Review article

Unveiling mechanisms of antimicrobial peptide: Actions beyond the membranes disruption

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A B S T R A C T

Background: Antimicrobial peptides (AMPs) are a critical component of the innate immune system, playing a key role in defending against a variety of pathogenic microorganisms. While many AMPs act primarily on the cell membrane of target pathogens, leading to lysis and subsequent cell death, less is known about their nonlytic membrane activity. This nonlytic activity allows AMPs to target and disrupt bacterial cells without causing lysis, leading to bacterial death through alternative mechanisms.Understanding these nonlytic properties of AMPs is crucial, as they present a promising alternative to traditional antibiotics, which can induce bacterial resistance and have adverse effects on human health and the environment. The mechanisms by which AMPs exhibit nonlytic membrane activity are still being explored. However, it is believed that AMPs penetrate the bacterial membrane and interact directly with internal cellular components such as DNA, RNA, and various enzymes essential for microbial survival and replication. This interaction disrupts metabolic homeostasis, ultimately resulting in bacterial death.The nonlytic activity of AMPs also results in minimal damage to host cells and tissues, making them attractive candidates for the development of new, more effective antibiotics. This review emphasizes the mechanisms by which AMPs nonlytically target cellular components, including DNA, proteins, RNA, and other biomolecules, and discusses their clinical significance. Understanding these mechanisms may pave the way for developing alternatives to conventional antibiotics, offering a solution to the growing issue of antibiotic resistance.

1. Background

Antimicrobial resistance in microorganisms has become a critical concern due to the misuse of antibiotics in medicine, agriculture, and animal husbandry, particularly in developing nations [1]. Pharmaceutical companies face significant challenges in developing new antibiotic drugs, primarily due to their low profitability. Consequently, industries are increasingly exploring alternative options to replace conventional antibiotics [2].The innate immune system, recognized as the more ancient and rudimentary form of immunity compared to the acquired or adaptive immune response [3], plays a fundamental role in protecting the host from a broad array of toxins and infectious agents, including bacteria, viruses, fungi, and parasites. Among the various components of innate immunity, antimicrobial peptides (AMPs) are integral element s [4]. AMPs are vital components in both prokaryotic and eukaryotic organisms

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List of abbreviations

AMPs	Antimicrobial peptides
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
NMR	Nuclear magnetic resonance
CD	Circular dichroism
FITC	Fluorescein isothiocyanate
ct	Calf thymus
IFNs	Interferons
PrAMPs	Proline-rich antimicrobial peptides
Prf	Peptide chain release factor
EF	Elongation factor
EB	Ethidium bromide
RF	Release factor
MIC	Minimum inhibitory concentration
POP	Human prolyl oligopeptidase
ARGO	Acquired Resistance induced by Gene Overexpression
WAP	Whey acidic protein
RTS	Rapid translation system
UDP-MurNAc	Uridine diphosphate N-acetylmuramic acid
FtsZ	Filamenting temperature-sensitive mutant Z
RNAP	RNA polymerase

[4]. These small peptides, typically consisting of 12–50 amino acids, exhibit broad-spectrum antibacterial, antifungal, and antiviral capabilities, making them highly valuable for therapeutic and preventive applications in pharmacology [5].AMPs possess unique properties that make it challenging for bacteria to develop resistance, unlike conventional antibiotics. This is due to their diverse range of targets and mechanisms of action [6]. AMPs primarily interact with bacterial cell membranes through electrostatic interactions [7]. There are four commonly recognized modes of action that describe the membrane activity of AMPs: the barrel-stave model, the carpet model, the aggregate/cluster model (micellization), and the toroidal-pore model [8].In the toroidal-pore model, the peptide first binds to the membrane, and then incoming monomer units aggregate, causing the inward folding of lipid moieties in both outer and inner membranes. This results in the formation of a continuous channel lined by multiple peptide units, with the peptide tightly associated with the lipid head groups of membrane phospholipids [9]. The carpet model suggests that the accumulation of peptides on the outer membrane triggers the formation of localized weaknesses, leading to the gradual disintegration of the membrane structure, which takes on a carpet-like pattern [10]. The barrel-stave model differs in that peptide monomers align parallel to the membrane's phospholipids, forming a transmembrane channel lined with the hydrophilic side of the peptides facing inward and the hydrophobic side facing outward, interacting with the lipid core of the bilayer [11]. In the aggregate/cluster model (micellization), AMPs aggregate on the membrane surface, forming non-specific clusters or micelle-like structures. These aggregates disrupt the membrane by extracting lipids, causing localized disturbances that lead to membrane thinning, curvature, and eventually cell lysis.Beyond their ability to disrupt cell membranes, research indicates that AMPs may also impact bacterial viability through intracellular mechanisms of action [12].

Numerous studies have explored the possibility that AMPs may target intracellular components within bacterial cells (Fig. 1). However, the precise mechanisms by which certain AMPs penetrate bacterial cells remain under active investigation [13]. Proposed mechanisms suggest that certain peptides, such as proline-rich AMPs, initially bind to the bacterial surface and subsequently translocate into the cell through the formation of transient pores, ultimately exerting their effects on intracellular targets [14]. Additionally, some research indicates that AMPs can cross bacterial membranes without forming pores, possibly through receptor-mediated transport [15,16]. Once inside, these molecules may target intracellular macromolecules and disrupt critical biological processes, including the inhibition of DNA replication, transcription, and translation [17].

Furthermore, AMPs have demonstrated the ability to inhibit key enzymes involved in various essential bacterial functions, such as protein folding, cell division, cell wall synthesis, ion transport, and other vital metabolic processes [15–19]. Table 1 illustrates the specific targets and modes of action of these antimicrobial peptides. There is growing interest in designing synthetic AMPs with enhanced stability, specificity, and reduced toxicity. While many AMPs function by disrupting microbial membranes, others specifically target intracellular components. Additionally, AMPs play a role in modulating the host immune response, highlighting their dual role in direct antimicrobial activity and immune modulation—an emerging area of interest. Given the rapid advancements and expanding applications of AMPs, an updated review is essential to synthesize recent findings, address current challenges, and identify future research directions. The objective of this review is to provide a comprehensive overview of the intracellular targets and non-lytic mechanisms exhibited by antimicrobial peptides, with a specific emphasis on their effectiveness against bacteria. Furthermore, the review aims to elucidate the clinical significance of AMPs in treating bacterial infections.
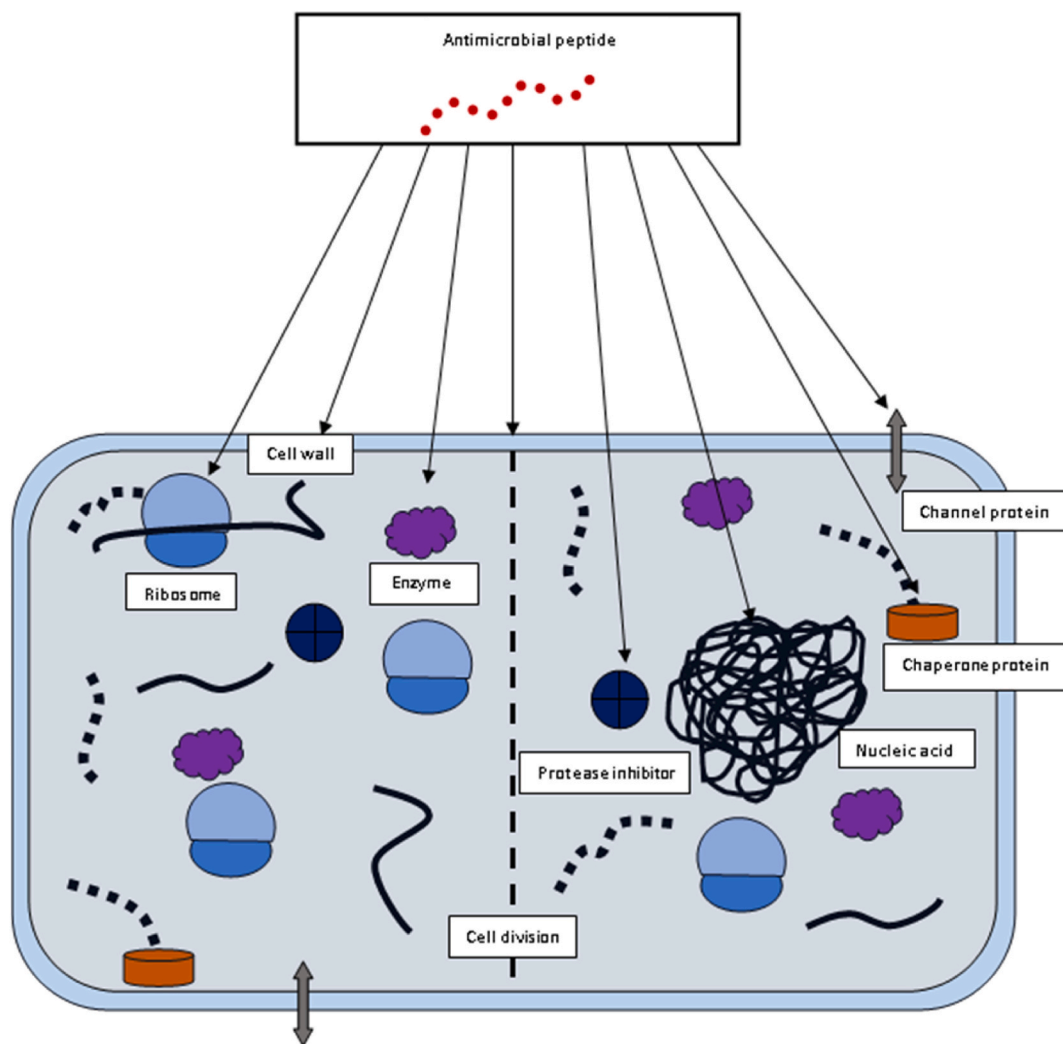
Fig. 1. Nonmembrane intracellular targets of the antimicrobial peptides.

2. Methodology

This systematic review was conducted by searching relevant literature published between 1998 and 2024, using key terms such as "AMPs acting on intracellular targets" and "non-lytic mechanisms of AMPs." To ensure accessibility and transparency, only freely available research articles were included in the review. No articles were intentionally selected or omitted to avoid bias, and the inclusion criteria were strictly based on the relevance of the content to the review's objectives.

Antimicrobial peptides (AMPs), more recently referred to as host defense peptides, are found across virtually all forms of life. These peptides are produced by organisms ranging from bacteria to plants, vertebrates, and invertebrates (Fig. 1). In bacteria, AMPs benefit individual species by eliminating competing bacterial species that vie for the same nutrients and environmental niches. Bacterial AMPs, known as bacteriocins, are classified into two categories: lantibiotics and non-lantibiotics. Lantibiotics are AMPs that contain the non-natural amino acid lanthionine.

Nisin, a well-known lantibiotic, was one of the first AMPs to be isolated and characterized, extracted from Lactococcus lactis in 1947 [1]. It exhibits potent activity against a variety of Gram-positive bacteria, with a minimum inhibitory concentration (MIC) in the nanomolar range, and has been used as a food preservative for 50 years without significant development of resistance [2]. Other bacteriocins, such as mersacidin, have also been studied for their potential use against antibiotic-resistant Gram-positive bacteria [3]. Most AMPs identified to date are of eukaryotic origin, derived from plants, animals, and fungi (Fig. 1). Since 1885, bodily fluids such as blood, sweat, saliva, plasma, white blood cell secretions, and granule extracts have been recognized for their antimicrobial properties [4]. However, it wasn't until 1981 that Hans Boman reported that the hemolymph (plasma and blood) of the silk moth (Hyalophora cecropia) contained AMPs known as cecropins [4]. These cationic and amphipathic peptides display broad-spectrum activity, being effective against a wide range of microorganisms, including Gram-positive and Gram-negative bacteria and fungi.The field expanded

Table 1
Nonmembrane target of the antimicrobial peptide and their mode of action.

AMPs	Sequences	Source	Mode of action/Target
Indolicidin (IR-13)	ILPWKWPWWPWRR	Neutrophils of cows	Inhibition of DNA replication and transcription
Buforin-II	TRSSRAGLQFPVGRVHRLLRK	Stomach tissue of Asian toad	Targeting DNA
Cathelicidins (LL-37)	LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES	Mammals	Nucleic acids of plasmacytoid dendritic cells
Chensinensi	SAVGRHGRRFGLRKHRKH	Skin secretions of the Chinese brown frog, *Rana chensinensis*	Electrostatics interaction with DNA
AN5-1	YSKSLPLSVLNP	Paenibacillus alvei	Intercalation binding with DNA
Apidaecin (Api-137)	GNNRPVYIPQPRPPHPRI	Honey bees	Release factor 1 (PrfA)/ribosome
Bactenecin (Bac-5 and Bac-7)	RFRPPIRRPPIRPPFYPPFRPPIRPPIFPPIRPPFRPPLGPFP	Mammalian neutrophils	Ribosome & DnaK
Drosocin	GKPRPYSPRPTSHPRPIRV	Fruit fly (*Drosophila melanogaster*)	Class 1 release factor of ribosome
Pyrrhocoricin	VDKGSYLPRPTPPRPIYNRN	Insects, arthropods, invertebrates, animals	Misreading of tRNA in ribosome
Oncocin (Onc112)	VDKPPYLPRPPPPRRIYNNR	Milkweed bug (*Oncopeltus fasciatus*)	Peptidyl transferase center of the ribosome
Micrococcin p1	SCTTCVCTCSCCTT	*Staphylococcus equorum*	Ribosomal protein L11
Wheat antimicrobial peptides (1a &1b)	AQRCGDQARGAKCPNCLCCGKYGFCGSGDAYCGAGSCQSQCRG/AQRCGDQARGAKCPNCLCCGKYGFCGSGDAYCGAGSCQSQCRGCR	Seeds of *Triticum kiharae*	Fungalysin
The human secretory leukocyte protease inhibitor (SLPI)	SGKSFKAGVCPPKKSAQCLRYKKPECQSDWQCPGKKRCCPDTCGIKCLDPVDTPNPTRRKPGKCPVTYGQCLMLNPPNFCEMDGQCKRDLKCCMGMCGKSCVSPVKA	Leukocyte of human	Serine proteases
Potamin-1 (PT-1)	DICTNCCAGTKGCNTTSANGAFICEGQSDPKKPKACPLNCDPHIAYA	Potato tubers.	Chymotrypsin, trypsin, and papain
Bovine pancreatic trypsin inhibitor (BPTI).	RPDFCLEPPYTGPCKARMIRYFYNAKAGLCQPFVYGGCRAKRNNFKSSEDCMRTCGGA	Bovine source	Serine protease enzymes
PSI-1.2	KACPRNCDTDIAYMVCPSSGERIIRKVCTNCCAAQKGCKLFRSNGSIKCTGT	Potato tubers	Trypsin and Chymotrypsin
Psysol 2	GLPICGESCVGGTCNTPGCTCTWPVCTRN	*Psychotria solitudinum*	Prolyl oligopeptidase

Table 1 (*continued*)

AMPs	Sequences	Source	Mode of action/ Target
Kunitzin	AAKIILNPKFRCKAAFC	Skin secretions of *Rana esculenta*	Trypsin
Bldesin	GWGCNIFGGNDYRCHRHCKSISGYKGGYCKLGGICKC	Blastomyces dermatitidis	Chymotrypsin protease, elastase, and serine protease
Histatin 5	DSHAKRHHGYKRKFHEKHHSHRGY	Primates	Clostripain
SWD	PTRHSKPRPQPLPRPGTCPDTSGIITTCEVTERNCFSDSQCGPGQKCCPLGCGRECLAVGPPYGKGRW	Hemocytes of the black tiger shrimp	Subtilisin A
Plectasin	GFGCNGPWDEDDMQCHNHCKSIKGYKGGYCAKGGFVCKCY	Insect source	Lipid II
Nisin	ITSISLCTPGCKTGALMGCNMKTATCNCSIHVSK	Streptococcus spp.	Lipid II
Copsin	QNCPTRRGLCVTSGLTACRNHCRSCHRGDVGCVRCSNAQCTGFLGTTCTCINPCPRC	*Basidiomycete coprinopsis*	Ljipid II
Mersacidin	CTFTLPGGGGVCTLTSECIC	Bacillus strain	Lipid II
Pep5	TAGPAIRASVKQCQKTLKATRLFTVSCKGKNGCK	*Staphylococcus epidermidis*	Teichoic and teichuronic acids
L27-11	TWLKKRRWKKAKPP	Synthetic	OstA protien
POL7001	–	Synthetic	Lipid II
Mutacin 1140	KSWSLCTPGCARTGSFNSYCC	Streptococcus spp	Lipid II
Temporin L	FVQWFSKFLGRIL	Skin of *Rana temporaria.*	FtsZ protein
Microcin 25	GGAGHVPEYFVGIGTPISFYG	*Escherichia coli*	Filamentation of the cell
Human α-defensin 5	ATCYCRTGRCATRESLSGVCEISGRLYRLCCR	Gut of human	Bleb formation, cellular elongation, and clumping
CRAMP	GLLRKGGEKIGEKLKKIGQKIKNFFQKLVPQPE	*Bacillus subtilis*	FtsZ protein
Pyrrhocoricin	VDKGSYLPRPTPPRPIYNRN	Insect source	DnaK and GroEL
Apidaecin	GNNRPVYIPQPRPPHPRI	Insect source	DnaK and GroEL
Drosocin	GKPRPYSPRPTSHPRPIRV	Fruit fly	DnaK
Abaecin	YVPLPNVPQGRRPFPTFPGQGPFNPKIKWPQGY	Bumblebees	DnaK
Bac7	RRIRPRPPRLPRPRPRPLPFPRPGPRPIPRPLPFPRPGPRPIPRPLPFPRPGPRPIPRP	Neutrophils of Mammals	DnaK
SPINK9	KQTKQMVDCSHYKKLPPGQQRFCHHMYDPICGSDGKTYKNDCFFCSKVKKTDGTLKFVHFGKC	Skin of human	SKP
LL-37	LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES	Mammals	Palmitoyl transferase
microcin J25 (MccJ25)	GGAGHVPEYFVGIGTPISFYG	*Escherichia coli*	Bacterial RNA polymerase
Melitti	GIGAVLKVLTTGLPALISWIKRKRQQ	*Honeybee venom*	Ca-ATPase
MIAP	GIGKFLHSAGKFGKAFVGEIMKS	Analogue of Magainin-I	F1F0-ATPase
Human neutrophil peptide	ACYCRIPACIAGERRYGTCIYQGRLWAFCC	Human neutrophil	Phospholipid/Ca2+ protein kinase

(*continued on next page*)

Table 1 (*continued*)

AMPs	Sequences	Source	Mode of action/Target
Lipid transfer proteins (LTP1& (LTP2)	ITCQQVTSELGPCVPYLTGQGIP/ITCQQVTAELEPCVPYLTQGIP	Plant source	α-amylase inhibiition
Hp-1935	KLSPSLGPVSKGKLLAGQR	Skin secretions of *Boana pulchella*	Acetylcholinesterase
Griselimycin	VPSLPLVPLG	Streptomyces	Sliding clamp DnaN
Microcin H47	GGAPATSANAAGAAAIVGALAGIPGGPLGVVVGAVSAGLTTGIGSTVGSGSASSSAGGGS	Human	FoF1 ATP synthase
β -KTx	KSTVGQKLKKKLNQAVDKVKEVLNKSEYMCPVVSSFCKQHCARLG	*Scorpion venom*	potassium channel
AtPDF2.3	RTCESKSHRFKGPCVSTHNCANVCHNEGFGGGKCRGFRRRCYCTRHC	Scorpion	blocks Kv1.2 and Kv1.6 potassium channels
Bldesin	GWGCNIFGGNDYRCHRHCKSISGYKGGYCKLGGICKCY	Fungaldefensin	Kv1.3 channel/chymotrypsin
BmKDfsin4	GFGCPFNQGQCHKHCQSIRRRGGYCDGFLKTRCVCYR	Scorpion defensin	Kv1.1, Kv1.2, and Kv1.3 potassium channels

further when researchers such as Robert Leher, Shunji Natori, and Michael Zasloff isolated and described defensins [5] (from mammalian macrophages), sarcotoxins [6] (from fly larvae), and magainins [7] (from the skin of frogs *Xenopus laevis*), respectively.

2.1. History of AMP

The discovery of AMPs dates back to 1939, when Dubos [20]. extracted an antimicrobial agent from a soil Bacillus strain. This extract was demonstrated to protect mice from pneumococci infection. In the following year, Hotchkiss and Dubos [21] fractionated this extract and identified an AMP named gramicidin. Despite some reported toxicity associated with intraperitoneal application, gramicidin was found effective for topical treatment of wounds and ulcers [22]. In 1941, another AMP, tyrocidine, was discovered and found to be effective against both Gram-negative and Gram-positive bacteria [23]. However, tyrocidine exhibited toxicity to human blood cells [24]. In the same year, another AMP was isolated from the plant *Triticum aestivum* [25]. 1942, which was later named purothionin and found effective against fungi and some pathogenic bacteria [26].

The first reported animal-originated AMP is defensin, which was isolated from rabbit leukocytes in 1956 [27]. In the following years, bombinin from epithelia [28] and lactoferrin from cow milk [31] were both described. During the same time, it was also proven that human leukocytes contain AMPs in their lysosomes [29].

In total, more than 5000 AMPs have been discovered or synthesized up to date [30]. Natural AMPs can be found in both prokaryotes (e.g., bacteria) and eukaryotes (e.g., protozoan, fungi, plants, insects, and animals) [31–34]. In animals, AMPs are mostly found in the tissues and organs that are exposed to airborne pathogens and are believed to be the first line of innate immune defense [38–39] against viruses, bacteria, and fungi [35]. Thus, AMPs play an important role in stopping most infections before they cause any symptoms. For example, frog skin is the source of more than 300 different AMPs [36].

Most AMPs are produced by specific cells at all times, while the production of some AMPs is inducible. For example, using silk moth as a model system, Hultmark and colleagues [37] demonstrated that P9A and P9B can be induced in hemolymph by vaccination with *Enterobacter cloacae*. In another study [38], epithelial cells from different tissues of mice showed an increased rate of mRNA transcription for defensin production after infection with *Pseudomonas aeruginosa* PAO1.

Several types of eukaryotic cells are involved in AMP production, such as lymphocytes, epithelial cells in gastrointestinal and genitourinary systems [39,40], phagocytes [41], and lymphocytes of the immune system [42]. In addition to direct involvement in innate immunity, AMPs have also been found to influence the host's inflammatory responses during an infection [43–45]. It is known that lipopolysaccharide (LPS) molecules, released from bacteria as a result of antibiotic treatment or host immunity, can induce AMP production in mammals [41]. For example, HEK293 cells produce defensin in response to LPS stimulation [46]. Some AMPs (e.g., CAP18 [47], CAP35 [48], and a lactoferrin-derivative [49]) can also block LPS-induced cytokine release by macrophages. Thus, these AMPs can reduce the inflammatory response. In comparison, antibiotics do not have this type of regulation on the inflammatory response of the host immune system; and LPS secretion following antibiotic treatment might cause over-reaction of the host immune system.

2.2. AMPs targeting nucleic acid and its biosynthesis

The mode of action of AMPs is widely recognized to involve the inhibition of bacterial DNA replication and transcription [50]. However, the precise mechanisms by which AMPs bind to DNA and interfere with transcription remain to be fully understood. Gel retardation assays commonly show that AMPs affect the mobility of DNA/RNA in agarose gels. In addition, various low-resolution methodologies, including micropatterned surface chemistry microscopy and spectroscopic techniques such as fluorescence and circular dichroism (CD), in conjunction with high-resolution NMR spectroscopy, are widely used for studying AMP and nucleic acid interactions [51].

This review discusses AMPs that target nucleic acids inside cells. Tachyplesin I, for instance, is a 17-amino-acid cationic antimicrobial peptide that adopts an antiparallel beta-sheet conformation connected by a beta-turn with two disulfide bridges. Tachyplesin I binds to the minor groove of DNA duplexes, and studies have presented initial findings regarding the interaction between DNA and small peptides with distinctive antiparallel β-sheet conformations [52].

Indolicidin is another short AMP, consisting of 13 amino acids with frequent tryptophan and proline residues, derived from the cytoplasmic granules of neutrophils in cows. Despite its bactericidal activity and ability to disrupt bacterial membranes, indolicidin does not cause cell lysis [53]. It hinders bacterial DNA synthesis, leading to bacterial cell filamentation. The PWWP motif in IR13 (a variant of Indolicidin) has a distinctive structural feature that enables it to bind and stabilize duplex B-type DNA structures. Substituting the central Trp-Trp pair with Ala-Ala, His-His, or Phe-Phe residues in the PXXP motif significantly impacts the peptide's capacity to stabilize duplex DNA. Microscopic investigations and spectroscopic data confirm that IR13 stabilizes the DNA duplex, leading to the inhibition of both DNA replication and transcription [54]. Indolicidin's binding to DNA has been shown in gel retardation and fluorescence quenching experiments, with further sequence selectivity towards certain DNA sequences, although it exhibited limited binding capability towards the ds [GT] sequence [55]. Additionally, indolicidin can inhibit topoisomerase I, a key enzyme involved in DNA replication, supporting the notion that AMPs commonly exhibit multiple actions affecting DNA at the molecular level [56].

Buforin I, a 39-amino-acid peptide initially identified in the stomach tissue of the Asian toad (Bufo garagriozans), has a derivative, Buforin II, consisting of 21 amino acids. Buforin II is more potent and devoid of significant hemolytic activity [61]. Buforin II shares complete sequence identity with the N-terminal region of the histone H2A protein and exhibits a helical structure [62]. Experimental findings using fluorescein isothiocyanate (FITC)-labeled Buforin II revealed its ability to hinder cellular processes by binding to the

DNA and RNA of cells upon membrane penetration, leading to rapid cell death. This mechanism notably differs from that of magainin 2, despite their structural similarities as linear amphipathic α-helical pept ides [63]. The evaluation of various Buforin II variants in relation to DNA binding affinity showed a correlation between antimicrobial activity and relative DNA binding affinity. Buforin IIb, however, displayed cytotoxic effects on mammalian cells, including human red blood cells (RBCs) and fibroblasts, at high concentrations, thereby reducing the therapeutic index. To address this, novel cell-penetrating AMPs derived from Buforin IIb, named Buforin III analogs, were designed based on structural information. Buforin IIIb and Buforin IIIc exhibited increased antimicrobial activity and decreased hemolytic activity, resulting in a seven-fold improvement in the therapeutic index. The enhanced antimicrobial activity of Buforin IIIb and IIIc is attributed to their higher helical content and DNA-binding affinity compared to Buforin IIb and other analogs [64].

Cathelicidins, a class of host defense peptides (HDPs), are encoded by a single gene consisting of four exons. These peptides are synthesized initially as pre-pro-peptides, featuring a signal peptide at the N-terminus that directs the peptide to secretory granules. Within the pre-pro-peptide lies the conserved cathelin domain and an active mature peptide at the C-terminus [65]. Upon release by neutrophils, cathelicidins, such as human cathelicidin LL-37, are in an inactive pro-peptide state and require extracellular enzymes like elastase or proteinase-3 to liberate the biologically active C-terminal peptide [66]. LL-37 functions by interacting with molecules on the bacterial cell wall, leading to perforations in the cytoplasmic membranes and eventual bacterial cell death [67]. Recent research indicates that LL-37 also facilitates the detection of unmethylated CpG motifs, resulting in significant IFN-α production by plasmacytoid dendritic cells. Type I IFNs, particularly IFN-α/β, are pivotal in the innate immune response, directly combating viral infections and promoting adaptive immune responses. Phylogenetic analysis reveals that porcine myeloid antimicrobial peptide (PMAP)-36, among porcine cathelicidins, shares the closest relationship with human LL-37. Porcine cathelicidins efficiently interact with nucleic acids in plasmacytoid dendritic cells, leading to robust IFN-α release, suggesting a role for nucleic acid-peptide interactions in immune modulatio n [68].

Chensinin-1 is a naturally occurring peptide extracted from the skin secretions of the Chinese brown frog (*Rana chensinensis*). A designed analogue, Chensinin-1b, exhibited potent broad-spectrum antimicrobial activity due to its enhanced hydrophobicity and amphipathicity. To further enhance anticancer properties, three aliphatic analogs were developed by attaching lipid acids to the 17th lysine residue within the sequence. The binding of Chensinin-1b and its lipo-analogs to calf thymus (ct) DNA has been demonstrated using multiple biophysical techniques, including UV–Visible absorbance, agarose gel electrophoresis, and dynamic light scattering measurements. Complex formation between ctDNA and the antimicrobial Chensinin-1b and its lipo-analogs has been observed, with significant conformational changes in ctDNA, including increased helicity and disruption of base pair stacking. Iodide quenching and viscosity measurements suggest an electrostatic binding mechanism between the peptides and ctDNA, rather than intercalation between DNA base p airs [69].

AN5-1 is an antimicrobial peptide discovered in the fermentation broth of Paenibacillus alvei strain AN5. It exhibits the ability to disrupt bacterial membranes, thereby hindering cellular functions. In addition, AN5-1 targets genomic DNA as its secondary target, as demonstrated by genomic DNA binding activity assays and spectroscopy. The decrease in emission intensity at 590 nm in the DNA–ethidium bromide (EB) system was observed upon the introduction of AN5-1 to DNA. The observed fluorescence quenching indicates that AN5-1 can displace EB from the DNA–EB complex, suggesting that AN5-1 may interact with DNA through intercalation [57]. Reduced mobility in gel retardation assays confirms the plausible interaction between several antimicrobial peptides and model DNA [58].

Despite these findings, the precise mechanisms behind the successful antimicrobial activity of AMPs, particularly from the perspective of AMP-DNA interactions, still require further elucidation. DNA or RNA plays a vital role in protein synthesis and cell proliferation, potentially acting as an intracellular target for various AMPs. Understanding these interactions can help develop new strategies to combat antibiotic resistance. In addition to the AMPs discussed above, peptides like human hepcidin, pseudin-2, ABP-CM4, Lasioglossin II, pBD2, AN5-1, YD1, Phibilin, and bacteriocin LFX01 have demonstrated the ability to interact with nucleic acids, as confirmed by gel retardation assays or ultraviolet spectrometry. Their impact on antimicrobial activity remains a promising area for further research [59–70].

2.3. AMPs targeting ribosome

The interaction between antimicrobial peptides (AMPs) and ribosomes is crucial for understanding the mechanism of action of AMPs and their potential as antimicrobial agents. Ribosomes, the molecular machines responsible for protein synthesis in cells, consist of two subunits: the small ribosomal subunit (30S in bacteria) and the large ribosomal subunit (50S in bacteria). These subunits work together to read the genetic information in messenger RNA (mRNA) and synthesize proteins by linking amino acids in the correct order [71].

AMPs interact with ribosomes in various ways, often disrupting protein synthesis at multiple stages. A key interaction involves AMPs binding to ribosomal RNA (rRNA) within the ribosome, particularly to the 16S rRNA in the small subunit of bacterial ribosomes. This binding can impair ribosomal function, leading to inhibition of protein synthesis and subsequent microbial cell death. AMPs can also interact with ribosomal proteins, further affecting protein synthesis and ribosomal integrity. The exact mechanisms of these interactions vary depending on the type of AMP and the microorganism involved [72].

2.4. Proline-rich antimicrobial peptides (PrAMPs)

Recent research has highlighted the effectiveness of proline-rich antimicrobial peptides (PrAMPs) in traversing bacterial

membranes and disrupting protein synthesis. PrAMPs are particularly effective against Gram-negative bacteria and exhibit minimal toxicity to eukaryotic cells, making them promising candidates for antimicrobial drug development [73]. Structural and biochemical studies have identified the binding sites of PrAMPs on the ribosome, revealing that most PrAMPs bind within the nascent polypeptide exit tunnel, though their mechanisms of action differ [74].

For instance, AMPs like Bac7, Onc112, pyrrhocoricin, and metalnikowin obstruct the transfer of aminoacyl-tRNA by EF-Tu to the ribosomal A-site, while others like apidaecin 1b and Api137 interfere during translation termination by capturing release factors on the 70S ribosome after the nascent polypeptide chain has been hydrolyzed [75].

2.5. Apidaecins

Apidaecins I and II, derived from honeybees, are small peptides with a high proline content (up to 33 %) that display strong antibacterial activity against Gram-negative bacteria without damaging cellular membranes [76]. Despite their high proline content, which theoretically limits helical structure formation, Apidaecins are potent antimicrobial agents. Research has shown that Apidaecins competitively bind to the A-site of ribosomes, specifically inhibiting the termination of translation associated with the UAG stop codon, recognized solely by peptide chain release factor 1 (PrfA) [77].

High-resolution cryo-electron microscopy (cryo-EM) studies have provided insights into the interaction between the ribosome, RF1 (PrfA), and Api137. These studies revealed that Api137 binds within the ribosomal exit tunnel, inhibiting the release of newly synthesized proteins by interacting with 23S rRNA nucleotides and ribosomal protein uL4 [78].

2.6. Bactenecin

Bactenecin, an antimicrobial peptide from bovine neutrophils, contains proline and arginine residues, accounting for over 65 % of its amino acid composition. Bac7(1–35), a functional segment of bactenecin, is known to inhibit protein synthesis by binding to bacterial ribosomes. It has been shown to enter *Escherichia coli* cells through the inner membrane protein SbmA and selectively hinders specific ribosomal subunits or associated proteins. This interaction suggests a primary inhibition of translation rather than other cellular processes like transcription or DNA replication [79,80].

The mode of action of another bactenecin fragment, Bac5, has also been studied. Bac5 exerts its inhibitory effect within the ribosomal tunnel, preventing the transition from initiation to elongation during translation. Interestingly, Bac5 shows species-specific inhibition, strongly affecting protein synthesis in *E. coli* but not in Thermus thermophilus, suggesting distinct modes of interaction despite sharing a binding site with Bac7 [81].

2.7. Drosocin

Drosocin, a 19-residue peptide from *Drosophila melanogaster*, binds to ribosomes and stalls them at stop codons, likely by capturing class 1 release factors associated with the ribosome, similar to the mechanism of apidaecin [82]. Recent studies have shown that while only a few amino acids in the C-terminal region of apidaecin are crucial for binding, Drosocin interacts with multiple residues throughout its sequence, indicating a more complex interaction with the ribosome [83].

2.8. Pyrrhocoricin

Pyrrhocoricin is another potent AMP known for its non-toxicity in healthy mammals and its effectiveness in eliminating resistant bacteria. Its mechanism involves binding to the bacterial heat shock protein DnaK, disrupting chaperone-assisted protein folding and inhibiting ATPase activity. However, bacterial strains lacking DnaK remain susceptible to pyrrhocoricin, suggesting additional intracellular targets. Studies using in vitro protein synthesis systems have shown that pyrrhocoricin primarily inhibits translation, similar to the antibiotic streptomycin [84].

2.9. Oncocin

Oncocin is a group of AMPs derived from the milkweed bug *Oncopeltus fasciatus*. These peptides inhibit protein synthesis not by membrane lysis but by binding to the bacterial ribosome. Onc112, a derivative of oncocin, blocks both the peptidyl transferase center and the peptide-exit tunnel, effectively halting translation [85].

2.10. Micrococcin P1

Micrococcin P1, a macrocyclic peptide antibiotic from Staphylococcus equorum (commonly found on French raclette cheese), targets the ribosome. X-ray crystallography has shown that Micrococcin P1 binds between ribosomal protein L11 and helices 43 and 44 of the 23S rRNA, disrupting the binding of translation factor EF-G. This disruption impairs the turnover of EF-G during protein synthesis, leading to the inhibition of protein synthesis [86,87].

2.11. Protease inhibitor antimicrobial peptides

Proteases serve as key virulence factors (VFs) in the progression of diseases caused by diverse pathogens. Their ability to cleave peptide bonds within proteins and peptides grants them significant virulence potential, facilitating the pathogen's invasion into host tissues. By targeting various host molecules, both intracellular and extracellular, proteases play a crucial role in evading host defenses [88].

Protease inhibitors are a broad and diverse group of proteins and peptides that effectively impede the proteolytic functions of proteases across various organisms [89]. Several antimicrobial peptides (AMPs) exhibit both protease-inhibiting and antimicrobial properties as part of the host defense mechanism, with many sourced from plants.

Wheat Antimicrobial Peptides (WAMPs) 1a and 1b, identified in the seeds of Triticum kiharae, a robust wheat variety, are noteworthy examples [90]. Upon fungal pathogen attack, fungi secrete compounds that compromise plant defenses. Among these, fungalysin, a proteinase from Fusarium verticillioides, degrades class IV chitinases, which are crucial plant defense proteins targeting fungal cell wall chitin. WAMPs, as novel protease inhibitors, antagonize fungalysin, thereby impeding Fusarium verticillioides activity. This inhibition underscores the significance of WAMPs as protease inhibitors in plant defense [91,92].

Potamin-1 (PT-1) is a small peptide derived from potato tubers, with a molecular weight of 5.6 kD. Sequence analysis reveals that PT-1 shares 62 % similarity with a serine protease inhibitor from the Kunitz family. Experimental studies have demonstrated that PT-1 inhibits chymotrypsin, trypsin, and papain. PT-1's polypeptide chains are interconnected by disulfide bridges, which are crucial for maintaining its protease inhibitory and antifungal properties, as reduction of these bridges significantly diminishes PT-1's activity [93].

Basic Pancreatic Trypsin Inhibitor (BPTI) is a small polypeptide composed of 58 amino acids with a mass of 6512 Da. It is stabilized by three disulfide bonds. In the family of BPTI-like peptides, the P1 site at position 15, occupied by Arginine (Arg) or Lysine (Lys), is crucial for its activity. BPTI shows high stability against various environmental factors, such as temperature, pH, organic solvents, and proteolytic degradation [94]. BPTI exhibits broad specificity in inhibiting serine protease enzymes, including trypsin, chymotrypsin, and elastase. The formation of stable enzyme-inhibitor complexes may involve multiple intermediates and discrete reaction steps. Additionally, BPTI inhibits nitric oxide synthase type-I and -II and interferes with the transport of potassium ions through calcium-activated potassium channels [95].

PSI-1.2, a 52-amino-acid cysteine-rich polypeptide from the potato type II inhibitor family, is characterized by its high cysteine content and exhibits inhibitory activity against trypsin and chymotrypsin enzymes [96].

Human prolyl oligopeptidase (POP) is an enzyme involved in memory and learning processes and is considered a therapeutic target for cognitive deficits in psychiatric and neurodegenerative diseases such as schizophrenia and Parkinson's disease. There is considerable scope for discovering and developing POP inhibitors with favorable pharmacokinetic properties [97]. Cyclotides, a class of peptides with protease inhibitory activity, could serve as promising starting points due to their stability in biological fluids and potential oral bioavailability. Several cyclotide extracts from Psychotria solitudinum, Psychotria poeppigiana, Psychotria capitata, and Viola tricolor have shown inhibitory effects on purified human POP, indicating their potential as therapeutic POP inhibitors [98].

Kunitzins, peptides from the skin secretions of the European frog species *Rana esculenta* and Chinese frog species Odorrana schmackeri, consist of 17 amino acids. These peptides, characterized by an N-terminal Ala (A) residue and a C-terminal Cys (C) residue connected by a disulfide bridge, exhibit potent inhibitory activity against bacteria [99]. Synthetic replicas of these peptides, kunitzin-RE and kunitzin-OS, have shown strong inhibitory effects on trypsin. When Lys-13, a key residue for protease inhibitory activity, is replaced with Phe (F), the trypsin inhibitory and antimicrobial activities against *Escherichia coli* decrease, though inhibition against chymotrypsin increases [100].

Bldesin, a fungal defensin, exhibits inhibitory activity against Kv1.3 channels and serine proteases. In antimicrobial assays, Bldesin is potent against Gram-positive *Staphylococcus aureus*, but not Gram-negative *Escherichia coli*. It uniquely inhibits chymotrypsin, elastase, and serine protease-associated coagulation activities [101].

Histatin 5 (Hst 5), a histidine-rich peptide produced exclusively in humans and higher primates, is a competitive inhibitor of clostripain, a cysteine proteinase from Clostridium histolyticum. With an inhibition constant (Ki) of 10 nM, Hst 5 remains resistant to proteolysis at physiological concentrations [102].

SWD proteins, found in the hemocytes of the black tiger shrimp *Penaeus monodon*, contain a single whey acidic protein (WAP) domain in their C-terminal region. Three isoforms, SWDPm1, SWDPm2, and SWDPm3, share characteristics with type III crustin proteins in shrimp, including a Pro-Arg region and a WAP domain. Purified recombinant SWDPm2 (rSWDPm2) has shown antibacterial activity against several Gram-positive bacteria and acts as a competitive inhibitor of subtilisin A, suggesting its role in regulating subtilisin A activity and protecting against Gram-positive bacterial infections [103].

2.12. AMPs inhibiting cell wall synthesis

Peptidoglycan, a key component of bacterial cell walls, is essential for maintaining bacterial integrity and survival. Lipid II, a crucial precursor in peptidoglycan synthesis, plays a significant role in this process. Certain antimicrobial peptides (AMPs) have a specific affinity for lipid II, inhibiting cell wall formation and making them effective therapeutic agents. Targeting lipid II is particularly advantageous because it is unique to bacterial cells and absent in eukaryotic cells, minimizing the risk of off-target effects in human cells. This specificity makes compounds that interfere with lipid II synthesis appealing as potential antibiotics, similar to traditional antibiotics [104,105].

Plectasin, a 40-amino acid fungal peptide with structural similarities to defensins found in various organisms (e.g., spiders,

scorpions, dragonflies, and mussels), has been extensively studied for its role in inhibiting cell wall biosynthesis. Plectasin exerts its antimicrobial activity by directly binding to lipid II. In vitro assays have confirmed that lipid II is its cellular target, forming a stoichiometric complex with plectasin. Nuclear magnetic resonance spectroscopy and computational modeling have further identified key residues involved in this interaction [105].

Defensin 3, another AMP, also targets cell wall biosynthesis, although its mechanism differs from that of plectasin. Transmission electron microscopy has revealed localized protrusions in the cytoplasmic contents of bacteria treated with Defensin 3. These findings suggest a buildup of the soluble final precursor UDP-MurNAc-pentapeptide, although Defensin 3 does not impair the overall biosynthetic capacity of cells or cause gross leakage of small cytoplasmic compounds [106].

Nisin, a member of the lantibiotic family, has a remarkable ability to form pores in the bacterial cell wall by binding to lipid II. This interaction significantly enhances nisin's membrane-permeabilizing activity by three orders of magnitude. The nisin-lipid II complex features a unique lipid II-binding motif, where the pyrophosphate moiety of lipid II is coordinated by the N-terminal backbone amides of nisin through intermolecular hydrogen bonds [107].

Copsin, a defensin from the basidiomycete Coprinopsis cinerea, has been shown to bind specifically to lipid II, interfering with cell wall biosynthesis. Unlike lantibiotics and other defensins, copsin's effective binding relies on the presence of a specific amino acid at the third position of the lipid II pentapeptide [108].

Mersacidin, a polycyclic peptide from the Bacillus strain HIL-Y 85,54728, has both antibiotic and immunosuppressive properties. It selectively targets the conversion of lipid II into polymeric nascent glycan strands through transglycosylation. Mersacidin's mechanism is distinct from that of vancomycin, as it inhibits peptidoglycan formation from UDP-N-acetylmuramoyl-tripeptide and remains active against Enterococcus faecium with the vanA resistance gene cluster, suggesting a unique molecular target [109–111].

Pep5, a lantibiotic produced by Staphylococcus epidermidis, indirectly causes cell wall lysis by activating lytic enzymes. Pep5's strong binding affinity to teichoic and teichuronic acids in Gram-positive bacteria's cell walls displaces cell wall-associated amidases, leading to premature autolytic enzyme release and subsequent cell lysis [112].

Two peptides, L27-11 and POL7001, have been developed to target LptD, a protein involved in outer membrane biogenesis in Gram-negative bacteria. These peptides do not significantly increase cell permeability but instead bind to LptD, disrupting its function. This leads to defects in the outer membrane structure, resulting in internal accumulation of membrane-like materials and alterations in lipid A composition [113].

Mutacin 1140 (MU1140), produced by Streptococcus mutans, shares structural similarities with nisin and also binds to lipid II. Molecular simulations of the MU1140-lipid II complex suggest that a single MU1140-lipid II complex can transport water molecules across the bacterial wall through a single-file water transport mechanism. At higher concentrations, this interaction can disrupt bacterial wall integrity, providing valuable insights for developing improved peptide variants with enhanced antimicrobial properties [114,115].

2.13. AMPs inhibiting cell division

Antimicrobial peptides (AMPs) can hinder bacterial cell division, leading to the formation of elongated cellular structures. This effect is often attributed to the interaction of AMPs with the transmembrane protein PhoQ, which, upon exposure, phosphorylates PhoP. This phosphorylation triggers the overexpression of QueE, a protein that obstructs the formation of the divisome complex, or suppresses outer membrane development, leading to structural irregularities in the membrane [116]. Researchers have used a combination of fluorescence microscopy, mass spectrometry, and bioinformatics analyses to investigate the impact of AMPs on cell division [117].

Several key proteins are involved in bacterial cell division, including FtsZ, FtsA, MurG, MukB, and MreB. These proteins work in a highly regulated cellular machinery to ensure the safe separation of the cell into two daughter cells. FtsZ, which shares similarities with bacterial tubulin, is crucial in this process and is expressed in both Gram-positive and Gram-negative cells. It initiates bacterial cell division by polymerizing into filaments using GTP molecules, leading to the formation of a ring-like structure called the Z ring at the division site. Subsequently, FtsZ recruits other proteins to create the divisome complex, which drives the constriction of the cell envelope, ultimately resulting in cell division. AMPs are reported to target these proteins, inhibiting cell division and causing filamentation [117].

Temporin L (TL), a naturally occurring peptide produced in the skin of the European frog *Rana temporaria*, exhibits significant activity against both Gram-positive and Gram-negative bacteria, including *E. coli* [85]. Temporin L can penetrate the bacterial outer membrane and specifically targets FtsZ, inhibiting its GTPase activity through competitive inhibition. This impairs bacterial cell division, leading to the formation of long cell filaments and eventual cell death. However, Temporin L's haemolytic activity limits its potential as a standalone antibiotic, although sequence optimization could reduce this drawback [118].

Microcin 25, a peptide antibiotic secreted by *Escherichia coli*, consists of approximately 20 amino acid residues. Exposure to microcin 25 causes bacterial cells to form filaments, indicating that it disrupts cell division [104].

Human α-defensin 5 (HD5), a 32-residue cysteine-rich host-defense peptide, is known for its broad-spectrum antimicrobial activity and plays a significant role in innate immunity. In its oxidized form (D5ox-HD5), it exhibits three regiospecific disulfide bonds, leading to distinct morphological changes in *Escherichia coli* and other Gram-negative bacteria, such as bleb formation, cellular elongation, and clumping. Fluorescence studies have shown that HD5ox specifically localizes to the site of cell division and cell pole formation, suggesting its involvement in the cell division process [84].

The α-helical peptide CRAMP, which shares sequence similarities with a 40-amino acid peptide found in Bacillus subtilis, has been studied for its effects on Salmonella cell division. CRAMP causes the formation of long filamentous structures in Salmonella, indicating

impaired cell division. Additionally, a related peptide from Bacillus subtilis inhibits the tubulin-like protein FtsZ, preventing improper Z-ring formation during sporulation. CRAMP has been found to bind to FtsZ and inhibit its assembly and GTPase activity in vitro. Computational analysis suggests that CRAMP binds to the cavity of the T7 loop of FtsZ, involving both hydrophobic and ionic interactions, leading to the malfunction of FtsZ [118].

2.14. AMPs inhibiting the chaperon proteins

Chaperones play a critical role in facilitating the correct folding and assembly of recently synthesized proteins. Among these, DnaK stands out as a major bacterial heat shock protein 70 (Hsp70) and serves as a vital component in the chaperone pathways in bacteria [119]. A group of AMPs are able to inhibit bacterial growth by interrupting the protein-folding pathway. AMPs interfere with protein folding are discussed here.

Short antimicrobial peptides (AMPs) derived from insects, ranging from 18 to 20 amino acids in length, have demonstrated the ability to inhibit bacterial growth by interfering with the protein-folding pathway. Some notable examples include pyrrhocoricin, which was isolated from the hemipteran insects. Pyrrhocoricin, apidaecin, and drosocin exhibit robust interactions with bacterial lipopolysaccharides (LPS) in their solution form, suggesting that their multiple inhibitory actions predominantly commence with the initial contact with a component of the bacterial cell wall [87]. Notably, both pyrrhocoricin and apidaecin effectively interact with DnaK and GroEL and disrupting the ATPase activity of DnaK (103–105). On the other hand, drosocin effectively inactivates DnaK and GroEF [87]. These AMPs prevent DnaK from refolding misfolded proteins by inducing a permanent closure of the DnaK peptide-binding cavity [120]. Importantly, the binding of these three antimicrobial peptides to DnaK exhibits a highly specific spatial orientation, rendering them advantageous in terms of cell selectivity, thereby preventing toxicity, as they do not target the human equivalent chaperone Hsp70 [121].

Abaecin, a linear peptide found in bumblebees, exhibits antimicrobial properties only when it associates with another antimicrobial peptide called hymenoptaecin from the same organism. The mechanism of action involves abaecin interacting with the bacterial chaperone DnaK. However, to effectively penetrate the bacterial membrane and access its intracellular target(s) to exert its full antimicrobial activity, abaecin relies on the pore-forming action of AMPs like hymenoptaecin. This cooperative action allows abaecin to display its complete efficacy against the target microorganism [122].

The peptide Bac7, belonging to the proline-rich cathelicidin group, effectively eliminates bacteria in a specific manner by entering target cells without causing any observable damage to the cell membrane. It achieves this antibacterial action by binding to intracellular targets that remain unidentified. A study using affinity chromatography identified a single protein with high affinity in *Escherichia coli* cytoplasmic protein lysates, which was later identified as DnaK. This suggests that DnaK might play a crucial role in mediating the antibacterial effect of Bac7 within the bacterial cells [123]. SPINK9 is a potent antibacterial peptide isolated from healthy human skin. Six different N-terminal variants of SPINK9 in the outermost layer of the skin. In *Escherichia coli* cells, a bacterial chaperone known as SKP has been identified as the primary interacting partner of SPINK9. When the SKP gene was deleted in mutant bacteria, they became more susceptible to the bactericidal effects of SPINK9 compared to the wild-type control. This finding suggests that the bactericidal activity of SPINK9 must overcome the resistance imposed by the bacterial chaperone SKP [124].

2.15. AMPs interfere with the activity of the different enzyme

Antibacterial peptides are recognized for their ability to inhibit bacterial enzymes through two primary mechanisms. First, they can function as pseudo-substrates, misleading the bacterial enzymes into binding with them rather than their natural substrates. Second, they can bind tightly to the active site of the bacterial enzymes, preventing the access of native substrates and disrupting normal enzymatic function. These actions contribute to combating bacterial infections and represent a promising avenue for developing novel antimicrobial treatments [125].

The mutant humanized antibacterial peptide LL-37 has demonstrated an enhanced antibacterial effect due to its inhibition of palmitoyl transferase. This enzyme is responsible for transferring a palmitate residue from a phospholipid to lipid A or its precursors in the outer membrane of certain Gram-negative bacteria, playing a crucial role in maintaining membrane integrity [126].

Microcin J25 (MccJ25), another antibacterial peptide, inhibits bacterial RNA polymerase (RNAP) by binding within its secondary channel, the natural target for this enzyme. This secondary channel is essential for the entry of NTP substrates into the active site of RNAP, where catalysis occurs. By obstructing the folding of the trigger loop, a vital step for efficient catalysis, MccJ25 hinders the binding of NTP substrates, thereby inhibiting RNA polymerase activity [127].

Melittin, a natural peptide sourced from bee venom, completely inhibits Ca-ATPase activity, as revealed through time-resolved phosphorescence and fluorescence spectroscopy. This inhibition is attributed to the restriction of microsecond protein rotational motion caused by Melittin. The presence of Melittin decreases the fraction of more mobile monomer/dimer species while increasing the fractions of larger oligomers and very large aggregates. The inhibitory effect of Melittin on Ca-ATPase is closely associated with its capacity to induce enzyme aggregation [128].

MIAP, a designed peptide and analog of magainin-I, was created with improved antimicrobial properties. When tested against *Mycobacterium tuberculosis*, MIAP demonstrated a two-fold increase in antimicrobial activity compared to magainin-I. Additionally, the presence of antimicrobial peptides (AMPs) reduced the basal ATPase activity of mycobacterial plasma membrane vesicles by approximately 24–30 %. MIAP, however, exhibited a more potent effect by completely abolishing the activity of the F1F0-ATPase, an enzyme responsible for H+ pumping across *M. tuberculosis* plasma membranes. This inhibition suggests that AMPs can interfere with bacterial internal pH regulation, potentially impacting bacterial viability [129].

Human neutrophil peptides (HNPs), another class of antimicrobial peptides, inhibit phospholipid/Ca2+-dependent protein kinase C (PKC), which is involved in the phosphorylation of endogenous proteins in the rat brain. HNP-2 is more potent than HNP-1 and HNP-3. HNPs inhibit PKC noncompetitively with respect to phosphatidylserine (a phospholipid cofactor), Ca2+ (an activator), ATP (a phosphoryl donor), and histone H1 (a substrate protein). HNPs have little or no effect on myosin light chain kinase (a calmodulin/Ca2+-dependent protein kinase) and the holoenzyme or catalytic subunit of cyclic AMP-dependent protein kinase, indicating a specificity of HNPs [130].

Lipid transfer proteins (LTPs) are a prominent class of AMPs found in plants. Among these, Coffea canephora seeds contain a specific peptide called *Cc*-LTP1, which exhibits potent fungicidal activity, particularly against pathogenic yeast. *Cc*-LTP1 also possesses α-amylase inhibitory properties, a novel finding for this peptide family [131].

Hp-1935, a naturally occurring peptide obtained from the skin secretions of the Argentinian frog Boana pulchella, is identified as an antimicrobial peptide with anti-acetylcholinesterase (AChE) properties. In the context of drug development for Alzheimer's disease (AD), traditional approaches have focused on targeting the formation of senile plaques and regulating acetylcholine (ACh) levels. However, ACh regulation crucially involves acetylcholinesterase, the enzyme responsible for its hydrolysis at the synaptic level. Compounds with inhibitory activity against acetylcholinesterase can enhance the duration of ACh after its release, offering potential relief from AD symptoms [132].

Griselimycin derivatives, produced by Streptomyces species, have shown remarkable efficacy against *Mycobacterium tuberculosis* in both laboratory conditions and live animal models. These derivatives inhibit the sliding clamp DnaN, an essential DNA polymerase component. Resistance to griselimycins is rare, as it is associated with the amplification of a specific chromosomal segment containing the dnaN gene and the ori site. These findings highlight the potential of griselimycins for tuberculosis treatment and validate DnaN as a promising target for antimicrobial therapy [133].

2.16. Channel protein modulating AMPs

Ion transporters are recognized as a distinct class of channel proteins crucial for regulating ion homeostasis. They traverse cell membranes by temporarily binding ions, facilitating their passage through the lipid barrier. Various synthetic and biomolecules can modulate the activity of ion transporters, with potential applications in cancer therapy and sensitization. These molecules include naturally occurring biomolecules such as peptides and antibiotics, as well as synthetically designed compounds like polyethers and crown ethers [134,135]. Additionally, ion transporters have been proposed as antimicrobial agents, offering the added benefit of immunomodulatory activity [136]. However, effective therapeutic application of these transporters faces challenges related to formulation and targeting. One potential strategy to overcome these hurdles involves selective modulation at pathological sites. Antimicrobial peptides with channel protein-modulating activity are summarized below [135].

The discovery of human β-defensin 2 (hBD2) as a Kv1.3 channel inhibitor has revealed a unique molecular mechanism and novel immune modulatory function, suggesting that human β-defensins represent a new class of channel ligands. hBD2 has been observed to suppress IL-2 secretion by blocking the Kv1.3 channel. Unlike hBD1, hBD2 interacts simultaneously with the extracellular S1-S2 linker and pore region of the Kv1.3 channel. Chimeric channel and mutagenesis experiments have shown that hBD1 binds only to the extracellular pore region, excluding the S1-S2 and S3-S4 linkers. These findings provide valuable insights into hBD2 as a novel immune-related Kv1.3 channel blocker and underscore the significant functional distinctions between hBD1 and hBD2 [137].

The antibiotic action of microcin H47 relies on the presence of the ATP synthase complex in *E. coli*, which consists of a membrane-bound Fo sector and a cytoplasmic F1 sector composed of eight distinct polypeptides. The presence of the FoF1 ATP synthase complex is essential for the antibiotic activity of microcin H47. Alterations in the Fo portion of ATP synthase impacted the antibiotic's effectiveness, with the Fo proton channel being essential for microcin H47 action, while the F1 catalytic portion was dispensable [105].

β-KTx peptides, a class of scorpine antimicrobial peptides, are characterized by three disulfide bridges and exhibit two identifiable domains: a flexible N-terminal sequence and a compact C-terminal region with a cysteine-stabilized α/β (CS-αβ) motif. These peptides display cytolytic, antimicrobial, and K+ channel-blocking activities, with a remarkable structural and functional diversity. The full-length peptide can induce cell lysis or combat microorganisms, while the C-terminal domain, containing the CS-αβ motif, is responsible for K+ channel blockade [138].

In *Arabidopsis thaliana*, the defensin AtPDF2.3 exhibits a toxin signature (K-C5-R-G) in its amino acid sequence, similar to scorpion toxins. Both AtPDF2.3 and scorpion toxins share a common CS-αβ motif, indicating a potential evolutionary relationship between antimicrobial plant defensins and scorpion toxins. Synthetically produced recombinant AtPDF2.3 blocks Kv1.2 and Kv1.6 potassium channels, similar to scorpion toxins. A recombinant variant, rAtPDF2.3 [G36N], with a KCXN toxin signature, shows higher potency in channel blocking, while the variant rAtPDF2.3 [K33A], lacking the toxin signature, exhibits reduced channel-blocking activity. However, the antifungal activity and Kv channel inhibitory function appear unrelated in these variants [138].

Bldesin, a fungal defensin, has been identified as a promising natural peptide with significant potential in antimicrobial drug development. It exhibits potent activity against Gram-positive *Staphylococcus aureus* and inhibits the Kv1.3 channel. Additionally, Bldesin demonstrates unique inhibitory effects on chymotrypsin, elastase, and serine protease-associated coagulation activities [139].

The scorpion defensin BmKDfsin4 from Mesobuthus martensii is a unique peptide with 37 amino acids, adopting a conserved CS-α/β structural fold. It has been found to inhibit Kv1.1, Kv1.2, and Kv1.3 potassium channels, similar to scorpion neurotoxins. Critical interactions between BmKDfsin4 and Kv1.3 involve basic residues Lys 13 and Arg 19. The peptide binds to the extracellular pore region of the channel, suggesting a mechanism akin to classical scorpion toxins. This discovery highlights defensins as a novel class of potassium channel blockers [139–141].

3. Predictive models explain mechanism of action of AMPs

3.1. Molecular dynamics (MD) simulations

Molecular Dynamics (MD) simulations have become a cornerstone in the study of antimicrobial peptides (AMPs) due to their ability to provide detailed, atomistic insights into the interactions between AMPs and microbial membranes. These simulations model the physical movements of atoms and molecules over time, allowing researchers to observe and predict how AMPs interact with lipid bilayers, proteins, and other cellular components in real time. The foundation of MD simulations is rooted in classical mechanics, where the forces between atoms are calculated based on potential energy functions (force fields), and these forces dictate the movement of atoms according to Newton's laws of motion.

MD simulations offer unparalleled insights into the mechanisms of action of AMPs, particularly in their ability to disrupt microbial membranes. The insertion and integration of AMPs into lipid bilayers are crucial steps in their mechanism of action. By simulating these processes, MD studies can reveal how specific structural features of AMPs, such as amphipathicity (the presence of both hydrophobic and hydrophilic regions), charge distribution, and secondary structure (e.g., alpha-helices or beta-sheets), contribute to their ability to destabilize membranes. For example, MD simulations have demonstrated that the amphipathic nature of AMPs allows them to align with the lipid bilayer, inserting their hydrophobic regions into the membrane's core while their hydrophilic regions interact with the polar head groups of the lipids [142]. This interaction often leads to the thinning of the membrane, pore formation, or complete membrane disruption.

One significant advancement in the application of MD simulations is the ability to model larger systems and longer time scales. Traditionally, MD simulations were limited by computational resources, restricting the size of the system and the duration of the simulation. However, with the advent of enhanced sampling techniques such as replica exchange molecular dynamics (REMD), metadynamics, and accelerated MD, researchers can now overcome energy barriers that would be insurmountable in conventional simulations. These techniques allow for the exploration of rare events, such as the transition states during pore formation, which are critical for understanding the full mechanism of AMP action [143].

Moreover, the development of polarizable force fields has improved the accuracy of MD simulations by allowing for a more realistic representation of electronic polarizability. This is particularly important in simulating the interactions between charged AMPs and the polar head groups of membrane lipids. Polarizable force fields account for the redistribution of electron density in response to the local electrostatic environment, leading to more accurate predictions of the binding affinities and insertion energies of AMPs [144].

Recent studies have also highlighted the utility of MD simulations in understanding the specificity of AMPs toward different microbial targets. For instance, by simulating the interaction of AMPs with membranes composed of different lipid compositions, researchers can predict which peptides are more effective against certain types of bacteria. Gram-positive and Gram-negative bacteria, for example, have distinct membrane compositions, and MD simulations can help design AMPs that preferentially target one over the other by optimizing interactions with specific lipid species, such as phosphatidylglycerol or cardiolipin [145].

Another exciting application of MD simulations is in the study of AMPs' effects on intracellular targets, such as nucleic acids or proteins. While the primary mode of action for many AMPs is membrane disruption, some AMPs can penetrate cells and interact with intracellular components. MD simulations have been used to model these interactions, providing insights into how AMPs can inhibit protein synthesis or induce oxidative stress by interacting with DNA or RNA [146].

In summary, MD simulations are a powerful tool for unraveling the complex mechanisms of action of AMPs. They provide a detailed, atomistic view of how these peptides interact with microbial membranes and other targets, leading to membrane disruption and cell death. The ongoing advancements in computational power, enhanced sampling techniques, and the development of more accurate force fields continue to expand the capabilities of MD simulations, making them an indispensable tool in the design and optimization of new AMPs.

3.2. Quantitative Structure-Activity Relationship (QSAR) models

Quantitative Structure-Activity Relationship (QSAR) models are an essential computational tool in the field of drug discovery and design, including the study of antimicrobial peptides (AMPs). QSAR models establish a quantitative link between the chemical structure of a compound and its biological activity, enabling the prediction of the activity of new compounds based on their structural features. These models rely on the concept that similar structures are likely to exhibit similar activities, and by identifying the key molecular descriptors—such as hydrophobicity, charge distribution, molecular weight, and specific functional groups—QSAR models can predict the antimicrobial potency of novel AMPs.

The development of QSAR models involves several critical steps: data collection, descriptor calculation, model building, and validation. Initially, a dataset of known AMPs with experimentally determined activities is compiled. Molecular descriptors that quantitatively describe the chemical structure of each peptide are then calculated. These descriptors might include physicochemical properties, such as hydrophobicity or hydrophilicity, electronic properties, such as charge distribution, and structural properties, such as the presence of alpha-helices or beta-sheets. Advanced computational tools and software, such as MOE (Molecular Operating Environment) or Dragon, are often used to calculate these descriptors.

Once the descriptors are calculated, statistical or machine learning methods are employed to build a predictive model. Techniques such as multiple linear regression (MLR), partial least squares (PLS), and more recently, machine learning algorithms like random forests, support vector machines (SVM), and neural networks, are used to establish the relationship between the molecular descriptors and the biological activity of the peptides [147]. The model is then validated using cross-validation techniques and tested on an

independent dataset to assess its predictive power.

QSAR models are particularly advantageous in the early stages of AMP design because they allow for the rapid screening of large libraries of peptides. Instead of synthesizing and testing each peptide experimentally, QSAR models can predict which peptides are likely to exhibit strong antimicrobial activity, significantly reducing the time and cost associated with experimental screening. Moreover, these models can provide insights into the structural features that are most critical for antimicrobial activity, guiding the rational design of new AMPs with enhanced efficacy and specificity.

Recent advancements in QSAR modeling have focused on incorporating more complex and informative descriptors, as well as utilizing machine learning techniques to improve prediction accuracy. For example, three-dimensional QSAR (3D-QSAR) models consider the spatial arrangement of atoms within the peptide, allowing for a more detailed representation of the peptide's interaction with microbial targets. Additionally, the use of deep learning techniques, which can handle large and complex datasets, has led to the development of QSAR models with significantly improved predictive capabilities [148].

One of the significant challenges in QSAR modeling is ensuring that the model is robust and generalizable. A model that is overfitted to the training data may perform well on the training set but fail to predict the activity of new peptides accurately. To address this, recent approaches have emphasized the importance of using diverse training datasets that cover a broad range of chemical space. Additionally, multitask QSAR models have been developed to predict multiple properties simultaneously, such as antimicrobial activity, toxicity, and stability, providing a more comprehensive evaluation of potential AMP candidates [148].

Another critical application of QSAR models is in predicting the toxicity and side effects of AMPs. While AMPs are generally considered to be less toxic than conventional antibiotics, some peptides can still exhibit cytotoxicity toward host cells or disrupt host cell membranes. QSAR models can be used to predict these potential side effects, enabling the design of AMPs that are both potent against pathogens and safe for host cells [147].

QSAR models are a powerful tool for the rational design and optimization of AMPs. By linking molecular structure to biological activity, these models allow for the rapid screening of peptide libraries, guiding the design of new AMPs with enhanced efficacy and reduced toxicity. The ongoing advancements in computational methods and the incorporation of machine learning techniques continue to expand the capabilities of QSAR models, making them an indispensable tool in the fight against multidrug-resistant pathogens.

3.3. Machine learning models

Machine learning (ML) has revolutionized the field of computational biology, offering powerful tools for analyzing complex datasets and uncovering patterns that may not be immediately apparent through traditional methods. In the context of antimicrobial peptides (AMPs), ML models have become increasingly important due to their ability to predict the antimicrobial activity, specificity, and potential toxicity of novel peptides based on large datasets of known AMPs. These models can learn from vast amounts of data, identifying key features that contribute to a peptide's activity and generalizing these findings to predict the properties of new, unseen peptides.

The application of machine learning in AMP research typically involves several steps: data collection, feature extraction, model training, and validation. Initially, a comprehensive dataset of AMP sequences and their corresponding antimicrobial activities is collected. These sequences are then transformed into numerical features that can be used by machine learning algorithms. Common features include amino acid composition, sequence length, physicochemical properties (e.g., hydrophobicity, charge), and structural motifs (e.g., alpha-helices, beta-sheets). Advanced techniques, such as natural language processing (NLP) tools, can also be employed to extract more complex features from peptide sequences [149].

Once the features are extracted, the next step is to train a machine learning model. Various algorithms can be used, including traditional methods like support vector machines (SVM), decision trees, and random forests, as well as more advanced techniques like neural networks and deep learning models. Deep learning, in particular, has shown great promise in AMP research due to its ability to automatically learn hierarchical features from raw sequence data, capturing complex patterns that may not be accessible through simpler models [150].

Deep learning models, such as convolutional neural networks (CNNs) and recurrent neural networks (RNNs), have been used to predict the antimicrobial activity of peptides with high accuracy. CNNs, which are particularly effective at detecting spatial patterns in data, can identify important motifs within peptide sequences that are associated with antimicrobial activity. RNNs, on the other hand, are well-suited for handling sequential data, making them ideal for analyzing peptide sequences and predicting their activity based on the order of amino acids [149].

One of the key advantages of machine learning models is their ability to handle large and complex datasets, which is crucial given the vast diversity of AMP sequences and the complexity of their interactions with microbial targets. Machine learning models can analyze these datasets to identify the most important features that contribute to antimicrobial activity, guiding the design of new peptides with optimized properties. For example, by training a model on a dataset of known AMPs and their activities, researchers can use the model to predict the activity of new peptides, identifying candidates that are likely to be effective against specific pathogens [150].

Recent advancements in machine learning have also focused on integrating these models with other computational tools, such as molecular docking and molecular dynamics (MD) simulations, to provide a more comprehensive understanding of AMP mechanisms. For instance, machine learning models can predict not only the antimicrobial activity of a peptide but also its binding affinity to specific microbial targets, such as membrane proteins or nucleic acids. This integrated approach allows for the design of peptides that are not only potent but also highly specific, reducing the risk of off-target effects [149].

Furthermore, machine learning models are increasingly being used to predict the potential toxicity and side effects of AMPs. While AMPs are generally considered to be safer than conventional antibiotics, some peptides can still exhibit cytotoxicity or cause undesirable immune responses. By training machine learning models on datasets that include both effective and toxic peptides, researchers can identify patterns that are associated with toxicity, guiding the design of safer AMPs [149].

Another important application of machine learning in AMP research is the identification of novel AMP sequences. By analyzing large databases of natural peptides or even generating synthetic sequences, machine learning models can identify candidates that exhibit similar features to known AMPs, but with potentially enhanced activity or stability. This approach has led to the discovery of several new AMPs with promising antimicrobial properties, offering new avenues for the development of antibiotics [147].

Machine learning models are a powerful tool in the study and design of antimicrobial peptides. By leveraging large datasets and advanced algorithms, these models can predict the activity, specificity, and toxicity of novel peptides, guiding the rational design of new AMPs with optimized properties. The integration of machine learning with other computational tools, such as molecular docking and MD simulations, further enhances the predictive power of these models, making them an indispensable resource in the fight against multidrug-resistant pathogens.

3.4. Bioinformatics tools

Bioinformatics has played a pivotal role in advancing our understanding of antimicrobial peptides (AMPs), providing essential tools for the prediction and analysis of their mechanisms of action. These tools leverage vast databases of peptide sequences and biological information, enabling researchers to identify conserved motifs, predict antimicrobial activity, and explore potential interactions between AMPs and their microbial targets. Bioinformatics tools are indispensable for the discovery and design of new AMPs, as well as for understanding the molecular basis of their activity.

One of the most valuable resources in AMP research is the availability of comprehensive databases that catalog known AMPs and their properties. Databases such as the Antimicrobial Peptide Database (APD3) and the Collection of Antimicrobial Peptides (CAMP) provide extensive information on the sequences, structures, and activities of AMPs from a wide range of organisms [151]. These databases serve as a foundation for bioinformatics analyses, allowing researchers to compare novel peptides with known AMPs, identify conserved motifs that are critical for activity, and predict the antimicrobial potential of new sequences.

Sequence alignment tools are among the most commonly used bioinformatics tools in AMP research. These allow for the comparison of peptide sequences to identify regions of similarity and conservation, which can provide insights into the structural features that are important for antimicrobial activity. Multiple sequence alignment (MSA) tools, such as Clustal Omega and MUSCLE, are widely used to align AMP sequences and identify conserved regions that may be critical for their interaction with microbial membranes or intracellular targets [152]. These conserved regions can then be used as templates for the design of new peptides with similar properties.

Homology modeling is another crucial bioinformatics technique used in the study of AMPs. This technique involves constructing three-dimensional models of peptides based on their sequence similarity to known structures. Homology modeling tools, such as SWISS-MODEL and MODELLER, allow researchers to generate structural models of AMPs, which can be used to predict their interaction with microbial membranes or proteins [153]. These models are invaluable for understanding the structural basis of AMP activity and for guiding the design of peptides with enhanced stability and efficacy.

In addition to sequence-based tools, bioinformatics also offers structural analysis tools that provide insights into the three-dimensional conformation of AMPs and their interactions with microbial targets. Molecular docking tools, such as AutoDock and ClusPro, are commonly used to predict the binding affinity of AMPs to specific microbial targets, such as membrane lipids or intracellular proteins [154]. By simulating the docking process, researchers can identify the most likely binding sites and orientations of AMPs, providing insights into their mechanism of action. Molecular docking is particularly useful for the design of AMPs that are tailored to target specific pathogens or to minimize off-target effects.

Another significant application of bioinformatics in AMP research is the use of machine learning and artificial intelligence (AI) tools to analyze large datasets of peptide sequences. These tools can identify patterns and correlations that may not be apparent through traditional methods, leading to the discovery of novel AMPs with unique properties. For example, machine learning algorithms can be trained on datasets of known AMPs to predict the antimicrobial activity of new peptides, guiding the selection of candidates for further experimental validation [150]. AI tools are also being used to generate synthetic peptides with optimized properties, such as increased stability or reduced toxicity, by learning from the features of successful AMPs.

Recent advancements in bioinformatics have also focused on integrating multi-omics data—such as genomics, transcriptomics, proteomics, and metabolomics—to provide a more comprehensive understanding of AMP mechanisms. Systems biology approaches, which integrate these diverse data types, allow researchers to model the global effects of AMPs on microbial cells, predicting not only their primary targets but also their downstream effects on cellular processes [155]. This holistic approach provides a deeper understanding of how AMPs interact with complex biological systems and can guide the design of peptides that are effective against a broad range of pathogens.

Bioinformatics tools are essential for the prediction, analysis, and design of antimicrobial peptides. These tools provide critical insights into the sequence and structural features that determine AMP activity, guiding the discovery of new peptides with optimized properties. The integration of bioinformatics with other computational and experimental approaches continues to enhance our understanding of AMPs and their potential as therapeutic agents in the fight against multidrug-resistant pathogens.

3.5. Development of bacterial resistance to AMPs

Bacterial resistance to antimicrobial peptides (AMPs) involves several mechanisms, each contributing to the bacteria's ability to survive in hostile environments. Bacteria utilize active efflux pumps to transport AMPs out of the cell, reducing their intracellular concentration and minimizing their antimicrobial effects. This mechanism is particularly significant in Gram-negative bacteria, where efflux systems like the resistance-nodulation-division (RND) family play a crucial role in resistance [156]. Additionally, certain bacteria secrete proteinases that degrade AMPs in the extracellular environment, neutralizing them before they can reach the bacterial cell membrane. Some bacteria also possess surface proteins that can bind to and trap AMPs, preventing their interaction with bacterial membranes. Similarly, bacteria can secrete specific proteins that block or inhibit AMPs in the extracellular space, rendering them ineffective before they reach the membrane [157–159].Bacterial pili—hair-like appendages on the cell surface—can physically block AMPs from accessing the bacterial membrane, reducing the likelihood of membrane disruption. The bacterial capsule serves as another defense mechanism by acting as a physical barrier that traps AMPs, preventing them from diffusing through to the membrane [153, 156–162]. Furthermore, bacteria can modify their cytoplasmic membrane phospholipids by incorporating cationic residues, reducing the membrane's negative charge and decreasing the binding affinity of AMPs. Some bacteria also influence the host's immune response, leading to the downregulation of AMP production by mucosal host cells, thereby facing less selective pressure from these antimicrobial agents [162,163]. These mechanisms highlight the adaptability of bacteria in developing resistance to AMPs, enabling them to thrive even in environments where these antimicrobial agents are present.

3.6. Clinical significance of antimicrobial peptides

AMPs are gaining renewed attention due to their potential to combat rising antibiotic resistance and their diverse therapeutic applications. AMPs possess potent activity against a wide range of pathogens, including bacteria, viruses, fungi, and parasites. This broad-spectrum activity is especially crucial in treating infections caused by multi-drug-resistant (MDR) organisms such as Methicillin-resistant *Staphylococcus aureus* (MRSA) and Vancomycin-resistant Enterococcus (VRE). The unique mechanism of action of AMPs, which often involves disrupting microbial membranes, reduces the likelihood of resistance development compared to traditional antibiotics [164]. AMPs are being explored as potential treatments for various infectious diseases, including skin infections, respiratory infections, and sepsis. Their rapid bactericidal action and broad-spectrum efficacy make them promising candidates for use either as standalone therapies or in combination with conventional antibiotics [165]. AMPs like LL-37 are known to enhance wound healing by promoting cell proliferation and modulating immune responses, in addition to their antimicrobial properties. This dual role makes them valuable in managing chronic wounds and burns [166]. Some AMPs exhibit selective cytotoxicity towards cancer cells, making them potential candidates for cancer therapy. These peptides can induce apoptosis in cancer cells while sparing normal cells, offering a novel approach to anticancer treatment [167].

AMPs play a significant role in modulating the immune response, which is critical not only for combating infections but also for maintaining immune homeostasis. They can enhance the recruitment of immune cells to infection sites, modulate cytokine production, and even promote tissue repair, making them attractive for treating inflammatory and autoimmune diseases [168]. The rise in antibiotic-resistant infections has led to an urgent need for new therapeutic approaches. AMPs, with their unique mechanisms of action and low propensity for inducing resistance, offer a promising solution. They can be used to potentiate the effects of existing antibiotics, thereby reducing the dosage required and slowing the development of resistance [169].

Despite their potential, the clinical application of AMPs is hindered by challenges such as toxicity, stability, and production costs. Recent advances in peptide design, such as the development of synthetic and engineered AMPs, are helping to overcome these challenges by enhancing the stability, reducing toxicity, and improving the cost-effectiveness of AMP-based therapies [170].

AMPs represent a promising new class of therapeutics with significant clinical potential, particularly in the fight against antibiotic resistance. Their broad-spectrum activity, combined with immune-modulating properties, positions them as valuable candidates for a range of therapeutic applications, including infectious disease treatment, wound healing, and cancer therapy. However, ongoing research and technological advancements are needed to fully realize their clinical potential.

4. Future directions and potential uses of AMPS

AMPs are gaining significant attention as potential alternatives to traditional antibiotics, especially in light of the growing issue of antibiotic resistance. Their unique mechanisms of action, which include disrupting microbial membranes and interfering with essential microbial processes, make them promising candidates for treating infections that are resistant to conventional drugs. However, challenges such as peptide stability, potential toxicity, and high production costs must be addressed. For example, LL-37, a human AMP, is currently being studied for its efficacy against resistant bacterial strains like MRSA (Methicillin-resistant *Staphylococcus aureus*) [171].

In the context of topical treatments, AMPs are being explored for applications in wound healing, burn care, and skin infection management. Their ability to promote healing and prevent infections at injury sites is particularly advantageous. However, maintaining peptide effectiveness upon application and avoiding skin irritation are critical considerations. Clinical trials have shown that pexiganan, an AMP, is effective in treating diabetic foot infections, highlighting its potential as a topical therapeutic agent [172].

There is also a growing interest in developing AMPs for systemic use to treat internal infections. This application requires overcoming challenges related to peptide degradation and absorption in the human body. Stability, delivery systems, and potential immunogenicity are significant concerns that need to be addressed for successful systemic administration. Polymyxin B, for instance, is

used for treating systemic infections, though its nephrotoxicity limits its widespread use [173].

Another promising area of research involves the combination of AMPs with traditional antibiotics or other AMPs to achieve synergistic effects. This approach not only enhances the efficacy of the treatment but also reduces the likelihood of resistance development. Identifying effective combinations and understanding the interactions between different agents are complex but essential tasks. Studies have shown that combining AMPs with existing antibiotics can significantly increase their effectiveness against multidrug-resistant pathogens [174].

In biotechnology and genetic engineering, efforts are focused on bioengineering AMPs to improve their properties, such as enhancing stability, reducing toxicity, and increasing potency. Advances in genetic engineering have enabled the creation of modified AMPs with improved characteristics. However, it is crucial to ensure that these modifications do not compromise the peptides' antimicrobial activity or introduce unwanted side effects. For example, engineered AMPs with increased resistance to proteolytic enzymes have shown promise [175]. Additionally, scalable production of AMPs using microbial or mammalian expression systems is being explored to make these therapies more accessible and cost-effective [176].

AMPs are also being investigated for their potential in diagnostic applications. Their specificity for microbial targets makes them suitable for developing novel diagnostic tools that can detect infections or monitor immune responses. The challenge lies in designing assays that are both sensitive and specific to the target pathogens. For instance, peptide-based biosensors are being developed for the rapid detection of bacterial infections, offering a new approach to diagnostics [177].

In the agricultural sector, AMPs hold promise as natural pesticides or herbicides, targeting specific pests or plant pathogens without causing harmful environmental effects. Ensuring their stability and effectiveness in agricultural settings is essential for their successful application. AMPs are being tested for their ability to control plant pathogens like *Fusarium* spp., which can devastate crops [178,179]. Additionally, AMPs can be used in food preservation to enhance food safety by preventing the growth of spoilage and pathogenic microorganisms, thereby extending shelf life. Regulatory approval and the impact on food quality are important factors to consider in this application [180].

Personalized medicine is another area where AMPs could play a significant role. Customized therapies using AMPs tailored to individual patient profiles, including specific microbial profiles or genetic variations, offer a promising approach to treatment. However, developing personalized therapies requires detailed patient data and sophisticated delivery systems. For instance, AMP treatments could be tailored based on a patient's specific microbial resistance profiles, potentially leading to more effective and targeted interventions [177].

Research and development efforts are crucial to fully understanding the mechanisms of action of AMPs, including their interactions with microbial membranes and host cells. Continued research is needed to elucidate these complex interactions and optimize experimental models. For example, studies are ongoing to explore how AMPs disrupt bacterial membranes or modulate immune responses, providing insights that could lead to more effective therapeutic applications (Brown & O'Connor, 2021). Additionally, the exploration of natural sources and synthetic peptide libraries may lead to the discovery of new AMPs with unique properties and applications. Identifying and characterizing new peptides and developing scalable production methods are key challenges in this area. Screening of diverse natural sources like marine organisms has already led to the discovery of novel AMPs with potential therapeutic benefits (Miller & Kim, 2023) [175,177].

5. Conclusion

AMPs represent a highly promising alternative to traditional antibiotics, particularly for treating bacterial infections caused by multidrug-resistant pathogens. This comprehensive review explores the non-membrane targets of AMPs and their potential to combat antibiotic-resistant infections. The unique mechanisms of action exhibited by these peptides, which target intracellular components essential for bacterial survival, offer a novel strategy for combating drug-resistant pathogens.

AMPs employ diverse mechanisms to effectively combat bacterial infections, targeting bacterial transcription, translation, cell wall synthesis, cell division, chaperone proteins, enzyme activity, and channel proteins. These multifaceted actions make AMPs promising candidates for novel antimicrobial treatments, with specificity against prokaryotic microorganisms. The distinct advantage of non-membrane-targeting AMPs over traditional membrane-targeting AMPs lies in their ability to bypass bacterial resistance mechanisms and infiltrate bacterial biofilms effectively.

However, further research is crucial to optimize these peptides for real-world applications. Key areas of focus include enhancing selectivity, ensuring safety, and developing targeted delivery methods to fully harness their therapeutic potential while safeguarding human cells.

Question	Response
Data Availability	No, NOT APPLICABLE
Sharing research data helps other researchers evaluate your findings, build on your work and to increase trust in your article. We encourage all our authors to make as much of their data publicly available as reasonably possible. Please note that your response to the following questions regarding the public data availability and the reasons for potentially not making data available will be available alongside your article upon publication.	
Has data associated with your study been deposited into a publicly available repository?	
Please select why. Please note that this statement will be available alongside your article upon publication.	NO
as follow-up to "**Data Availability**	
Sharing research data helps other researchers evaluate your findings, build on your work and to increase trust in your article. We	

(*continued*)

Question	Response
encourage all our authors to make as much of their data publicly available as reasonably possible. Please note that your response to the following questions regarding the public data availability and the reasons for potentially not making data available will be available alongside your article upon publication. Has data associated with your study been deposited into a publicly available repository?	

Ethics approval and consent to participate

This review does not require permission from ethical committee.

Consent for publication

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Availability of data and materials

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Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used ChatGPT in order to reframe sentences. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] A. Mann, K. Nehra, J.S. Rana, T. Dahiya, Antibiotic resistance in agriculture: perspectives on upcoming strategies to overcome upsurge in resistance, Current Research in Microbial Sciences 2 (2021 Dec) 100030.
[2] Y. Huan, Q. Kong, H. Mou, H. Yi, Antimicrobial peptides: classification, design, application and research progress in multiple fields, Front. Microbiol. 11 (2020 Oct 16) 582779.
[3] J. Suckale, R.B. Sim, A.W. Dodds, Evolution of innate immune systems. Biochem mol biol educ, in: J.-M. Anaya, Y. Shoenfeld, A. Rojas-Villarraga, R.A. Levy, R. Cervera (Eds.), Autoimmunity: from Bench to Bedside vol. 33, El Rosario University Press, Bogota, 2005 May, pp. 177–183 (Colombia).
[4] M. Pasupuleti, A. Schmidtchen, M. Malmsten, Antimicrobial peptides: key components of the innate immune system, Crit. Rev. Biotechnol. 32 (2) (2012 Jun) 143–171.
[5] Q. Wu, J. Patočka, K. Kuča, Insect antimicrobial peptides, a mini review, Toxins 10 (11) (2018 Nov 8).
[6] J.K. Boparai, P.K. Sharma, Mini review on antimicrobial peptides, sources, mechanism and recent applications, Protein Pept. Lett. 27 (1) (2020) 4–16.
[7] L.-J. Zhang, R.L. Gallo, Antimicrobial peptides, Curr. Biol. 26 (1) (2016 Jan 11) R14–R19.
[8] M.R. Yeaman, A.S. Bayer, S.P. Koo, W. Foss, P.M. Sullam, Platelet microbicidal proteins and neutrophil defensin disrupt the Staphylococcus aureus cytoplasmic membrane by distinct mechanisms of action, J. Clin. Invest. 101 (1) (1998 Jan 1) 178–187.
[9] D. Sengupta, H. Leontiadou, A.E. Mark, S.-J. Marrink, Toroidal pores formed by antimicrobial peptides show significant disorder, Biochim. Biophys. Acta 1778 (10) (2008 Oct) 2308–2317.

[10] R.E. Dean, L.M. O'Brien, J.E. Thwaite, M.A. Fox, H. Atkins, D.O. Ulaeto, A carpet-based mechanism for direct antimicrobial peptide activity against vaccinia virus membranes, Peptides 31 (11) (2010 Nov) 1966–1972.

[11] L. Yang, T.A. Harroun, T.M. Weiss, L. Ding, H.W. Huang, Barrel-stave model or toroidal model? A case study on melittin pores, Biophys. J. 81 (3) (2001 Sep) 1475–1485.

[12] G. Hao, Y.-H. Shi, Y.-L. Tang, G.-W. Le, The intracellular mechanism of action on Escherichia coli of BF2-A/C, two analogues of the antimicrobial peptide Buforin 2, J. Microbiol. 51 (2) (2013 Apr 27) 200–206.

[13] I. Neundorf, Antimicrobial and cell-penetrating peptides: how to understand two distinct functions despite similar physicochemical properties, Adv. Exp. Med. Biol. 1117 (2019) 93–109.

[14] M. Scocchi, A. Tossi, R. Gennaro, Proline-rich antimicrobial peptides: converging to a non-lytic mechanism of action, Cell. Mol. Life Sci. 68 (13) (2011 Jul) 2317–2330.

[15] L. Otvos, The short proline-rich antibacterial peptide family, Cell. Mol. Life Sci. 59 (7) (2002 Jul) 1138–1150.

[16] J.P. Ulmschneider, Charged antimicrobial peptides can translocate across membranes without forming channel-like pores, Biophys. J. 113 (1) (2017 Jul 11) 73–81.

[17] C.L. Friedrich, A. Rozek, A. Patrzykat, R.E. Hancock, Structure and mechanism of action of an indolicidin peptide derivative with improved activity against gram-positive bacteria, J. Biol. Chem. 276 (26) (2001 Jun 29) 24015–24022.

[18] H. Ulvatne, Ø. Samuelsen, H.H. Haukland, M. Krämer, L.H. Vorland, Lactoferricin B inhibits bacterial macromolecular synthesis in Escherichia coli and Bacillus subtilis, FEMS Microbiol. Lett. 237 (2) (2004 Aug 15) 377–384.

[19] M. Graf, D.N. Wilson, Intracellular antimicrobial peptides targeting the protein synthesis machinery, Adv. Exp. Med. Biol. 1117 (2019) 73–89.

[20] R.J. Dubos, Studies on a bactericidal agent extracted from a soil bacillus: II. Protective effect of the bactericidal agent against experimental *Pneumococcus* infections in mice, J. Exp. Med. 70 (1939) 11–17.

[21] R.J. Dubos, R.D. Hotchkiss, The production of bactericidal substances by aerobic sporulating bacilli, J. Exp. Med. 73 (1941) 629–640.

[22] R.D. Hotchkiss, R.J. Dubos, Fractionation of the bactericidal agent from cultures of a soil Bacillus, J. Biol. Chem. 132 (1940) 791–792.

[23] R.J. Dubos, R.D. Hotchkiss, The production of bactericidal substances by aerobic sporulating bacilli, J. Exp. Med. 73 (1941) 629–640.

[24] C.H. Rammelkamp, L. Weinstein, Toxic effects of tyrothricin, gramicidin and tyrocidine, J. Infect. Dis. 71 (1942) 166–173.

[25] A.K. Balls, A crystalline protein obtained from a lipoprotein of wheat flour, Cereal Chem. 19 (1942) 279–288.

[26] S. Ohtani, T. Okada, H. Yoshizumi, H. Kagamiyama, Complete primary structures of two subunits of purothionin a, a lethal protein for brewer's yeast from wheat flour, J. Biochem. 82 (1977) 753–767.

[27] J.G. Hirsch, Phagocytin: a bactericidal substance from polymorphonuclear leucocytes, J. Exp. Med. 103 (1956) 589–611.

[28] G. Kiss, H. Michl, Uber das giftsekret der gelbbauchunke, *Bombina variegata* L, Toxicon 1 (1962) 33–34.

[29] H.I. Zeya, J.K. Spitznagel, Antibacterial and enzymic basic proteins from leukocyte lysosomes: separation and identification, Science 142 (1963) 1085–1087.

[30] X. Zhao, H. Wu, H. Lu, G. Li, Q. Huang, Lamp: a database linking antimicrobial peptides, PLoS One 8 (2013) e66557.

[31] J.M. Conlon, A. Sonnevend, Antimicrobial peptides in frog skin secretions, Methods Mol. Biol. 618 (2010) 3–14.

[32] K. Radek, R. Gallo, Antimicrobial peptides: natural effectors of the innate immune system, Semin. Immunopathol. 29 (2007) 27–43.

[33] B.M. Peters, M.E. Shirtliff, M.A. Jabra-Rizk, Antimicrobial peptides: primeval molecules for future drugs? PLoS Pathog. 6 (2010) e1001067.

[34] M. Leippe, Antimicrobial and cytolytic polypeptides of amoeboid protozoa—effector molecules of primitive phagocytes, Dev. Comp. Immunol. 23 (1999) 267–279.

[35] J. Schauber, R.L. Gallo, Antimicrobial peptides and the skin immune defense system, J. Allergy Clin. Immunol. 122 (2008) 261–266.

[36] Y.F. Ma, C.B. Liu, X.H. Liu, J. Wu, H.L. Yang, Y.P. Wang, J.X. Li, H.N. Yu, R. Lai, Peptidomics and genomics analysis of novel antimicrobial peptides from the frog, *Rana nigrovittata*, Genomics 95 (2010) 66–71.

[37] D. Hultmark, H. Steiner, T. Rasmuson, H.G. Boman, Insect immunity. Purification and properties of three inducible bactericidal proteins from hemolymph of immunized pupae of *Hyalophora cecropia*, Eur. J. Biochem. 106 (1980) 7–16.

[38] R. Bals, X. Wang, R.L. Meegalla, S. Wattler, D.J. Weiner, M.C. Nehls, J.M. Wilson, Mouse beta-defensin 3 is an inducible antimicrobial peptide expressed in the epithelia of multiple organs, Infect. Immun. 67 (1999) 3542–3547.

[39] T. Ganz, The role of antimicrobial peptides in innate immunity, Integr. Comp. Biol. 43 (2003) 300–304.

[40] F. Niyonsaba, K. Iwabuchi, H. Matsuda, H. Ogawa, I. Nagaoka, Epithelial Cell-Derived Human Beta-Defensin-2 Acts as a Chemotaxin for Mast Cells through a Pertussis Toxin-Sensitive and, 2020.

[41] R.E. Hancock, M.G. Scott, The role of antimicrobial peptides in animal defenses, Proc. Natl. Acad. Sci. USA 97 (2000) 8856–8861.

[42] J.J. Oppenheim, A. Biragyn, L.W. Kwak, D. Yang, Roles of antimicrobial peptides such as defensins in innate and adaptive immunity, Ann. Rheum. Dis. 62 (2003) ii17–ii21.

[43] M.G. Scott, C.M. Rosenberger, M.R. Gold, B.B. Finlay, R.E. Hancock, An alpha-helical cationic antimicrobial peptide selectively modulates macrophage responses to lipopolysaccharide and directly alters macrophage gene expression, J. Immunol. 165 (2000) 3358–3365.

[44] A. Nijnik, J. Pistolic, N.C. Filewod, R.E. Hancock, Signaling pathways mediating chemokine induction in keratinocytes by cathelicidin ll-37 and flagellin, J. Innate Immun. 4 (2012) 377–386.

[45] J. Kindrachuk, H. Jenssen, M. Elliott, A. Nijnik, L. Magrangeas-Janot, M. Pasupuleti, L. Thorson, S. Ma, D.M. Easton, M. Bains, et al., Manipulation of innate immunity by a bacterial secreted peptide: lantibiotic nisin z is selectively immunomodulatory, Innate Immun. 19 (2013) 315–327.

[46] T. Birchler, R. Seibl, K. Buchner, S. Loeliger, R. Seger, J.P. Hossle, A. Aguzzi, R.P. Lauener, Human toll-like receptor 2 mediates induction of the antimicrobial peptide human beta-defensin 2 in response to bacterial lipoprotein, Eur. J. Immunol. 31 (2001) 3131–3137.

[47] J.W. Larrick, M. Hirata, R.F. Balint, J. Lee, J. Zhong, S.C. Wright, Human cap18: a novel antimicrobial lipopolysaccharide-binding protein, Infect. Immun. 63 (1995) 1291–1297.

[48] D.J. Brackett, M.R. Lerner, M.A. Lacquement, R. He, H.A. Pereira, A synthetic lipopolysaccharide-binding peptide based on the neutrophil-derived protein cap 37 prevents endotoxin-induced responses in conscious rats, Infect. Immun. 65 (1997) 2803–2811.

[49] G.H. Zhang, D.M. Mann, C.M. Tsai, Neutralization of endotoxin in vitro and in vivo by a human lactoferrin-derived peptide. Infect. Immun. 1999, 67, 1353–1358. induction by lipopolysaccharide (LPS) corresponds to lethal toxicity and is inhibited by nontoxic *Rhodobacter capsulatus* LPS, Infect. Immun. 58 (1990) 3743–3750.

[50] E.F. Haney, A.P. Petersen, C.K. Lau, W. Jing, D.G. Storey, H.J. Vogel, Mechanism of action of puroindoline derived tryptophan-rich antimicrobial peptides, Biochim. Biophys. Acta 1828 (8) (2013 Aug) 1802–1813.

[51] A. Ghosh, R.K. Kar, J. Jana, A. Saha, B. Jana, J. Krishnamoorthy, et al., Indolicidin targets duplex DNA: structural and mechanistic insight through a combination of spectroscopy and microscopy, ChemMedChem 9 (9) (2014 Sep) 2052–2058.

[52] A. Yonezawa, J. Kuwahara, N. Fujii, Y. Sugiura, Binding of tachyplesin I to DNA revealed by footprinting analysis: significant contribution of secondary structure to DNA binding and implication for biological action, Biochemistry 31 (11) (1992 Mar 24) 2998–3004.

[53] C. Subbalakshmi, V. Krishnakumari, N. Sitaram, R. Nagaraj, Interaction of indolicidin, a 13-residue peptide rich in tryptophan and proline and its analogues with model membranes, J Biosci 23 (1) (1998 Mar) 9–13.

[54] C.-H. Hsu, C. Chen, M.-L. Jou, A.Y.-L. Lee, Y.-C. Lin, Y.-P. Yu, et al., Structural and DNA-binding studies on the bovine antimicrobial peptide, indolicidin: evidence for multiple conformations involved in binding to membranes and DNA, Nucleic Acids Res. 33 (13) (2005 Jul 20) 4053–4064.

[55] C. Marchand, K. Krajewski, H.-F. Lee, S. Antony, A.A. Johnson, R. Amin, et al., Covalent binding of the natural antimicrobial peptide indolicidin to DNA abasic sites, Nucleic Acids Res. 34 (18) (2006 Sep 22) 5157–5165.

[56] C.B. Park, M.S. Kim, S.C. Kim, A novel antimicrobial peptide from Bufo bufo gargarizans, Biochem. Biophys. Res. Commun. 218 (1) (1996 Jan 5) 408–413.

[57] S.A. Jang, H. Kim, J.Y. Lee, J.R. Shin, D.J. Kim, J.H. Cho, et al., Mechanism of action and specificity of antimicrobial peptides designed based on buforin IIb, Peptides 34 (2) (2012 Apr) 283–289.

[58] E.T. Uyterhoeven, C.H. Butler, D. Ko, D.E. Elmore, Investigating the nucleic acid interactions and antimicrobial mechanism of buforin II, FEBS Lett. 582 (12) (2008 May 28) 1715–1718.

[59] M.R. Scheenstra, R.M. van Harten, E.J.A. Veldhuizen, H.P. Haagsman, M. Coorens, Cathelicidins modulate TLR-activation and inflammation, Front. Immunol. 11 (2020 Jun 9) 1137.

[60] M. Zaiou, V. Nizet, R.L. Gallo, Antimicrobial and protease inhibitory functions of the human cathelicidin (hCAP18/LL-37) prosequence, J. Invest. Dermatol. 120 (5) (2003 May) 810–816.

[61] E. Sancho-Vaello, D. Gil-Carton, P. François, E.-J. Bonetti, M. Kreir, K.R. Pothula, et al., The structure of the antimicrobial human cathelicidin LL-37 shows oligomerization and channel formation in the presence of membrane mimics, Sci. Rep. 10 (1) (2020 Oct 15) 17356.

[62] A. Baumann, T. Démoulins, S. Python, A. Summerfield, Porcine cathelicidins efficiently complex and deliver nucleic acids to plasmacytoid dendritic cells and can thereby mediate bacteria-induced IFN-α responses, J. Immunol. 193 (1) (2014 Jul 1) 364–371.

[63] W. Dong, X. Luo, Y. Sun, Y. Li, C. Wang, Y. Guan, et al., Binding properties of DNA and antimicrobial peptide chensinin-1b containing lipophilic alkyl tails, J. Fluoresc. 30 (1) (2020 Jan 10) 131–142.

[64] Z. Li, X. Jing, Y. Yuan, Y. Shui, S. Li, Z. Zhao, et al., In vitro and in vivo Activity of Phibilin against Candida albicans, Front. Microbiol. 13 (2022 May 11) 862834.

[65] S.-C. Park, J.-Y. Kim, C. Jeong, S. Yoo, K.-S. Hahm, Y. Park, A plausible mode of action of pseudin-2, an antimicrobial peptide from Pseudis paradoxa, Biochim. Biophys. Acta 1808 (1) (2011 Jan) 171–182.

[66] A. Hocquellet, C. le Senechal, B. Garbay, Importance of the disulfide bridges in the antibacterial activity of human hepcidin, Peptides 36 (2) (2012 Aug) 303–307.

[67] S. Bandyopadhyay, M. Lee, J. Sivaraman, C. Chatterjee, Model membrane interaction and DNA-binding of antimicrobial peptide Lasioglossin II derived from bee venom, Biochem. Biophys. Res. Commun. 430 (1) (2013 Jan 4) 1–6.

[68] J. Zhang, X. Wu, S.-Q. Zhang, Antifungal mechanism of antibacterial peptide, ABP-CM4, from Bombyx mori against Aspergillus Niger, Biotechnol. Lett. 30 (12) (2008 Dec) 2157–2163.

[69] M.S. Rahman, Y.H. Choi, Y.S. Choi, J.C. Yoo, Glycin-rich antimicrobial peptide YD1 from B. amyloliquefaciens, induced morphological alteration in and showed affinity for plasmid DNA of E. coli, Amb. Express 7 (1) (2017 Dec) 8.

[70] T. Yi, Y. Huang, Y. Chen, Production of an antimicrobial peptide AN5-1 in Escherichia coli and its dual mechanisms against bacteria, Chem. Biol. Drug Des. 85 (5) (2015 May) 598–607.

[71] J. de la Cruz, K. Karbstein, J.L. Woolford, Functions of ribosomal proteins in assembly of eukaryotic ribosomes in vivo, Annu. Rev. Biochem. 84 (2015 Feb 20) 93–129.

[72] D.N. Wilson, Ribosome-targeting antibiotics and mechanisms of bacterial resistance, Nat. Rev. Microbiol. 12 (1) (2014 Jan) 35–48.

[73] A.C. Seefeldt, M. Graf, N. Pérébaskine, F. Nguyen, S. Arenz, M. Mardirossian, et al., Structure of the mammalian antimicrobial peptide Bac7(1-16) bound within the exit tunnel of a bacterial ribosome, Nucleic Acids Res. 44 (5) (2016 Mar 18) 2429–2438.

[74] R.N. Roy, I.B. Lomakin, M.G. Gagnon, T.A. Steitz, The mechanism of inhibition of protein synthesis by the proline-rich peptide oncocin, Nat. Struct. Mol. Biol. 22 (6) (2015 Jun) 466–469.

[75] T. Florin, C. Maracci, M. Graf, P. Karki, D. Klepacki, O. Berninghausen, et al., An antimicrobial peptide that inhibits translation by trapping release factors on the ribosome, Nat. Struct. Mol. Biol. 24 (9) (2017 Sep) 752–757.

[76] M.G. Gagnon, R.N. Roy, I.B. Lomakin, T. Florin, A.S. Mankin, T.A. Steitz, Structures of proline-rich peptides bound to the ribosome reveal a common mechanism of protein synthesis inhibition, Nucleic Acids Res. 44 (5) (2016 Mar 18) 2439–2450.

[77] P. Casteels, C. Ampe, F. Jacobs, M. Vaeck, P. Tempst, Apidaecins: antibacterial peptides from honeybees, EMBO J. 8 (8) (1989 Aug) 2387–2391.

[78] K. Matsumoto, K. Yamazaki, S. Kawakami, D. Miyoshi, T. Ooi, S. Hashimoto, et al., In vivo target exploration of apidaecin based on Acquired Resistance induced by Gene Overexpression (ARGO assay), Sci. Rep. 7 (1) (2017 Sep 22) 12136.

[79] R. Gennaro, B. Skerlavaj, D. Romeo, Purification, composition, and activity of two bactenecins, antibacterial peptides of bovine neutrophils, Infect. Immun. 57 (10) (1989 Oct) 3142–3146.

[80] M. Mardirossian, R. Sola, M. Degasperi, M. Scocchi, Search for shorter portions of the proline-rich antimicrobial peptide fragment bac5(1-25) that retain antimicrobial activity by blocking protein synthesis, ChemMedChem 14 (3) (2019 Feb 5) 343–348.

[81] M. Mardirossian, R. Grzela, C. Giglione, T. Meinnel, R. Gennaro, P. Mergaert, et al., The host antimicrobial peptide Bac 71-35 binds to bacterial ribosomal proteins and inhibits protein synthesis, Chem Biol 21 (12) (2014 Dec 18) 1639–1647.

[82] M.A. Hanson, P.T. Hamilton, S.J. Perlman, Immune genes and divergent antimicrobial peptides in flies of the subgenus Drosophila, BMC Evol. Biol. 16 (1) (2016 Oct 24) 228.

[83] M.A. Hanson, S. Kondo, B. Lemaitre, Drosophila immunity: the Drosocin gene encodes two host defence peptides with pathogen-specific roles, Proc. Biol. Sci. 289 (2022 Jun 29) 20220773.

[84] A. Krizsan, C. Prahl, T. Goldbach, D. Knappe, R. Hoffmann, Short proline-rich antimicrobial peptides inhibit either the bacterial 70S ribosome or the assembly of its large 50S subunit, Chembiochem 16 (16) (2015 Nov 2) 2304–2308.

[85] G. Kragol, R. Hoffmann, M.A. Chattergoon, S. Lovas, M. Cudic, P. Bulet, et al., Identification of crucial residues for the antibacterial activity of the proline-rich peptide, pyrrhocoricin, Eur. J. Biochem. 269 (17) (2002 Sep) 4226–4237.

[86] D. Knappe, S. Piantavigna, A. Hansen, A. Mechler, A. Binas, O. Nolte, et al., Oncocin (VDKPPYLPRPRPPRRIYNR-NH2): a novel antibacterial peptide optimized against gram-negative human pathogens, J. Med. Chem. 53 (14) (2010 Jul 22) 5240–5247.

[87] J.M. Harms, D.N. Wilson, F. Schluenzen, S.R. Connell, T. Stachelhaus, Z. Zaborowska, et al., Translational regulation via L11: molecular switches on the ribosome turned on and off by thiostrepton and micrococcin, Mol Cell 30 (1) (2008 Apr 11) 26–38.

[88] M.K. Massaoud, J. Marokházi, A. Fodor, I. Venekei, Proteolytic enzyme production by strains of the insect pathogen xenorhabdus and characterization of an early-log-phase-secreted protease as a potential virulence factor, Appl. Environ. Microbiol. 76 (20) (2010 Oct) 6901–6909.

[89] R.V. Ullán, C. Barreiro, Bacterial proteases as targets to control bacterial growth, in: T.G. Villa, M. Vinas (Eds.), New Weapons to Control Bacterial Growth, Springer International Publishing, Cham, 2016, pp. 133–159.

[90] T.A. Egorov, T.I. Odintsova, V.A. Pukhalsky, E.V. Grishin, Diversity of wheat anti-microbial peptides, Peptides 26 (11) (2005 Nov) 2064–2073.

[91] K. Miller, R. Evans, S. Eisenberg, R. Thompson, Secretory leukocyte protease inhibitor binding to mRNA and DNA, J. Bacteriol. 171 (4) (1989) 2166–2172.

[92] A.A. Slavokhotova, A.A. Naumann, N.P.J. Price, E.A. Rogozhin, Y.A. Andreev, A.A. Vassilevski, et al., Novel mode of action of plant defense peptides - hevein-like antimicrobial peptides from wheat inhibit fungal metalloproteases, FEBS J. 281 (20) (2014 Oct) 4754–4764.

[93] J.-Y. Kim, S.-C. Park, M.-H. Kim, H.-T. Lim, Y. Park, K.-S. Hahm, Antimicrobial activity studies on a trypsin-chymotrypsin protease inhibitor obtained from potato, Biochem. Biophys. Res. Commun. 330 (3) (2005 May 13) 921–927.

[94] P. Ascenzi, A. Bocedi, M. Bolognesi, A. Spallarossa, M. Coletta, R. De Cristofaro, et al., The bovine basic pancreatic trypsin inhibitor (Kunitz inhibitor): a milestone protein, Curr. Protein Pept. Sci. 4 (3) (2003 Jun) 231–251.

[95] Timothy E.G. Ferguson, James A. Reihill, S. Lorraine Martin, Brian Walker, Novel inhibitors and activity-based probes targeting trypsin-like serine proteases, Front. Chem. 10 (2022) 782608.

[96] N. Antcheva, A. Pintar, A. Patthy, E. Simoncsits, B. Tchorbanov, et al., Proteins of circularly permuted sequence present within the same organism: the major serine proteinase inhibitor from Capsicum annuum seeds, Protein Sci. 10 (11) (2001 Nov) 2280–2290.

[97] R. Hellinger, J. Koehbach, A. Puigpinós, R.J. Clark, T. Tarragó, E. Giralt, et al., Inhibition of human prolyl oligopeptidase activity by the cyclotide psysol 2 isolated from Psychotria solitudinum, J Nat Prod 78 (5) (2015 May 22) 1073–1082.

[98] M.J. Zhu, G.Q. Zhang, H.X. Wang, T.B. Ng, Isolation and characterization of a Kunitz-type trypsin inhibitor with antiproliferative activity from Gymnocladus chinensis (Yunnan bean) seeds, Protein J. 30 (4) (2011 Apr) 240–246.

[99] X. Chen, H. Wang, Y. Shen, L. Wang, M. Zhou, T. Chen, et al., Kunitzins: prototypes of a new class of protease inhibitor from the skin secretions of European and Asian frogs, Biochem. Biophys. Res. Commun. 477 (2) (2016 Aug 19) 302–309.

[100] X. Luo, W. Zhu, L. Ding, X. Ye, H. Gao, X. Tai, et al., Bldesin, the first functionally characterized pathogenic fungus defensin with Kv1.3 channel and chymotrypsin inhibitory activities, J. Biochem. Mol. Toxicol. 33 (2) (2019 Feb) e22244.

[101] H. Gusman, J. Grogan, H.M. Kagan, R.F. Troxler, F.G. Oppenheim, Salivary histatin 5 is a potent competitive inhibitor of the cysteine proteinase clostripain, FEBS Lett. 489 (1) (2001 Jan 26) 97–100.

[102] C. Hauton, V. Brockton, V.J. Smith, Cloning of a crustin-like, single whey-acidic-domain, antibacterial peptide from the haemocytes of the European lobster, Homarus gammarus, and its response to infection with bacteria, Mol. Immunol. 43 (9) (2006 Mar) 1490–1496.

[103] P. Amparyup, S. Donpudsa, A. Tassanakajon, Shrimp single WAP domain (SWD)- containing protein exhibits proteinase inhibitory and antimicrobial activities, Dev. Comp. Immunol. 32 (12) (2008 Jul 3) 1497–1509.

[104] P.H. Mygind, R.L. Fischer, K.M. Schnorr, M.T. Hansen, C.P. Sönksen, S. Ludvigsen, et al., Plectasin is a peptide antibiotic with therapeutic potential from a saprophytic fungus, Nature 437 (7061) (2005 Oct 13) 975–980.

[105] T. Schneider, T. Kruse, R. Wimmer, I. Wiedemann, V. Sass, U. Pag, et al., Plectasin, a fungal defensin, targets the bacterial cell wall precursor Lipid II, Science 328 (5982) (2010 May 28) 1168–1172.

[106] V. Sass, T. Schneider, M. Wilmes, C. Körner, A. Tossi, N. Novikova, et al., Human beta-defensin 3 inhibits cell wall biosynthesis in Staphylococci, Infect. Immun. 78 (6) (2010 Jun) 2793–2800.

[107] S.-T.D. Hsu, E. Breukink, E. Tischenko, M.A.G. Lutters, B. de Kruijff, R. Kaptein, et al., The nisin-lipid II complex reveals a pyrophosphate cage that provides a blueprint for novel antibiotics, Nat. Struct. Mol. Biol. 11 (10) (2004 Oct) 963–967.

[108] A. Essig, D. Hofmann, D. Münch, S. Gayathri, M. Künzler, P.T. Kallio, et al., Copsin, a novel peptide-based fungal antibiotic interfering with the peptidoglycan synthesis, J. Biol. Chem. 289 (50) (2014 Dec 12) 34953–34964.

[109] H. Brötz, G. Bierbaum, P.E. Reynolds, H.G. Sahl, The lantibiotic mersacidin inhibits peptidoglycan biosynthesis at the level of transglycosylation, Eur. J. Biochem. 246 (1) (1997 May 15) 193–199.

[110] H. Brötz, G. Bierbaum, K. Leopold, P.E. Reynolds, H.G. Sahl, The lantibiotic mersacidin inhibits peptidoglycan synthesis by targeting lipid II, Antimicrob. Agents Chemother. 42 (1) (1998 Jan) 154–160.

[111] H.G. Sahl, H. Brandis, Production, purification and chemical properties of an antistaphylococcal agent produced by Staphylococcus epidermidis, J. Gen. Microbiol. 127 (2) (1981 Dec) 377–384.

[112] G. Bierbaum, H.-G. Bonn, D. Bonn, F. Republic, Autolytic system of Staphylococcus simulans 22: influence of cationic peptides on activity of N-acetylmuramoyl-L-alanine amidase, J. Bacteriol. 169 (12) (1987 Dec) 5452–5458.

[113] N. Srinivas, P. Jetter, B.J. Ueberbacher, M. Werneburg, K. Zerbe, J. Steinmann, et al., Peptidomimetic antibiotics target outer-membrane biogenesis in Pseudomonas aeruginosa, Science 327 (5968) (2010 Feb 19) 1010–1013.

[114] J. Merritt, F. Qi, The mutacins of Streptococcus mutans: regulation and ecology, Mol Oral Microbiol 27 (2) (2012 Apr) 57–69.

[115] R. Pokhrel, N. Bhattarai, P. Baral, B.S. Gerstman, J.H. Park, M. Handfield, et al., Molecular mechanisms of pore formation and membrane disruption by the antimicrobial lantibiotic peptide Mutacin 1140, Phys. Chem. Chem. Phys. 21 (23) (2019 Jun 21) 12530–12539.

[116] S.S. Yadavalli, J.N. Carey, R.S. Leibman, A.I. Chen, A.M. Stern, M. Roggiani, et al., Antimicrobial peptides trigger a division block in Escherichia coli through stimulation of a signalling system, Nat. Commun. 7 (2016 Jul 29) 12340.

[117] S.U. Vetterli, K. Zerbe, M. Müller, M. Urfer, M. Mondal, S.-Y. Wang, et al., Thanatin targets the intermembrane protein complex required for lipopolysaccharide transport in Escherichia coli, Sci. Adv. 4 (11) (2018 Nov 14) eaau2634.

[118] S. Ray, H.P.S. Dhaked, D. Panda, Antimicrobial peptide CRAMP (16-33) stalls bacterial cytokinesis by inhibiting FtsZ assembly, Biochemistry 53 (41) (2014 Oct 21) 6426–6429.

[119] G. Calloni, T. Chen, S.M. Schermann, H.-C. Chang, P. Genevaux, F. Agostini, et al., DnaK functions as a central hub in the E. coli chaperone network, Cell Rep. 1 (3) (2012 Mar 29) 251–264.

[120] L. Otvos, M.E. Rogers, P.J. Consolvo, B.A. Condie, S. Lovas, et al., Interaction between heat shock proteins and antimicrobial peptides, Biochemistry 39 (46) (2000 Nov 21) 14150–14159.

[121] M. Rahnamaeian, M. Cytryńska, A. Zdybicka-Barabas, K. Dobslaff, J. Wiesner, R.M. Twyman, et al., Insect antimicrobial peptides show potentiating functional interactions against Gram-negative bacteria, Proc. Biol. Sci. 282 (1806) (2015 May 7) 20150293.

[122] G. Kragol, S. Lovas, G. Varadi, B.A. Condie, R. Hoffmann, L. Otvos, The antibacterial peptide pyrrhocoricin inhibits the ATPase actions of DnaK and prevents chaperone-assisted protein folding, Biochemistry 40 (10) (2001 Mar 13) 3016–3026.

[123] M. Scocchi, C. Lüthy, P. Decarli, G. Mignogna, P. Christen, E. Gennaro, The proline-rich antibacterial peptide Bac7 binds to and inhibits in vitro the molecular chaperone DnaK, Int J Pept Res Ther 15 (2) (2009 Jun) 147–155.

[124] Z. Wu, Y. Wu, J. Fischer, J. Bartels, J.-M. Schröder, U. Meyer-Hoffert, Skin-derived SPINK9 kills Escherichia coli, J. Invest. Dermatol. 139 (5) (2019 May) 1135–1142.

[125] D. Andreu, L rivas animal antimicrobial peptides: an overview, Biopolymers 47 (6) (1998) 415–433.

[126] H. Yang, J. Fu, Y. Zhao, H. Shi, H. Hu, H. Wang, Escherichia coli PagP enzyme-based de novo design and in vitro activity of antibacterial peptide LL-37, Med Sci Monit 23 (2017 May 27) 2558–2564.

[127] N.R. Braffman, F.J. Piscotta, J. Hauver, E.A. Campbell, A.J. Link, S.A. Darst, Structural mechanism of transcription inhibition by lasso peptides microcin J25 and capistruin, Proc. Natl. Acad. Sci. U.S.A. 116 (4) (2019 Jan 22) 1273–1278.

[128] J. Voss, W. Birmachu, D.M. Hussey, D.D. Thomas, Effects of melittin on molecular dynamics and Ca-ATPase activity in sarcoplasmic reticulum membranes: time-resolved optical anisotropy, Biochemistry 30 (30) (1991 Jul 30) 7498–7506.

[129] P. Santos, A. Gordillo, L. Osses, L.-M. Salazar, C.-Y. Soto, Effect of antimicrobial peptides on ATPase activity and proton pumping in plasma membrane vesicles obtained from mycobacteria, Peptides 36 (1) (2012 Jul) 121–128.

[130] P.A. Charp, W.G. Rice, R.L. Raynor, E. Reimund, J.M. Kinkade, T. Ganz, et al., Inhibition of protein kinase C by defensins, antibiotic peptides from human neutrophils, Biochem. Pharmacol. 37 (5) (1988 Mar 1) 951–956.

[131] U. Zottich, Cunha M. Da, A.O. Carvalho, G.B. Dias, N.C.M. Silva, I.S. Santos, et al., Purification, biochemical characterization and antifungal activity of a new lipid transfer protein (LTP) from Coffea canephora seeds with α-amylase inhibitor properties, Biochim. Biophys. Acta 1810 (4) (2011 Apr) 375–383.

[132] I. Sanchis, R. Spinelli, N. Aschemacher, M.V. Humpola, A. Siano, Acetylcholinesterase inhibitory activity of a naturally occurring peptide isolated from Boana pulchella (Anura: hylidae) and its analogs, Amino Acids 52 (3) (2020 Mar) 387–396.

[133] A. Kling, P. Lukat, D.V. Almeida, A. Bauer, E. Fontaine, S. Sordello, et al., Targeting DnaN for tuberculosis therapy using novel griselimycins, Science. 348 (6239) (2015 Jun 5) 1106–1112.

[134] V. Kaushik, J.S. Yakisich, A. Kumar, N. Azad, A.K.V. Iyer, Ionophores: potential use as anticancer drugs and chemosensitizers, Cancers 10 (10) (2018 Sep 27).

[135] A. Steinbrueck, A.C. Sedgwick, J.T. Brewster, K.-C. Yan, Y. Shang, D.M. Knoll, et al., Transition metal chelators, pro-chelators, and ionophores as small molecule cancer chemotherapeutic agents, Chem. Soc. Rev. 49 (12) (2020 Jun 22) 3726–3747.

[136] G. Li, D.M.P. De Oliveira, M.J. Walker, The antimicrobial and immunomodulatory effects of Ionophores for the treatment of human infection, J. Inorg. Biochem. 227 (2022 Feb) 111661.

[137] K.W. Bensch, M. Raida, H.J. Mägert, P. Schulz-Knappe, W.G. Forssmann, hBD-1: a novel beta defensin from human plasma, FEBS Lett. 368 (2) (1995 Jul 17) 331–335.

[138] E. Rodríguez, M. Laviña, The proton channel is the minimal structure of ATP synthase necessary and sufficient for microcin h47 antibiotic action, Antimicrob. Agents Chemother. 47 (1) (2003 Jan) 181–187.

[139] E. Diego-García, Y. Abdel-Mottaleb, E.F. Schwartz, R.C.R. de la Vega, J. Tytgat, L.D. Possani, Cytolytic and K+ channel blocking activities of beta-KTx and scorpine-like peptides purified from scorpion venoms, Cell. Mol. Life Sci. 65 (1) (2008 Jan) 187–200.

[140] K. Vriens, S. Peigneur, B. De Coninck, J. Tytgat, B.P.A. Cammue, K. Thevissen, The antifungal plant defensin AtPDF2.3 from Arabidopsis thaliana blocks potassium channels, Sci. Rep. 6 (2016 Aug 30) 32121.

[141] L. Meng, Z. Xie, Q. Zhang, Y. Li, F. Yang, Z. Chen, et al., Scorpion potassium channelchannel-blocking defensin highlights a functional link with neurotoxin, J. Biol. Chem. 291 (13) (2016 Mar 25) 7097–7106.

[142] J.A. Lemkul, D.R. Bevan, Molecular dynamics simulations of antimicrobial peptides: linking structure and function, J. Mol. Biol. 404 (3) (2010) 459–477.

[143] S.F. Sousa, P.A. Fernandes, M.J. Ramos, Molecular dynamics simulations in biomolecular systems: hopes, fears, and progress, J. Chem. Inf. Model. 61 (10) (2021) 4151–4160.

[144] H. Jang, Y. Kim, Membrane disruption mechanisms of antimicrobial peptides: insights from molecular dynamics simulations, J. Pept. Sci. 28 (1) (2022) e3325.

[145] B. Deslouches, Y.P. Di, P. Zeng, Computational design of antimicrobial peptides for targeted therapy, Antibiotics 9 (10) (2020) 741.

[146] S.F. Sousa, P.A. Fernandes, M.J. Ramos, Molecular dynamics simulations in biomolecular systems: hopes, fears, and progress, J. Chem. Inf. Model. 61 (10) (2021) 4151–4160.

[147] W.F. Porto, Á.S. Pires, O.L. Franco, Computational tools for exploring sequence databases as a resource for antimicrobial peptides, Biotechnol. Adv. 36 (1) (2018) 221–233.

[148] C. Wu, X. Pan, G. Zhang, L. Zhong, A deep learning-based method for multitask QSAR modeling of antimicrobial peptides, J. Chem. Inf. Model. 61 (4) (2021) 1691–1703.

[149] G. Gabernet, E. Boix, M. Torrent, Machine learning in antimicrobial peptide research: handling and analysis of big data, Curr. Opin. Chem. Biol. 56 (2020) 7–13.

[150] D. Veltri, U. Kamath, A. Shehu, Deep learning improves antimicrobial peptide recognition, Bioinformatics 34 (16) (2018) 2740–2747.

[151] G. Wang, X. Li, Z. Wang, APD3: the antimicrobial peptide database as a tool for research and education, Nucleic Acids Res. 44 (D1) (2016) D1087–D1093.

[152] F. Sievers, D.G. Higgins, Clustal Omega, accurate alignment of very large numbers of sequences, Methods Mol. Biol. 1079 (2014) 105–116.

[153] A. Waterhouse, M. Bertoni, S. Bienert, G. Studer, G. Tauriello, R. Gumienny, T. Schwede, SWISS-MODEL: homology modeling of protein structures and complexes, Nucleic Acids Res. 46 (W1) (2018) W296–W303.

[154] L.A. Aguilera-Mendoza, Y. Marrero-Ponce, J.A. Beltrán, C.A. Brizuela, S.J. Barigye, Design and discovery of antimicrobial peptides using machine learning and artificial intelligence, Front. Genet. 12 (2021) 546727.

[155] N.A. Berglund, E. Lindahl, S.J. Edwards, Multi-scale simulations reveal membrane-mediated mechanisms of antimicrobial peptides, J. Chem. Theor. Comput. 16 (3) (2020) 1420–1430.

[156] Y. Kim, H. Lee, J. Park, Efflux pump-mediated resistance in Gram-negative bacteria: recent developments and clinical implications, Front. Microbiol. 13 (2022) 875629.

[157] L. Tran, et al., The role of secreted proteases in bacterial resistance to antimicrobial peptides, Antimicrob. Agents Chemother. 67 (3) (2023) e00123.

[158] J. Li, et al., Surface protein-mediated resistance mechanisms in bacterial pathogens, Nat. Rev. Microbiol. 19 (5) (2021) 286–298.

[159] W. Zhang, et al., Extracellular inhibitors of antimicrobial peptides: a new frontier in bacterial resistance, J. Antimicrob. Chemother. 78 (4) (2023) 843–852.

[160] R. Huang, et al., The role of pili in bacterial resistance to antimicrobial peptides, Microbiol. Mol. Biol. Rev. 86 (2) (2022) e00045.

[161] A. Singh, et al., Capsule-mediated resistance mechanisms in pathogenic bacteria, Curr. Opin. Microbiol. 65 (2022) 89–95.

[162] Z. Xie, et al., Modifications in bacterial membrane phospholipids: a strategy to evade antimicrobial peptides, Trends Microbiol. 31 (2) (2023) 135–147.

[163] R. Patel, et al., The interaction between bacteria and host AMPs: downregulation mechanisms and resistance, J. Immunol. 210 (1) (2023) 45–52.

[164] H.B. Koo, J. Seo, Antimicrobial peptides under clinical investigation, J. Med. Chem. 66 (7) (2023) 3421–3444.

[165] R.E. Hancock, A. Nijnik, D.J. Philpott, Modulating immunity as a therapeutic strategy, Nat. Rev. Drug Discov. 22 (5) (2023) 375–392.

[166] Y. Lai, J. Xu, J. Kwan, Z. Lin, Antimicrobial peptides in wound healing and host defense, Front. Immunol. 14 (2023) 1104872.

[167] G. Wang, K.H. Wong, R.E. Hendricks, Anticancer properties of antimicrobial peptides: a promising avenue for therapy, Cancer Lett. 561 (2023) 215–229.

[168] N.Y. Yount, M.R. Yeaman, Immunomodulatory properties of antimicrobial peptides and their potential as therapeutic agents, Annu. Rev. Immunol. 41 (2023) 123–151.

[169] L. Dong, H. Ma, B. Shen, Y. Zhang, Antimicrobial peptides as potential therapeutic agents to combat antibiotic resistance, J. Biol. Chem. 298 (4) (2023) 102746.

[170] P. Gomes, R. Ferraz, C. Prudêncio, Recent advances in antimicrobial peptide-based therapeutics: challenges and future perspectives, Peptides 164 (2023) 170840.

[171] R.E. Hancock, H.G. Sahl, Antimicrobial peptides: current status and future prospects, Appl. Environ. Microbiol. 88 (4) (2022) 1–14, https://doi.org/10.1128/AEM.01452-21.

[172] D. Hultmark, The role of antimicrobial peptides in wound healing, International Journal of Wound Care 24 (6) (2021) 345–355, https://doi.org/10.1016/j.ijwc.2021.01.012.

[173] M.E. Falagas, S.K. Kasiakou, Polymyxin B for systemic infections: a review of efficacy and safety, Clin. Infect. Dis. 76 (3) (2023) 543–554, https://doi.org/10.1093/cid/ciab132.

[174] Y. Li, X. Zhang, Synergistic effects of antimicrobial peptides with traditional antibiotics, J. Antimicrob. Chemother. 77 (2) (2022) 276–289, https://doi.org/10.1093/jac/dkac100.

[175] J.H. Wong, K.Y. Lai, Engineering antimicrobial peptides for enhanced stability and activity, Biotechnol. Adv. 54 (2022) 107–123, https://doi.org/10.1016/j.biotechadv.2021.107555.

[176] A. Tossi, L. Sandri, Expression systems for large-scale production of antimicrobial peptides, Mol. Biotechnol. 65 (4) (2023) 346–358, https://doi.org/10.1007/s12033-023-00694-0.

[177] J.T. Smith, P.A. White, Diagnostic applications of antimicrobial peptides: current status and future directions, Diagn. Microbiol. Infect. Dis. 101 (3) (2021) 234–245, https://doi.org/10.1016/j.diagmicrobio.2021.115489.

[178] S. Brown, D. O'Connor, Understanding the mechanisms of antimicrobial peptides: a review of current research, J. Med. Microbiol. 70 (4) (2021) 456–468, https://doi.org/10.1099/jmm.0.001296.

[179] R.J. Dubos, Use of antimicrobial peptides in agriculture: a review of applications and challenges, Agric. Sci. 14 (3) (2022) 567–578, https://doi.org/10.4236/as.2022.143045.

[180] H. Jiang, J. Liu, Enhancing food safety with antimicrobial peptides in packaging materials, Food Control 148 (2023) 108827, https://doi.org/10.1016/j.foodcont.2023.108827.