



Review Article

Antimicrobial Peptides in Poultry Farming: A Multi-Omics Approach to Combat Antimicrobial Resistance

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ABSTRACT

The current rise of antimicrobial resistance (AMR) in poultry farming is a serious concern for the health of both birds and people. Overuse of antibiotics in the concentrated animal feeding operations have contributed greatly to the emergence of resistant bacteria and thus requiring innovation. Antimicrobial peptides (AMPs) are the potential alternatives to antibiotics as, owing to their action on microbial cell membranes, microbial resistance to them is less credible. Other than the direct impacts of addressing AMR, the use of AMPs would also bring favorable effects including; intestinal health optimization and production efficiency in poultry farming. This review aims at discussing the possibility of utilizing AMPs as a new source of sustainable antimicrobial drugs different from antibiotics, with special emphasis on their strategies of action and possibility to discover them by the help of multi-omics strategies. The integration of these tools helps to increase the yield of AMP production, breed for expression of AMPs, and to screen for the microbes capable of producing AMPs in the poultry microbiome. The increased incidence of AMR is investigated in this review with regards to the future of poultry farming and the possibilities to achieve untapped antibiotic-free poultry production through the utilization of AMPs.

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Key words

Antimicrobial peptides, AMR, Poultry, Human Health, Antibiotics, Microbiome

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INTRODUCTION

The increased frequency of antimicrobial peptides (AMPs) in poultry has been observed as a major threat to human health. In recent years, encouraged by results of the ever-increasing demand for poultry products, the three intensive poultry farming systems have greatly relied on antibiotics for treatment, growth promotion, and for

disease prevention. But this extensive and subsequently unrestrained prescription of antibiotics has not only amplified the pace at which bacteria develop resistance but is a serious threat to animal and human health (de Mesquita Souza Saraiva *et al.*, 2022). This is true with particular reference to the administration of sub-therapeutic antibiotic doses in the feeds given to poultry. Such practices have led to the chronic elucidation of bacterial species to low and sub-lethal concentrations of antibiotics so that resistant strains emerge and replicate. Pathogenic bacteria can be transmitted by several routes: contaminated meat, water, soil and through direct contact with infected persons and animals (Alegbeleye and Sant'Ana, 2020). This puts into perception the correlation between intensive poultry farming and the worldwide emergence of resistant bacteria, the consequences of which for sustainable non-antibiotic solutions.

Over the last few years, antimicrobial peptides (AMPs) have been identified as potential sources of alternatives to antibiotics used in poultry production. AMPs are short proteins that are part of the host immune response and are active against bacteria, fungi and viruses. While most antibiotics act upon certain structures of bacteria and consequently resistance is likely to occur, AMPs act on microbial's cell membranes (Seyfi *et al.*, 2020). The health benefits it brings to poultry farming include the decrease of AMR, support of good gut health, improved immunity, and improvement of productivity all without the dissemination of the antibiotic resistance issue (Mudenda *et al.*, 2023). For these reasons, AMPs have been considered as the new hopes in the war against AMR. Poultry farmers have a high perception towards quick action against pathogens while less chances of resistance development also make poultry a very reasonable substitution in present poultry farming. The use of AMPs can help change the way disease control or prevention is viewed and practiced within the growing problem of AMR in the poultry sector.

ANTIMICROBIAL RESISTANCE IN POULTRY

Bacterial pathogens in poultry are some of the biggest risks to both animal and personnel's health. The most common of them include extended spectrum beta-lactamase (ESBLs) from *Escherichia coli*, *Salmonella* and *Campylobacter*, which affects different diseases in poultry and pose potential health risks to human beings (Balta *et al.*, 2021). Not only have these pathogens learned how to exist within various conditions, but they have also found ways to defeat the antimicrobial treatments applied to them, mainly leading to the emergence of AMR to the poultry industry.

E. coli is a leading cause of colibacillosis in poultry and many have developed resistance to tetracycline and sulfonamide antibiotics. Likewise, *Salmonella* species causing the salmonellosis has developed resistance to fluoroquinolone, which is a crucial antibiotic to tackle severe forms of infection (Assoumy *et al.*, 2021). Foodborne infections in human by *Campylobacter* are becoming difficult to treat due to the increased resistance in the bacteria to macrolides and fluoroquinolones (Ammar *et al.*, 2021).

There is a need for alternate strategies to fight against infections such as antimicrobial peptides, bacteriophage therapy or enhanced biosecurity in poultry production. Studying the patterns of resistance inside these pathogens is significant in the course of understanding procedures to protect poultry from infections and to counteract the advancement of AMR.

There are several aspects of genetic and molecular basis of antibiotic resistance in bacteria can be discussed. A popular route is the gene transfer by horizontal gene transfer (HGT) in which new resistance genes in bacteria can be obtained from the environment or other organisms (Brito, 2021). This process happens mainly through conversion, shifting, or transference, which allow the quick surveillance of resistance traits by stroke through bacterial populaces.

Another important issue for consideration is the presence and function of efflux pumps as part of the underlying cause for resistance development. These membranes bound transport proteins pump antibiotics out of the bacterial cell and thus decreases antibiotic concentration and efficacy within bacterial cell. This mechanism is widespread in Gram-negative bacteria because the structure of their cell wall makes the solution of the problem even more complicated (Lorusso *et al.*, 2022).

ANTIMICROBIAL PEPTIDES (AMPS): MECHANISMS OF ACTION

What are AMPs?

AMPs are small molecular weight peptides that have intrinsic specificity and possess an inherent ability to kill microbes and bacteria. Taking a molecular weight of between 12 and 50 amino acids, AMPs are inherently designed to selectively recognize and destruct microbial pathogens of both bacterial, fungal, viral, and parasitic origin (Garvey, 2023). They are classified depending on their structure, as well as the specific amino acid components of the peptide and charge this imparts on the RBC's surface. AMPs can be categorized into three main types: cationic, anionic and neutral peptides. Defensins

and cathelicidin based C-AMPs are C-typed and being positively charged, whereas non-cationic AMPs, on the contrary, bear a negative charge (Huan *et al.*, 2020). Neutral peptides show variable specificity and specificity of action with microbial membranes.

Mechanisms of action of AMPs on bacterial cells

AMPs are fundamental parts of the intrinsic host defense and have distinct antibacterial modes compared to traditional antibiotics. Among the primary actions is undermining of bacterial membranes. AMPs as having the propensity of being amphipathic in structure in a bid to enable it interact with lipid bilayers. This interaction leads to membrane destabilization, to the formation of pores which cause cellular leakage and result in cell death (Guilhelmelli *et al.*, 2013).

Apart from disrupting the membrane, AMPs may interfere with cell wall formation within bacterial cells. It has foldamers that hinder the synthesis of layered layers to offer bacteriophage structural support (Pontes *et al.*, 2022). Since basic model of action of AMPs implies interference with the biosynthesis of enzymes constituting the cell wall; disruption of the regular bacterial cell wall synthesis inhibits bacteria growth and division, making them susceptible to osmotic pressure and what follows lysis (Assoni *et al.*, 2020).

AMPs are however, involved in the regulation of immune responses. They can stimulate many immune cells, including the macrophages and neutrophils that will boost the host's defense mechanism as shown in Figure 1. This immunomodulatory effect also improves not only the direct pathogen killing activity but also supports the general immune response to infections. Taken together, these mechanisms anchored on the capability of AMPs in countering antibiotic-resistant bacteria.

Multi-targeting nature of AMPs: Combating resistance

This multiple targeting behavior of AMPs makes them suitable for use in the fight against antibiotic resistance in a way that other antimicrobial agents cannot achieve. Compared with most of the venational antibiotics targeting one or many bacterial molecules, AMPs engage the microbial cell structures inclusive of cell membranes, proteins and nucleic acids. This diverse interaction strategy increases their effectiveness against a wide range of pathogens and at the same time minimizing the chances of bacteria acquiring resistance to the antimicrobial agents (Li *et al.*, 2021).

Furthermore, the penetration of the bacterial membrane is not affected by the changes in the bacterial cell wall structure or its ability to produce efflux pumps. Such compounds existed in their cationic forms, which

lead to the disruption of bacterial membrane coating hence causing cell lysis even in resistant strains. This is a novel mode of action not only extends the life for AMPs in treating infections but also provides a compelling solution to one of the most challenging issues within the poultry farming industry, the antimicrobial resistance.

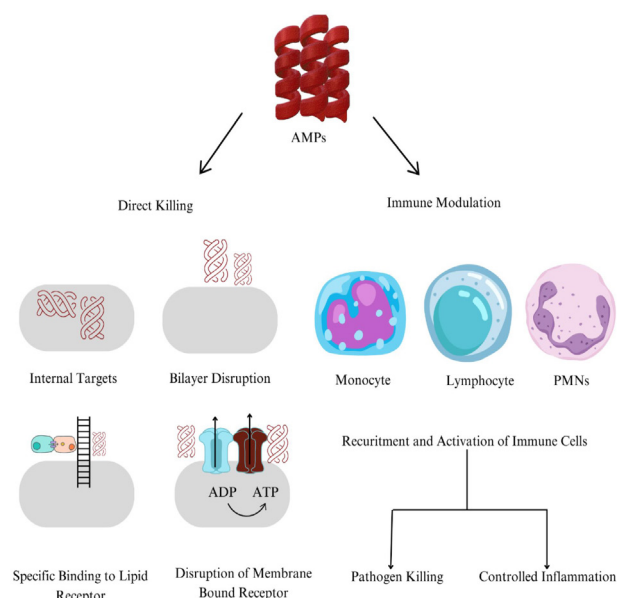


Fig. 1. Mechanism of action of AMPs and their immunomodulatory strategies.

MULTI-OMICS APPROACHES IN POULTRY SCIENCE: INTEGRATION FOR AMP DISCOVERY AND DEVELOPMENT

Microbiomics: Exploring the poultry gut microbiome

It is quite clear that gut microbiome in poultry has significant importance in promoting health and production among birds. Not only does this dense population of microorganisms play a role in nutrient acquisition but also immune regulation is also heavily influenced by it. A healthy composition of the microorganisms in the gut can in fact help the host to counter the pathogens and help restrain the growth of pathogenic microbes at the site. Research has also identified certain microbial strains that act as a chiropodist for the immune system to form and function in the most efficient way so far for infection controlling in poultry (Nazeer *et al.*, 2021).

Genomics and transcriptomics: Discovering AMPs and resistance genes

Genomics and transcriptomics are key descriptors in expanding knowledge of AMPs and resistance genes in poultry. It means that with the help the high-throughput

sequencing, one can discover new AMPs in various breeds of poultry (Silva *et al.*, 2020; Yakovlev *et al.*, 2020). They help to carry out investigation on the complete genome which centrals to the identification of hitherto unknown sequences of peptides that possess antibacterial features.

Proteomics: Analyzing AMP interactions with pathogens

AMPs involved in immunity as direct agents of bacteria-virus interactions and part of the first line of host defense. Since the target of AMPs are pathogens, it is crucial to develop knowledge on PPIs between the AMPs and pathogens in order to understand the way in which antimicrobial functions. Interactions between these two molecules occur at particular receptor sites on both the AMP and the pathogen, resulting in these effects and others such as destruction of microbial membranes or preclusion of critical cellular processes (Mücke *et al.*, 2020). The methods of proteomics have proved to be instrumental in unraveling these interactions, enabling investigators to locate the intricacy of interactions between AMPs and various microbial organisms.

Metabolomics: AMP metabolism and pathogen metabolic pathways

Small metabolite profiling strategy called metabolomics serves to be instrumental in learning about AMPs and pathogen interactions. It is understood that the AMPs, because of their membrane disruptive properties, can interfere with the basic cell functions (Kieler *et al.*, 2021). Information on the metabolic process of AMP molecules is derived from the study of the mechanism of action of the enzymes involved in the synthesis, modification, and degradation of these peptides by microbial organisms.

Thus, the general model is that when the bacteria come into contact with AMPs, they die or, in some cases adapt metabolically to support the bacterium. Such changes may include: increased synthesis of enzymes that break down AMPs that can otherwise harm the invading pathogens.

Bioinformatics in AMP design and optimization

On the efficiency of design and optimization of AMPs, bioinformatics serves an important function, mostly increasing the speed of their development. When the structural and functional properties of synthetic AMPs are considered, computational approaches allow estimating their effectiveness (Ramezanzadeh *et al.*, 2021). For example, molecular dynamics simulations yielded information on the stability and interaction between the AMPs and microbial membranes, which steer the optimization process.

Several other databases and computational tools have been designed for the purpose of identification and

characterization of AMPs. There are much larger libraries of known AMPs available at public databases such as the Antimicrobial Peptide Database (APD) and the CAMP (Collection of Antimicrobial Peptides).

AMPS AND POULTRY IMMUNOLOGY: ENHANCING HOST DEFENSES

Role of AMPs in modulating innate and adaptive immunity

AMPs represent a versatile class of molecules that are involved in the regulation of immune responses that are innate and acquired immunity systems. These small, positively charged peptides are not only antimicrobial with activity against a wide range of microbes, but they also modulate immune functions as shown in Figure 1.

AMPs modulate different immune cells, such as macrophages, dendritic cells, and neutrophils as well as improve the antibacterial effects. Direct activation influences the synthesis of a great number of pro-inflammatory cytokines using conventional examples, for instance, TNF- α , IL-1 β , and IL-6, which direct the immune response (Ma *et al.*, 2020). Apart from its ability to recruit other immune cells to the site of infection it also generates the inflammation necessary for pathogen elimination.

Cross-talk between AMPs and the poultry gut-immune axis

AMPs are essential for the defense of the digestive tract and regulation of the immune system in animals as it is in poultry. Not only are these endogenous molecules capable of exhibiting strong antimicrobial properties but they also modulate the relationship of the gut with the immune system. AMPs are able to control the microbiota through the mechanism of the suppression of pathologic microbes and at the same time the stimulation of beneficial microbes to contribute to their proper colonization (Krsek and Baticic, 2024). This balance is critical since the gut needs to be strong at the same time as the absorption of macronutrients is to occur optimally.

AMPS IN POULTRY BREEDING: TARGETING GENETIC TRAITS FOR RESISTANCE

Incorporating AMP production into poultry breeding programs

The inclusion of AMPs in genetically improved chickens provides opportunities to improve disease resistance (Rodrigues *et al.*, 2021). AMPs are part of the poultry's innate defenses against different kinds of attacks by pathogens. Thus, breeders now know specific genetic markers indicating potential for high levels of AMP production and therefore improved bacterial resistance in

their poultry stocks.

Promoting the selection of AMP related characters also gives promising while using antibiotics which has some issues with AMR. Successful breeding for birds that express higher AMPs could enhance their ability to fight bacterial invading pathogens like *E. coli* and *Salmonella* (Murugaiyan *et al.*, 2022). Such an approach is concurrent with the industry's requirement of minimizing antibiotic use for the welfare of animals and the outcome of agriculture production.

Genetic engineering and CRISPR approaches for AMP optimization

Application of modern methods of genetic engineering, such as CRISPR/Cas9 technology, creates new opportunities for regulating the indicators of AMPs in poultry breeds. AMPs are important for immune system protecting humans naturally against different kinds of infectious agents (Alok *et al.*, 2021). It can take long periods for traditional breeding techniques to help boost the levels of AMP expression in plants, but CRISPR offers clear and effective ways of modulating AMP-related gene characteristics inherited in plants (Getahun *et al.*, 2022).

Poultry genes can now be modified using the CRISPR/Cas9 system to increase the expression of other AMP-related genes. When CRISPR technology is incorporated into selection programs, Breyer and his team will be able to produce chickens with the best AMP scores to ensure they are highly resistant to bacterial infections (Khwatenge and Nahashon, 2021).

BIOTECHNOLOGICAL ADVANCES IN AMP PRODUCTION FOR POULTRY FARMING

Synthetic biology and AMP engineering

To this effect, advances in biotechnology have presented other channels for increasing the efficacy and output of AMPs in poultry farming. The conventional techniques of synthesizing AMP from natural sources are not affluent in yield and frequently appear instability (Zhao *et al.*, 2023). Though, in this field, biotechnology, especially synthetic biology is a vast improvement. This is one of the major advantages of using technology to come up with peptides as compared to natural peptides the ability to come up with peptides with better potency, stability and specificity than we have seen in natural products.

Use of recombinant DNA technology for AMP production

Biotechnology that has boosted the production of AMPs is Recombinant DNA technology which allows for reflection of these peptides in microbial systems. Of them, one technology is a method for cloning the genetic

sequences of AMPs into bacterial or yeasts, so they can produce the peptides in mass quantities (Sampaio de Oliveira *et al.*, 2020). When the recombinant AMPs are expressed in bacterial hosts, for instance *Escherichia coli*, or yeast system, such as *Saccharomyces cerevisiae*, the benefits include the ability to create large quantities of the AMP which are important for uses in the poultry industry (Fig. 2).

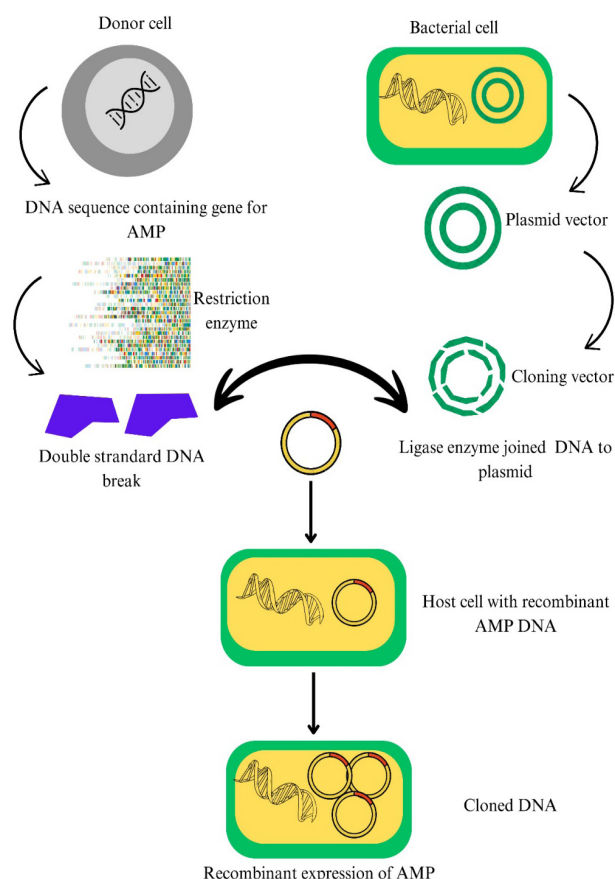


Fig. 2. Schematic representation of recombinant DNA technology for AMPs production: A stepwise approach illustrating the cloning of AMP genes into bacterial expression systems, optimization of gene expression, and large-scale AMP production.

In bacterial systems, the AMP genes are incorporated into the plasmid and the later they are transferred in the host bacteria. The bacteria are manipulated to form biofilms which secrete the target AMPs identified in this research; these biofilms are then harvested where the AMPs are extracted and purified (Jiang *et al.*, 2020). Yeast systems can be seen as an alternative especially for expression of structure-matured AMPs or those that require post-translational modifications.

Biotechnological approaches for enhancing AMP stability and delivery

New bio-hybrid materials that have gained prominence in the poultry industries, can also be used in the biochemical stabilization of AMPs required in chicken production. Owing to their susceptibility to degradation, challenges associated with stability of AMPs when in feed or on the skin surface have been known to occur (Yadav *et al.*, 2024). This problem has been solved by newer methods in the delivery of AMPs as well as enhancement of their bioavailability by methods like encapsulation.

CHALLENGES AND OPPORTUNITIES IN IMPLEMENTING AMPs IN POULTRY FARMING

Challenges in AMP development and use

This paper investigates the potential opportunities and challenges engaged with the application of AMPs in poultry farming. One of the main challenges of developing AMPs is largely based on the production mode and its feasibility (Chen and Lu, 2020). Although, most of the AMPs have the potency of the conventional Antibiotics, the problem associated with the synthesis of the peptides is still a limitation in terms of affordable mass production. However, the large-scale cultivation, though possible, demands the efficiency, reduction of cost of biotechnology techniques so as to obtain high yields.

Another problem is the stability and bioavailability of the compound. Disadvantages of AMPs include a short half-life in the gastro-intestinal tract of poultry, and are easily degradable by the harsh conditions existing in the tract (Bisht *et al.*, 2024). Several of the identified peptides need to exercise their antimicrobial functions long enough and may need additional technologies more advanced than encapsulation to keep them safe from degradation enroute to their targets.

Other barriers to the adoption of AMPs in poultry farming include; Other challenges which also affect the use of AMPs include the following: Governmental agencies are very sensitive to sanction new antimicrobial agents to the market, and AMPs, therefore, have to pass several safety and efficacy tests. This takes some time and a lot of money.

Potential synergy between AMPs and traditional antibiotics

The use of AMPs organized with traditional antibiotics is a shift in the accepted ways of animal husbandry so as to fight AMR as a contagious problem (Taheri-Araghi, 2024). For targeting of bacterial cells, AMPs operate

architecturally unlike mediocre antibiotics which tend to focus on targeting a certain biological process. Such differences in mechanisms allow for an improvement in effectiveness of therapy when other antibiotics are included since there is a combined action effect.

E. coli, *Salmonella*, and *Campylobacter* are some of the resistant pathogens that could be carried under control by the combined action of antibiotics and AMPs. Since the drugs diminish the chances of the bacteria becoming resistant by hitting at many points, the bacteria become less able to survive the drugs, hence, the risk of resistance mutation being very low (El-Baaboua *et al.*, 2022). Also, AMPs can make it possible for antibiotics to enter the bacteria more proficiently by making their membranes permeable.

FUTURE PROSPECTS AND CONCLUSION

The role of multi-omics in the future of AMP research

The potential of antimicrobial peptides with regards to their research applications in poultry farming, especially using multi-omics approaches, seems to be limitless. These tools for Amp studies such as genomics, proteomics, transcriptomics and metabolomics are a novel way for studying AMPs at various levels within different organisms (O'Donnell *et al.*, 2020). When deployed, these technologies can enable the researchers to identify new AMPs more accurately, hence enhancing their effectiveness and precision of target engagement. Multi-omics does not only improve the rate of discovery of new AMPs but improves our understanding of their engagement with pathogens and host organisms.

The future of AMP research, aided by the availability to use multi-omics tools will enable turning TEM from a vague concept into an effective method. They can help us to design AMPs targeted at a particular pathogen or resistance mechanisms, thereby optimizing their efficacy and stability. In addition, a holistic approach such as combined synthetic biology and bioengineering strategies by using wealth of multi-omics data may enable us to design AMPs that can be perfectly tailored with high activity and stability.

AMPs as a sustainable solution to AMR in poultry farming

The sustainable solution offered by means of AMPs is an innovative method to address the menace caused due to AMR in poultry farming thus envisaging antibiotic free production systems (Rokade *et al.*, 2023). With all the attention nationwide against antibiotic use in poultry, AMPs could be a more natural, built-in alternative to fighting off those unwanted pathogens.

AMPs are synthesized by different sources and show antimicrobial activity across a wide range of organisms

(bacteria, fungi, viruses). Its mechanism of action is unique, such as chewing away microbial membranes willy-nilly, so it inhibits the rate of resistance compared with traditional antibiotics (Erdem-Büyükkiraz and Kesmen, 2022). The inclusion of AMPs in poultry farming can improve health, growth and productivity while reducing the use of antibiotics that confer a risk for resistance development.

In addition, AMPs can improve basic flock health which results in better production and lower mortality. In addition to being an animal welfare improvement, this will also address consumer demand for cleaner and less polluting meat production (Annunziato and Costantino, 2020).

Call for integrated research and policy framework

The full potential of AMPs combating AMR is now realizable by the integration between researchers and policymakers. Microbiologists, immunologists and biotechnologist may work together to offer an overview ranging from their fundamental aspects to fruition in avian farming.

These cross-disciplinary relationships may also inspire new ideas and strategies, thus terminating in the identification of emerging AMPs together with new methods for their delivery. Besides, the early involvement of policy advisors in research is a way to provide suggestion that can help translate findings into workable recommendations and regulations needed for responsible use of AMPs on farms.

CONCLUSION

All things considered; AMPs have now become a creative adjuvant in the fight against AMR in poultry farming. Their specific mechanism of action, broad-spectrum activity and minimal resistance potential make them important substitutes for conventional antibiotics. By using alimentary AMPs within the poultry production systems antibiotics and other therapeutics can be minimized thus extending healthy and sustainable use of animal agriculturists towards the mission of curtailing antibiotic use in the world.

The next chapter in the evolution of research on AMPs is dedicated to finding new peptides specific for certain pathogens and improving their characteristics using modern multi-omics tools. This will further our appreciation of AMPs and how they function and function in the complex milieu that is biological tissues.

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The authors declare that no generative artificial intelligence (AI) or AI-assisted technologies were used in the writing or analysis of this manuscript.

Statement of conflict of interest

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