DOI: 10.56511/JIPBS.2017.v4.i01.pp25-32

e-ISSN: 2349-2759 p-ISSN: 2395- 1095

Journal of Innovations in Pharmaceutical and Biological Sciences (JIPBS)

www.jipbs.com



Mini Review

Antimicrobial peptides: Therapeutic potential as an alternative to conventional antibiotics

Imran safder[1*], Amjad Islam[2, 3]

[1]*H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi-72570, Pakistan.*
[2]*Ningbo Institute of Materials Technology and Engineering, Chinese Academy of Sciences, Ningbo 315201, P. R. China.*
[3]*University of Chinese Academy of Sciences, Beijing-100049, P.R. China.*

Key words: Antimicrobial peptides, antimicrobial resistance, antibiotics, host defense peptide, drug resistant bacteria, alternative antibiotic drug development, synthetic antimicrobial peptide.

***Corresponding Author: Imran Safder,** H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi-72570, Pakistan.

Abstract

Antibiotics are among the most important class of therapeutics to treat life threatening diseases, however, the emergence of multi-drug resistance bacteria is a serious threat to public health. Since conventional antibiotics are becoming resistant to all approved antimicrobial drugs, there is an urgent need to find alternative therapeutic agents. Antimicrobial peptides (AMPs) are naturally occurring host peptides produced by various organisms, as part of the non-specific immune response to defend against microbes. They have received great attention serving as a new class of antimicrobial agents, to counter multidrug resistance because of their broad spectrum activities against microorganism and low propensity to develop resistance.
By focusing on developing optimized peptide designs using computer assisted approaches and applying advanced bioinformatic tools to consider factors such as peptides physical stability in physiological condition, and reduced toxicity to host cells, AMP can fulfill in the future as promising next generation antibiotics to fight drug resistant bacteria.

Introduction

Antibiotics are one of the fascinating discoveries of the twentieth century. Since the development of first antibiotic penicillin, they are widely used as a magic bullet against various life threatening infections for over 100 years and have improved the quality of life with the availability of a diverse range of antibiotics to people [1-3]. However, their misuse including the application of powerful and broad spectrum drugs, the presence of antibiotics in the food industry has led to the bacterial resistance against various antimicrobial agents and caused the emergence of multi-drug resistance microorganisms (resistance against three or many antimicrobial drugs) [4]. Strains of the important human pathogen as *Mycobacterium Tuberculosis*, *pseudomonas aeruginosa* and *Acinobacter baumannii* have showed increased resistance to almost all the available conventional antibiotics [5,6].
A lot of mechanisms have been involved in bacterial resistance process including the mutation or enzymatic activation that results in the altered target protein, acquiring genes from other bacteria to express less susceptible targeted proteins, and also acquire mobile genetic elements including transposons or plasmids [7]. Meanwhile, a synthetic antibiotic Linezolid was also developed, to tackle the outbreaks of *M. Tuberculosis* and *S. aureaus*, but, the reported resistance cases have diminishes the hopes that were relied on it [8,9]. Despite continuous efforts, multiple drug resistance is still a serious global concern, particularly a severe medical threat to developing nations resulting in economic burden and increase mortality rate. It is estimated that global death due to antimicrobial resistance would reach to 10 million by 2050[10]. Another alarming factor in this regard is the markedly low number of Food and Drug Administration FDA) approved antibiotics in last 20 years [11] and the antibiotic research is declined to such an extent that only two antibiotics have been approved since 2008. Only four pharmaceutical companies are operating drug development process, in contrast to 18 in 1990 [12]. Therefore, it is critical to seek and develop alternative antimicrobial agents on an urgent basis.
Antimicrobial peptides (AMPs) have become a promising candidate and have received notable attention as a novel class of antibiotics. They are peptides naturally produced

©JIPBS, All rights reserved

by all organisms including prokaryotes to human beings in response to foreign microbes and have a role in innate specific defense system and provide instant non-specific defense against infections [13, 14]. They have a broad spectrum of activity against a wide range of microorganism including Gram negative and Gram positive bacteria, fungi, parasites and viruses [13-16].
In antimicrobial peptide database, about 2300 AMPs have been reported, around 500-600 candidates are in pre-clinical processes [17] however, none has received FDA approval for therapeutic use, except few approved only for tropical use. Although there is no drug currently in the market based on AMP, however, their selectivity, natural antimicrobial properties, low propensity to develop antimicrobial resistance make them an attractive candidate for clinical development as new antibiotics [18]. In this review, we discuss their potential as a therapeutic agent, different strategies including chemical, computational and bioinformatic tools used to improve their antibiotic drug development process.

Structure and general characteristics of AMPs

Antimicrobial peptides are short polypeptides with less than 50 amino acid residues, typically between 15-40 amino acid residues [19], net positive charge mainly due to an excess of positively charged amino acids arginine, lysine, and histidine, contain 50 % hydrophobic amino acids and constitute an amphipathic structure [20].
In humans, AMPs activity was initially explored in early 1960's, that cationic peptides were responsible for assisting neutrophils in eradicating bacterial cell [21]. Synthesized in various parts of organisms, they act as regulators and effectors of innate immunity and perform a broad range of activities; such as in the production of chemokine and its release by epithelial and immune cells, exert anti-apoptotic effects on certain immune cells, stimulate wound healing and angiogenesis, and involved in adjuvant activity to increase antibody production[22-24]. AMPs are also able to kill biofilm production, and attract phagocytes chemotactically and induce non-opsonic phagocytosis [25-27].
Due to their cationic nature, they selectively interact with the negatively charged membrane of microorganism, disturbing the membrane structure [28, 29]. Cationic AMPs diffused to the lipopolysaccharide and teichoic acid based negatively charged surfaces, in the initial stage as shown in Figure 2. [24, 30]. After binding the membrane, they undergo a conformational change, which allows the peptide to translocate into the interior of the bacterial cell [31].
According to the chemical structures and sequence diversity reported they are categorized into one of the four main structural groups: Linear structure mostly alpha helical peptides, β-strand/sheet peptides having two or more disulfide bridges, extended non-helical linear/sheet peptides rich in Lys, Trp, and His residues, and mixed helical sheet peptides structures (Figure 1). Among these structures, AMPs adopt mostly alpha helical conformation. Most peptides undergo a transformation from a flexible unstructured structure to a particular structured or fixed conformation when they interact with a membrane. A change in single or double amino acid has a substantial effect on the secondary structure of peptide that also influences its activity [54, 79].

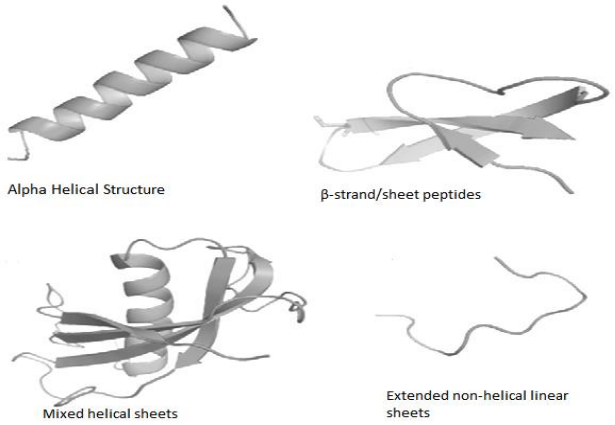


Figure 1. Structural forms of Antimicrobial peptides

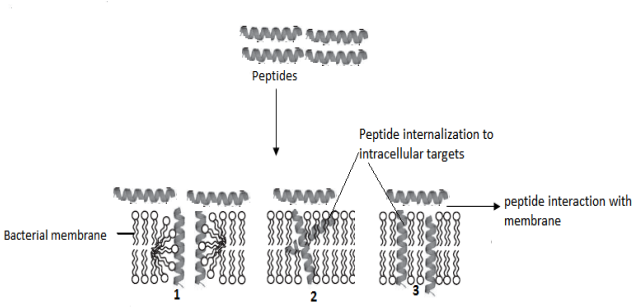


Figure 2. Antimicrobial peptides mechanism of action. Cationic AMPs binds the negatively charged bacterial membrane, diffuse and internalize into intracellular targets leading to cell death and use any of these mechanisms. (1) Toroidal pore model (2) Aggregate model of action (3) barrel-stave.

Mechanism of Action

There are many proposed mechanisms of action for AMPs, but the exact mechanism is still unclear [32]. A better understanding of the molecular mechanism for the mode of action will assist to develop better drugs.
Different studies suggested that their mode of action is predominantly based on their structural features. The hydrophobicity, cationic charge, amino acid sequence, size, influence their interaction with negatively charged bacterial membranes [33].
In fact, the difference between the bacterial and eukaryotic membranes is the reason why only bacterial cells are vulnerable to AMPs and are killed. As bacterial

membranes are densely populated by negatively charged phospholipids, whereas eukaryotic cells are less susceptible to these peptides because they contain neutral phospholipids [1, 34]. Interaction with bacterial membrane leads to unsettle membrane, this perturbation eventually results in disturbing membrane associated events including cell division or cell wall synthesis and restriction in translocation process. As a result of this interaction, AMP is inserted into the lipid bilayer and in turn, lead to lipid displacement. Change in bacterial membrane structure includes pore formation, electrostatic modifications, curvature modification and severe perturbations that shuffle the membrane peptide molecules. Hence, the peptide bypasses the membrane and ultimately reach the cytoplasm to influence intracellular targets [35].

Several mechanisms have been proposed that depicts a method of pore formation such as; the toroidal pore model, the barrel-stove model, the carpet model and aggregate model as shown in figure 2. [36-38]. In the toroidal model, the pore is formed due to the interaction between hydrophobic residues of the peptide with hydrophobic residues of bacterial membrane. In the barrel-stave model, AMP interacts laterally with the membrane forming a stave in a barrel-shaped cluster, as the peptide consistently move, pore size increases resulting in the release of the cellular content of the cell [37]. Carpet model includes the aggregation of AMP on the membrane surface mainly through electrostatic interaction. As the peptide concentration reaches a specific threshold, changes in membrane structure occur, either a reduction in membrane barrier or in fluidity results in permeabilization [39, 40]. However, there is not a specific model that is applicable to a particular peptide-membrane interaction, but various factors lead to pore formation. Cell death, as a result of pore formation, occurs due to; membrane dysfunction, inhibition of extracellular biopolymer synthesis or intracellular functions [40].

Potential as therapeutics

Due to complex and multi target mechanism of action, AMPs are distinguishable from conventional antibiotics and make them an ideal candidate to generate new antimicrobials [41]. A number of factors back this strong narrative such as their synergistic effects with conventional antibiotics, relatively small size, neutralizing endotoxin ability, considerable minimum inhibitory concentration [MIC], manifest that they are highly prone to kill bacterial cells. They have demonstrated potent activity against bacterial biofilms too, which also showed resistance to traditional antibiotics. In addition, AMPs have also shown a wide range of antiviral properties in vitro studies [42].

In malignant cancer cells, multidrug resistance (MDR) is a major mechanism of drug resistance. Few AMPs displayed cytotoxic activity against MDR cancer cells. They were able to kill cancer cells rapidly, had lower side effects with easier absorption [17]. For their therapeutic use in cancer, they are mandatory to perform specific anti-cancer activity alongside stability in serum [34, 43]. A better understanding in this area would definitely contribute cancer research.

On the commercial side, several companies are conducting clinical studies. Around 40 compounds are in active clinical development. [44]. A number of synthetic peptides have entered in clinical trial phase and about 15 peptides are in crucial decisive stages of these clinical trials as antimicrobial agents. [35].

Though several AMPs have developed for commercial applications, but large efforts have only restricted to develop tropical agents. A major reason would be the safety of topical application and possible consequences of long term side effects may cause due to systematically administered peptide[34]. Few of the extensively researched peptides in clinical trials are P-113(Histatin), MSI-78(Pexiganan), MBI-226 (Omiganan), IB-367 (Iseganan), rBP121 (Nuprex), but none have yet received FDA approval to use as a systemic therapeutic agent and are limited only to administered topically (Table 1). Most of them passed phase I clinical trials but faced difficulties in phase II and phase III trials [45]. In 2003, FDA approved Daptomycin, to treat skin infections. It is a cyclic lipopeptide and is marketed under trade name CUBIN ®. Moreover, Novozyme company found an active antimicrobial peptide, Plectasin NZ2114 peptide derivative, and granted Sanofi-Aventis license to produce and commercialize this compound in yeast *Pichia pastoris*. This is expected to be the first AMP expressed in yeast to be approved for market in future [34,46].

Limitations of AMPs in clinical studies

AMPs have several advantages that make them ideal candidate as therapeutic agents for new antimicrobial development, but they have certain limitations which need to overcome prior to their therapeutic usage.

The first hurdle is their possible toxicity to host cell. Although, bacterial membrane contains negatively charged phospholipids, a direct target for AMP, whereas the human membrane is composed of zwitterionic phospholipids. However, there are reports where AMPs have directly interacted with host cells and have lysed them [47]. Moreover, AMP can also attack human microflora and cause infection due to the absence of normal flora[48]. They can also bind to various components of host cell surfaces including membranes, extracellular matrix. As a result, clinical trials are only restricted to topical applications instead of oral administration [49]. Another notable hurdle is their

physical stability under physiological conditions. They can be degraded by proteases and are susceptible to serum, salt, and pH [45]. As AMPs need electrostatic attraction with microbial membranes to bind and this step can be effected by the ionic strength of the solution. Therefore human fluids carrying high salt concentration neutralize AMPs and restrict their electrostatic interactions to bind [50]. Furthermore, their linear structure is easily prone to proteolysis by host proteases, also the serum proteolysis capacity is very high which provide strong resistance to AMP activity, another reason why they are only administered topically to skin infections and other physical maladies.[51]. Lastly, a major limitation that impedes the development of AMPs is their high production cost, in contrast to conventional antibiotics. Other technical issues including the synthesis and purification process also facilitate high cost [52].

Strategies and Physical factors to consider for designing new anti-infective therapeutic drugs

Literature data suggest, there is marked diversity in the AMPs mode of action, host selection and their intensity of activity even with similar structures. Therefore their physiochemical properties such as size, charge, solubility, hydrophobicity are important for their antimicrobial activities and at the same time these factors need to consider alongside different strategies to combat their major hurdles and limitations including physical stability, toxicity, high production cost in the therapeutic development process. We review steps considered in addressing these factors.

Length and net charge

Length is crucial to peptide activity, as it needs minimum7-8 amino acids to form an amphipathic structure carrying hydrophobic and hydrophilic faces. Length also influences the 3-D structure, mode of action and cytotoxicity, therefore it needs to consider during drug development process [53]. Besides length, modification in net charges of AMPs, that includes the addition or removal of certain ionizable groups of peptides influences antimicrobial and hemolytic activities [54].

Enhancing Physical stability of AMPs

Cyclization of AMPs by linking their C- and N- terminus is a known method to enhance their bactericidal activity. This bulkiness due to cyclization makes peptide less prone to proteases as compared to a linear structure, which is more susceptible to proteases [55]. Vogel et al investigated cyclization and end capping effects on the stability and found that cyclization was highly effective for peptides antimicrobial activity and serum stability, whereas end capping had contradictory effects against proteolytic stability [56]. Incorporation of chemical compounds including the fluorinated derivatives of buforin and magainin is said to improve AMPs stability with two fold increase against the proteolytic action. It is assumed that steric occlusion could be the reason behind this stability, but further investigation in this regard would validate the exact reason [57].

Several modification strategies have taken to prevent peptide degradation. Reducing cationic residue content, since fast degradation of AMP often results from cationic residues primarily Arg and Lys in sequence [58].Modification by changing amino acid content is another important strategy used, based on exploiting physiochemical properties of specific amino acids, for instance, high proline content is supposed to have a negative influence on penetration in cell membrane due to its less tendency to form alpha helical structure [59]. Similarly, the introduction of D- or non-natural amino acids protect the peptides from proteolytic degradation, as host proteases easily hydrolyze existing L- amino acids [60]. Furthermore, the end modification of N-terminus and C- terminus through amidation or acetylation can also increase stability against proteases [61]. In addition, the development in peptide synthesis has made it possible to incorporate specific chemical groups at the end of the peptide. A modified PMAP-23 with amidation at C-terminus showed 10 fold high cellular uptake and fast interaction with bacterial cells in contrast to original PMAP-23 [62]. Also, several amino acids have more tendencies to form helix structure, therefore modification of the amphipathic ratio in AMPs will improve their stability.

Reduce Toxicity

Toxicity is attributed to high hydrophobicity which leads to hemolysis. Change in hydrophobicity can confer AMP selective activity against microorganism [45]. The ratio of antimicrobial activity and hemolytic activity is expressed as a therapeutic index, so a high therapeutic index is needed to avoid hemolysis of host cells. Therefore, it is crucial to utilize non-hemolytic AMPs as the seed compounds [63].

Computational methods using knowledge based designs create highly selective AMPs, based on information from previous known AMPs, provide a promising strategy to develop high bacterial selectivity peptides [64, 65]. Further advancement in antimicrobial databases would assist researchers to retrieve a vast amount of information on the basis of important peptide parameters such as charge, amino acid content, composition, hydrophobicity to design novel AMPs [66].

Gram nature-selective AMPs can be used to thwart Gram negative bacteria by attacking the outer membrane lipopolysaccharides. These cyclic AMPs bind and interfere at LPS-binding sites to inhibit membrane synthesis [67]. Fusion peptides are also used to target

specific species, providing high selectivity. These fusion peptides comprised of two domains, one provides specific binding to the desired pathogen and the other render bactericidal action. Specificity towards pathogen is based on cell wall structure, membrane receptor, or hydrophobicity . This peptide also selectively eliminates normal flora from pathogenic bacteria [66]. Similarly, protease activated AMPs and environmental sensing AMPs have been designed that are activated when virulent proteases are secreted by pathogenic bacteria or sense environment changes like acidic or physiologic pH change respectively [68, 69].

To overcome toxicity and stability issues other techniques are also deployed including; polymeric nanoencapsulation of AMPs using nanoparticles and nanospheres; PEGylating peptides; liposomal formulations and induction of new drug delivery system would strengthen AMPs therapeutic activity and stability [53, 70, 71].

High production Cost

High production cost can be resolved by the production of smaller peptides with high stability. Also, machine learning approaches have produced bioactive peptides alongside broad spectrum activity. Further exploration would certainly provide positive outcomes [35].Moreover, development of compatible expression hosts, for instance, yeast in the case of plectasin, will also help to reduce production cost.

Synthetic AMPs

Antimicrobial peptide databases have provided a valuable knowledge base for quantitative and qualitative prediction models. These models have contributed to design synthetic AMPs, like adepantins [72]. These approaches utilize previous data or predicted sequences to design peptides with desired properties. Three of these studies including template based strategies, biophysical studies, and virtual screening studies are discussed here.

In template based study, a known AMP template sequence is used to create peptides with high antibacterial activity based on modifying amino acid sequence. Single amino acid is changed or their position is altered to explore superior peptide performance. [73] Biophysical study seeks to study AMP activity and different variant designs by analyzing peptide structures through biophysical modeling peptides at the atomic level or their performance in hydrophobic environment. These computational based studies include thermodynamic calculations of AMP interaction, molecular dynamics simulation between the peptide and bacterial membrane interactions [74]. These approaches have successfully applied in optimizing drug designs [75]. Virtual screening offer cost-effective alternative when synthetic and biological science strategies are exhaustive to apply. They only require primary sequence to imply peptide structures. The result is not model based as in the case of computational studies, but it utilize mathematical models to infer quantifiable peptide properties including hydrophobicity, charge, and then subsequently relate with peptides biological activity using virtual screening models including quantitative structure–activity relationship models (QSAR models). These numerical models decipher biological activity of peptide as output variable [76, 77].

In addition to synthetic design strategies discussed, bioinformatics has also emerged as a remarkable tool in discovering new drug targets. These computer science, mathematical, statistical, high-throughput computational and bioinformatic tools are merged and are providing substantial benefits to researchers [78].These bioinformatic tools carry multiple options to retrieve primary data as homology searches, phylogenies, sequence alignment and then analyzed with different tools such as Swiss Model, Rosetta, HHpred, and I-Tasser to predict secondary structures. Using these powerful bioinformatic tools predicts a promising role in antimicrobial therapeutic development [79].

Synthetic peptides are becoming interested in drug development domains because short, synthetic peptides can be developed and they can meet systemic regulation objectives, are short so reduce cost and stability issues. This interest was initiated in early 1992 when a synthetic peptide containing Leu and Lys were reported and active against Gram negative and gram positive bacteria. Many of the synthetic AMPs have also entered in clinical development phases and at least 15 peptides are in different stages for instance adepantins (automatically designed peptide antibiotics) is already developed. Some of the synthetic short peptides such as Pac-525, MP196, Anoplin showed the ability to permeabilize and penetrate bacterial membranes, also were highly specific. Recently arylated-amino acids such as Trp exhibited low hemolytic activity with improved performance than its counterpart [81]. There are various reports that reveal 29 AMPs had potent activity against *Mycobacterium tuberculosis, Enterococcus faecalis*, and *Salmonella typhimurium* with MIC values in the 0.8-11.5uM range, three synthetic AMPs particularly (WKWLKKWIK, WRKFWKYLK, and RRWRVIVKW) found non-toxic to human cells [82]. A synthetic peptide (APKAMKLLK KLLKLQKKGI) showed stability and maintained its structure in a high salt concentration. Two tripeptides lysine-Dproline-tyrosine-NH2 and lysine-proline-valine-NH2 were initially inactive against *E.coli, S. aureus* and yeast, but when modified by N-terminal acetylation, a 2000 fold increase activity was observed [83]. Eckert inducted a D-amino acid enantiomer, G10KHc-D, to protect the peptide against serine proteases [83].

Probability of bacterial resistance

We can't ignore the probability of bacterial resistance. Although they have a low propensity to develop resistance, but modifications in the bacterial membrane have shown resistance mechanism [80]. These include cell surface modifications with reduced anionic charge, which inhibits peptide to aggregate on the membrane, degradation by proteolytic enzymes, variation in cell wall hydrophobicity, membrane fluidity and membrane bound efflux pump cause expulsion of the peptides [7].

Table 1. Important Antimicrobial peptides in clinical trials

Name and peptide sequence	Company	Application	Clinical trial phase, outcome and recent events
Pexiganan (MSI 78) GIGKFLKK AKKFGKA FVKILKK	Genera Plymoth, Dipexium-Pharmaceuticals	Topical Antibiotic	Phase III trials demonstrated no advantage over existing therapies. Another phase III onestep-1 and onestep-2 trials by Dipexium Pharmaceuticals completes for Diabetic foot ulcer in the USA (NCT01594762; NCT01590758)
Omiganan ILRWPWW PWRRK	Migenix/ Cutanea-Life Sciences	Severe Acne,	Phase III trials unsuccessful, Phase II showed notable efficacy. Cutanea Life Sciences completes a phase II trial for Acne vulgaris recently (NCT02571998) and plans a phase II trial for Vulvar intraepithelial neoplasia (NCT02596074).Phase II clinical trials as a topical agent in genital wart in Netherland (EudraCT2015-005553-13)
Iseganan (IB-367) RGGLCY CRGRFC VCVGR	Ardea Biosciences	Oral mucositis, Pneumoniae	Phase III trials showed no advantage over existing therapies
Neuprex KLFR-(D-naptho-Ala)-QAK-(D-naphtho-Ala)	Xoma	Impetigo, meningococcemia	Phase III trials of meningococcemia in children and no development reported yet.
Histatins (P-113) AKRHHG YKRKFH	Demegen	Oral candidiasis	Phase IIb trials with candidiasis demonstrated positive results, phase IIb trials increase in 34% endpoint efficacy level. Phase III trials not initiated yet.
hLF1-11 GRRRRS VQWCA	AM-Pharma	Bacteraemia, Fungal infection	Positive phase I. Under phase II trials for bacterial and fungal infections
CZEN-002 $(CKPV)_2$	Zengen	Vulvovaginal Candidiasis	Still no development reported in phase-II clinical trials
OP-145 IGKEFKRIVERIK RFLRELVRPLR	Leiden University/ Octoplus	Otitis media	Efficacy in phase II trials completed (ISRCTN84220089) but phase III trials still not Initiated
Delmitide RXXXRX XXGY (X=norleucine)	Genzyme	Bacterial infections	Phase II trials completed (ISRCTN84220089).

In contrast to these mechanisms, there is an optimistic perspective that even if resistance occur, AMP would tend to develop very low level induced resistance since cationic peptides directly bind to the negatively charged membrane through electrostatic binding. Secondly, their function as a modulator of innate immunity where they have a broader role, as compared to acting only against bacterial membrane suggests increased resistance is hard to develop [10].

Conclusion

The desperate need to tackle multidrug resistance bacteria has thrived Antimicrobial peptide research to develop them as a new class of antibiotics is highly promising. Their mode of action, broad spectrum activity, ease to synthesize, along with additional biological functions in immune response make them a potential candidate for anti-infective therapeutic development and use. However, there are few limitations that demand attention prior to their clinical use, and only after resolving these issues their significance as an alternative antibiotic would be realized. These factors include high production cost, toxicity, and solubility in physiological conditions. The Scientific community and pharmaceutical companies are working aggressively to cope these issues. Therefore, we are optimistic that with technological advancements, innovative computer-assisted peptide design strategies, high-throughput genomics, and advanced bioinformatic tools will facilitate to identify more cost effective peptide sequences that are highly active without associated toxicity, will significantly boost using AMPs as next generation therapeutic antibiotics.

References

1. Zasloff M: Antimicrobial peptides of multicellular organisms. Nature 2002; 415:89-95
2. Demain AL, Sanchez S: Microbial drug discovery: 80 years of progress. The Journal of antibiotics 2009; 62:5-16.
3. Jenssen H, Hamill P, Hancock RE: Peptide antimicrobial agents. Clinical microbiology reviews 2006; 19:491-511.
4. Kaufmann BB, Hung DT: The fast track to multidrug resistance. Molecular cell 2010; 37:297-8.
5. Aloush V, Navon-Venezia S, Seigman-Igra Y, Cabili S, Carmeli Y: Multidrug-resistant Pseudomonas aeruginosa: risk factors and clinical impact. Antimicrobial agents and chemotherapy 2006; 50:43-8.
6. Manchanda V, Sanchaita S, Singh NP: Multidrug resistant acinetobacter. Journal of global infectious diseases 2010; 2:291.
7. Baltzer SA, Brown MH: Antimicrobial peptides–promising alternatives to conventional antibiotics. Journal of molecular microbiology and biotechnology 2011; 20:228-35.
8. Lee M, Lee J, Carroll MW, Choi H, Min S, Song T, Via LE, Goldfeder LC, Kang E, Jin B, Park H: Linezolid for treatment of chronic extensively drug-resistant tuberculosis. New England Journal of Medicine 2012; 367:1508-1518.
9. García MS, De la Torre MÁ, Morales G, Peláez B, Tolón MJ, Domingo S, Candel FJ, Andrade R, Arribi A, García N, Sagasti FM: Clinical outbreak of linezolid-resistant Staphylococcus aureus in an intensive care unit. JAMA 2010; 303:2260-2264.
10. Wang S, Zeng X, Yang Q, Qiao S: Antimicrobial peptides as potential alternatives to antibiotics in food animal industry. International journal of molecular sciences 2016; 17:603.
11. Alanis AJ: Resistance to antibiotics: Are we in the post-antibiotic era?. Archives of medical research 2005; 36:697-705.
12. Cooper MA, Shlaes D: Fix the antibiotics pipeline. Nature 2011; 472:32.
13. Brown KL, Mookherjee N, Hancock RE. Antimicrobial, host defence peptides and proteins. Encyclopedia of Life Sciences. Chichester, Wiley, 2007; 1–11.
14. Hancock RE. Cationic peptides: effectors in innate immunity and novel antimicrobials. The Lancet infectious diseases. 2001; 1:156-64.
15. Andreu D, Rivas L: Animal antimicrobial peptides: an overview. Peptide Science 1998; 47:415-33.
16. Bradshaw JP: Cationic antimicrobial peptides. BioDrugs 2003; 17:233-40.
17. Parachin NS, Franco OL: New edge of antibiotic development: antimicrobial peptides and corresponding resistance. Frontiers in microbiology 2014; 5.
18. Fox JL: Antimicrobial peptides stage a comeback. Nature biotechnology 2013; 31:379.
19. Hancock RE, Scott MG: The role of antimicrobial peptides in animal defenses. Proc Nat Acad Sci. 2000; 97:8856-61.
20. Brown KL, Hancock RE: Cationic host defense (antimicrobial) peptides. Current opinion in immunology 2006; 18:24-30.
21. Zeya HI, Spitznagel JK: Cationic proteins of polymorphonuclear leukocyte lysosomes II. Composition, properties, and mechanism of antibacterial action. Journal of bacteriology 1966; 91:755-62.
22. Boman HG: Antibacterial peptides: basic facts and emerging concepts. Journal of internal medicine 2003; 254:197-215.
23. Zasloff M: Antibiotic peptides as mediators of innate immunity. Current opinion in immunology 1992; 4:3-7.
24. Hoskin DW, Ramamoorthy A: Studies on anticancer activities of antimicrobial peptides. Biochimica et Biophysica Acta (BBA)-Biomembranes 2008; 1778:357-75.
25. Chung YS, Kocks C: Recognition of pathogenic microbes by the Drosophila phagocytic pattern recognition receptor Eater. Journal of Biological Chemistry 2011; 286:26524-32.
26. Alalwani SM, Sierigk J, Herr C, Pinkenburg O, Gallo R, Vogelmeier C, Bals R: The antimicrobial peptide LL- 37 modulates the inflammatory and host defense response of human neutrophils. European journal of immunology 2010; 40:1118-26.
27. Van der Does AM, Bogaards SJ, Ravensbergen B, Beekhuizen H, van Dissel JT, Nibbering PH: Antimicrobial peptide hLF1-11 directs granulocyte-macrophage colony-stimulating factor-driven monocyte differentiation toward macrophages with enhanced recognition and clearance of pathogens. Antimicrobial agents and chemotherapy 2010; 54 :811-6.
28. Shai Y: Mode of action of membrane active antimicrobial peptides. Peptide Science 2002; 66:236-48.
29. Dawson RM, Liu CQ: Properties and applications of antimicrobial peptides in biodefense against biological warfare threat agents. Critical reviews in microbiology 2008; 34:89-107.
30. Hancock RE, Lehrer R: Cationic peptides: a new source of antibiotics. Trends in biotechnology 1998; 16:82-8.
31. Straus SK, Hancock RE: Mode of action of the new antibiotic for Gram-positive pathogens daptomycin: comparison with cationic antimicrobial peptides and lipopeptides. Biochimica et Biophysica Acta (BBA)-Biomembranes 2006; 1758:1215-23.
32. Appelt C, Schrey AK, Söderhäll JA, Schmieder P: Design of antimicrobial compounds based on peptide structures. Bioorganic & medicinal chemistry letters 2007; 17:2334-7.
33. Keymanesh K, Soltani S, Sardari S: Application of antimicrobial peptides in agriculture and food industry. World Journal of Microbiology and Biotechnology 2009; 25:933-44.
34. Giuliani A, Pirri G, Nicoletto S: Antimicrobial peptides: an overview of a promising class of therapeutics. Open Life Sciences 2007; 2:1-33.
35. Fjell CD, Hiss JA, Hancock RE, Schneider G: Designing antimicrobial peptides: form follows function. Nature reviews Drug discovery 2012; 11:37-51.
36. Ludtke SJ, He K, Heller WT, Harroun TA, Yang L, Huang HW: Membrane pores induced by magainin. Biochemistry 1996; 35:13723-8.
37. Rapaport D, Shai Y: Interaction of fluorescently labeled pardaxin and its analogues with lipid bilayers. Journal of Biological Chemistry 1991; 266:23769-75.
38. Gazit E, Miller IR, Biggin PC, Sansom MS, Shai Y: Structure and orientation of the mammalian antibacterial peptide cecropin P1 within phospholipid membranes. Journal of molecular biology 1996; 258:860-70.

39. Wimley WC, Hristova K: Antimicrobial peptides: successes, challenges and unanswered questions. The Journal of membrane biology 2011; 239:27-34.
40. Yeaman MR, Yount N:. Mechanisms of antimicrobial peptide action and resistance. Pharmacological reviews 2003; 55:27-55.
41. Hancock RE, Nijnik A, Philpott DJ: Modulating immunity as a therapy for bacterial infections. Nature Reviews Microbiology 2012; 10:243-54.
42. Steckbeck J, Deslouches B, Montelaro R: Antimicrobial peptides: new drugs for bad bugs? Expert Opinion on Biological Therapy 2013; 14:11-14.
43. Chen Y, Xu X, Hong S, Chen J, Liu N, Underhill CB, Creswell K, Zhang L: RGD-Tachyplesin inhibits tumor growth. Cancer research 2001; 61:2434-8.
44. Butler MS, Cooper MA: Antibiotics in the clinical pipeline in 2011. The Journal of antibiotics 2011; 64:413-25.
45. Kang SJ, Park SJ, Mishig-Ochir T, Lee BJ: Antimicrobial peptides: therapeutic potentials. Expert review of anti-infective therapy 2014; 12:1477-86.
46. Ahmad M, Hirz M, Pichler H, Schwab H: Protein expression in Pichia pastoris: recent achievements and perspectives for heterologous protein production. Applied microbiology and biotechnology 2014; 98:5301-17.
47. Helmerhorst EJ, Reijnders IM, van't Hof W, Veerman EC, Nieuw Amerongen AV: A critical comparison of the hemolytic and fungicidal activities of cationic antimicrobial peptides. FEBS letters 1999; 449:105-10.
48. Hancock R: Cationic antimicrobial peptides: towards clinical applications. Expert Opinion on Investigational Drugs 2000; 9:1723-1729.
49. Mylne JS, Wang CK, van der Weerden NL, Craik DJ: Cyclotides are a component of the innate defense of Oldenlandia affinis. Peptide Science 2010; 94:635-46.
50. Tam JP, Lu YA, Yang JL: Correlations of cationic charges with salt sensitivity and microbial specificity of cystine-stabilized β-strand antimicrobial peptides. Journal of Biological Chemistry 2002; 277:50450-6.
51. Chan DI, Prenner EJ, Vogel HJ: Tryptophan-and arginine-rich antimicrobial peptides: structures and mechanisms of action. Biochimica et Biophysica Acta (BBA)-Biomembranes 2006; 1758:1184-202.
52. Li Y: Recombinant production of antimicrobial peptides in Escherichia coli: a review. Protein expression and purification 2011; 80:260-7.
53. Bahar AA, Ren D: Antimicrobial peptides. Pharmaceuticals 2013; 6(12):1543-75.
54. Jiang Z, Vasil AI, Hale JD, Hancock RE, Vasil ML, Hodges RS: Effects of net charge and the number of positively charged residues on the biological activity of amphipathic α-helical cationic antimicrobial peptides. Peptide Science 2008; 90:369-83.
55. Pakkala M, Hekim C, Soininen P, Leinonen J, Koistinen H, Weisell J, Stenman UH, Vepsäläinen J, Närvänen A: Activity and stability of human kallikrein- 2- specific linear and cyclic peptide inhibitors. Journal of Peptide Science 2007; 13:348-53.
56. Nguyen LT, Chau JK, Perry NA, De Boer L, Zaat SA, Vogel HJ: Serum stabilities of short tryptophan-and arginine-rich antimicrobial peptide analogs. PloS one 2010; 5:e12684.
57. Meng H, Kumar K: Antimicrobial activity and protease stability of peptides containing fluorinated amino acids. Journal of the American Chemical Society 2007; 129:15615-22.
58. Kim H, Jang JH, Kim SC, Cho JH: De novo generation of short antimicrobial peptides with enhanced stability and cell specificity. Journal of Antimicrobial Chemotherapy 2014; 69:121-32.
59. Zhang L, Benz R, Hancock RE: Influence of proline residues on the antibacterial and synergistic activities of α-helical peptides. Biochemistry 1999; 38:8102-11.
60. Strömstedt AA, Pasupuleti M, Schmidtchen A, Malmsten M: Evaluation of strategies for improving proteolytic resistance of antimicrobial peptides by using variants of EFK17, an internal segment of LL-37. Antimicrobial agents and chemotherapy 2009; 53593-602.
61. Nguyen LT, Schibli DJ, Vogel HJ: Structural studies and model membrane interactions of two peptides derived from bovine lactoferricin. Journal of Peptide Science 2005; 11:379-89.
62. Kim JY, Park SC, Yoon MY, Hahm KS, Park Y: C-terminal amidation of PMAP-23: translocation to the inner membrane of Gram-negative bacteria. Amino Acids 2011; 40:183-95.
63. Malmsten M, Kasetty G, Pasupuleti M, Alenfall J, Schmidtchen A: Highly selective end-tagged antimicrobial peptides derived from PRELP. PloS one 2011; 6:e16400.
64. Chou HT, Kuo TY, Chiang JC, Pei MJ, Yang WT, Yu HC, Lin SB, Chen WJ: Design and synthesis of cationic antimicrobial peptides with improved activity and selectivity against Vibrio spp. International journal of antimicrobial agents 2008; 32:130-8.
65. Juretić D, Vukičević D, Petrov D, Novković M, Bojović V, Lučić B, Ilić N, Tossi A: Knowledge-based computational methods for identifying or designing novel, non-homologous antimicrobial peptides. European biophysics journal 2011; 40:371-85.
66. Aoki W, Ueda M: Characterization of antimicrobial peptides toward the development of novel antibiotics. Pharmaceuticals 2013; 6:1055-81.
67. Su Y, Waring AJ, Ruchala P, Hong M: Structures of β-hairpin antimicrobial protegrin peptides in lipopolysaccharide membranes: mechanism of Gram selectivity obtained from solid-state nuclear magnetic resonance. Biochemistry 2011; 50:2072-83.
68. Svensäter G, Sjögreen B, Hamilton IR: Multiple stress responses in Streptococcus mutans and the induction of general and stress-specific proteins. Microbiology 2000; 146:107-17.
69. Aoki W, Kitahara N, Miura N, Morisaka H, Kuroda K, Ueda M: Design of a novel antimicrobial peptide activated by virulent proteases. Chemical biology & drug design 2012; 80:725-33.
70. Brandelli A: Nanostructures as promising tools for delivery of antimicrobial peptides. Mini reviews in medicinal chemistry 2012; 12:731-41.
71. Yount NY, Yeaman MR: Emerging themes and therapeutic prospects for anti-infective peptides. Annual review of pharmacology and toxicology 2012; 52:337-60.
72. de Azevedo J, Walter F, Dias R: Computational methods for calculation of ligand-binding affinity. Current drug targets 2008; 9:1031-9.
73. Robinson JA: Protein epitope mimetics as anti-infectives. Current opinion in chemical biology 2011; 15:379-86.
74. Matyus E, Kandt C, Tieleman DP: Computer simulation of antimicrobial peptides. Current medicinal chemistry 2007; 14:2789-98.
75. Jorgensen WL: Efficient drug lead discovery and optimization. Accounts of chemical research 2009; 42:724-33.
76. Engel T: Basic overview of chemoinformatics. Journal of chemical information and modeling. 2006; 46:2267-77.
77. Schneider G, Baringhaus KH: Molecular design: concepts and applications. (John Wiley & Sons) 2008.
78. Koch U, Hamacher M, Nussbaumer P: Cheminformatics at the interface of medicinal chemistry and proteomics. Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics 2014; 1844:156-61.
79. Da Costa JP, Cova M, Ferreira R, Vitorino R: Antimicrobial peptides: an alternative for innovative medicines? Applied microbiology and biotechnology 2015; 99:2023-40.
80. Marr AK, Gooderham WJ, Hancock RE: Antibacterial peptides for therapeutic use: obstacles and realistic outlook. Current opinion in pharmacology 2006; 6:468-72.
81. Ramesh S, Govender T, Kruger HG, Torre BG, Albericio F: Short AntiMicrobial Peptides (SAMPs) as a class of extraordinary promising therapeutic agents. Journal of Peptide Science 2016; 22:438-51.
82. Hilpert K, Mikut R, Ruden S, Roland Berecz: Antimicrobial peptides for treatment of infectious diseases WO2013053772 A1.
83. Kang HK, Kim C, Seo CH, Park Y: The therapeutic applications of antimicrobial peptides (AMPs): a patent review. Journal of Microbiology 2017; 55:1-2.