

REVIEW ARTICLE

Probiotics as sustainable tools for mitigating antimicrobial resistance in aquaculture: A narrative review

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Abstract

The rising crisis of antimicrobial resistance (AMR) in global aquaculture threatens food security and public health, calling for a shift away from prophylactic antibiotic use. Probiotics offer a potentially viable and sustainable alternative through competitive exclusion, pathogen suppression, and immunomodulation, thereby helping to reduce disease burden. This review integrates current evidence on the application of probiotics to mitigate AMR within a One Health framework, examining their use across feed-based, water-based, and larval-rearing systems. Across diverse species and production contexts, probiotics may help decouple aquaculture intensification from reliance on antibiotics. However, widespread adoption is hindered by strain-specific efficacy, variable performance across environmental conditions, and logistical challenges associated with maintaining and delivering viable organisms. The field also suffers from a paucity of standardized, long-term, multisite trials, which creates uncertainty about efficacy, durability, and safety under commercial conditions. To address these gaps, emerging innovations such as fermentation-based delivery approaches, synbiotics, postbiotics, and microbiome engineering show promise for enhancing probiotic stability and shaping favorable microbial communities. Probiotics should therefore be viewed as an important component of holistic health management, with the potential to influence resistome dynamics while complementing vaccination and biosecurity measures. Realizing these benefits requires transparent reporting, rigorous ecological monitoring, harmonized regulatory standards, and targeted capacity building, particularly in low- and middle-income countries, to promote equitable access.

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1. Introduction

Aquaculture has become one of the fastest-growing food production sectors worldwide and is increasingly recognized as an essential component of global food systems. It contributes substantially to food security, nutritional health, employment, and

economic development while helping to meet the growing global demand for aquatic products as capture fisheries approach their production limits.¹ The rapid expansion and intensification of aquaculture have significantly increased production efficiency. However, these advances have also heightened the incidence of infectious diseases, which remain among the most important constraints to aquaculture productivity, profitability, and long-term sustainability.^{2,3}

To reduce production losses associated with disease outbreaks, antimicrobial agents have become important tools for disease prevention and treatment in intensive aquaculture systems.⁴ While antibiotics have played a critical role in controlling bacterial infections and improving production outcomes, their indiscriminate and prolonged use has accelerated the emergence and dissemination of antimicrobial resistance (AMR) within aquatic environments.^{5,6} Resistant bacteria and antimicrobial resistance genes (ARGs) can accumulate in cultured organisms, sediments, biofilms, and surrounding water bodies, creating environmental reservoirs that facilitate the persistence and transmission of resistance determinants across ecological and biological boundaries.^{7,8} Consequently, AMR not only compromises the effectiveness of disease treatment in aquaculture but also poses significant threats to food safety, environmental health, and public health through interconnected food chains and environmental exposure pathways.

The aquatic environment provides unique ecological conditions that favor the emergence, persistence, and dissemination of AMR. High microbial diversity, continuous exposure to antimicrobial residues, and the widespread occurrence of mobile genetic elements, including plasmids and integrons, can accelerate the evolution and horizontal transfer of resistance determinants among microbial communities.⁹ In addition, biofilm formation and complex microbial interactions promote the long-term persistence of resistant microorganisms and resistance genes, making AMR in aquaculture a highly dynamic and multifaceted challenge.^{10–12} Because aquatic ecosystems are closely linked to terrestrial environments, wildlife, livestock, and human populations, the spread of AMR in aquaculture extends beyond farm boundaries and represents a broader One Health concern that requires integrated and sustainable management strategies.

The growing recognition of AMR as a global threat has intensified efforts to identify sustainable alternatives

that maintain aquatic animal health while reducing dependence on antibiotics. Among the various approaches investigated, probiotics have emerged as a promising biological intervention for modern aquaculture.^{13,14} Probiotics, defined as live microorganisms that confer health benefits on the host when administered in adequate amounts, have been extensively investigated in a wide range of cultured fish and shellfish species.¹³ Unlike conventional antimicrobial therapies, probiotics can promote host health through multiple complementary mechanisms while potentially exerting less selective pressure for the development of AMR.

The beneficial effects of probiotics are mediated through several biological mechanisms, including competitive exclusion of pathogenic microorganisms, production of antimicrobial metabolites, enhancement of innate and adaptive immune responses, and modulation of the gut microbiota.¹⁴ These mechanisms can improve nutrient utilization, growth performance, survival, and disease resistance in cultured aquatic organisms. By promoting microbial homeostasis and reducing pathogen colonization, probiotics can decrease disease incidence and consequently reduce the frequency of antibiotic use. This reduction in antibiotic use may lower the selective pressure driving the emergence and spread of resistant microorganisms and ARGs in aquaculture systems.¹⁵

Despite the rapidly expanding body of research on probiotic applications in aquaculture, their contribution to mitigating AMR remains incompletely understood and is often inconsistently reported. Most studies have primarily focused on improvements in growth performance, feed utilization, and disease resistance, whereas comparatively fewer investigations have critically examined how probiotics influence ARGs, microbial community dynamics, horizontal gene transfer, and the overall aquaculture resistome. Furthermore, substantial variability in probiotic strains, administration methods, host species, culture systems, and environmental conditions has resulted in inconsistent outcomes across studies, limiting the translation of experimental findings into standardized management practices.¹⁶ These inconsistencies underscore the need for a comprehensive synthesis of current evidence to better understand the potential of probiotics in antimicrobial stewardship within aquaculture.

Therefore, this review critically synthesizes current knowledge on the emergence, dissemination, and impacts of AMR in aquaculture systems while evaluating the

potential of probiotics as a sustainable strategy for disease prevention and antimicrobial stewardship. Specifically, the review examines the mechanisms underlying AMR development and transmission, explores the functional roles of probiotics in promoting aquatic animal health and microbial homeostasis, and evaluates current evidence regarding their capacity to reduce antibiotic dependence and influence resistance dynamics. By integrating findings from aquatic microbiology, animal health, and environmental sciences, this review identifies key knowledge gaps, highlights existing challenges, and proposes future research priorities to effectively integrate probiotics into sustainable aquaculture practices.

2. Review methodology

This narrative review synthesizes peer-reviewed evidence on AMR, antibiotic use, probiotics, and alternative disease-control approaches in aquaculture. Literature searches were conducted in Scopus, Web of Science, PubMed, and Google Scholar, covering publications from 2000 to May 2026, with the search finalized in May 2026. The searches employed Boolean operators AND and OR across topics including aquaculture, fish and shrimp, probiotics and beneficial bacteria, AMR and antibiotics, fish health and disease resistance, microbiota and probiotic mechanisms, and One Health. Backward reference screening was also conducted to identify additional relevant studies.

As a narrative rather than a systematic review, this study did not use a Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)-style flow diagram or provide a numerical accounting of records retrieved and screened. English-language sources were eligible for inclusion and comprised original research articles, systematic reviews, meta-analyses, and narrative reviews addressing probiotics, AMR, antibiotic use, bacterial disease management, host health, immune responses, gut microbiota, or alternative disease-control approaches in aquaculture. Non-aquaculture studies, duplicate records, conference abstracts, editorials, commentaries, and articles lacking relevant evidence were excluded. Primary experimental studies were distinguished from secondary literature, with priority given to evidence on probiotic efficacy, antimicrobial use, immune responses, disease resistance, and potential antibiotic replacement. Reviews and meta-analyses were used to provide contextual information and identify relevant primary studies.

Relevance screening proceeded from titles and abstracts

to full texts, where necessary, and the most complete and directly relevant record was prioritized when multiple publications reported findings from the same study. Data extracted from primary studies included probiotic strain, host species, dose, antibiotic comparator and dose, treatment duration, experimental sample size, health and immune endpoints, disease-challenge conditions, and statistical or qualitative results. Studies without an antibiotic-treated group were not considered direct evidence of probiotic equivalence to, or replacement of, antibiotics. Findings were synthesized qualitatively, with attention to variation in probiotic effects according to strain, host, dose, duration, challenge conditions, and comparator. Particular emphasis was placed on distinguishing improvements in health or disease-resistance outcomes from direct evidence supporting antibiotic reduction or replacement.

3. Antibiotic use in aquaculture: Practices and concerns

3.1. Common classes of antibiotics used in aquaculture

A narrow range of antibiotic classes dominates aquaculture, including sulfonamides, tetracyclines, macrolides, and quinolones.^{17,18} In many systems, these drugs are selected for their broad-spectrum activity and cost-effectiveness and are often administered through feed or added to water. However, reliance on these antibiotic classes reflects concerning patterns of use, including overlapping applications across species and geographic regions, sometimes in settings with limited veterinary oversight. This widespread use can select for resistant bacteria and resistance genes that may persist in the environment or potentially be transferred across microbial populations, including those of public health significance.¹⁹ In some production systems, alternatives such as vaccines, improved biosecurity, and probiotics remain underutilized when economic incentives favor rapid and relatively inexpensive antibiotic-based interventions. The resulting variation in antimicrobial-use practices can sustain antibiotic exposure even where stewardship programs exist but are inconsistently implemented.²⁰ Table 1 shows the regulatory classification, documented applications, and AMR concerns associated with antibiotics used in aquaculture.

3.2. Prophylactic versus therapeutic applications

Prophylactic use involves administering antibiotics to healthy stocks to prevent disease and remains common

Table 1. Regulatory classification, documented applications, and antimicrobial resistance (AMR) concerns associated with antibiotics used in aquaculture

Regulatory status	Antibiotic	Antimicrobial class	Aquaculture application	Major target pathogens/ pathogen groups	Major AMR or public health concern	References
Licensed therapeutic use	Oxytetracycline	Tetracycline	Treatment of bacterial diseases under veterinary prescription	<i>Aeromonas</i> spp., <i>Vibrio</i> spp., <i>Flavobacterium</i> spp., <i>Edwardsiella</i> spp.	Widespread tetracycline resistance genes (tet genes); reduced therapeutic efficacy following repeated use	17,21,22
	Florfenicol	Amphenicol	Treatment of systemic bacterial infections	<i>Piscirickettsia salmonis</i> , <i>Aeromonas</i> spp., <i>Edwardsiella</i> spp., <i>Streptococcus</i> spp.	Emergence of <i>floR</i> resistance genes; increasing multidrug resistance	17
	Sulfadimethoxine + ormetoprim	Sulfonamide + diaminopyrimidine	Therapeutic treatment of bacterial infections	Gram-negative fish pathogens	Selection of <i>sul</i> and <i>dftr</i> resistance genes	17,21
Restricted or regulated therapeutic use	Oxolinic acid	Quinolone	Licensed or restricted in selected countries; veterinary prescription only	<i>Vibrio</i> , <i>Aeromonas</i> , <i>Yersinia</i>	Selection for quinolone-resistant bacteria	23
	Enrofloxacin	Fluoroquinolone	Restricted veterinary use in some jurisdictions; prohibited in food fish in others	Broad-spectrum bacterial infections	Selection of fluoroquinolone-resistant pathogens; critically important for human medicine	24
	Sarafloxacin	Fluoroquinolone	Limited veterinary use in selected countries	Gram-negative pathogens	Cross-resistance among fluoroquinolones	25
Historically used but now limited or phased	Chloramphenicol	Amphenicol	Former treatment of bacterial infections	Broad spectrum	Serious human toxicity (aplastic anemia); resistance development	17
	Furazolidone	Nitrofurans	Previously used against bacterial infections	Broad spectrum	Carcinogenic residues; AMR selection	17
	Nitrofurazone	Nitrofurans	Former antibacterial treatment	Broad spectrum	Carcinogenicity and illegal residues	17
	Malachite green	Triphenylmethane dye (not an antibiotic)	Former antifungal treatment in aquaculture	<i>Saprolegnia</i>	Toxicity and carcinogenicity	17,26
Experimental or research applications	Rifampicin	Rifamycin	Experimental studies only	Experimental bacterial infections	Rapid resistance development; critical human medicine	17
	Vancomycin	Glycopeptide	Laboratory studies only; not approved for aquaculture	Gram-positive bacteria	Critical importance in human medicine	23
	Gentamicin	Aminoglycoside	Experimental treatment and laboratory research	Gram-negative bacteria	Aminoglycoside resistance genes	17
Prohibited or banned in food-producing aquaculture	Chloramphenicol	Amphenicol	Prohibited because of public health concerns	-	Human toxicity; zero tolerance for residues	27
	Nitrofurans	Nitrofurans	Banned in food animals	-	Carcinogenic metabolites	17
	Fluoroquinolones (certain jurisdictions)	Fluoroquinolones	Prohibited in aquaculture in some countries because of AMR concerns	-	Preservation of critically important antimicrobials	17

in many aquaculture operations.²³ While this pre-emptive approach can reduce short-term production losses, it may increase selection pressure for AMR and can obscure underlying stressors such as crowding, poor water quality, and suboptimal nutrition.²⁸ Therapeutic use targets confirmed or clinically suspected infections and, in principle, represents a more prudent approach; however, in practice, it may be delayed or misapplied because of diagnostic limitations, regulatory gaps, traceability challenges, or economic pressures, allowing disease to spread and potentially necessitating higher drug doses later.²⁹ The distinction is important: prophylactic use can increase selection pressure, whereas timely and appropriately dosed therapeutic use can help control disease spread, although it may still contribute to resistance when antibiotics are used unnecessarily, as first-line measures without appropriate diagnostic justification, or when treatment protocols and withdrawal periods are not properly followed.³⁰ Effective antimicrobial stewardship therefore depends on timely diagnostics, appropriate veterinary oversight, and transparent records of antimicrobial use and disease events, enabling targeted interventions and reducing unnecessary antimicrobial exposure.

3.3. Drivers of antibiotic misuse

Multiple factors drive inappropriate antimicrobial use in aquaculture. Regulatory gaps persist in some regions, allowing over-the-counter access, inadequate adherence to withdrawal periods, and limited monitoring of antimicrobial residues.^{31,32} Economic pressures, including narrow profit margins, high stocking densities, and the need to maintain disease-free stocks, can make preventive measures appear costly relative to the immediate perceived benefits of antibiotic use.³³ Access to veterinary expertise is also uneven, particularly among small-scale farmers and remote operations, leading some producers to rely on personal experience or peer networks rather than science-based disease-management protocols.³⁴ Taken together, these factors can create a cycle in which antibiotics are used as a rapid response to disease rather than as a targeted intervention when clinically justified, undermining long-term aquaculture sustainability and public health objectives.

3.4. Environmental dissemination

Antibiotics and their metabolites enter aquatic ecosystems through effluents, pond leakage, and inadequate waste management.^{35,36} In sediments and water, some antimicrobial compounds can persist and exert selective pressure on native microbial communities, potentially promoting the enrichment and dissemination of resistance genes. This can create environmental reservoirs of AMR

that may persist even after aquaculture activities cease.³⁷ Aquaculture effluents can transport antimicrobial residues downstream, affecting aquatic wildlife and potentially contributing to human exposure through contaminated water sources or aquaculture products.^{38,39} Compounding the issue, environmental matrices differ in their capacity to adsorb, transform, and degrade antimicrobial compounds, meaning that some substances may persist longer and at higher concentrations in sediments than in the water column. These dynamics complicate environmental risk assessment and highlight the need for integrated management approaches, including improved waste treatment, appropriate discharge standards, and routine surveillance of both antimicrobial residues and resistance markers.

4. Mechanisms of antimicrobial resistance in aquatic systems

4.1. Selection pressure in aquaculture environments

Intensive antibiotic use and high stocking densities create strong selective pressures that favor the emergence, persistence, and proliferation of AMR bacteria in aquaculture systems.⁴⁰ When combined with environmental stressors such as poor water quality, elevated organic loads, hypoxia, and temperature fluctuations, these conditions can further promote the enrichment and dissemination of AMR.⁴¹ Importantly, resistance is not confined to pathogenic bacteria. Several non-pathogenic environmental microorganisms, including *Pseudomonas* spp., *Acinetobacter* spp., *Shewanella* spp., environmental *Aeromonas* spp., and *Bacillus* spp., have been reported to harbor diverse ARGs, including genes conferring resistance to tetracyclines (*tet*), sulfonamides (*sul*), β -lactams (*bla*), and quinolones (*qnr*).⁴² Although these microorganisms are generally not considered major pathogens of fish or humans, they can serve as important environmental reservoirs of ARGs within aquaculture ecosystems.⁴³

These resistance determinants are frequently associated with mobile genetic elements, including plasmids, transposons, and integrons, which facilitate horizontal gene transfer among microbial communities.⁴⁴ Consequently, environmental bacteria can act as intermediaries in the transfer of ARGs to opportunistic and pathogenic bacteria under favorable environmental conditions.⁴² Aquaculture water, sediments, biofilms, and farm-associated microbiota can therefore function as interconnected reservoirs that facilitate the persistence and dissemination of AMR within production systems and surrounding aquatic environments.⁴⁵ This dynamic poses challenges for disease management, food safety, and broader One Health efforts to mitigate AMR.

4.2. Horizontal gene transfer (plasmids, integrons, transposons)

Horizontal gene transfer accelerates the dissemination of AMR beyond the bacterial populations in which resistance determinants initially arise.^{46,47} Plasmids can transfer resistance genes between bacteria, integrons can capture and express gene cassettes, and transposons can move resistance determinants between chromosomes and plasmids, thereby facilitating the rapid restructuring of microbial resistomes.^{48,49} In aquatic systems, these mobile genetic elements can disseminate through water, biofilms, sediments, and fecal inputs, contributing to a complex and interconnected resistome that spans multiple bacterial species and environmental compartments.

4.3. Role of biofilms in resistance persistence

Biofilms can protect resident microorganisms from antimicrobial exposure, enhancing their survival during treatment and creating conditions that facilitate horizontal gene transfer.⁵⁰ Their extracellular matrix can impede antimicrobial penetration and establish localized microenvironments in which resistant microorganisms may persist and proliferate.⁵¹ In aquaculture, biofilms develop not only on abiotic surfaces such as tanks, pipes, nets, and sediments but also on biotic surfaces, including the skin, gills, shells, and gastrointestinal mucosa of aquatic animals.⁵² These biofilm-associated microbial communities can function as reservoirs of ARGs and opportunistic pathogens, thereby facilitating horizontal gene transfer and recurrent infections.⁵³ Consequently, biofilms may contribute to the persistence and dissemination of AMR within aquaculture systems, including after antimicrobial use has ceased.⁵⁴

4.4. Aquatic microbiomes as reservoirs of resistance genes

The aquatic microbiome represents a substantial reservoir of ARGs, including many whose potential to contribute to resistance in human pathogens remains poorly characterized.⁵⁵ Diverse microbial communities harbor a wide range of resistance determinants that may become enriched or mobilized under antimicrobial selective pressure. Aquaculture ponds, receiving waters, sediments, and connected aquatic ecosystems can therefore function as reservoirs and potential dissemination pathways for resistance determinants, facilitating their movement across microbial communities and environmental compartments and potentially increasing opportunities for transmission to bacteria associated with animals and humans.⁵⁶

4.5. Cross-resistance and co-selection mechanisms

Cross-resistance occurs when a single resistance mechanism confers reduced susceptibility to multiple antimicrobial agents, whereas co-selection occurs when resistance to one antimicrobial is selected alongside resistance to other agents through linked resistance determinants.⁵⁷ Key mechanisms include multidrug efflux pumps, target modification, enzymatic inactivation, and alternative metabolic pathways that reduce antimicrobial susceptibility. In practice, exposure to one antimicrobial or other selective agents can inadvertently favor microorganisms carrying multiple resistance determinants, potentially contributing to multidrug resistance and complicating treatment strategies and antimicrobial stewardship in aquaculture and connected ecosystems.⁵⁸

5. Impacts of antimicrobial resistance in aquaculture

Antimicrobial resistance in aquaculture represents a complex, multidimensional challenge that extends beyond farm boundaries, generating interconnected consequences for treatment efficacy, economic viability, environmental integrity, and public health, as illustrated in [Figure 1](#).

5.1. Reduced efficacy of disease treatment in fish and shellfish

The rise and spread of AMR in aquaculture increasingly undermine bacterial disease management by reducing the efficacy of antimicrobials against resistant pathogens and narrowing therapeutic options.^{59,60} Aquaculture-specific studies have documented AMR among important bacterial pathogens, including *Aeromonas* and *Vibrio* species, with resistance reported across multiple antimicrobial classes used in aquatic animal production.^{61,62} A recent systematic review and meta-analysis of *Aeromonas hydrophila* in aquatic food animals reported a pooled prevalence of 30.7% (95% confidence interval: 17.0–46.3%), with high resistance to penicillins (80.7%), oxytetracycline (69.9%), and macrolides (67.8%), as well as frequent detection of the resistance genes *bla*TEM (67.0%) and *tetA* (63.7%).⁶³ These findings underscore the widespread occurrence of AMR bacteria in aquatic food-animal production and their potential to compromise disease management.

The continued emergence of resistant pathogens can complicate the selection and effective use of antimicrobials in aquaculture, particularly where therapeutic options are already limited.^{59,60} The persistence and dissemination of resistant bacteria and AMR determinants in aquaculture-associated environments may further sustain resistance within production systems.^{61–64} Consequently, AMR

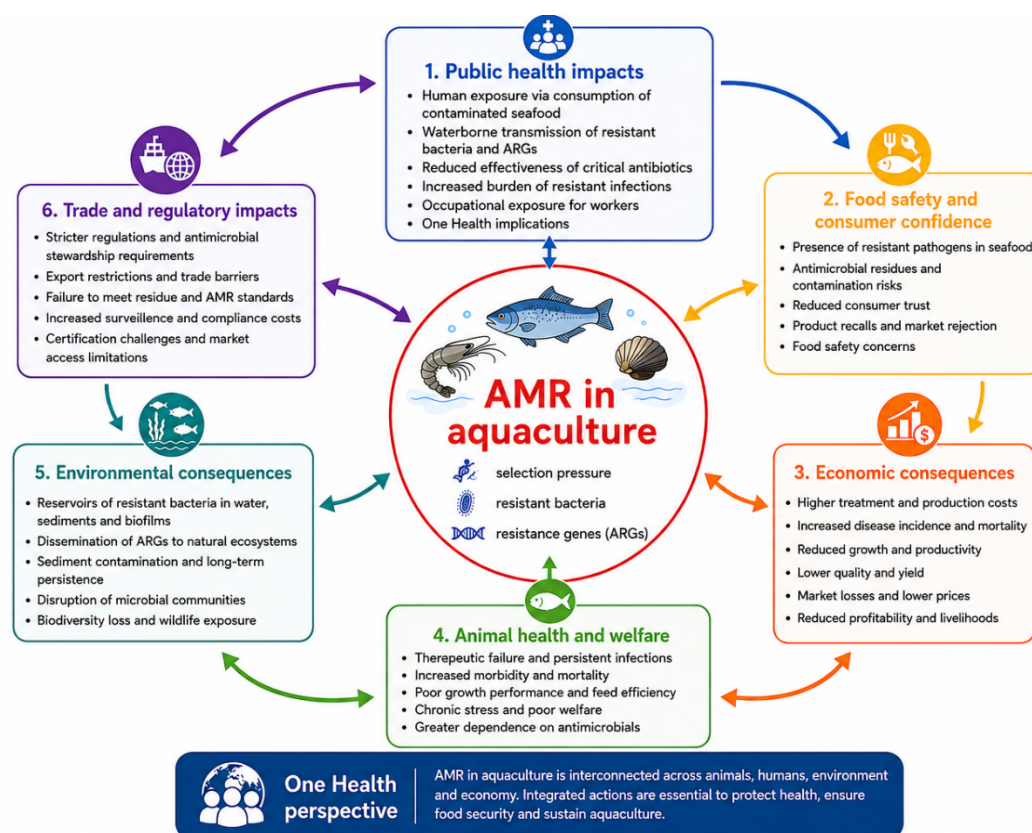


Figure 1. One Health impacts of AMR in aquaculture. Figure created by the authors with ChatGPT (OpenAI, GPT-5.6 Luna).
Abbreviations: AMR: antimicrobial resistance; ARGs: Antimicrobial resistance genes.

remains a major challenge to sustainable aquaculture and reinforces the need for integrated disease-prevention and management strategies, including enhanced biosecurity, good husbandry practices, vaccination, prudent antimicrobial use, and complementary approaches such as probiotics and prebiotics.^{59,60,64} Importantly, these approaches should be considered components of integrated disease management rather than direct substitutes for antibiotics, because their effectiveness and potential to reduce antimicrobial use depend on the pathogen, host species, production system, and specific intervention evaluated.⁶⁰

5.2. Economic losses and production inefficiencies

The economic consequences of AMR can extend throughout the aquaculture production chain. As antimicrobial treatments become less effective, production costs may increase because of repeated or alternative treatments, prolonged grow-out periods, and additional labor required to manage diseased stocks.³³ AMR-associated disease can also contribute to poorer feed utilization, reduced growth, increased mortality, and lower production efficiency,

potentially diminishing overall yield and economic returns. These financial burdens may disproportionately affect small-scale producers, who often have limited financial resources to absorb production losses or access alternative disease-management options, potentially widening disparities between large-scale operations and resource-constrained farmers.

5.3. Environmental consequences (ecosystem imbalance)

Aquaculture environments can act as important reservoirs and dissemination pathways for ARGs. Antimicrobial residues and resistant bacteria may enter surrounding waters and sediments through effluents, runoff, and other waste streams, where some resistance determinants can persist after antimicrobial treatment has ceased.^{65,66} These inputs can alter microbial community structure and create conditions that favor the persistence, enrichment, and horizontal transfer of resistance determinants among environmental microorganisms. Such processes may extend beyond farm boundaries and contribute to the establishment of environmental reservoirs of AMR,

potentially affecting aquatic biodiversity and complicating long-term efforts to control its dissemination.

5.4. Public health implications

Public health implications arise when AMR associated with aquaculture reaches humans through contaminated aquatic products, water, or occupational and handling pathways, potentially complicating clinical management and reinforcing the need for effective regulatory controls, surveillance, and testing throughout the supply chain.^{67,68} Resistance determinants may move from farm to fork through contaminated feed, water, processing environments, and handling practices, with cross-contamination risks potentially increasing during harvesting, processing, and distribution. These risks underscore the importance of stringent hygiene practices, effective traceability, and appropriate compliance with antimicrobial withdrawal periods. Moreover, resistant bacteria and mobile resistance genes circulating in aquatic environments may be transmitted across animal, environmental, and human interfaces, particularly where these compartments are closely connected.⁶⁹ A One Health approach is therefore essential for monitoring, preventing, and responding to AMR across interconnected human, animal, and environmental systems.

5.5. Trade and regulatory implications

In an increasingly globalized aquaculture market, antimicrobial stewardship and compliance with food-safety requirements can influence market access.⁷⁰ Importing countries may impose stringent residue limits and require testing, documentation, and certification for aquaculture products. Non-compliance with applicable requirements or detection of unacceptable antimicrobial residues or microbial hazards can result in shipment rejection, trade restrictions, and economic losses for exporting countries. Regulatory harmonization, transparent antimicrobial-use practices, and effective stewardship programs are therefore increasingly important for maintaining international

market access, protecting consumer confidence, and supporting the reputation and competitiveness of the aquaculture sector.⁷¹

6. Probiotics in aquaculture: Concepts and applications

6.1. Definition and selection criteria of probiotics

Probiotics in aquaculture are live microorganisms that, when administered in adequate amounts, confer health benefits on the host.⁷² Although definitions and selection criteria vary, probiotic candidates should demonstrate measurable health benefits, an acceptable safety profile, and sufficient viability during production, storage, and application. Importantly, probiotic effects are often strain-specific, meaning that different strains within the same species may exhibit distinct functional properties. Therefore, accurate strain identification is a key consideration when selecting probiotics for specific aquaculture species, life stages, and production systems.^{72,73} Selection criteria should include antagonistic activity against pathogens, enhancement of host immune responses, improved digestion or nutrient utilization, and compatibility with existing husbandry practices.¹⁴ A major challenge is variability in probiotic performance; for example, a *Bacillus* strain that performs well in one hatchery may show limited efficacy in another because of differences in water chemistry, temperature, management practices, or resident microbial communities.⁷⁴ Moreover, safety and regulatory considerations, including the absence of transferable AMR determinants and harmful toxin production, should be incorporated into probiotic selection and evaluation. In practice, robust and adequately replicated *in vivo* trials conducted under realistic farm conditions are essential before large-scale application. However, many studies rely on small sample sizes, limited controls, or inconsistent reporting, which can reduce confidence in the reproducibility and generalizability of reported benefits. The key differences between probiotics and antibiotics in aquaculture are summarized in [Table 2](#).

Table 2. The differences between antibiotics and probiotics in aquaculture

Aspect	Antibiotics	Probiotics
Definition	Chemical agents used to kill or inhibit pathogenic microorganisms ⁷⁵	Live beneficial microorganisms administered to improve host health ¹⁴
Primary purpose	Treatment and prevention of bacterial diseases ⁷⁵	Disease prevention, growth promotion, and microbiome modulation ¹⁴
Mode of action	Direct killing or inhibition of pathogens ⁷⁵	Competitive exclusion, antimicrobial metabolite production, and immune stimulation ¹⁴

(Cont'd...)

Table 2. (Continued)

Aspect	Antibiotics	Probiotics
Target specificity	Often broad-spectrum ⁷⁵	Usually selective and ecological ¹⁴
Impact on microbiota	Disrupts beneficial microbiota ⁷⁶	Enhances beneficial microbial balance ¹⁴
Risk of antimicrobial resistance	High ²⁴	Very low/negligible ⁷⁷
Residue accumulation	Possible residues in fish tissues and environment ⁷⁸	Generally regarded as safe ⁷⁹
Environmental impact	Potential ecosystem contamination ⁶⁶	Environmentally friendly ⁷⁸
Effect on fish immunity	Limited long-term immune enhancement ⁸⁰	Enhances innate and adaptive immunity ¹⁴
Sustainability	Unsustainable with excessive use ⁶⁵	Sustainable disease management strategy
Regulatory concerns	Increasing restrictions globally ⁸¹	Increasing acceptance and commercialization ¹⁴
Consumer acceptance	Concerns over food safety	Increasingly encouraged globally
Examples	Oxytetracycline, florfenicol and sulfonamides	<i>Lactobacillus</i> , <i>Bacillus</i> , <i>Pediococcus</i> , and <i>Saccharomyces</i>

6.2. Common probiotic microorganisms

Lactic acid bacteria are valued for their ability to acidify the intestinal environment, compete with pathogens, and modulate host immune responses.⁸² However, their efficacy in fish and crustaceans can be inconsistent and may depend strongly on host species, diet, probiotic strain, and rearing conditions.⁸³ *Bacillus* spp., particularly spore-forming strains, are widely used in aquaculture because of their stability, ease of incorporation into feeds, and capacity to produce digestive enzymes that may enhance feed utilization.^{84–86} Nevertheless, their effectiveness varies with strain, dosage, host species, and rearing conditions, and some products have shown limited efficacy *in vivo*. Yeasts, including *Saccharomyces* spp., may provide nutritional benefits, modulate immune responses, and contribute to competitive exclusion, although their

persistence in aquatic systems and interactions with the resident gut microbiota can vary.⁷² Across these probiotic groups, a recurring concern is that some products rely more heavily on commercial claims than on transparent, independently verified evidence. Greater attention is also needed to interactions with native microbiota, biosafety considerations, and the potential for horizontal transfer of AMR or other relevant genetic traits.²⁹ Overall, while probiotics hold promise for disease mitigation, improved growth performance, and reduced reliance on antibiotics, their real-world effectiveness depends on careful strain selection, rigorous farm-level validation, consistent manufacturing quality, and integration with comprehensive management practices, including biosecurity, nutrition, and environmental stewardship. Table 3 summarizes commonly used probiotics in aquaculture, their modes of application, and their functional roles.

Table 3. Common probiotics used in aquaculture, their host species, functional roles, and application forms

Probiotic microorganism	Aquaculture species	Major functional role	Common application form	References
<i>Bacillus subtilis</i>	Nile tilapia, shrimp, carp	Enhances digestion and immunity	Food additive	19,87
<i>Bacillus licheniformis</i>	Catfish, tilapia	Improves feed utilization	Feed and water additive	84,88
<i>Bacillus pumilus</i>	Shrimp, carp	Disease prevention	Pond water treatment	85
<i>Bacillus coagulans</i>	Tilapia	Gut microbiota stabilization	Feed additive	86
<i>Lactobacillus plantarum</i>	Carp, tilapia, shrimp	Competitive exclusion of pathogens	Feed supplementation	89-91
<i>Lactobacillus acidophilus</i>	Catfish, trout	Immune stimulation	Feed additive	92,93
<i>Lactobacillus rhamnosus</i>	Rainbow trout	Enhances mucosal immunity	Dietary supplement	94
<i>Lactobacillus paracasei</i>	Tilapia	Enhances disease resistance	Feed additive	95
<i>Pediococcus acidilactici</i>	Salmon, shrimp	Improves feed conversion ratio	Commercial probiotic feed	96,97
<i>Enterococcus faecium</i>	Tilapia, carp	Improves growth and immunity	Feed additive	98
<i>Saccharomyces cerevisiae</i>	Shrimp, tilapia, catfish	Immune enhancement	Yeast-based feed additive	99,100
<i>Debaryomyces hansenii</i>	Marine fish larvae	Improves larval survival	Live feed enrichment	101

Table 3. (Continued)

Probiotic microorganism	Aquaculture species	Major functional role	Common application form	References
<i>Pseudomonas fluorescens</i> AH2	Rainbow trout (<i>Oncorhynchus mykiss</i>)	Antagonism against <i>Vibrio anguillarum</i> and reduced disease-associated mortality	Bath/immersion treatment	102
<i>Clostridium butyricum</i>	Tilapia, shrimp	Enhances intestinal integrity	Feed additive	103
<i>Nitrosomonas</i> spp.	Shrimp ponds	Ammonia reduction	Bioaugmentation	104
<i>Nitrobacter</i> spp.	Intensive aquaculture systems	Nitrite oxidation	Water probiotic	105
<i>Rhodobacter</i> spp.	Marine shrimp	Water quality management	Pond inoculant	106
<i>Shewanella putrefaciens</i>	Marine fish	Enhances digestive activity	Feed additive	107

6.3. Methods of administration

The successful integration of probiotics into aquaculture depends on selecting an appropriate administration strategy for the target species, developmental stage, and production system. The chosen delivery method should also be compatible with farm management practices and operational conditions. Delivery strategies influence probiotic viability and the dose that reaches the host, thereby affecting efficacy, cost-effectiveness, and potential environmental impacts (Figure 2). Three main administration routes are commonly used: feed-based supplementation, in-water application, and integration into larval-rearing systems. Each approach has distinct advantages and management requirements for achieving effective probiotic delivery and promoting health benefits. However, inappropriate strain selection, poor handling and storage practices, and inadequate monitoring can reduce probiotic effectiveness regardless of the administration route.

6.3.1. Feed-based supplementation

Probiotics are commonly delivered through functional feeds, making feed-based administration a practical approach for aquaculture production. The effectiveness of this strategy depends partly on maintaining probiotic viability during feed preparation, incorporation, and storage.¹⁰⁸ Encapsulation or protective coating systems can enhance probiotic stability and facilitate delivery through the gastrointestinal tract, as demonstrated in aquaculture studies using alginate- and chitosan-encapsulated probiotics and probiotic-coated feed pellets.¹⁰⁹ Dosage, frequency, and duration should be tailored to the target species, life stage, and disease risk, while probiotic viability and potential effects on the resident microbiota should be monitored.^{108,109} Feed-based delivery is scalable and practical for commercial operations, though its effectiveness may vary with feed composition, storage conditions, environmental temperature, and feeding practices.

6.3.2. Water application

Waterborne administration can expose a broader proportion of the cultured population, including species or life stages with limited feed intake, but it also poses challenges related to dilution, fluctuating probiotic concentrations, and potential environmental release.¹¹⁰ Effective water-based application therefore requires appropriate dosing and exposure periods while maintaining water quality and minimizing potential ecological impacts.

6.3.3. Larval rearing systems

During larval stages, probiotics may be administered through micro-diets, moist feeds, or direct immersion in rearing tanks.¹¹¹ Early-life probiotic exposure may influence gut maturation and disease resistance, although responses are highly species- and strain-specific. Practical considerations include maintaining suitable salinity, temperature, and pH to support probiotic viability, while minimizing cross-contamination from hatchery personnel, equipment, or water systems. In larval-rearing systems, rigorous validation under realistic hatchery conditions is essential to determine whether probiotic administration improves survival, growth, and disease resistance without compromising larval welfare or biosecurity.¹¹²

7. Mechanisms of probiotic action in disease control

7.1. Competitive exclusion of pathogenic bacteria

Probiotics can occupy ecological niches in the gut or rearing environment, limiting pathogen establishment through competition for nutrients, adhesion sites, and other available microhabitats.¹¹³ This competitive interaction may reduce pathogen colonization, attenuate pathogen-associated effects, and contribute to a more stable microbial community. However, the effectiveness of competitive exclusion is context-dependent and influenced by strain-specific adhesion properties, growth rates,

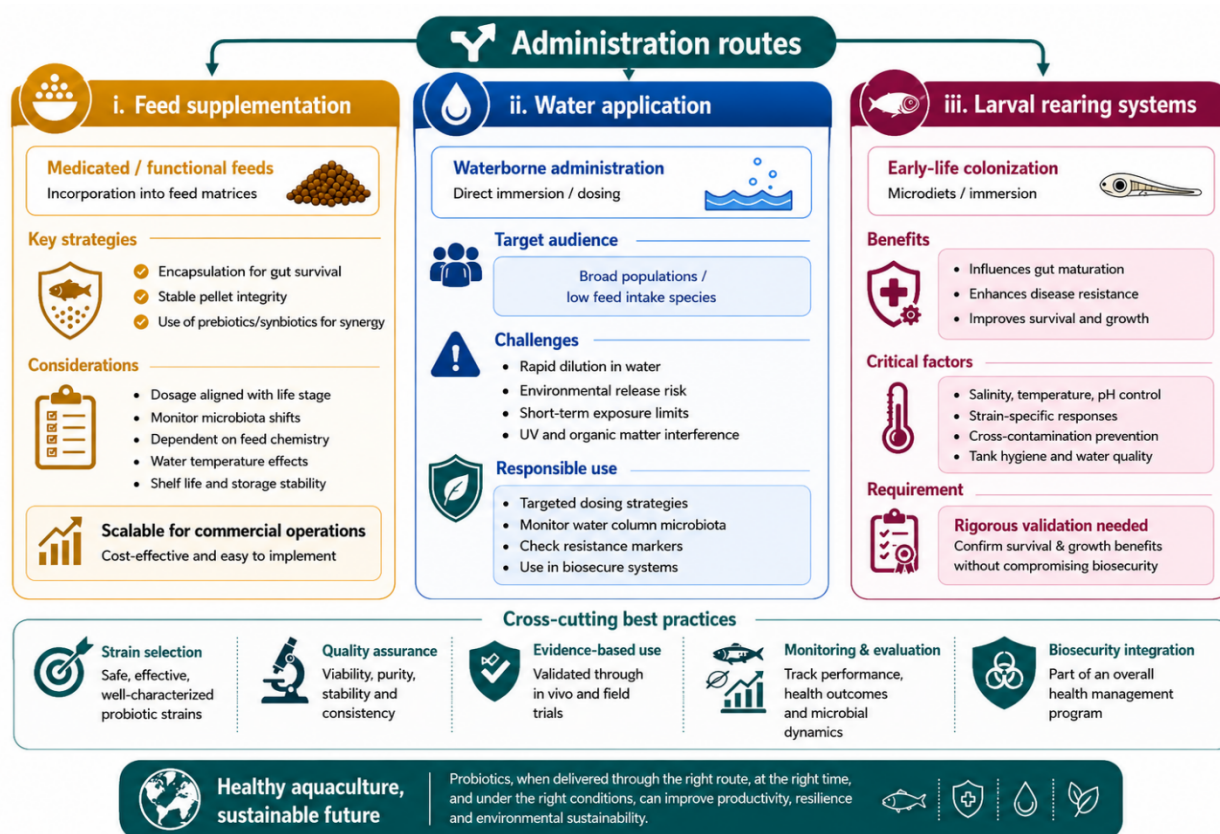


Figure 2. Major routes of probiotic administration in aquaculture and key considerations for their effective application. Figure created by the authors with ChatGPT (OpenAI, GPT-5.6 Luna).

and compatibility with the host's resident microbiota.¹¹⁴ For example, a probiotic consortium comprising *Vibrio diabolicus*, *Vibrio hepatarius*, and *Bacillus cereus* colonized the cuticle and intestinal lining of *Penaeus vannamei* larvae and was associated with reduced colonization by pathogenic *Vibrio parahaemolyticus* and increased larval survival following an acute hepatopancreatic necrosis disease challenge.¹¹⁵ Similarly, *Pseudovibrio denitrificans* isolates (Ps17 and Ps18) demonstrated competitive exclusion against pathogenic *Vibrio* spp. and improved the survival and production performance of *P. vannamei* in laboratory challenge and pond-culture trials.¹¹⁶

7.2. Production of antimicrobial substances

Many probiotic candidates produce antimicrobial compounds, including bacteriocins, organic acids, and enzymes, that can suppress competing microorganisms.¹¹⁷ Bacteriocins primarily inhibit susceptible bacteria, particularly closely related species; organic acids can reduce local pH and create less favorable conditions for

pathogens; and extracellular enzymes may interfere with pathogen cell structures or metabolic processes.¹¹⁸ These antimicrobial activities are generally localized and may have relatively narrow spectra, with their effectiveness influenced by buffering capacity, feed composition, and interactions with the surrounding microbial community. Although probiotics are generally considered safe when appropriately selected and administered, unsuitable strains or excessive doses may alter resident microbial communities and potentially disrupt ecological balance.¹¹⁹ Therefore, probiotic administration should be guided by scientific evidence, appropriate strain selection, validated dosage, and recommended application protocols.

For example, dietary supplementation with the bacteriocin-producing probiotic *Paenibacillus ehimensis* NPUST1 significantly enhanced growth performance and innate immune responses in Nile tilapia (*Oreochromis niloticus*). The bacteriocin-like substance produced by this strain exhibited broad-spectrum antimicrobial activity against *A. hydrophila*, while probiotic-fed fish showed

significantly higher survival following challenge with *A. hydrophila* and *Streptococcus iniae* than fish receiving the control diet.^{120,121} These findings demonstrate the potential of probiotic-derived antimicrobial compounds as one mechanism contributing to disease prevention in aquaculture.

7.3. Modulation of host immune responses

Probiotics can prime mucosal defenses, modulate cytokine profiles, and regulate both innate and adaptive immune responses. Enhanced barrier integrity, improved phagocytic activity, and balanced inflammatory signaling may help fish and crustaceans respond more effectively to pathogenic challenges.¹²² However, immune modulation is not universal; its effects depend on probiotic strain, dose, administration route, and timing, while excessive or inappropriate immune activation may impose physiological stress or energy costs. For example, dietary supplementation with host-associated *Bacillus velezensis*, *Bacillus subtilis*, and *Bacillus amyloliquefaciens* enhanced skin mucus and intestinal mucosal immunity in Nile tilapia by increasing nitric oxide, immunoglobulin M, lysozyme, and alkaline phosphatase activities.¹²³ Probiotic supplementation also improved intestinal morphology, reduced the abundance of potentially pathogenic *Aeromonas* and *Staphylococcus* spp., and enhanced resistance to *A. hydrophila*. The combined *Bacillus* treatment reduced mortality from 86.67% in the control group to 30.00% following bacterial challenge.¹²³

7.4. Enhancement of gut microbiota balance

A balanced gut microbiome supports intestinal health and disease resilience. Probiotics can modify gut microbial communities and their metabolites, including short-chain fatty acids (SCFAs), which are associated with intestinal morphology and barrier integrity.^{124–126} However, interactions at the microbe–host–diet–environment interface are dynamic and should be validated across different production systems. Dietary supplementation with host-derived probiotics in Malaysian mahseer (*Tor tambroides*) significantly altered the gut microbiota, influencing intestinal morphology and volatile SCFA production.¹²⁴ Similarly, supplementation with *Pediococcus pentosaceus* in Nile tilapia (*Oreochromis niloticus*) altered the gut microbiota, increased intestinal propionate concentrations, and contributed to partial restoration of diet-induced intestinal barrier injury.¹²⁵ A probiotic mixture of *Bacillus subtilis*, *Bacillus licheniformis*, and *Bacillus pumilus* in Nile tilapia improved intestinal morphology, enhanced serum lysozyme activity, and modified the gut microbiome.¹²⁶ Together, these findings support modulation of the gut microbial ecosystem and its metabolites as a potential mechanism through which

probiotics contribute to intestinal health in aquaculture.

7.5. Improvement of nutrient utilization and gut health

Beyond disease resistance, probiotics may improve feed efficiency by supporting digestion, digestive enzyme activity, and nutrient utilization. Improvements in intestinal morphology, lipid and protein metabolism, and energy utilization may contribute to enhanced growth and reduced nutrient losses.¹²⁷ However, these benefits depend on host species, digestive physiology, probiotic strain, diet composition, and husbandry conditions.¹²⁸ For example, dietary supplementation with *Pediococcus acidilactici* in Nile tilapia improved intestinal health by promoting probiotic colonization, increasing intraepithelial leukocyte abundance and tumor necrosis factor- α expression, and stimulating innate immune cell populations, suggesting enhanced intestinal integrity and mucosal immune function.⁷⁰

8. Role of probiotics in mitigating antimicrobial resistance

8.1. Reduction in antibiotic usage

Probiotics offer a potential strategy for reducing reliance on medicinal antibiotics in aquaculture by improving host health, enhancing disease resistance, modulating the intestinal microbiota, and reducing pathogen-associated disease pressure.^{129,130} Fewer or less severe disease outbreaks may reduce the need for therapeutic antimicrobial interventions; however, direct evidence quantifying reductions in antibiotic consumption at the farm level remains limited. Probiotic efficacy is also context-dependent and varies according to host species, probiotic strain, dose, life stage, pathogen pressure, and farming conditions.^{130,131} Recent comparative feeding studies have shown that selected probiotic treatments can produce health and performance outcomes comparable to, or better than, those achieved with antibiotic-supplemented diets under experimental conditions. These findings support the potential use of probiotics as components of antibiotic-reduction strategies rather than demonstrating their universal replacement of antibiotics.^{129–131} Real-world adoption therefore requires long-term, large-scale studies that quantify changes in antimicrobial use while simultaneously evaluating disease outcomes, economic benefits, manufacturing consistency, and ecological safety.

8.2. Suppression of resistant pathogens

Probiotics can reduce pathogen loads and, through competitive exclusion and antagonistic activity, potentially mitigate resistance. However, caution is warranted

because pathogen suppression observed under controlled experimental conditions may not translate directly to complex farm ecosystems, where microbial communities, environmental conditions, and management practices differ.¹³² The antimicrobial susceptibility and genetic safety of probiotic candidates should also be considered, as strains used in aquaculture may differ in their resistance profiles and capacity to harbor resistance determinants.¹³³ Recent investigations in shrimp aquaculture have therefore emphasized systematic screening of candidate probiotics for antimicrobial susceptibility, resistance-associated genes, and other safety characteristics before application.^{133,134} In a recent *P. vannamei* larval study, supplementation with shrimp-derived *Lactobacillus casei* HD1 and *Bacillus licheniformis* WZ1 altered the aquatic microbial community but did not significantly change the abundance of the ARGs assessed, suggesting that probiotic administration does not necessarily increase ARG abundance under the conditions tested.¹³⁴ Rigorous monitoring of probiotic safety, pathogen suppression, antimicrobial susceptibility, and resistance determinants is therefore essential to support credible claims that probiotics can contribute to AMR mitigation.

8.3. Influence on resistome dynamics in aquaculture systems

Probiotics may influence the resistome by reshaping microbial community structure and gene exchange patterns. Potential benefits include reductions in reservoirs of highly mobilizable resistance genes when pathogenic or resistant bacteria are competitively suppressed.^{135,136} Conversely, potential risks include horizontal gene transfer involving probiotic strains and resident microorganisms, as well as co-selection of resistance traits associated with other probiotic characteristics.¹³⁷ Assessing the net effects of probiotics on the aquaculture resistome requires integrated metagenomic and resistome profiling, with careful consideration of other sources of selective pressure and resistance genes, including feed, water, sediments, and farm inputs. Short-term studies may fail to capture downstream or delayed effects; therefore, long-term, system-wide investigations are needed to determine the persistence, ecological significance, and safety of probiotic-associated changes in resistance dynamics.

8.4. Evidence from experimental and field studies

Experimental studies frequently report favorable health and growth outcomes following probiotic supplementation, but field studies often show more modest or variable effects because of environmental complexity, management practices, and differences in implementation. Previous studies evaluating probiotics for mitigation

AMR in aquaculture have been limited by inadequate controls, insufficient replication, or other methodological limitations, which may weaken causal inference regarding treatment efficacy.¹³⁸ A credible evidence base requires standardized protocols, transparent reporting of strain identity and dosing, clearly defined treatment durations, and replication across multiple sites and production seasons. The experimental studies summarized in Table 4 demonstrate that probiotics can improve selected health, immune, growth, and disease-resistance outcomes in aquaculture. However, the limited number of studies incorporating direct antibiotic comparators means that probiotics cannot yet be considered universally established alternatives to antibiotics.¹³⁹⁻¹⁴⁵

9. Synergistic approaches enhancing probiotic effectiveness

9.1. Probiotic-enriched feeds

Embedding live beneficial microorganisms directly into feed can streamline delivery and improve dose consistency, but maintaining probiotic viability during pelleting and storage remains a major challenge.¹⁴² Fermentation-based approaches provide opportunities to pre-condition feed using compatible microbial cultures, protective carriers, and controlled moisture and temperature conditions.^{72,146} Key challenges include maintaining shelf stability, minimizing viability losses during feed processing and storage, and preventing adverse interactions with feed components such as fiber, fats, and binders. Opportunities include developing strain-specific delivery systems, such as lipid-based carriers and microencapsulation, as well as controlled fermentation processes that enrich the feed matrix with metabolites that support intestinal health.¹⁴⁷ Such approaches may also facilitate the co-delivery of probiotics with prebiotics and other bioactive compounds, potentially enhancing probiotic persistence and functional outcomes while reducing reliance on antimicrobial interventions.

9.2. Fermented feed systems as delivery vehicles

Feeds can be produced through controlled fermentation to create bioactive matrices that deliver probiotics, prebiotic substrates, and beneficial metabolites. This approach may enhance probiotic survival during storage, support more consistent gut colonization, and facilitate the in-situ production of organic acids or enzymes during consumption.¹⁴⁸ Practical considerations include selecting starter cultures that are compatible with the target probiotic strains, maintaining hygienic fermentation conditions to prevent contamination and spoilage, and ensuring consistent product quality across batches.

Table 4. Experimental evidence on probiotic effects on health, immunity, and disease resistance in aquaculture species, including studies with direct antibiotic comparisons

Study	Aquaculture species	Experimental design	Sample size	Probiotic strain(s)	Probiotic dose	Direct antibiotic comparator (type)	Antibiotic dose	Feeding duration	Quantitative/statistical findings
Aly <i>et al.</i> ¹³⁹	Nile tilapia (<i>Oreochromis niloticus</i>)	Feeding trial evaluating dietary probiotics, immune responses, and resistance to bacterial challenge, supported by <i>in vitro</i> antimicrobial assays.	NR	<i>Bacillus subtilis</i> and <i>Lactobacillus acidophilus</i> , administered individually and as a mixture	<i>Bacillus subtilis</i> : 1×10^7 bacteria/g diet; <i>Lactobacillus acidophilus</i> : 1×10^7 bacteria/g diet; mixture: 0.5×10^7 bacteria/g diet of each strain	No	N/A	1 and 2 months	Dietary supplementation significantly enhanced innate immune responses, including respiratory burst (NBT activity), neutrophil adherence, lysozyme activity, and serum bactericidal activity ($p < 0.05$). Probiotic supplementation also significantly improved survival following challenge with <i>Aeromonas hydrophila</i> , <i>Pseudomonas fluorescens</i> and <i>Streptococcus iniae</i> ($p < 0.05$). The probiotics additionally showed <i>in vitro</i> inhibitory activity against the tested pathogens.
Won <i>et al.</i> ¹⁴⁰	Nile tilapia (<i>Oreochromis niloticus</i>)	Feeding trial comparing a basal control diet, four probiotic treatments containing <i>Bacillus subtilis</i> WB60 and <i>Lactococcus lactis</i> at two concentrations, and an oxytetracycline-treated group.	3 replicate groups/treatment; 20 fish/tank	<i>Bacillus subtilis</i> WB60 and <i>Lactococcus lactis</i>	<i>Bacillus subtilis</i> : 1×10^7 and 1×10^8 CFU/g diet; <i>Lactococcus lactis</i> : 1×10^7 and 1×10^8 CFU/g diet	Yes (oxytetracycline)	4 g/kg diet (0.4%)	8 weeks	Supplementation with <i>Bacillus subtilis</i> WB60 and <i>Lactococcus lactis</i> significantly enhanced innate immune responses, including lysozyme, superoxide dismutase, and myeloperoxidase activities, compared with the control ($p < 0.05$). Higher-dose probiotic treatments significantly improved intestinal villus development and muscular layer thickness compared with the control and, for selected outcomes, the oxytetracycline group ($p < 0.05$). Selected probiotic treatments also improved survival following <i>Aeromonas hydrophila</i> challenge compared with the control and, for some outcomes, oxytetracycline ($p < 0.05$).

(Cont'd...)

Table 4. (Continued)

Study	Aquaculture species	Experimental design	Sample size	Probiotic strain(s)	Probiotic dose	Direct antibiotic comparator (type)	Antibiotic dose	Feeding duration	Quantitative/statistical findings
Pirarat <i>et al.</i> ¹⁴¹	Nile tilapia (<i>Oreochromis niloticus</i>)	Feeding trial evaluating dietary probiotic supplementation on intestinal morphology and mucosal, humoral, and cellular immune responses.	200 fish/experimental run; 6 fish/group for specific analyses	<i>Lactobacillus rhamnosus</i> GG (ATCC 53103)	1×10^{10} CFU/g feed	No	N/A	30 days	<i>Lactobacillus rhamnosus</i> GG significantly improved intestinal morphology and several immune responses compared with the control ($p < 0.05$), including intestinal villus height, intraepithelial lymphocytes, acidophilic granulocytes, and distal intestinal mucous cells. Probiotic supplementation also significantly enhanced head-kidney leukocyte activity, serum alternative complement activity and expression of IL-1 and TNF- α ($p < 0.05$). Serum bactericidal activity against <i>Streptococcus iniae</i> was also enhanced.
Zokaiefar <i>et al.</i> ⁸⁷	White shrimp (<i>Litopenaeus vannamei</i>)	Eight-week feeding trial evaluating dietary probiotics, immune gene expression and disease resistance following <i>Vibrio harveyi</i> challenge.	NR	<i>Bacillus subtilis</i> strains L10 and G1, administered as an equal-proportion mixture	1×10^5 CFU/g feed (BM5) and 1×10^8 CFU/g feed (BM8)	No	N/A	8 weeks, followed by <i>Vibrio harveyi</i> challenge	Both probiotic treatments significantly upregulated the immune-related genes proPO, PE, LGBP, and SP compared with the control ($p < 0.05$). Following <i>Vibrio harveyi</i> challenge, cumulative mortality was 63.3% in the control compared with 33.3% and 20.0% in BM5 and BM8, respectively, with significantly greater survival in probiotic-treated shrimp.
Standen <i>et al.</i> ⁷⁰	Nile tilapia (<i>Oreochromis niloticus</i>)	Six-week feeding trial comparing a control diet with a <i>Pediococcus acidilactici</i> -supplemented diet to evaluate intestinal health and haematological immunological responses under non-challenged conditions.	320 fish; 160 fish/treatment; 4 replicate tanks/treatment (40 fish/tank)	<i>Pediococcus acidilactici</i> MA 18/5 M (Bactocell [®])	2.81×10^6 CFU/g diet	No	N/A	6 weeks	<i>Pediococcus acidilactici</i> significantly increased intestinal lactic acid bacteria levels ($p = 0.014$) and intraepithelial leukocyte abundance ($p = 0.039$) compared with the control. Intestinal TNF- α expression was significantly upregulated at weeks 3 ($p = 0.042$) and 6 ($p = 0.005$), while circulating neutrophil and monocyte abundance increased significantly at week 6 ($p < 0.05$).

(Cont'd...)

Table 4. (Continued)

Study	Aquaculture species	Experimental design	Sample size	Probiotic strain(s)	Probiotic dose	Direct antibiotic comparator (type)	Antibiotic dose	Feeding duration	Quantitative/statistical findings
Brunt <i>et al.</i> ¹⁴³	Rainbow trout (<i>Oncorhynchus mykiss</i>)	Fourteen-day feeding trial evaluating dietary probiotics against multiple bacterial pathogens through challenge tests and assessment of innate immune responses.	NR	JB-1 (<i>Bacillus</i> spp.) and GC2 (<i>Aeromonas sobria</i>)	2×10^8 cells/g feed	No	N/A	2 weeks	Both probiotic treatments markedly reduced mortality following bacterial challenge compared with controls. JB-1 reduced mortality to 0%–13% and GC2 to 0%–16%, compared with 80%–100% mortality in control fish. The probiotics also enhanced innate immune responses, including lysozyme activity, phagocytosis, and respiratory burst.
Choi <i>et al.</i> ¹⁴⁴	Olive flounder (<i>Paralichthys olivaceus</i>)	Eight-week feeding trial comparing a control diet, three probiotic-supplemented diets and two antibiotic-supplemented diets, followed by a 15-day <i>Edwardsiella tarda</i> challenge.	450 fish; 75 fish/treatment; 3 replicate groups/treatment	<i>Bacillus subtilis</i> WB60 (Pro-A), <i>Bacillus subtilis</i> SJ10 (Pro-B), and <i>Enterococcus faecium</i> SH30 (Pro-C)	Pro-A: 1×10^8 CFU/g diet; Pro-B: 1×10^8 CFU/g diet; Pro-C: 1×10^7 CFU/g diet	Yes (amoxicillin and oxytetracycline)	4 g/kg diet each	8 weeks, followed by a 15-day <i>Edwardsiella tarda</i> challenge	Pro-A, Pro-B, and Pro-C significantly increased lysozyme activity compared with the control, amoxicillin, and oxytetracycline groups ($p < 0.05$). Selected probiotic treatments also increased myeloperoxidase and IL-1 β and IL-10 expression ($p < 0.05$). All three probiotic treatments produced significantly greater intestinal villus length than the control, amoxicillin, and oxytetracycline groups ($p < 0.05$). Following <i>Edwardsiella tarda</i> challenge, all probiotic treatments significantly improved cumulative survival compared with the control ($p < 0.05$); superiority over antibiotic groups was not established.

(Cont'd...)

Table 4. (Continued)

Study	Aquaculture species	Experimental design	Sample size	Probiotic strain(s)	Probiotic dose	Direct antibiotic comparator (type)	Antibiotic dose	Feeding duration	Quantitative/statistical findings
Rhee <i>et al.</i> ¹⁴⁵	Olive flounder (<i>Paralichthys olivaceus</i>)	Eight-week feeding trial comparing a basal commercial diet, an oxytetracycline-supplemented diet and three probiotic-supplemented diets, followed by a 7-day disease challenge.	300 fish; 20 fish/tank; 3 replicate tanks/treatment	<i>Bacillus subtilis</i> ; commercial microbial product (CES); PI mixture of <i>Groenewaldozyma salmanticensis</i> and <i>Gluconacetobacter liquefaciens</i>	NR	Yes (oxytetracycline)	5 g/kg diet (5,000 mg/kg)	8 weeks, followed by a 7-day challenge	The PI probiotic mixture significantly enhanced NBT activity, while the CES treatment significantly increased lysozyme activity compared with the control ($p < 0.05$). Following the challenge, PI-fed fish showed cumulative survival equivalent to the oxytetracycline group, indicating comparable disease protection under the experimental conditions. The PI diet also increased intestinal microbial-community diversity.

Notes: Values and findings are reported as described in the original studies. "Direct antibiotic comparator" indicates whether an antibiotic-treated group was included in the same experimental study; "No" indicates that the study compared probiotics with an untreated/basal control only and therefore does not provide direct evidence of probiotic-antibiotic equivalence or superiority. "NR" indicates that the relevant information was not reported or could not be reliably extracted from the original study, whereas "N/A" indicates that the parameter was not applicable because no antibiotic treatment was included. Probiotic and antibiotic doses are expressed as reported by the respective authors. Statistical findings are reported as provided in the original studies, with $p < 0.05$ generally considered statistically significant. Findings in the "Quantitative/statistical findings" column are restricted primarily to fish health, immune responses, intestinal health, and pathogen-resistance outcomes. The inclusion of a direct antibiotic comparator does not itself demonstrate that probiotics can replace antibiotics; conclusions regarding equivalence, superiority, or replacement are limited to the specific outcomes and experimental conditions reported in each study. Abbreviations: CFU: Colony-forming units; IL: Interleukin; NBT: Nitroblue tetrazolium; TNF- α : Tumor necrosis factor- α .

Fermentation can also modify pH, redox potential, and nutrient availability, potentially creating conditions that favor probiotic activity in the gastrointestinal tract and support broader health and growth outcomes.

9.3. Synbiotics (probiotics and prebiotics)

Combining live microorganisms with specific substrates that selectively support their growth may enhance probiotic persistence and functional activity. Critical considerations include selecting prebiotics that can be efficiently utilized by the target probiotic strain without disproportionately promoting opportunistic microorganisms.^{149,150} Fermentation-based approaches can help identify compatible prebiotic substrates, such as selected oligosaccharides and resistant starches, that remain sufficiently stable during feed processing and reach the gastrointestinal tract. Potential risks include unintended alterations in the resident microbiota, excessive stimulation of specific metabolic pathways, and variable responses across host species and life stages. The potential benefits include improved probiotic persistence and more consistent effects at practical inclusion levels under farm conditions.¹⁵¹

9.4. Postbiotics and bioactive metabolites

The approach involves using non-viable microbial cells, cellular components, or microbial metabolites, such as bacteriocins, organic acids, SCFAs, and enzymes, to elicit beneficial host responses without administering live microorganisms. Fermentation-based processes can facilitate the production of more consistent postbiotic preparations with defined compositions, potentially improving reproducibility and safety.^{152,153} Key considerations include standardizing concentrations of bioactive components, maintaining their activity during feed processing and storage, and understanding species- and life-stage-specific responses. Potential advantages include improved shelf stability and reduced concerns associated with the use of live microorganisms, whereas limitations may include narrower activity spectra and the need for precise dose optimization.

10. Challenges and limitations of probiotic applications

10.1. Strain specificity and inconsistent performance

Strain specificity and variable performance can undermine the reliability of probiotic interventions. Their benefits are highly context-dependent; a strain that performs well in one host species, life stage, or production environment may underperform or fail under different conditions.^{154,155} This heterogeneity reflects complex interactions among

the probiotic strain, host, resident microbiome, diet, water quality, and farm-management practices. Relying on genus- or species-level descriptions without adequate strain-level characterization can compromise reproducibility and limit the translational value of findings.¹⁵⁶ To improve reliability, future studies should prioritize rigorous strain-level characterization, standardized and validated efficacy endpoints, and replication across multiple sites and production contexts. Without such standards, uncertainty surrounding probiotic performance may hinder adoption, commercial investment, and scalable implementation.

10.2. Survival and stability in aquatic environments

Probiotics must remain viable during feed processing, storage, water exposure, and temperature fluctuations. Many strains are sensitive to pelleting temperatures, humidity changes, and dilution in large-scale production systems.¹⁵⁷ Encapsulation, protective carriers, and preconditioning strategies can help improve probiotic stability and viability, but these approaches may increase production costs and potentially affect feed palatability or release characteristics.¹⁵⁷ Environmental factors, including salinity, pH, ultraviolet exposure, and competition with resident microorganisms, can further influence probiotic persistence and consistent colonization of the gastrointestinal tract.

10.3. Dosage and administration challenges

Probiotic dosing is challenging because insufficient doses may fail to produce measurable benefits, whereas excessive doses may provide limited additional benefit while increasing costs.^{158,159} The delivery method, whether through feed, water, or immersion, can also substantially influence probiotic exposure, residence time, and bioavailability, with effects varying across production systems. Real-time monitoring of probiotic colonization and health outcomes at the farm level is often impractical. Therefore, dose optimization should rely on standardized efficacy endpoints, validated indicators of probiotic establishment or activity, and context-specific trials that account for host species, life stage, and production environment.¹⁶⁰

10.4. Limited long-term and large-scale studies

Most available evidence comes from short-term, tightly controlled trials or small-scale hatchery experiments. However, field conditions introduce additional sources of variability, including seasonality, stock movement, mixed-species production systems, and differences in management practices, which may attenuate or obscure probiotic effects. Establishing the durability of these effects, assessing potential resistance risks, and determining

economic viability under real-world conditions therefore requires longitudinal, multi-site studies conducted under representative production environments.

10.5. Regulatory and commercialization barriers

Regulatory pathways for safety, efficacy, and strain provenance vary considerably across jurisdictions. Certification, good manufacturing practice-compliant manufacturing, and accurate labeling impose additional compliance costs, while intellectual property protections may restrict smallholders' access to beneficial strains.¹⁶¹ Market fragmentation and inconsistent quality control further hinder widespread adoption. Harmonization of regulatory standards could facilitate cross-border trade while strengthening consumer and producer confidence.

11. Future perspectives and research directions

11.1. Genomics and precision probiotics

Advances in genomics are transforming the selection and optimization of probiotics. Whole-genome sequencing enables precise strain identification, detection of ARGs, and prediction of safety and functional traits. When integrated with omics-based approaches, including metagenomics, transcriptomics, and metabolomics, these technologies facilitate the development of "precision probiotics" tailored to specific host species, life stages, diets, and environmental conditions. The overarching goal is to move beyond broad efficacy claims toward predictive, dose-optimized interventions with clearly defined mechanisms of action and measurable outcomes, while maintaining rigorous biosafety and traceability standards.

11.2. Microbiome engineering and synthetic microbial communities

Beyond the introduction of individual probiotic strains, microbiome engineering has emerged as a promising strategy for manipulating entire microbial communities to enhance host resilience, disease resistance, and production performance. Potential approaches include the rational design of microbial consortia with complementary functional traits, such as improved nutrient utilization, pathogen exclusion, immune modulation, and enhanced microbiome stability. Synthetic microbial communities (SynComs) have shown considerable promise in broader microbiome research by enabling the assembly of functionally complementary microbial consortia.¹⁵⁸ However, direct experimental evidence supporting the rational design and application of SynComs in aquaculture remains limited, and their use is still largely

at the conceptual and early research stages. Future studies should therefore prioritize the identification of compatible probiotic combinations, elucidation of host–microbiome–environment interactions, evaluation of the stability and persistence of engineered microbial communities under commercial farming conditions, and assessment of potential ecological risks, including horizontal gene transfer and disruption of native microbial communities, before large-scale implementation.

11.3. Integration into sustainable aquaculture systems

Future probiotic strategies will increasingly be integrated into holistic and sustainable production systems that combine biosecurity, nutrition, water quality management, and waste reduction. Probiotics could serve as integral components of closed-loop recirculating aquaculture systems, integrated multi-trophic aquaculture, and biofloc-based systems, where microbial activity can enhance water quality and feed efficiency while reducing reliance on chemical inputs. Successful implementation will depend on system-level modeling, real-time monitoring, and adaptive management approaches that balance production economics with environmental sustainability and animal health.

11.4. Policy and regulatory frameworks

Progress depends on coherent, science-based policy frameworks that balance innovation with safety and consumer confidence. Harmonized standards for strain characterization, efficacy validation, labeling, and withdrawal guidance are essential to facilitate cross-border trade and investment. Policymakers should promote transparent data sharing, post-market surveillance, and risk-based regulatory approvals, while supporting capacity building and knowledge transfer among producers and smallholders.

11.5. Scaling up in low- and middle-income countries

Translating scientific advances into global impact requires targeted strategies tailored to resource-limited settings. Key priorities include the development of affordable, locally adaptable probiotic formulations; technology transfer and capacity building; accessible diagnostic and monitoring tools; and financing models that reduce risks for smallholders. Emphasizing co-development with local stakeholders and aligning probiotic interventions with food security and public health priorities will help ensure that their benefits reach diverse communities without exacerbating existing inequities.

12. Conclusion

Probiotics offer meaningful, context-dependent benefits in aquaculture, including enhanced disease resilience, improved gut health, and reduced reliance on antibiotics. However, their effectiveness depends on rigorous strain-level validation, robust delivery systems, and evaluation under practical farm conditions across diverse species and production systems. Probiotics should therefore be integrated into broader disease management strategies that incorporate vaccination, biosecurity, appropriate nutrition, and environmental stewardship. Such an integrated approach can improve feed efficiency, enhance animal welfare, and strengthen the resilience and sustainability of aquaculture production.

Sustainable adoption also requires transparent reporting, standardized protocols, and continuous monitoring of AMR and potential ecological impacts. In addition, harmonized regulatory frameworks and capacity-building initiatives are essential to support the responsible use of probiotics, particularly in low- and middle-income countries. Collectively, these efforts can contribute to mitigating AMR while maintaining productivity, protecting livelihoods, and safeguarding environmental integrity.

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