

Review Article

Antimicrobial Peptides: Promising Alternatives to Antibiotics in Aquaculture

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Received 27 October 2025; Revised 17 November 2025; Accepted 18 November 2025

Abstract

The increasing global demand for sustainable protein has intensified aquaculture production, leading to excessive antibiotic use and a corresponding rise in antimicrobial resistance (AMR). This threatens aquatic animal health, ecosystem stability, and public health, prompting the Food and Agriculture Organization (FAO) to implement a five-year plan (2021–2025) to reduce antibiotic use in animal agriculture. Antimicrobial peptides (AMPs), naturally occurring oligopeptides with broad-spectrum antimicrobial activity and immunomodulatory properties, have emerged as promising alternatives to conventional antibiotics in aquaculture. This review highlights the potential of antimicrobial peptides (AMPs) in aquatic animal health by examining their sources, types, and mechanisms against major pathogens. It evaluates studies on AMP efficacy, safety, and impacts on non-target organisms. Innovative approaches to enhance AMP stability and reduce costs for aquaculture use are discussed, along with their role in reducing antibiotic resistance and environmental impact. Challenges including production costs, inconsistent effectiveness, and regulatory issues are also addressed. Future research should focus on enhancing AMP specificity, optimizing large-scale production, and assessing long-term ecological impacts. This review provides valuable insights for researchers, policymakers, and stakeholders aiming to integrate AMPs into sustainable aquaculture practices.

Keywords: Antimicrobial peptides (AMPs), Aquaculture, Antibiotic resistance (AMR), Disease management, Immunomodulatory.

1. Introduction

The rising global population and increasing demand for nutritious food have positioned aquaculture as a sustainable solution for protein production. In 2018, aquaculture reached a record 82.1 million tonnes of fish across 425 species FAO (2020). However, excessive antibiotic use has led to drug-resistant pathogens and antibiotic residue accumulation (Cabello, 2006; Karunasagar et al., 2020). In response, the FAO introduced a five-year plan (2021–2025) to curb antibiotic use in animal production FAO (2021). Antimicrobial peptides (AMPs) have emerged as a promising alternative to antibiotics in aquaculture. These naturally occurring peptides, typically under 100 amino acids, have broad-spectrum antimicrobial properties due to their positive charge and hydrophobic residues (Zhang & Gallo, 2016). AMPs enhance immune responses, stimulate phagocytosis, and help break down pathogens, making them valuable in disease control (Tassanakajon et al., 2018).

Despite their potential, AMPs' mechanisms are not fully understood. Ongoing research aims to optimize their physicochemical properties for cost-effective and safe applications (Lazzaro et al., 2020). Future studies should focus on improving AMP specificity, synergistic potential, and antimicrobial efficacy to promote sustainable aquaculture practices while reducing antibiotic dependency.

2. Classification of Antimicrobial Peptides

As of January 2025, the Antimicrobial Peptide Database (APD) contains a total of 5,099 peptides, comprising 3,306 natural antimicrobial peptides (AMPs) derived from six life kingdoms. This includes 410 bacteriocins/peptide antibiotics from bacteria, 5 from archaea, 8 from protists, 29 from fungi, 268 from plants, and 2,580 from animals. In addition, the database features 1,299 synthetic AMPs and 231 predicted AMPs, all exhibiting a range of functions and biological activities (UNMC, 2025).

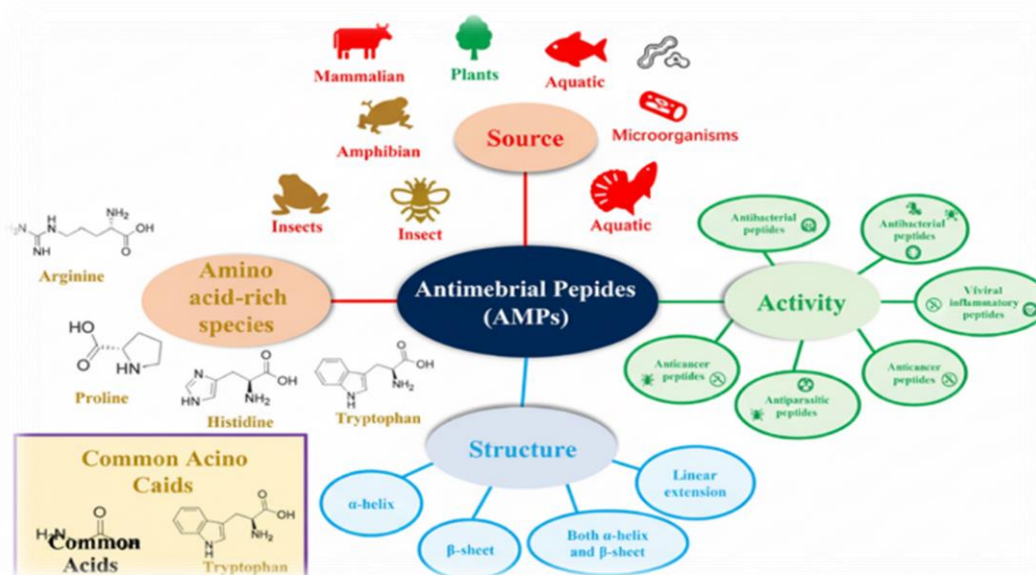


Figure 1: Classification of AMPs

The vast diversity of natural AMPs makes their classification challenging. They are categorized based on four main criteria: (1) their source, (2) biological activity, (3) structural characteristics, and (4) amino acid composition (Figure 1).

2.1 Classification of AMPs based on sources

AMPs originate from mammals, amphibians, insects, microorganisms, plants, and marine organisms.

2.1.1 Mammalian AMPs

Cathelicidins and defensins, found in humans, cattle, and other vertebrates, protect against infections. Defensins are categorized into α -, β -, and θ -defensins based on disulfide bonds (Reddy et al., 2004). Human host defense peptides (HDPs) vary by life stage, with LL-37 in newborns and hBD-2 in the elderly (Gschwandtner et al., 2014). AMPs in breast milk enhance infant immunity (Field, 2005). Dairy-derived AMPs like lactoferricin B (LfcinB) may aid dairy preservation (Sibel Akalin, 2014). HDPs also regulate immunity, apoptosis, and wound healing (Wang, 2014).

2.1.2 Amphibian AMPs

Frogs are a primary source of AMPs, including magainins from skin secretions (Conlon & Mechterska, 2014). Cancrin, found in *Rana cancrivora*, expands the diversity of amphibian AMPs (Lu et al., 2008).

2.1.3 Insect AMPs

Insects synthesize AMPs in fat bodies and blood cells for survival (Vilcinskas, 2013). Cecropins, found in silkworms, bees, and *Drosophila*, exhibit antimicrobial properties (Dutta et al., 2019). AMP count varies by species, with *Harmonia axyridis* and *Hermetia illucens* producing up to 50 AMPs, while *Acyrtosiphon pisum* lacks them (Shelomi et al., 2020). Jellein, from bee royal jelly, has antibacterial and antifungal activity (Zahedifard et al., 2020).

2.1.4 Microbial AMPs

Bacteria and fungi produce AMPs like nisin and gramicidin (*Lactococcus lactis*, *Bacillus subtilis*, *Bacillus brevis*) (Cao et al., 2018). Biological expression methods in *Pichia pastoris*, *Saccharomyces cerevisiae*, and *E. coli* are gaining interest, though challenges like toxicity necessitate fusion tags (Parachin et al., 2012; Yu et al., 2015).

2.1.5 Plant and Marine AMPs

Plants produce AMPs like thionins and defensins (Tang et al., 2018). Marine AMPs show promising potential, with As-CATH4 boosting immunity (Semreen et al., 2018), Myticusin-beta as an antibiotic alternative (Oh et al., 2020), and GE33 (pardaxin) enhancing antitumor immunity (Huang et al., 2013).

2.2 Classification based on activity

AMPs exhibit diverse bioactivities, categorized into 18 groups according to the ADP3 database. These can be broadly classified as antibacterial, antifungal, antiviral, antiparasitic, anti-HIV, and anti-tumor peptides.

2.2.1 Antibacterial peptides

Antibacterial AMPs effectively inhibit various pathogens, including MRSA, VRE, *Acinetobacter baumannii*, *S. aureus*, *E. coli*, and Salmonella. Natural and synthetic AMPs like nisin, cecropins, and defensins target both Gram-positive and Gram-negative bacteria. Notably, synthetic peptides P5 and P9, inspired by *Aristiclutluthys nobilia* interferon-I, show strong MRSA inhibition with low cytotoxicity (Li et al., 2019).

2.2.2 Antifungal peptides (AFPs)

AFPs combat drug-resistant fungal infections, targeting *Candida albicans*, *Aspergillus*, and food-related molds. Peptides such as brevinin, ranatuerin, and cecropins exhibit antifungal properties. AurH1, derived from aurein 1.2, effectively treats *C. albicans* (Madanchi et al., 2020). Additionally, AFPs from *Lactobacillus plantarum* reduce *A. flavus* spore formation in maize, while radish-derived

AMPs inhibit yeast species like *Zygosaccharomyces bailii* (Shwaiki et al., 2020).

2.2.3 Antiviral peptides (AVPs)

AVPs target viruses by preventing attachment, disrupting envelopes, or inhibiting replication (Jung et al., 2019). They offer potential solutions against COVID-19, HIV, foot-and-mouth disease virus, and avian influenza virus. Epi-1 peptide effectively inactivates foot-and-mouth disease virus (Huang et al., 2018), while swine intestinal AMPs significantly reduce mortality in IBV-infected chicken embryos (Sun et al., 2010). Anti-HIV peptides, including defensins, LL-37, gramicidin D, and dermaseptins, have been widely studied, with enfuvirtide (Fuzeon™) being commercially available as an HIV treatment (Ashkenazi et al., 2011).

2.2.4 Antiparasitic peptides

Parasitic protozoa spread through various contact routes, posing health risks (Chalmers et al., 2020). With increasing drug resistance, new treatments are needed. AMPs like cathelicidin and temporins-SHd inhibit malaria and leishmaniasis-causing parasites (Mangoni et al., 2005; Abbassi et al., 2013). Epi-1 disrupts *Trichomonas vaginalis* membranes, while Jellein and KDEL show strong anti-*Leishmania* activity (Neshani et al., 2019; Cao et al., 2019). Unlike eukaryotic AMPs, cyanobacterial peptides target specific proteins for parasite selectivity (Rivas & Rojas, 2019).

Anticancer peptides (ACPs)

ACPs fight cancer by recruiting immune cells, inducing tumor cell death, inhibiting angiogenesis, and disrupting gene regulation (Ma et al., 2020). Peptides like tritrpticin, indolicidin, and puroindoline A show strong anticancer effects (Arias et al., 2020). Optimizing their efficacy requires balancing net charge and hydrophobicity.

2.2.5 Other bioactive peptides

Peptides with anti-inflammatory, anti-diabetic, and spermicidal properties differ from AMPs. Anti-

inflammatory peptides inhibit cytokines and signaling pathways (Meram & Wu, 2017). Anti-diabetic peptides modulate GRK 2/3 or activate GLP-1 and glucagon receptors (Khan et al., 2018). Due to their broader functions, they are classified as bioactive rather than antimicrobial peptides.

2.3 Classification of AMPs based on amino acid composition

2.3.1 Proline-rich peptides (PrAMPs)

Unlike most AMPs, PrAMPs enter bacterial cells via the SbmA transporter rather than disrupting membranes. Inside, they inhibit protein synthesis by targeting ribosomes (Mattiuzzo et al., 2007; Seefeldt et al., 2015). Examples include Tur1A, which interferes with translation, and Bac5 and Bac7, which contain repeating proline-arginine motifs (Mardirossian et al., 2019). Though primarily effective against Gram-positive bacteria, some, like pPR-AMP1 from *Scylla paramamosain*, also target Gram-negative bacteria (Imjongjirak et al., 2017). PrAMPs may also enhance immune responses (Li W. et al., 2016).

2.3.2 Tryptophan- and arginine-rich peptides

Tryptophan (Trp) and arginine (Arg) enhance peptide interactions with bacterial membranes. Trp facilitates membrane penetration, while Arg provides charge and bonding properties (Walrant et al., 2020). Well-known AMPs such as indolicidin and tritrpticin, along with Octa 2 (RRWWRWR), show strong antibacterial effects (Chan et al., 2006). Short Trp- and Arg-rich peptides derived from lactoferricin also exhibit potent antimicrobial activity (Bacalum et al., 2017).

2.3.3 Histidine-rich peptides

Histidine-rich AMPs improve membrane permeability, leading to bacterial cell death. HV2, designed with specific histidine patterns, disrupts bacterial membranes and reduces bacterial motility while also exhibiting anti-

inflammatory properties (Dong et al., 2019). Modified versions of Octa 2 and magainin-based peptides, such as L4H4, further enhance antibacterial activity and cell penetration (Bacalum et al., 2017; Lointier et al., 2020).

2.3.4 Glycine-rich peptides

Glycine-rich AMPs, such as attacins and dipterocins, commonly occur in nature and play a structural role in peptide function (Kwon et al., 2008). Some, like those from salmonid cathelicidins, activate immune responses instead of directly killing bacteria (D'Este et al., 2016). GG3, a glycine-rich synthetic peptide, is a promising candidate for treating Gram-negative infections (Wang et al., 2015).

2.4 Classification of AMPs based on structure

Antimicrobial peptides are classified into four structural types: linear α -helical peptides, β -sheet peptides, extended linear peptides, and those containing both α -helices and β -sheets (Lei et al., 2019). Additionally, more complex structures, such as cyclic peptides, lasso peptides, and thioether-bridged AMPs, have been identified (Koehbach & Craik, 2019).

3. Modes of Action of Antimicrobial Peptides

The potential mechanisms of AMPs include membrane-active and non-membrane-active pathways.

3.1 Membrane-active pathway

Membrane-active AMPs exert their antimicrobial effects by disrupting the cell membranes of bacteria, fungi, and other pathogens (Huan et al., 2020). Their cationic nature facilitates binding to the negatively charged lipopolysaccharides on microbial membranes through electrostatic interactions. This interaction enables AMPs to assume an amphipathic conformation, increasing their membrane affinity. Classified as permeabilizing membrane peptides, these AMPs induce pore formation in the microbial membrane, causing leakage of cellular

contents and leading to rapid cell death (Sengupta et al., 2008).

3.2 Non-membrane-active pathway

These mechanisms function by disrupting essential intracellular processes. They encompass several key actions: (a) interference with crucial stages of protein biosynthesis, including transcription and translation (Mardirossian et al., 2014); (b) suppression of nucleic acid synthesis by targeting vital enzymes like DNA topoisomerase I, thereby accelerating nucleic acid degradation (Subbalakshmi and Sitaram, 1998); (c) significant inhibition of protease enzyme activity in various bacterial cells (Huan et al., 2020); and (d) obstruction of cellular division by disrupting the DNA replication process (Lutkenhaus, 1990).

4. Antibiotic resistance in aquatic ecosystem

Regional aquaculture practices and the types of species farmed lead to variations in antibiotic levels found in farmed water. Despite these geographical differences, the types of antibiotics most frequently detected tend to be consistent across regions. Tetracyclines, macrolides, fluoroquinolones, and sulfonamides are commonly found in aquaculture water. The solubility of the antibiotics themselves mainly influences the presence of antibiotic residues in water, how often they are used, and the extent of their application (García-Galán et al., 2010). The age and development stage of the farmed organisms also play a role, with younger animals often requiring more antibiotics for disease prevention and to promote growth because they are typically more vulnerable (Hedberg et al., 2018). The widespread and sometimes inappropriate use of antibiotics has led to bacterial resistance, increasing the presence of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) in various aquatic environments. This pollution from antibiotics, ARB, and ARGs has become a major concern.

Table 1: List of AMPs isolated and identified from fish

S. No.	AMPs	Source	Expression site	Activity	References
1.	Pleurocidin	<i>Pleuronectes americanus</i>	Gills, skin, Spleen, kidney, Intestine	Antibacterial, Antifungal, Antiviral, Antiparasitic, Antitumor, Immunomodulation & chemotaxis.	Cole & Diamond, 1997
2.	Cathelicidin	<i>Oncorhynchus mykiss</i>	Gills, liver, Spleen, Intestine	Antibacterial against Gram-positive & Gram-negative bacteria, Antifungal, Immunomodulation.	Uzzell et al., 2003
3.	β -defensin	<i>Danio rerio</i>	Skin, mucosal membrane of Gut and urinogenital tract, kidney, spleen, eyes	Antibacterial, Antiviral against SGIV, VNVV etc, chemotaxis, immunomodulation, nuclear & mitochondrial fragmentation in <i>Trypanosoma cruzi</i>	Bulet et al., 2004
4.	Piscidin	<i>Morone chrysops</i>	Skin, gills, Blood Intestine, Kidney, Spleen	Antibacterial, Antifungal, Antiviral, Haemolytic and antitumor	Silphaduang et al., 2001
5.	Hepcidin	<i>Channa maculate</i>	Liver, spleen, Kidney and intestine	Anti-Gram-positive, Anti-tumor, Anti- Gram-negative, Antiviral, Immunomodulation, iron regulation	Shike et al., 2002
6.	Epinecidin	<i>Epinephelus coiodes</i>	Gills, Intestine	Anti-Gram-positive, Antifungal, Anti- Gram-negative, Anti-cancer, Antiviral, Wound healing	Lauth et al., 2002
7.	Hipposin	<i>Hippoglossus hippoglossus</i>	Skin	Anti-Gram-positive and Gram-negative	Birkemo et al., 2003
8.	Histone-derived	<i>Salmo salar</i>	Gills, liver, Spleen, Intestine	Antibacterial against Gram-positive & Gram-negative bacteria, Antifungal, Immunomodulation.	Richards et al., 2001
9.	Moronecidin	<i>Morone sp.</i>	Skin, gills, Blood Intestine, Kidney, Spleen	Antibacterial, Antifungal and Causes necrosis	Lauth et al., 2002
10.	Misgurin	<i>Misgurnus sp.</i>	Skin	Anti-Gram-positive, Antifungal and Anti-Gram –negative	Park et al., 1997
11.	Pardaxin	<i>Pardachirus sp.</i>	Skin	Anti-Gram-positive and Gram-negative, Haemolytic, Anticancer	Porcelli et al., 2004
12.	Oncorhynchin II	<i>Onchorhynchus mykiss</i>	Skin, Gills	Anti-Gram positive and Gram-negative, Poorly hemolytic.	Fernandes et al., 2004

The contamination of ARGs and their distribution in microbial populations across the biosphere is a consequence of continuous selection driven by heavy antibiotic use in human activities over recent decades (Knapp et al., 2010). High demand for aquaculture products results in greater antibiotic use in farmed products than human applications, exacerbating antibiotic resistance in pathogens (Tang et al., 2017). Antibiotics commonly found in aquaculture and aquatic ecosystems include fluoroquinolones (enrofloxacin, norfloxacin, and ofloxacin), sulfonamides (sulfamethoxazole and trimethoprim), macrolides (erythromycin) and tetracyclines etc (Okeke et al., 2022). The spread of ARB and ARGs in aquaculture environments, as a novel form of pollution, may pose greater harm than the antibiotics themselves (Amarasiri et al., 2020).

The distribution of antimicrobial resistance among fish pathogens highlights *Vibrio* (23%) and *Aeromonas* (20%) as the most resistant, posing significant risks in aquaculture. Enterobacteriaceae (10%) and biofilm-forming bacteria like *Pseudomonas*, *Acinetobacter*, and *Flavobacterium* (5% each) show moderate resistance. Lower resistance is observed in *Salmonella* and *Staphylococcus* (4%), while emerging resistance is noted in marine bacteria *Photobacterium* and *Pseudoalteromonas* (3%). Least prevalent resistant pathogens ($\leq 2\%$) include *Edwardsiella*, *Arthrobacter*, *Streptococcus*, and *Mycobacterium*, which still pose challenges due to their pathogenicity.

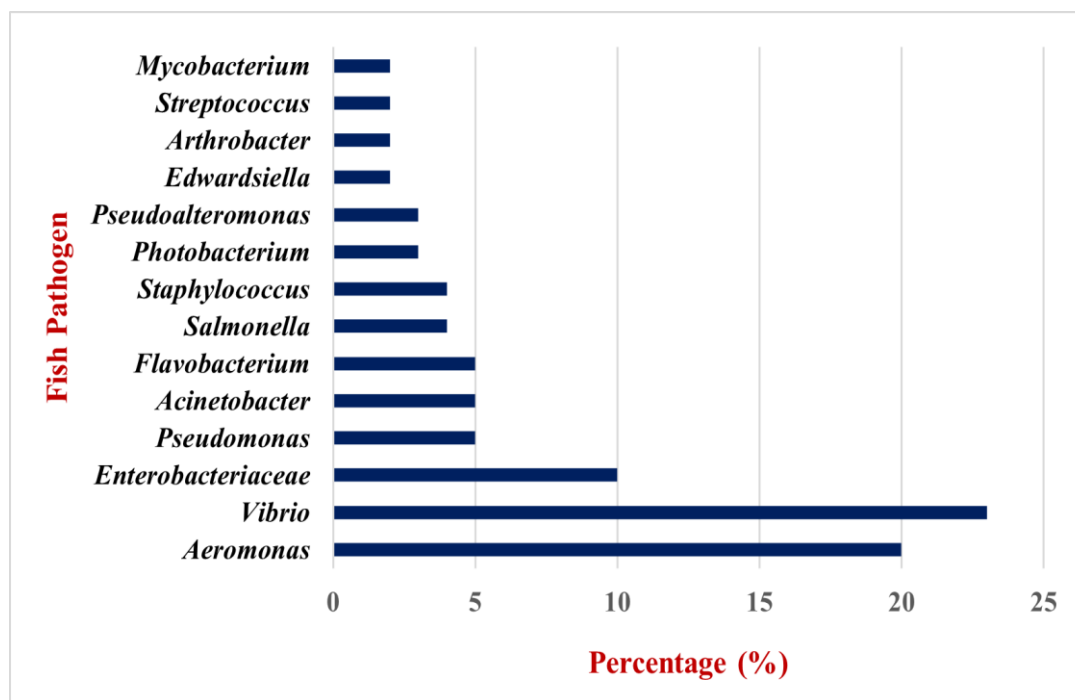


Figure 2: Graph representing percentage distribution of antimicrobial resistance levels in fish pathogens

5. Antimicrobial peptides as alternatives of antibiotics in aquaculture

Antimicrobial peptides (AMPs) can be derived from a variety of sources, including mammals, amphibians, insects, aquatic organisms, plants, and microorganisms (Naiel et al., 2022). They are increasingly recognized as viable substitutes for antibiotics in aquaculture, addressing issues such as antibiotic resistance and environmental pollution. These peptides play a crucial role in the innate immune defenses of fish, providing broad-spectrum antimicrobial action and modulating immune responses (Naiel et al., 2023). AMPs have demonstrated effectiveness against bacteria, parasites, and fungi, suggesting their potential to reduce the reliance on conventional antibiotics in aquaculture. Furthermore, their ability to enhance immune responses and improve disease resistance in aquatic animals underscores their importance in maintaining aquatic animal health (Shabir et al., 2018).

AMPs comprise a diverse group of molecules that are integral to the host defense mechanisms of various species. Fish express several major classes of AMPs, including defensins, cathelicidins, hepcidins, and piscidins (Naiel et al., 2023). Piscidins, unique to fish, show significant homology to linear, amphipathic AMPs found in other species. Like other vertebrate AMPs, fish AMPs exhibit broad-spectrum antimicrobial activities and immunomodulatory functions, though they have evolved specific differences to address the unique aquatic environments inhabited by these species (Naiel et al., 2023). AMPs can neutralize pathogens, migrate to infection sites, and recruit immune cells to affected tissues. Their distinct structures present therapeutic options against infectious diseases in fish, with the added benefit that bacteria are less likely to develop resistance to them.

AMPs are essential bioactive compounds with well-documented antibacterial, antifungal, antiviral, and

antiparasitic properties against a wide range of fish-associated pathogens. Compared to conventional antibiotics, AMPs offer several advantages. First, they help circumvent common antibiotic resistance mechanisms (Wang et al., 2015). Second, their synthesis is relatively straightforward due to their short amino acid sequences (Hansen et al., 2020). Third, AMPs exhibit rapid bactericidal activity (Raheem and Straus, 2019). Additionally, they remain effective against bacterial strains regardless of resistance phenotypes since they are not influenced by standard antibiotic resistance mechanisms (Lee et al., 2020). Lastly, AMPs do not disrupt gut-associated microbial communities, which is a common drawback of traditional antibiotics (Kim et al., 2020). These advantages position AMPs as promising alternatives to antibiotics in aquaculture.

6. Conclusion

The rising threat of antibiotic resistance and the need for sustainable aquaculture call for innovative solutions. Antimicrobial peptides (AMPs) offer a promising alternative to antibiotics due to their broad-spectrum activity, ability to enhance immune responses, and environmentally safe, biodegradable nature. By reducing antibiotic dependence, AMPs can help mitigate resistance, improve animal health, and promote responsible aquaculture practices. To realize their full potential, further research should focus on enhancing their specificity, synergistic potential, and antimicrobial efficacy, supported by greater investment and adoption in comprehensive health management strategies for a sustainable and resilient aquaculture industry.

7. Statements and Declarations

Ethical Approval: Not Applicable

Conflict of Interest Declaration: The authors declare that they have no conflict of interest.

Funding Statement: Not Applicable

Author Contributions:

7. References:

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How to Cite: Pandey, S., Singh, G., Gupta, R.K., Dahiya, T., Khan, M.I.R., Padmanabha, A., Soni, V., Sharma, S. 2026. Antimicrobial Peptides: Promising Alternatives to Antibiotics in Aquaculture. *Journal of Fisheries and Zoological Sciences*, 1(1), 1-16.