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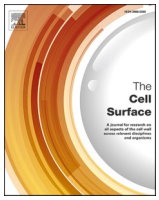

Antimicrobial peptides and proteins against drug-resistant pathogens

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ABSTRACT

The rise of drug-resistant pathogens, driven by the misuse and overuse of antibiotics, has created a formidable challenge for global public health. Antimicrobial peptides and proteins have garnered considerable attention as promising candidates for novel antimicrobial agents. These bioactive molecules, whether derived from natural sources, designed synthetically, or predicted using artificial intelligence, can induce lethal effects on pathogens by targeting key microbial structures or functional components, such as cell membranes, cell walls, biofilms, and intracellular components. Additionally, they may enhance overall immune defenses by modulating innate or adaptive immune responses in the host. Of course, development of antimicrobial peptides and proteins also face some limitations, including high toxicity, lack of selectivity, insufficient stability, and potential immunogenicity. Despite these challenges, they remain a valuable resource in the fight against drug-resistant pathogens. Future research should focus on overcoming these limitations to fully realize the therapeutic potential of antimicrobial peptides in the infection control.

1. Introduction

Throughout history, pathogenic microorganisms have exerted a profound and complex impact on human health. For example, the plague, which ravaged Europe and Asia between the 13th and 17th centuries, resulted in massive population declines(Yadav and Chauhan, 2024). The advent of antibiotics in the 20th century revolutionized modern medicine by not only saving countless lives but also enabling high-risk medical procedures such as organ transplantation, open-heart surgery, and cancer treatments(Cook and Wright, 2022). However, the overuse and misuse of antibiotics have fostered the emergence of drug-resistant pathogens(Durand-Reville et al., 2021). As the 21st century unfolds, the rapid evolution of multidrug-resistant (MDR) pathogens has emerged as a pressing issue requiring immediate resolution. These pathogens cause millions of deaths or disabilities annually and impose a heavy socioeconomic burden. According to World Bank projections, by 2050, MDR pathogens could result in as much as $1 trillion in additional healthcare expenditures and a global GDP loss of $3.44 trillion(Yan et al., 2024). In this context, researchers are actively exploring innovative avenues to develop new antimicrobial agents to confront this severe challenge. Therefore, sustained and substantial investment in the research and development of novel antimicrobials is both critical and urgent.

Exploring novel antimicrobial targets has garnered significant attention as a promising alternative strategy to combat MDR pathogens (Chang et al., 2022). However, developing antimicrobials with entirely new mechanisms of action is undoubtedly more challenging than chemically modifying existing drugs(Lewis, 2020). The ongoing threat posed by MDR pathogens has heightened concerns about reliance on traditional antibiotics and underscored the urgent need for new therapeutic strategies. In this context, functional peptides and proteins have garnered widespread interest owing to their unique biological properties (Chiu et al., 2022; Fernández de Ullivarri et al., 2020). Typically, molecules composed of fewer than 50 amino acids are referred to as peptides, while those composed of more than 50 amino acids are classified as proteins. These molecules not only have the potential to target a broad spectrum of pathogens but also effectively modulate the immune system, demonstrating great promise in treating infections caused by MDR pathogens(Duarte-Mata and Salinas-Carmona, 2023). Numerous studies have demonstrated that functional peptides and proteins possess the ability to directly kill bacteria or fungi and inhibit their growth. For instance, some peptides and proteins can directly target bacterial or fungal cell membranes or cell walls, disrupting their stability and ultimately leading to cell death(Luo and Song, 2021). Moreover, other peptides and proteins can target specific intracellular components, interfering with key metabolic processes and resulting in microbial

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death(Le et al., 2017). In addition to their direct antimicrobial effects, the role of functional peptides and proteins in modulating the immune system is also well-recognized(He et al., 2024; Liu et al., 2024). This dual mechanism of action—direct antimicrobial activity and immune modulation—makes them ideal candidates for developing novel antimicrobial therapies (Mercer and O'Neil, 2020). Several peptide compounds have entered clinical trials or received approval for clinical use. In 1948, bacitracin was approved for market release by the U.S. Food and Drug Administration (FDA). Subsequently, peptide antibiotics such as colistin, polymyxin B, daptomycin, and vancomycin were approved in the 20th century. In recent years, research on antimicrobial peptides (AMPs) has advanced significantly, with multiple studies progressing to clinical trials. Examples include the first-in-human study of the antimicrobial peptide PL-18 vaginal suppository in 2023 and a Phase II clinical trial of AMP peptide PL-5 targeting mild infections in diabetic foot ulcers in 2024. Industry forecasts suggest that by 2031, the peptide drug market will reach an estimated $64.3 billion, with a projected compound annual growth rate (CAGR) of 6.8 %(Cresti et al., 2024). Consequently, research interest in functional peptides and proteins is intensifying, with an increasing number of scholars focusing on their potential applications in treating MDR pathogen infections. This undoubtedly paves new pathways and directions for the future development of anti-infective drugs.

2. Peptides and proteins with antimicrobial properties

Functional peptides and proteins demonstrate a diverse array of strategies in combating infections. These biomolecules can directly kill pathogens and disrupt the integrity of their cellular structures. Moreover, they can synergistically assist the host in resisting pathogen invasion through multiple mechanisms, such as precise targeting of specific molecules, inhibition of biofilm formation, and modulation of the host immune response. Typically, antimicrobial peptides and proteins exhibit broad-spectrum antimicrobial activity, effectively targeting a wide range of pathogens, including those resistant and non-resistant to conventional treatments (See Table 1).

2.1. Targeting cell membranes and cell walls

Many peptides and proteins with antimicrobial properties can directly act on the cell membrane or cell wall of pathogens, disrupting the integrity of their cellular structures and leading to their death(Luo and Song, 2021).

Cytolysins containing membrane attack complex/perforin (MACPF) domains can disrupt cell integrity by forming pores in the target cell membrane(O'Neill et al., 2020). Perforin-2, encoded by the MPEG1 gene, plays a crucial role in eliminating intracellular bacteria (McCormack and Podack, 2015; O'Neill et al., 2020). Additionally, phospholipases are effective candidate molecules targeting the cell membrane; phospholipase A2 (PLA2) secreted by Paneth cells in the intestine preferentially targets anionic phospholipid layers, rapidly degrading bacterial phospholipids(van Hensbergen et al., 2020). Among the functional peptides and proteins targeting the cell wall, lysozyme is a well-known antibacterial enzyme. It primarily degrades the peptidoglycan layer of bacterial cell walls by hydrolyzing β-1,4-glycosidic bonds, leading to cell lysis and subsequent death(Leśnierowski and Yang, 2021). Besides lysozyme, lectins are characterized by their ability to simultaneously attach to various carbohydrate units on target pathogens. They play a significant role in microbial inhibition by targeting specific carbohydrates—mostly cell wall components—on the microbial surface(Breitenbach Barroso Coelho et al., 2018).

In addition to proteins that disrupt cell membrane structures, there are numerous antimicrobial peptides (AMPs) with amphipathic structures that function by targeting the cell membrane(Luo and Song, 2021; Satchanska et al., 2024). Most AMPs are cationic peptides, while the surfaces of Gram-positive and Gram-negative bacteria contain teichoic acids or lipopolysaccharides, respectively, resulting in a net negative charge on the membrane surface(Poxton, 2015). Therefore, a mutual attraction develops between bacterial surfaces and cationic AMPs, promoting their aggregation on the cell membrane(Savini et al., 2020). This aggregation subsequently leads to membrane structural disruption or allows peptide molecules to penetrate the cell interior to exert their biological functions(Savini et al., 2020). As the concentration of peptide molecules increases, peptide molecules then freely diffuse or preassemble on the membrane surface, inducing damage to the pathogen's cell membrane(Luo and Song, 2021). Defensins are one of the earliest discovered families of membrane-disrupting AMPs in mammals(Ramazi et al., 2022). These peptides range in size from 2 to 4 kDa and exhibit broad-spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria, fungi, protozoa, and enveloped viruses(Tavares et al., 2023). Cathelicidins are a large class of peptides found in humans and other species; they are cationic and possess broad-spectrum antimicrobial properties, with LL-37 being the human-derived cathelicidin(Alford et al., 2020). In addition to the action on cell membranes, certain AMPs—such as vancomycin—can selectively bind to lipid II, a precursor molecule in cell wall synthesis, thereby inhibiting the synthesis of the cell wall (Malin and de Leeuw, 2019). LL-37 and vancomycin not only exhibits bactericidal activity against common pathogens but also demonstrates potent efficacy against multidrug-resistant strains, such as multidrug-resistant *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*(Feng et al., 2013; Gardete and Tomasz, 2014; Geitani et al., 2019; Haisma et al., 2014).

2.2. Anti-biofilm

Biofilms are microbial communities that cooperate within organized systems associated with suitable substrates. This phenomenon results from certain microorganisms acquiring evolutionary adaptations that allow them to protect themselves from environmental threats that individual microbes cannot withstand alone(Ciofu et al., 2022). The formation of biofilms is one of the main virulence determinants in many bacterial or fungal infections, significantly increasing the pathogens' resistance to antibiotics and innate host defenses(Rather et al., 2021).

Many functional peptides and proteins possess the ability to inhibit biofilm formation and are considered potential alternatives to traditional antibiotics. For instance, HPA3NT3-A2, designed by Park and colleagues, can significantly reduce the secretion of extracellular polymeric substances (EPS) in *Pseudomonas aeruginosa* ATCC strains 15692 and 3241 and clinical isolates of drug-resistant *Pseudomonas aeruginosa* (Lee et al., 2019; Silveira et al., 2021). A carboxyl-terminal fragment of crotalicidin (Ctn15–34), identified in the venom glands of the South American rattlesnake, can inhibit biofilm formation and eradicate preformed biofilms at concentrations 100 folds of the minimum inhibitory concentration (MIC) against *Candida* strains(Fontanot et al., 2024). At the same time, it also exhibits activity against multidrug-resistant yeasts, including *Candida albicans* and *Candida glabrata(*de Aguiar et al., 2020*)*. IDR-1018, derived from a modification of the bovine neutrophil host defense peptide bactenecin, can directly interact with the signaling molecule ppGpp in methicillin-resistant *Staphylococcus aureus* (MRSA) to prevent ppGpp accumulation and accelerate its degradation(Jiale et al., 2021). Moreover, it demonstrates activity against multidrug-resistant *Mycobacterium tuberculosis* isolates(Mansour et al., 2015).

2.3. Targeting intracellular components

Although most peptides and proteins with antimicrobial activity exert their biological effects primarily by disrupting cell membrane or cell wall structures, some antimicrobial peptides can directly act on the intracellular molecular mechanisms of pathogenic microorganisms to exert their antimicrobial efficacy(Le et al., 2017). For example, members of the proline-rich antimicrobial peptide family—including but not limited to pyrrhocoricin, apidaecin, and drosocin—have been shown to achieve their antibacterial effects by interacting with the helical lid

Table 1
Antimicrobial peptides and proteins.

Name	Origin	Protein or peptide	Mechanistic Pathway	Specific Mechanism	Target Organisms	References
Perforin-2	Human	Protein	Targeting Cell Membranes	/	G+ and G− bacteria	(O'Neill et al., 2020; Strbo et al., 2020)
Phospholipase A2	Human	Protein	Targeting Cell Membranes	/	G+ and G− bacteria	(van Hensbergen et al., 2020)
Lysozyme	Human	Protein	Targeting Cell Walls	/	G+ bacteria	(Leśnierowski and Yang, 2021)
Cyanobacterial lectin	*Cyanobacterial*	Protein	Targeting Cell Walls	/	*Cryptococcus*	(Breitenbach Barroso Coelho et al., 2018)
LL-37	Human	Peptide	Targeting Cell Membranes Immunomodulation Anti-Biofilm Combined Use with Conventional Antibiotics	regulating the activities of MAPK p38 and extracellular signal-regulated kinase (ERK)	broad-spectrum activity against bacteria, fungi, viruses	(Alford et al., 2020; Wang et al., 2019a)
Vancomycin	*Amycolatopsis orientalis*	Peptide	Targeting Cell Walls	Targeting lipid II	G+ bacteria	(Malin and de Leeuw, 2019)
HPA3NT3-A2	*Helicobacter pylori*	Peptide	Anti-Biofilm	/	*Pseudomonas aeruginosa*	(Silveira et al., 2021)
Ctn15–34	South American rattlesnake	Peptide	Anti-Biofilm	/	*Candida*	(Fontanot et al., 2024)
IDR-1018	Human	Peptide	Anti-Biofilm	Targeting ppGpp	*Staphylococcus aureus*	(Jiale et al., 2021)
Pyrrhocoricin,	sap sucking bugs	Peptide	Targeting Intracellular Components	Targeting DnaK heat shock protein	*Escherichia coli*	(Welch et al., 2020)
Apidaecin	*Apis mellifera*	Peptide	Targeting Intracellular Components	Targeting DnaK heat shock protein	*Escherichia coli*	(Welch et al., 2020)
Drosocin	*Fruit fly*	Peptide	Targeting Intracellular Components	Targeting DnaK heat shock protein	*Escherichia coli*	(Welch et al., 2020)
Microcin B17	*Enterobacteriaceae*	Peptide	Targeting Intracellular Components	Targeting DNA gyrase	*Escherichia coli* *Enterobacter* *Pseudomonas* *Shigella*	(Collin and Maxwell, 2019)
Buforin II	*Bufo bufo garagriozans*	Peptide	Targeting Cell Membranes Targeting Intracellular Components	Targeting DNA and RNA	*Escherichia coli*	(C. B. Park et al., 1998)
PR-39	*Sus scrofa*	Peptide	Targeting Cell Membranes Targeting Intracellular Components	Targeting protein and DNA synthesis	*Escherichia coli*	(Boman et al., 1993b)
tPMP	Human	Protein	Targeting Cell Membranes Targeting Intracellular Components	Targeting protein synthesis	*Staphylococcus aureus*	(Xiong et al., 2002)
Histatin 5	Human	Peptide	Targeting Intracellular Components	Targeting protease	*Candida albicans* *Candida glabrata* *Candida krusei* *Cryptococcus neoformans* *Saccharomyces cerevisiae*	(Sharma et al., 2021)
interleukin-17	Human	Protein	Immunomodulation	induce the expression of AMPs	/	(Boodhoo et al., 2024)
IFN-γ	Human	Protein	Immunomodulation	promote the efficiency and diversity of peptide presentation	/	(Shaykhiev et al., 2010)
HNP1–3	Human	Peptide	Immunomodulation	activate macrophages	/	(Guryanova and Ovchinnikova, 2022)
IDR-1002	Human	Peptide	Immunomodulation	modulate cytokine production and to reduce the inflammatory response induced by LPS	*Escherichia coli* *Saccharomyces cerevisiae*	(Piyadasa et al., 2018)
mBD2	Human	Peptide	Immunomodulation	enhances the delivery of the antigen into the MHC class II antigen-presentation pathway in DCs	/	(Yang et al., 2007)
PMAP-36	*Sus scrofa*	Peptide	Targeting Cell Membranes Combined Use with Conventional Antibiotics	increasing membrane permeability	*Escherichia coli* *Saccharomyces cerevisiae*	(N. Wang et al., 2022)
PRW4	*Sus scrofa*	Peptide	Targeting Cell Membranes Combined Use with Conventional Antibiotics	increasing membrane permeability	*Escherichia coli* *Saccharomyces cerevisiae*	(N. Wang et al., 2022)
indolicidin	*Bos taurus*	Peptide	Anti-Biofilm Combined Use with Conventional Antibiotics	/	*Saccharomyces cerevisiae*	(Mataraci and Dosler, 2012)
CAMA	Artificial Design	Peptide	Anti-Biofilm Combined Use with Conventional Antibiotics	/	*Saccharomyces cerevisiae*	(Mataraci and Dosler, 2012)

(*continued on next page*)

Table 1 (*continued*)

Name	Origin	Protein or peptide	Mechanistic Pathway	Specific Mechanism	Target Organisms	References
nisin	*Lactococcus lactis*	Peptide	Anti-Biofilm Combined Use with Conventional Antibiotics	/	*Saccharomyces cerevisiae*	(Mataraci and Dosler, 2012)
Pt5-1c	zebrafish	Peptide	Anti-Biofilm Combined Use with Conventional Antibiotics	/	*Escherichia coli* *Saccharomyces cerevisiae* *Klebsiella pneumoniae*	(Duan et al., 2021)
SPR741	*Bacillus polymyxa*	Peptide	Targeting Intracellular Components Combined Use with Conventional Antibiotics	Targeting efflux pumps	*Escherichia coli* *Acinetobacter baumannii* *Klebsiella pneumoniae*	(MacNair and Brown, 2020)
PAS8-b-PDM12	Artificial Design	Peptide	Targeting Intracellular Components Combined Use with Conventional Antibiotics	Targeting efflux pumps	*Escherichia coli* *Pseudomonas aeruginosa* *Saccharomyces cerevisiae* *Acinetobacter baumannii*	(Si et al., 2020)
Lactoferrin	Human	Protein	Other Indirect Antimicrobial Effects	sequestering iron in the bacterial environment	G+ and G− bacteria	(Wang et al., 2019b)

region of the bacterial DnaK heat shock protein(Welch et al., 2020). Specifically, the C-terminus of pyrrhocoricin can traverse into the bacterial cytoplasmic environment, while its N-terminus inhibits the ATPase activity of the DnaK protein(Moravej et al., 2018). Additionally, microcin B17, a ribosomally synthesized antimicrobial peptide produced by *Enterobacteriaceae*, inhibits DNA replication by interfering with the function of DNA gyrase (Collin and Maxwell, 2019). Moreover, there are AMPs such as buforin II, PR-39, and tPMP that are capable of successfully traversing the bacterial cell wall to interfere with internal metabolic pathways(S.-C. Park et al., 2011). Research by Chan and colleagues revealed that buforin II kills bacteria through direct interaction with nucleic acid molecules, without significant changes in membrane permeability during this process(Cho et al., 2009). PR-39, isolated from porcine small intestine, requires an approximately 8-min delay phase before penetrating the outer membrane, after which it rapidly kills actively growing *Escherichia coli* cells by interrupting protein and DNA synthesis(Boman et al., 1993a). Additionally, peptide substances such as histatin 5 exert effects by inhibiting protease activity in both the host and pathogens, thereby affecting the virulence and survival capabilities of microorganisms(Sharma et al., 2021).

2.4. Immunomodulation

Functional peptides and proteins not only possess the ability to directly combat pathogens but can also indirectly enhance the body's immune defense mechanisms by regulating innate and adaptive immune responses(Duarte-Mata and Salinas-Carmona, 2023; Mercer and O'Neil, 2020). Specific molecules may exhibit both of these functions or exhibit only one. For example, innate immune peptides such as α-defensins and cathelicidins can not only directly kill invading pathogens but also effectively recruit various immune cells to the site of infection, including neutrophils, eosinophils, mast cells, monocytes, and lymphocytes (Auvynet and Rosenstein, 2009). Additionally, interleukin-17 (IL-17), mainly secreted by Th17 cells, can induce the expression of AMPs, thereby enhancing epithelial barrier functions and strengthening antimicrobial defense mechanisms(Boodhoo et al., 2024). Under the stimulation of IL-17, the expression of β-defensins is increased, which enhances mucosal immunity efficacy. Similarly, cytokines like interferon-gamma (IFN-γ) can promote the efficiency and diversity of peptide presentation by major histocompatibility complex (MHC) class I and II molecules on the cell surface(Shaykhiev et al., 2010). Further studies have shown that human neutrophil peptides (HNP1-3) can activate macrophages in vitro and in animal models, enhancing their phagocytic functions(Guryanova and Ovchinnikova, 2022). LL-37 promotes the production of interleukin-8 (IL-8) by regulating the activities of MAPK p38 and extracellular signal-regulated kinase (ERK), thereby enhancing the phagocytic capacity of neutrophils(Y. Zhu et al., 2022). Moreover, some AMPs also exhibit the ability to regulate immune responses, such as inducing the generation of reactive oxygen species and promoting the formation of neutrophil extracellular traps (NETs) (Melbouci et al., 2023; Oyinloye et al., 2015). Notably, some AMPs have been observed to have anti-inflammatory effects; for example, when IDR-1002 is applied in vivo, it can reduce the inflammatory response at the site of infection and decrease the infiltration of alveolar macrophages(Piyadasa et al., 2018).

As key molecules connecting innate and adaptive immunity, antimicrobial peptides act as a bridge during infections. Different types of defensins and the human cathelicidin LL-37 can promote the migration of antigen-presenting cells (APCs), such as immature dendritic cells (iDCs), B lymphocytes, and macrophages, to the site of infection(Duarte-Mata and Salinas-Carmona, 2023). Additionally, AMPs can affect the differentiation and polarization of T lymphocytes; for instance, in the presence of peptides like LL-37 and mBD2, a Th1-type immune response can be promoted(Yang et al., 2007). Experiments have shown that under the influence of LL-37, the endocytic activity of immature monocyte-derived dendritic cells is significantly enhanced(Bandholtz et al., 2006).

2.5. Combined use with conventional antibiotics

A substantial amount of in vitro experimental data supports the potential of combining AMPs with conventional antibiotics. The mechanisms of this combination therapy mainly encompass three aspects: increasing membrane permeability, inhibiting biofilm formation, and targeting the mechanisms of drug resistance.

Firstly, increasing membrane permeability is one of the most common synergistic mechanisms. By elevating the concentration of drugs entering the cells, the effectiveness of antibiotics is enhanced. For example, when LL-37 is used in combination with colistin, it can significantly reduce the MIC values against multidrug-resistant *Escherichia coli*, achieved through increasing cell membrane permeability (Morroni et al., 2021). Similarly, PMAP-36 and PRW4, when combined with aminoglycoside antibiotics, can promote effective antibiotic delivery by enhancing membrane permeability, thereby achieving synergistic antibacterial effects(N. Wang et al., 2022).

Secondly, since AMPs can inhibit or disrupt biofilm structures, pathogens that were previously protected become exposed, increasing

their sensitivity to antibiotic treatments. Antibiotics such as daptomycin, linezolid, teicoplanin, azithromycin, and ciprofloxacin, as well as antimicrobial cationic peptides (including indolicidin, CAMA [cecropin (1-7)–melittin A (2-9) amide], and nisin) have all demonstrated effective intervention against biofilms formed by MRSA ATCC 43300, whether used alone or in combination. Particularly noteworthy is that almost all combinations of antibiotics and AMPs exhibited synergistic effects against MRSA biofilms(Mataraci and Dosler, 2012). Another study found that peptide Pt5-1c, when used in combination with vancomycin and streptomycin, not only disrupts biofilms but also restores antibiotic sensitivity in multidrug-resistant strains, providing a dual therapeutic advantage(Duan et al., 2021).

Furthermore, AMPs can inhibit the mechanisms of drug resistance in pathogens. For instance, SPR741 enhances antibiotic efficacy by bypassing bacterial resistance mechanisms—such as efflux pumps in *E. coli*—to increase intracellular antibiotic concentrations(MacNair and Brown, 2020). Additionally, PAS8-b-PDM12 can inhibit efflux pump systems by dissipating the transmembrane electrochemical potential (Si et al., 2020).

2.6. Other indirect antimicrobial effects

In addition to functional proteins and peptides that directly impact pathogens, there are also proteins and peptides that indirectly inhibit pathogen growth by modulating their growth environment. For example, lactoferrin is a multifunctional iron-binding glycoprotein belonging to the transferrin family. It exerts its antimicrobial effect by sequestering iron in the bacterial environment—that is, by depriving bacteria of iron, a critical nutrient required for their growth (Wang et al., 2019b). Due to its broad-spectrum activity affecting both Gram-negative and Gram-positive bacteria, lactoferrin is considered an effective antimicrobial agent.

3. Sources of antimicrobial peptides and proteins

The discovery of AMPs dates back to 1939 when René J. Dubos extracted an antibacterial substance from a *Bacillus* strain in soil(Dubos, 1939). Since then, research on AMPs has gradually become a significant branch in the field of biomedicine. To date, the Database of Antimicrobial Activity and Structure of Peptides (DBAASP) has cataloged over 22,575 peptide entries, the vast majority of which are peptides no longer than 50 amino acid residues(Pirtskhalava et al., 2021).

3.1. Naturally occurring antimicrobial peptides

AMPs are among the oldest known molecular defense components of the innate immune system and are found across a wide range of organisms, including plants, arthropods, microorganisms, and various animals. According to data from the Database of Antimicrobial Activity and Structure of Peptides (DBAASP), approximately 68 % of AMP records originate from animals, 17 % from bacteria, while plants and fungi account for 8 % and 7 % respectively(Pirtskhalava et al., 2021).

In vertebrates, defensins are initially synthesized and are further classified into α, β, and θ types. These peptides typically consist of 18–45 amino acids, carry a positive charge, and contain three intramolecular disulfide bonds(Hollox and Abujaber, 2017). Mammals can produce various AMPs such as cathelicidins, defensins, and hepcidin(Shin and Jo, 2011). Amphibians can produce magainins and cancrins, while reptiles and birds primarily synthesize cathelicidins and defensins(Islam et al., 2023; Song et al., 2009; van Dijk et al., 2023). Fish possess multiple AMPs, including β-defensins, hepcidins and piscidins(Masso-Silva and Diamond, 2014). Invertebrates enhance their humoral defense mechanisms by synthesizing AMPs, which usually contain six to eight cysteine residues and exhibit cysteine-stabilized α/β structural motifs(Wu et al., 2021). Evolutionarily and structurally, β-defensins in invertebrates share similarities with those in vertebrates(S. Zhu and Gao, 2013). Defensins produced by insects, arthropods, and mollusks also contain six cysteine residues(Dini et al., 2022). Gram-positive microorganisms can synthesize various bacteriocins, which can be further classified based on their structural characteristics into lantibiotics, non-lantibiotics, large-sized bacteriocins, and uniquely structured bacteriocins(Somase et al., 2024). Fungi can produce peptaibols and fungal defensins. The antimicrobial peptides found in plants are even more diverse, including thionins, hevein-like peptides, defensins, knottins, stable-like peptides, snakins, lipid transfer proteins, cyclotides, and others(Shishupala, 2023).

3.2. Artificial design and optimization of antimicrobial peptides

Although AMPs exhibit excellent antimicrobial activity in vitro, their efficacy significantly decreases in the physiological environment of mammals due to factors such as high salt concentrations, serum proteins, divalent cations, and glycosaminoglycans(Malik et al., 2016). Additionally, the high sensitivity of AMPs to proteases affects their stability in bodily fluids and tissues, as well as their half-life in plasma; for example, LL-37 is extremely sensitive to endogenous enzymes present in the intestine, pancreas, and serum(Choonara et al., 2014). Coupled with challenges such as poor oral absorption, high production costs, potential toxicity to eukaryotic cells, and insufficient potency faced by natural AMPs, the artificial design of antimicrobial peptides has become a key strategy to overcome these obstacles. Artificial design of antimicrobial peptides is not merely a simple imitation of natural templates; rather, it is based on an in-depth study of the structure–function relationships of AMPs, utilizing modern biotechnologies and computational tools to develop novel AMPs with ideal antimicrobial activity, low toxicity, and high stability. Currently, the artificial design methods for AMPs mainly include site-directed mutagenesis, synthetic library screening, statistical approaches, molecular modeling, and display systems.

Site-directed mutagenesis allows researchers to adjust existing peptide sequences by adding, deleting, or replacing specific amino acids to improve their performance. For example, alanine or lysine scanning can be used to assess the impact of each amino acid side chain on the peptide's structure and function, enabling purposeful design. Shorter derivatives of LL-37 are a successful example; these short peptides exhibit higher stability and lower cytotoxicity(Rodríguez et al., 2021). Studies have shown that truncated versions of LL-37, such as LL-13 and LL-17, display stronger antimicrobial activity against clinically relevant resistant strains—including MRSA and vancomycin-resistant *Staphylococcus aureus* (VRSA)—whether used alone or in combination with vancomycin (Shurko et al., 2018). Synthetic peptide libraries are another powerful tool that can efficiently screen peptide sequences with desired activities. Cudic and colleagues constructed a positional scanning combinatorial library of cyclic lipohexapeptides, from which they identified analogs with enhanced antimicrobial activity without increasing nonspecific toxicity(Bionda et al., 2016). As more AMPs are discovered, scientists can extract important structural and biophysical properties from large databases; these data help predict and enhance the antimicrobial efficacy of peptides(G. Wang, 2020). Molecular dynamics simulations, as a powerful technical means, can reveal the details of interactions between AMPs and bacterial membranes at the atomic level(Allsopp et al., 2022; Y. Wang et al., 2016). If the simulation timescale is sufficiently long, it is even possible to directly observe the dynamic processes of membrane rupture or pore formation(Y. Wang et al., 2016). Additionally, bacterial surface display technology also provides possibilities for the de novo design of antimicrobial peptides(Randall et al., 2024).

3.3. Applications of artificial intelligence in antimicrobial peptide discovery

With the rapid advancement of artificial intelligence (AI) technologies, especially breakthroughs in generative models and large-scale

language models, the discovery process of AMPs has undergone revolutionary changes. The applications of AI in AMP design can be mainly categorized into two types: classification and generation.

Classification tasks involve representing peptides from one or multiple perspectives—including amino acid sequences, physicochemical properties, secondary structures, molecular fingerprints, and more—to predict various attributes of a given peptide, such as antimicrobial activity, cytotoxicity, and stability. This approach allows researchers to screen large pools of candidate sequences to identify AMPs that meet specific requirements for further experimental validation. For example, Wang et al. utilized this method to identify 181 peptides with antimicrobial potential from the human gut microbiome(Ma et al., 2022). Cesar de la Fuente-Núñez et al. mined a large number of antimicrobial peptides from the proteomes of extinct organisms(Wan et al., 2024). Additionally, Coelho et al. and De la Fuente-Núñez et al. discovered new AMPs from the global microbiome and the human microbiome, respectively(Santos-Júnior et al., 2024; Torres et al., 2024).

In contrast, generative tasks involve constructing peptide sequences from scratch that meet specific attribute requirements. As the peptide chain length increases, the computational resources required by traditional classification methods grow exponentially, making exhaustive searches of the entire chemical space impractical. Generative models, such as Generative Adversarial Networks (GANs) and Variational Autoencoders (VAEs), enable targeted exploration within the chemical space rather than blind enumeration, thus requiring relatively fewer computational resources. From a modeling framework perspective, one of the most popular methods for generating AMPs currently is the use of VAEs. Mojsilović et al. trained an encoder using a VAE and performed conditional sampling in its latent space to generate candidate AMP sequences(Das et al., 2021). On the other hand, Szczurek et al. combined conditional variational autoencoders (cVAEs) with classifiers to optimize given peptides, making them more likely to possess desired functional properties(Szymczak et al., 2023).

4. Current challenges

Functional peptides and proteins have shown great potential in the field of antifungal therapy; however, they also face a series of challenges. These issues mainly include high toxicity, lack of selectivity, insufficient stability, and potential immunogenicity(Sarkar et al., 2021). Peptides and proteins are easily degraded by proteases in the body, leading to low absorption rates and rapid clearance from the system—all of which reduce their effectiveness as therapeutic agents(Choonara et al., 2014). Additionally, the activity of functional peptides and proteins can be influenced by environmental factors such as pH, ionic strength, and serum proteins, which may alter their conformation and weaken their activity(Malik et al., 2016). Therefore, developing strategies to enhance their stability is crucial for the successful transition of these molecules from the laboratory to clinical applications.

When applying the direct antimicrobial activity of functional peptides and proteins to clinical therapy, one major challenge is their toxicity and selectivity (Takahashi et al., 2010; Torres et al., 2019). Although many peptides and proteins have demonstrated broad-spectrum antimicrobial activity, specificity remains an issue. Most functional peptides and proteins exert their effects by disrupting the cell membranes of pathogens; however, due to structural similarities between the membranes of host cells and pathogens, these peptides and proteins may also cause toxic damage to host cells. Therefore, balancing toxicity and pathogen selectivity is a key consideration in the design of antimicrobial peptides. Furthermore, the use of broad-spectrum antimicrobials may disrupt the microbial balance within the human body, leading to dysbiosis and other adverse health consequences (Rademacher et al., 2021; Ribeiro et al., 2020). Improving the selectivity of peptides and proteins so that they can specifically target certain pathogens without affecting beneficial microbes is thus an important current research direction. Another potential issue is the immunogenicity of functional peptides and proteins(Fernandez et al., 2018). Since they may be recognized as foreign substances by the host immune system, they can trigger unwanted immune responses. Such immune reactions may reduce the efficacy of the peptides and proteins and could even induce allergic reactions, inflammation, or other side effects. In clinical applications, these issues may limit the safety and feasibility of long-term use of peptides and proteins.

To address these challenges, scientists are exploring various strategies to improve functional peptides and proteins. These include optimizing their structures by modifying amino acid sequences or chemically modifying peptide chains to enhance their stability and reduce immunogenicity(Gentilucci et al., 2010; Han et al., 2021; Torres et al., 2019). The application of nanotechnology has also opened new avenues; by encapsulating peptides and proteins within nanocarriers for controlled release and targeted delivery to infection sites, it is possible to protect peptides from degradation and reduce immune responses (Fadaka et al., 2021). Laura Maria Duran Gleriani Primo and colleagues utilized nanoparticles to graft AMPs onto the surface of *N*-acetylcysteine-chitosan nanoparticles, enhancing the activity of rifampicin against *Mycobacterium tuberculosis*(Primo et al., 2024). Genetic engineering techniques similarly provide possibilities for producing more stable peptides and proteins, such as introducing genes encoding antimicrobial peptides into bacteria or fungi to produce modified, more stable peptides.(Drayton et al., 2020) Another strategy is to develop peptides and proteins derived from the host, such as human-derived peptides, which can significantly reduce their immunogenicity. The core of this approach is to utilize host endogenous proteins or peptides as a foundation and enhance their antimicrobial capabilities through appropriate engineering modifications. By overcoming these challenges, functional peptides and proteins are expected to become a viable clinical therapeutic option.

5. Conclusion and outlook

In recent years, as the issue of antibiotic resistance has gained increasing attention, there has been a renewed surge of interest in developing novel antimicrobial peptides and proteins. These peptides and proteins with antimicrobial functions are not only potential antimicrobial therapeutics but can also act as immunomodulators, exhibiting advantages of multifunctionality and durability, effectively addressing the challenges posed by antibiotic resistance. Furthermore, peptides and proteins can serve as templates or sources of inspiration, aiding researchers in designing more cost-effective and efficacious antimicrobial molecules(Sztukowska et al., 2019).

The rapid advancements in AI significant improvements in computational power, and the continuous enrichment of big data resources have provided robust support for rational design based on AI. The integration of AI technologies has not only accelerated the drug discovery process but has also facilitated the development of more efficient and targeted therapeutic strategies. Through these technological innovations, we hope to pave new pathways in combating infectious diseases and antibiotic resistance. Looking ahead, with the continuous progress of science and technology and the deepening of interdisciplinary collaborations, research on antimicrobial peptides and proteins will further expand their scope of application and may become crucial weapons against microbial infections. Simultaneously, by integrating cutting-edge technologies such as modern biotechnology, computational biology, and nanotechnology, we will continue to advance antimicrobial peptides and proteins from the laboratory to clinical applications, making significant contributions to public health endeavors.

CRediT authorship contribution statement

Yeji Wang: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation.

Minghui Song: Writing – review & editing, Writing – original draft. **Wenqiang Chang:** Writing – review & editing, Writing – original draft, Supervision, Resources, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Wenqiang Chang reports financial support was provided by National Natural Science Foundation of China (No. 82273975). If there are other authors, they 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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