if unnatural D-amino acids are introduced in the AMP sequences, these can cause the stereochemistry to be reversed, hindering protease destruction[47]. Peptido-mimetics, in which the main constituents resemble the architecture of peptides, are generally formed through modifications of existing peptides, like extension of chain or heteroatom inclusion[48]. Non-chemical modification also employs ornine, an unnatural positively-charged residue with a high resistance to protease action. Tryptophan residues can also be replaced with β-dihydrophenylalanine to enhance the stability of peptide structures and their antimicrobial activities[49].

Computer based- designing

Simple statistical modelling, structure activity relationship studies[50], deep learning[51], neural networks[52], machine learning and word embedding[53] are some of the techniques used in computer-based designing. For instance, a Matlab using machine learning algorithm, established on the idea of ranking the participation of antibacterial activity of each amino acid is proposed[54]. Guavalin 2, an amphiphilic α-helical peptide, which is composed of unusual amino acids (three and three tyrosine residues) was designed using the genetic algorithm, and it induces membrane hyperpolarization that shows a distinct method[55].On quantitative structure–activity relationships (QSAR)- based research, two techniques have evolved: AMP therapeutic index-based prediction technique, and the principles of highly conserved signal peptide subclasses linked to AMP based technique, called the identification of novel AMPs from the expressed sequence tag database[56].

Rational library- based designing

Currently, the discovery of novel AMPs has been eased through screening of library via the rational approach. Three considerable methodologies are used for rational design: template-based design methodology, physicochemical based methodology, and the denovo-based methodology aiming to develop novel potential peptides/or to improve pre-existing peptides. A wide range of AMP variants are acquired through this approach. The desired AMP can be obtained effectively when integrated with high-throughput screening. For example, melittin's combinatorial peptide library has been used to develop a few novel AMPs that have a greater antibacterial activity and a reduced cytotoxicity[57]. Among all these designing methodologies that are being used for overcoming the shortcomings of AMPs, and for improving their efficacy; addition of synthetic amino acids and peptide-mimetic methodologies are emerging as promising approaches in the development of AMP research.

VII. CONCLUSION AND FUTURE PERSPECTIVES

The AMP research has been driven by the fact that the past decade has witnessed a rapid rise in the spread of antibiotic-resistant bacteria, hence

creating the need to search for other new antimicrobials. In this direction, AMPs which have a broad-spectrum antimicrobial activity hold a high potential. However, infection control by AMPs is still hindered by several challenges including low specificity; toxicity to animal cells; easy proteolytic degradation; and above all their extraction, purification, characterization and production being highly, laborious, time-taking and expensive. So, in view of these shortcomings, various computational tools are currently being developed to narrow down the search for potential candidate AMPs, and also for providing a new direction in favour of designing and optimizing potential novel AMPs with improved efficacy. The use of these tools and methodologies, although in its infancy, is bound to play a major role in AMP research.

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