

# Promoting sustainable aquaculture: exploring antibiotic alternatives to fight against pathogens

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**Abstract** Aquaculture, a rapidly expanding industry, provides a diverse range of protein sources. However, disease outbreaks can significantly impact production, leading to the use of antibiotics to mitigate financial losses. Nevertheless, the overuse of antibiotics in aquaculture poses significant environmental and public health risks, contributes to antibiotic resistance, and disrupts fish metabolism. The rise of multidrug-resistant microorganisms demands the creation of innovative, alternative therapies. This comprehensive review aims to identify and evaluate the efficacy of alternative approaches for combating pathogens in aquaculture, focusing on sustainable and environmentally responsible practices. Promising alternatives include immunotherapeutics, vaccinations, probiotics, prebiotics, herbal plant-based interventions, organic acids, bacteriophages, and quorum quenching. By exploring these innovative approaches, this review endeavors to promote the development of sustainable aquaculture practices that prioritize environmental stewardship, animal health, and human well-being.

**Keywords** Antibiotics . Antibiotic resistance . Disease prevention . Alternative treatment

## Introduction

Aquaculture is a diverse and complex industry, encompassing a wide range of practices, from small-scale, traditional pond-based systems to large-scale, technologically advanced operations producing high-value aquatic species. The significance of aquaculture has grown rapidly over the years. In the

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1980s, Asian countries, which currently account for 89% of global aquaculture production, drove the industry's expansion to meet the demand for aquatic products (FAO 2019). Aquaculture has shown unprecedented growth, surpassing other food production sectors globally. Recent estimates from 2020 indicate that global production of farmed food fish has reached 82.1 million tons, highlighting the ongoing importance of aquaculture in developing improved animal protein sources (Pradeepkiran 2019; FAO 2020). Aquaculture systems operate in diverse environments, including brackish, freshwater, coastal, riverine, saltwater, and terrestrial locations, spanning temperate to tropical climates. The industry farms a variety of species, such as seaweeds, mollusks, crustaceans, and finfishes, using extensive, semi-intensive, and intensive farming techniques. Farming practices utilize a spectrum of feed inputs, from natural to formulated diets, resulting in a variety of products from high-value finfish to low-value invertebrates (Bondad-Reantaso et al. 2005; 2023).

The growth of intensive aquaculture practices has led to environmental pollution, disease outbreaks and slowed fish growth, resulting in significant economic losses (Lafferty et al. 2015). The industry faces numerous challenges, including dissemination of infectious diseases, emergence of novel pathogens, escalating disease severity, and the far-reaching consequences of globalization. Additionally, aquatic animals cohabitate with a diverse range of microorganisms, which can include pathogens that pose health risks. The presence of these microorganisms depends on factors specific to the pathogen, host, and environment. To mitigate these risks, controlling the aquaculture environment is crucial. However, the selection of suitable control methods depends on various considerations, including the target species, economic viability, and existing infrastructure at national and commercial levels. In aquaculture, antibiotics play a vital role in preventing and treating bacterial infections (Selamoglu 2018). Most antibiotics target gram-negative bacteria, which are responsible for most bacterial illnesses in aquatic animals. Gram-negative rods are a common cause of bacterial infections. Antibiotics are substances produced by certain microorganisms that exhibit bacteriostatic or bactericidal properties. This broader group includes semi-synthetic drugs. There are various classes of antibiotics, including the amphenicol class, which was introduced with the discovery of chloramphenicol in 1947. Macrolides represent another prominent class of antibiotics frequently used in medical treatments (Petty and Francis-Floyd 2020). In agricultural disease management, the most widely employed antibiotics include oxytetracycline, trimethoprim, and florfenicol (Alday-Sanz et al. 2012).

Any exposure to antibiotics, whether during treatment or through prolonged subtherapeutic exposure, can select for resistant mutants, enabling their natural development (Nesse and Simm 2018). The rise of antimicrobial resistance (AMR) has compromised the control of common bacterial aquaculture diseases, such as furunculosis (*Aeromonas salmonicida*) and edwardsiellosis (*Edwardsiella tarda*). Furthermore, the presence of antimicrobial residues in food has raised significant concerns regarding food safety, public health, and animal health. The primary public health concern associated with antimicrobial residues is the development of antibiotic resistance. Additionally, residues can cause allergies (e.g., penicillin), nephropathy (e.g., gentamicin), carcinogenicity (e.g., sulfamethazine, furazolidone and oxytetracycline), mutagenicity, anaphylactic shock, teratogenicity, disruption of normal intestinal flora and bone marrow depression (Okocha et al. 2018).

To safeguard consumer health, controlling antibiotic use in aquaculture is necessary. The overuse of antimicrobial drugs in aquaculture leads to disease resistance and adverse health consequences for humans. Two primary concerns associated with antibiotic use in aquaculture are the presence of antimicrobial residues in industry-produced products and the spread of AMR. Alternatively, aquatic animal pathogens may develop resistance, rendering treatments ineffective (Milijasevic et al. 2024).

AMR poses a significant threat to global health, as it can transcend geographical and phylogenetic boundaries, facilitating the exchange of resistant genes between human pathogens and animal diseases. Gene flow can occur through various mechanisms. Unlike terrestrial environments, where animals receive injectable antimicrobials, aquatic animals are primarily treated with medicated feed. Nevertheless, this method has limitations. Reduced feed intake by sick animals can compromise treatment efficacy, and unused medicated feed can accumulate in sediments. This accumulation can foster the growth of resistant bacteria, expanding the pool of resistance in aquatic environments. Consequently, exploring alternatives to antibiotics is crucial (Bondad-Reantaso et al. 2023; Elgendy et al. 2024). This comprehensive review seeks to investigate and identify effective antibiotic alternatives for combating pathogens in aquaculture, with a



focus on promoting sustainable and environmentally responsible practices.

### Controlling fish infections with antibiotics

Antibiotic therapy is a vital tool in the prevention and control of fish infections, particularly when employed prophylactically (Chen et al. 2018; Binh et al. 2018). By incorporating antibiotics into feed formulations, farmers can therapeutically treat and prevent bacterial diseases in both juvenile and adult fish. Additionally, antibiotics provide a defense against illnesses that emerge during the early stages of fish development. While antibiotics can significantly lower morbidity and mortality rates, encouraging the expansion of aquaculture, their continual small-scale use is also driven by economic factors. Farmers perceive antibiotics as a cost-effective measure, as they believe these drugs help minimize production costs, thereby motivating their continued use in aquaculture (Ringø 2020).

### Adverse effects of antibiotics

The use of antibiotics in aquaculture is crucial for maintaining fish health, as emphasized by Limbu et al. (2020). However, administering antibiotics to cultured fish can have unintended consequences. Following ingestion, antibiotics are absorbed into the fish's gastrointestinal tract, distributed to various tissues, and can accumulate, exerting their effects. The impact of antibiotics on fish varies depending on factors such as species, growth stage, type, dosage, and mode of action. Consequently, antibiotic-induced effects can significantly influence the productivity and sustainability of fish aquaculture (Ljubojević Pelić et al. 2024; Table 1).

The global misuse and uncontrolled use of antibiotics have escalated, driven by the misconception that they can reduce manufacturing losses and accelerate growth rates, thereby minimizing the spread of harmful pathogens (Muteeb et al. 2023). Thus, the excessive use of antibiotics in animal protein production poses a significant risk to human health (Romero et al. 2012). According to Guardabassi and Kruse (2010), the misuse of antibiotics can facilitate the zoonotic transfer of resistance genes into the human microbiome. This disruption can have far-reaching consequences, including the killing of beneficial microorganisms, weakened immunity, and impaired nutrition. Furthermore, the widespread use of antibiotics in intensive production systems contributes to environmental pollution and chemical accumulation (Kakoolaki et al. 2013; Su et al. 2017; Saxena et al. 2018).

### Antimicrobial resistance in aquaculture

The overuse and misuse of antibiotics in aquaculture have accelerated the development of antimicrobial resistance, rendering the treatment of infectious diseases increasingly challenging. The emergence and dissemination of antibiotic-resistant genes pose a significant threat to global health (Bhat et al. 2020). Anti-

**Table 1** The adverse effects of antibiotics on fish species

| Antibiotics      | Exposure method, dosage and duration                                  | Species                        | Effects   | References               |
|------------------|---|--------------------------------|---|--------------------------|
| Tetracycline     | Exposure 0.02, 0.01, 0.1, 100 µg; 120 h                               | <i>Danio rerio</i>             | Survival rates remained unchanged, with no significant variations in body length in treated fishes  | Qiu et al. (2020)        |
| Cefalexin        | Exposure; 1.3, 2.5, 5 and 10 mg; 96h                                  | <i>Pomatoschistus microps</i>  | No significant differences were observed in total length and weight between the treated and control groups. However, the treated fish showed reduced predatory performance compared to the control group. | Fonte et al. (2016)      |
| Sulfamethoxazole | Dietary; 100 mg kg <sup>-1</sup> diet; 84 days                        | <i>Oreochromis niloticus</i>   | Weight gain was significantly lower in treated fish than in controls  | Limbu et al. (2018)      |
| Norfloxacin      | Exposure; 0.0001, 0.1, 1.0, 5.0 and 10.0 mg L <sup>-1</sup> ; 34 days | <i>Cyprinus carpio</i>         | No significant differences in survival rates  | Charvatova et al. (2015) |
| Florfenicol      | Dietary; 20 g; 28 days  | <i>Ctenopharyngodon idella</i> | Disrupt both luminal and mucosal microbiota   | Sun et al. (2021)        |
| Chloramphenicol  | Exposure; 2.5, 5.0 and 10.0 mg L <sup>-1</sup> ; 15 days              | <i>Clarias gariepinus</i>      | Treatment-induced behavioral changes in fish included disorientation, erratic swimming, and hyperactive responses.  | Nwani et al. (2014)      |

microbials can select for antibiotic-resistant bacteria and facilitate the transmission of resistance genes (Liu et al. 2017). Furthermore, the presence of antibiotic residues in aquaculture products poses a health risk to consumers. These residues can persist in the environment due to their stability and resistance to biodegradation, subsequently accumulating in shellfish and fish. Studies have detected antibiotic residues, including sulphonamides, tetracyclines, and macrolides, in farmed fish species such as tilapia (Bortolotte et al. 2021), trout (Adel et al. 2017), and salmon (Miranda et al. 2018) from various countries (Done and Halden 2015; Almashhadany et al. 2024).

The consumption of aquaculture products contaminated with antibiotic residues can have carcinogenic and teratogenic effects, disrupting the natural intestinal flora in humans and animals. This issue is not limited to specific regions, as studies from Iran (Mahmoudi et al. 2014), India (Swapna et al. 2012), Bangladesh (Hassan et al. 2013), and Nigeria (Olatoye and Basiru 2013) have reported the presence of antibiotic residues in aquaculture products. The economic implications of antimicrobial resistance in aquaculture are substantial. Notably, the US and EU have rejected a considerable proportion of imported aquaculture products due to antibiotic residues, with rejection rates reaching 20% and 28%, respectively (FAO 2020). This highlights the need for sustainable and responsible use of antibiotics in aquaculture to mitigate the risk of antimicrobial resistance.

### **Mechanisms of horizontal gene transfer across bacteria to acquire antibiotic resistance**

Antibiotic-resistant bacterial strains are rapidly emerging, posing a substantial risk to global health and wellbeing. Research reveals that bacteria can develop resistance to antibiotics through various mechanisms, including horizontal gene transfer (HGT) (Chokshi et al. 2019; Murray et al. 2022). HGT is a pivotal process in bacterial evolution, facilitating the exchange of genetic material between cells. HGT enables bacteria to acquire antibiotic resistance genes (ARGs) through conjugation, transduction, and transformation.

During transformation, donor bacteria release DNA fragments containing antibiotic resistance genes (ARGs) into the environment. These fragments can be taken up by recipient bacteria, which then integrate the ARGs into their genomes, enabling the production of resistance-conferring proteins or enzymes. Transduction, mediated by bacteriophages, allows for the transfer of ARGs from donor to recipient bacteria. The recipient bacterium can then integrate these genes into its genome, acquiring antibiotic resistance. Conjugation occurs through direct cell-to-cell contact between donor and recipient bacteria, facilitated by a specialized structure called a pilus. This process enables the transfer of plasmid-borne ARGs from donor to recipient bacteria, where they are integrated into the chromosome (Chen 2022).

Outer membrane vesicles have been identified as a mechanism for HGT, but the majority of HGT events are mediated by mobile genetic elements (MGEs). MGEs comprise a diverse array of genetic entities, including conjugative DNA elements, transposable DNA elements, and bacteriophages (Tokuda and Shintani 2024). Conjugative DNA elements, such as plasmids and integrative and conjugative elements (ICEs), enable direct transfer of genetic material between bacterial cells through conjugation, a process mediated by type IV secretion systems (Delavat et al. 2017). Transposable DNA elements, including transposons and integrons, facilitate intracellular and inter-replicon gene mobility. Transposons can transpose to new genomic locations, whereas integrons capture and integrate gene cassettes, often conferring antibiotic resistance (Tansirichaiya et al. 2022). Bacteriophages, or phages, are bacterial viruses that can transfer genetic material between cells through transduction. During the phage replication cycle, host DNA can be inadvertently packaged into phage particles and transported to new host cells, facilitating HGT (Schneider 2021). These HGT mechanisms play a crucial role in the dissemination of antibiotic resistance among bacterial populations, highlighting the need for effective strategies to combat the spread of resistance.

### **Different alternatives to antibiotics**

The mechanisms and causes mentioned earlier highlight the need for innovative solutions to combat bacterial infections. Several options have emerged as potential replacements for antibiotics (Figure 1).

#### **Use of probiotics**

Probiotics are beneficial microorganisms that, when administered in sufficient amounts, can positively im-



pact the health and well-being of their host (Diwan et al. 2022). Despite debate surrounding their efficacy in aquaculture, probiotics offer a promising alternative to antibiotics in promoting fish health. In aquaculture, physiological stress responses triggered by chemical and physical factors can lead to disease. These factors include handling-related injuries, fish density, temperature, pH, oxygen and CO<sub>2</sub> concentrations, and the presence of organic matter in the water (Verschuere et al. 2000).

Stress and environmental changes can cause immunosuppression, hindering fish growth. Probiotic interventions can help mitigate these effects by promoting a balanced microbial community, enhancing fish resilience to stress and disease (Dawood et al. 2018; Riaz et al. 2024).

Probiotics provide various benefits, including improved development, health, disease resistance, feed utilization, stress response, and overall vigor (Dawood et al. 2016). Their primary functions include boosting the host’s immune system, increasing feed utilization, modifying microbial populations, and enhancing the surrounding environment. In aquatic animals, probiotics are particularly important due to the constant interaction between gut microbiota, surroundings, and host activities (Tong et al. 2023; Riaz et al. 2024).

Various microorganisms, including bacteria, yeasts, and algae, have been explored as probiotics in aquaculture (Irianto and Austin 2002; Ghezil et al. 2019). Probiotic supplements and live microbial feed products help stabilize the host’s gastrointestinal microbiota, enhancing aquatic species’ survival, growth, and overall health (Hai 2015; Figure 2). In fish aquaculture, probiotics play a significant role, offering financial and operational benefits. For example, *Pseudomonas fluorescens* has been investigated as a pro-

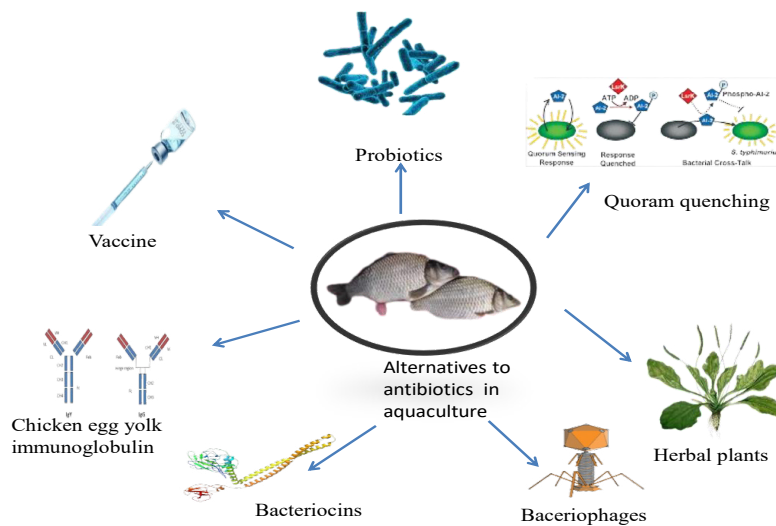


Fig. 1 Some alternatives to antibiotics

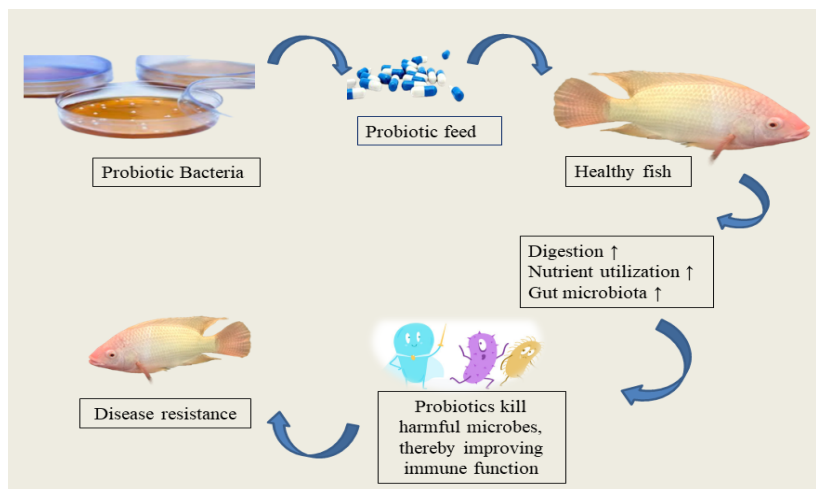


Fig. 2 Probiotics improve disease resistance in aquaculture (Okeke et al. 2022)

biotic agent against two major fish pathogens: *Streptococcus faecium* and *Pseudomonas anguilliseptica*. The latter is a notorious opportunistic pathogen affecting farmed fish globally (Eissa et al. 2014; Table 2).

### Mode of action of probiotics

Probiotics exert their role in disease resistance through various mechanisms (Figure 3). They generate various bioactive compounds, including organic acids and antimicrobial substances, which hinder the attachment of harmful bacteria to intestinal binding sites (Tiwari et al. 2012). Additionally, probiotics interact with the host's immune system by regulating immune cells, such as T cells and antigen-presenting cells, to promote a balanced immune response (Oelschlaeger 2010). Furthermore, probiotics alter the phenotype and function of dendritic cells, modulate the NF- $\kappa$ B pathway and AP-1, and control the production of pro-inflammatory cytokines (Roselli et al. 2005). Moreover, they stimulate the production of IgA antibodies, increase natural killer cell activity, and regulate apoptosis and nitric oxide release. They also selectively impact T helper cells and stimulate specific immune cells (Fong et al. 2016).

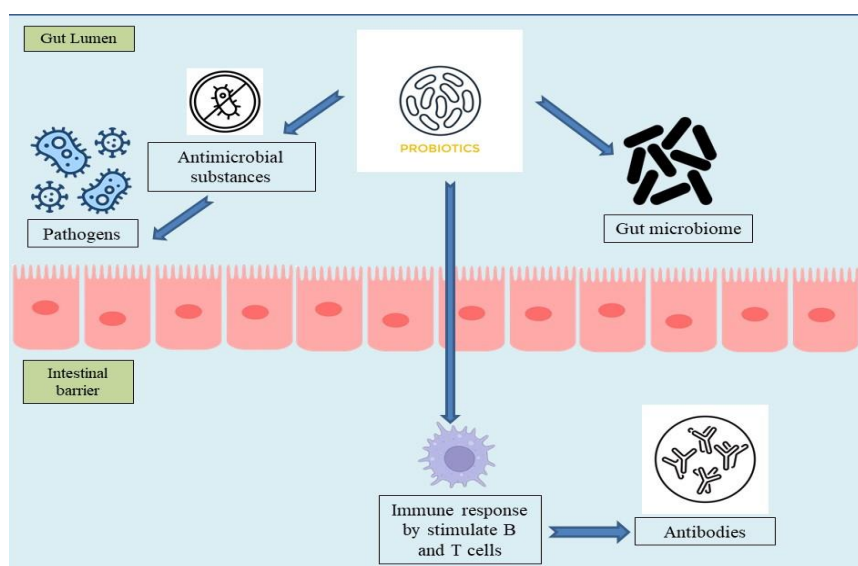
### Use of prebiotics in aquaculture

Prebiotics consist of non-digestible dietary fibers or carbohydrates that foster a favorable gut microbiome by stimulating the growth and activity of beneficial bacteria. By promoting a healthy gut microbiota prebiotics confer benefits to the host's immune system, digestion, and overall well-being. Prebiotics have emerged as a promising strategy to boost disease resistance in aquatic species. By modifying the microbial ecology in the gastrointestinal tract, prebiotics can enhance non-specific immune responses and promote a balanced gut microbiota (Song et al. 2014; Dawood et al. 2016).

Oligosaccharides, a type of prebiotic, selectively ferment, supporting the growth of beneficial anaerobic bacteria while suppressing pathogenic bacteria (Roberfroid 2005). This selective fermentation significantly alters the gut environment, leading to improved immunity and disease resistance (Dawood et al. 2016). Incorporating prebiotics in aquaculture has been shown to increase the production of volatile fatty acids in

**Table 2** The effects of probiotics against harmful bacteria in fish

| Fish species | Probiotic strains                | Bacterial pathogens            | Effects   | References          |
|--------------|----------------------------------|--------------------------------|---|---------------------|
| Tilapia      | <i>B. licheniformis</i>          | <i>Streptococcus iniae</i>     | Improved the disease resistance   | Han et al. (2015)   |
| Common carp  | <i>Flavobacterium sasangense</i> | <i>Aeromonas hydrophila</i>    | Enhance immune response and disease resistance                            | Chi et al. (2014)   |
| Crucian carp | <i>Lactobacillus acidophilus</i> | <i>Helicobacter pylori</i>     | Upgrade the protein frame of the skin mucus<br>To reduce in fatality rate | Adeshina (2018)     |
| Nile tilapia | <i>Lactobacillus rhamnosus</i>   | <i>Pseudomonas fluorescens</i> | Enhance in hematological parameters, total protein and globulin           | Eissa et al. (2014) |



**Fig. 3** Mechanism of probiotics as alternatives to antibiotics in combating disease resistance (Zhang et al. 2023)

the GI tract, inhibiting the growth of potentially harmful microorganisms and providing energy to the host (Ganguly et al. 2013). Studies have demonstrated the potential of prebiotics to modulate the gut microbiota and enhance disease resistance in various aquatic species (Patel and Goyal 2012; Iwashita et al. 2022). Further research is necessary to elucidate the mechanisms by which prebiotics exert their beneficial effects and to identify the most effective prebiotic compounds for specific aquatic species.

### Organic acids

Organic acids, including citric, lactic, benzoic, and formic acid, are volatile, short-chain fatty acids with antibacterial properties that can improve growth, disease resistance and nutrient utilization in aquatic animals (Ng and Koh 2017). Despite their benefits, research on organic acids in aquaculture is limited, particularly compared to their use in livestock feeds.

Several fish species, such as carp, tilapia, salmon, and rainbow trout, have been the subject of research examining how dietary organic acids influence their growth rates, nutrient uptake, and ability to fight off disease (Reda et al. 2016; Ng and Koh 2017). The findings suggest that organic acids have beneficial effects on aquaculture species, particularly in enhancing disease resistance. The dietary salts of formic acid, such as K-diformate (KDF), have been shown to improve growth, feed utilization, and nutrient digestibility in tilapia (Ramli et al. 2005; Lim et al. 2010; Ng et al. 2009). Furthermore, organic acids have been found to exhibit antibacterial properties, inhibiting the growth of Gram-negative bacteria and modulating gut microbiota (Bai et al. 2015; Ng and Koh 2016). These findings indicate that organic acids have potential as a natural alternative to antibiotics in aquaculture.

### Mechanism of action

Organic acids exhibit antibacterial properties by disrupting bacterial cell membranes and releasing protons into the cytoplasm, ultimately inhibiting the growth of Gram-negative bacteria (Bai et al. 2015). To maintain intracellular pH equilibrium, bacteria expend energy (ATP) to extrude protons, leading to cellular energy depletion and eventual death (Defoirdt et al. 2009). The antibacterial mechanism of organic acids involves a multi-step process: disruption of bacterial cell membranes, release of protons into the cytoplasm, inhibition of bacterial growth and depletion of cellular energy (ATP). Notably, organic acids are most effective against bacteria in their undissociated state, particularly at low pH levels (Brul and Coote 1999; Lambert and Stratford 1999; Ng and Koh 2017; Figure 4).

### Phytotherapy: Medicinal plants

Phytotherapy, which utilizes compounds derived from medicinal plants for therapeutic purposes, has gained attention in aquaculture. Medicinal herbs have demonstrated broad antiparasitic, antibacterial, and antifungal properties of medicinal herbs in humans and animals (Silva and Fernandes Junior 2010). Herbal rem-

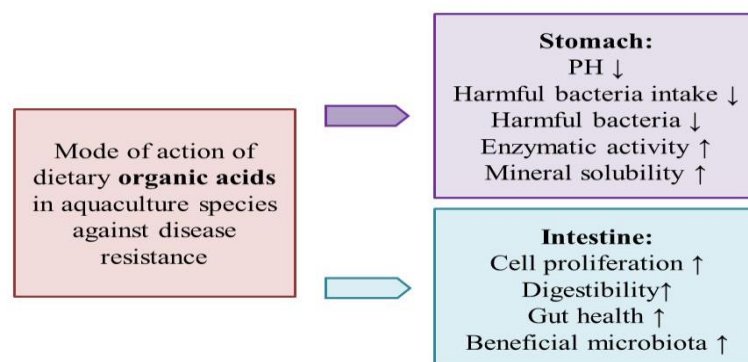


Fig. 4 Mechanism of organic acids as alternatives to antibiotics in combating disease resistance (Bai et al. 2015)

edies have been used for centuries to treat human ailments, and their potential application in aquaculture has only recently been explored (Li et al. 2022). The demand for alternatives to conventional medications, which pose environmental hazards, has driven the development of phytotherapeutic approaches in aquaculture. Strategies for managing and preventing fish infections include the development of prophylactic treatments, such as immunostimulant diets (Skalli et al. 2013). Natural compounds have shown promising preventive effects and improved fish immune responses (Samad et al. 2014; Vaseeharan and Thaya 2014).

To combat pathogen-caused mortality, therapeutic products must be employed during production. Phytotherapeutic ingredients are often administered to fish through water immersion. Medicinal herbs are proposed as a potential substitute for preventing fish disease, given the widespread negative impacts of antimicrobial resistance in aquaculture (Freitas et al. 2020; Iqbal et al. 2024).

Medicinal plants contain antioxidant chemicals that capture free radicals, interfering with regular cell activity (Moreno et al. 2020). Various Asian medicinal plants, such as *Andrographis paniculata*, *Cinnamomum zeylanicum*, and *Curcuma xanthorrhiza*, have demonstrated efficacy in treating ailments, including metabolic abnormalities and fever (Ismail et al. 2017; Yousaf et al. 2024; Table 3). Herbal medicines contain antibacterial compounds, including proteins, terpenoids, alkaloids, and phenolic compounds, which have shown resistance against bacterial diseases such as *Aeromonas hydrophila*, *Streptococcus spp*, *Vibrio spp*, *Edwardsiella ictaluri* (Jafarzadeh et al. 2020; Rodianawati et al. 2015).

Despite their potential, the use of phytotherapeutics in aquaculture feeds to treat parasitic diseases remains understudied. However, their efficacy as a feed additive suggests a promising application in aquaculture. Further research is warranted to explore this mode of administration, particularly against parasites like *Ichthyophthirius multifiliis* that complete part of their life cycle within host tissues (Valladão et al. 2015).

## Mechanism of action

Medicinal plants modulate the immune system by targeting the kidneys, spleen, and thymus, enhancing the growth and maturation of immune organs. They stimulate non-specific immunity by activating white blood cells and the monocyte-macrophage system, while also promoting specific immune responses through antibody production. Medicinal plants induce the release of cytokines, including interferon, tumour necrosis factor, and interleukin, regulating both non-specific and specific immunity. Their bioactive compounds, such as essential oils, saponins, phenolics, and polysaccharides, exhibit immunopotential, antioxidant activity, and stress mediation (Hoseinifar et al. 2020; Anal et al. 2023).

These compounds interact with proteins and DNA, altering their recognition, binding, catalytic activity, and turnover. Phenols and terpenoids in medicinal plants scavenge reactive oxygen species and oxygen free radicals, protecting cells and tissues from oxidative damage (Chong et al. 2020; Figure 5).

## Vaccines

Vaccines are mixtures of modified or inactivated pathogens and their metabolites, administered to prevent infectious diseases (Lillehaug et al. 2018). Prophylactic vaccination offers dual benefits: protecting tissues and organs before they become compromised (low pathogen burden) and preventing bacterial growth after initial infection. This proactive approach significantly reduces the likelihood of resistance mutations emerging and spreading. Unlike medications, vaccines contain multiple immunogenic epitopes, making it harder for pathogens to develop resistance (Bagnoli et al. 2017). Consequently, more mutations are required for pathogens to confer resistance against vaccines. Although vaccine resistance can occur, vaccines are less likely to induce it. Vaccines can be administered orally, intramuscularly, intraperitoneally, or through

**Table 3** Effects of medicinal plants with antibacterial properties

| Pathogen                            | Host   | Active compound        | Plants used                    | References                 |
|-------------------------------------|--|------------------------|--------------------------------|----------------------------|
| <i>Aeromonas hydrophila</i>         | <i>Clarias gariepinus</i>  | Allicin                | <i>Allium sativum</i>          | Eirima-Liza et al. (2018)  |
| <i>A. hydrophila</i>                | <i>Labo rohita</i>   | Sitosterols            | <i>Indian ginseng</i>          | Sharma et al. (2010)       |
| <i>Miamiensis avidus</i>            | <i>Paralichthys olivaceus</i>                                      | Saponins               | <i>Herbaceous seepweed</i>     | Harikrishnan et al. (2012) |
| <i>Edwardsiella ictaluri</i>        | <i>Ctenopharyngodon idella</i>                                     | Alkaloids; (berberine) | <i>Berberis aristata</i>       | Ji et al. (2012)           |
| <i>Ichthyophthirius multifiliis</i> | <i>Ictalurus punctatus</i>   | Pentagalloylglucose    | <i>Galla chinensis</i>         | Valladão et al. (2015)     |
| <i>A. hydrophila</i>                | <i>Labo rohita</i> , <i>Cirrhinus mrigala</i> , <i>Catla catla</i> | Terpenoids             | <i>Andrographis paniculate</i> | Basha et al. (2013)        |
| <i>Staphylococcus aureus</i>        | <i>Salmo trutta</i>  | Menthol                | <i>Mentha piperita</i>         | Adel et al. (2015)         |





water-based solutions. Injectable vaccinations are crucial for preventing disease in salmon and trout, while water-based vaccinations have been effective against diseases like *Listonella anguillarum* and *Vibrio salmonicida* (Bondad-Reantaso et al. 2023).

The salmonid vaccine industry emerged in the late 1980s with water-based vaccinations. Oil-based vaccinations introduced in the 1990s reduced furunculosis outbreaks (Duff 1942). Today, vaccinations are available for over 22 bacterial infections and six viral diseases in more than 17 fish species, with availability in over 40 countries. However, large-scale industrial vaccination is not widely practiced in major fish-producing nations like China, which accounts for 61% of the world’s aquaculture production (Bondad-Reantaso et al. 2023).

Mode of a vaccine action against facultative intracellular bacteria

Immunity against pathogens is typically acquired through two mechanisms: antibodies and cell-mediated immunity (Figure 6). However, antibodies are less effective against intracellular and facultatively intracellular bacteria, such as *Salmonella sp.*, *Listeria monocytogenes*, and *Mycobacterium sp.*, which can evade antibody detection by multiplying inside host cells. Although these pathogens can evade antibodies, most

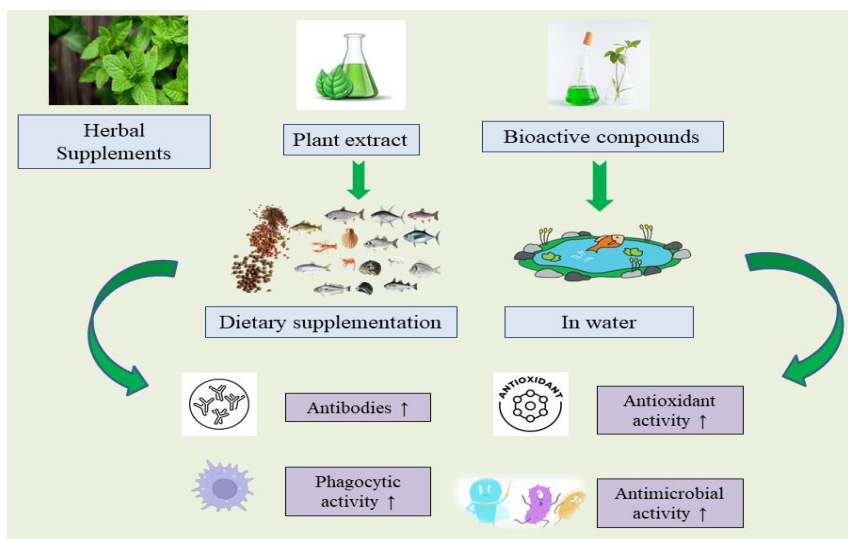


Fig. 5 Mechanism of medicinal plants as alternatives to antibiotics in combating disease resistance (Elgendy et al. 2024)

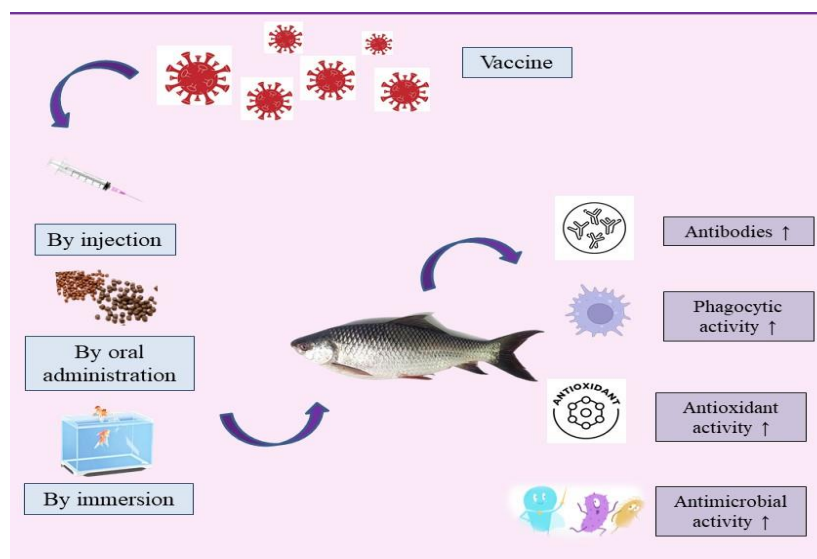


Fig. 6 Mechanism of vaccine as alternatives to antibiotics in combating disease resistance (Bondad-Reantaso et al. 2023)

intracellular pathogens spend part of their infectious cycle in the extracellular space, where they are vulnerable to antibody activity (Haesebrouck et al. 2004; Pridgeon and Klesius 2012).

Cell-mediated immune responses, mediated by Th1-produced lymphokines stimulate cytotoxic T-lymphocytes, which activate macrophages. This activation enhances the ability of macrophages to eliminate facultatively intracellular bacteria. Cytotoxic T-lymphocytes destroy infected host cells, releasing bacteria into the extracellular space where they can be eliminated. Live-attenuated vaccines are generally more effective against facultatively intracellular bacteria, such as *Salmonella sp.*, due to their ability to induce cell-mediated immunity. However, research suggests that inactivated vaccines can also elicit cell-mediated immunity, depending on the adjuvant used (Mondal and Thomas 2022).

### Reverse vaccinology

Reverse vaccinology has transformed the vaccine development landscape by harnessing the power of computational genomics and bioinformatics to pinpoint promising vaccine targets within pathogen genomes. This approach identifies specific protein regions (epitopes) that trigger strong immune responses, enabling the creation of multi-epitope vaccines. The benefits of reverse vaccinology include targeting multiple epitopes simultaneously to enhance vaccine efficacy against rapidly evolving antibiotic-resistant bacterial strains, and faster and more precise vaccine development that can potentially overcome the limitations of traditional methods. As antibiotic resistant bacteria remains a pressing global health concern (Enayatkhani et al. 2021; Herrera 2021; Tobuse et al. 2022), reverse vaccinology offers a promising solution for developing effective vaccines. Ongoing research is exploring the application of reverse vaccinology in aquaculture, focusing on developing effective vaccines against major bacterial pathogens such as *Vibrio sp.*, *Aeromonas sp.*, and *Streptococcus sp* (Mondal and Thomas 2022).

### Bacteriophages

Phages or bacteriophages, are viruses that target bacteria, making them harmless to humans. They are among the most abundant viruses, with estimates suggesting approximately  $10^{31}$  phages in the biosphere. Phages comprise ten distinct groups, including sessile and tailed phages, each with unique structures. (Abedon et al. 2011). As obligatory bacterial parasites, phages possess a complete genetic makeup, enabling them to multiply within suitable hosts. However, they lack ribosomes and energy-producing machinery, relying on host cells for protein synthesis.

Phage therapy, a medical treatment alternative to antibiotics, has been extensively explored since the

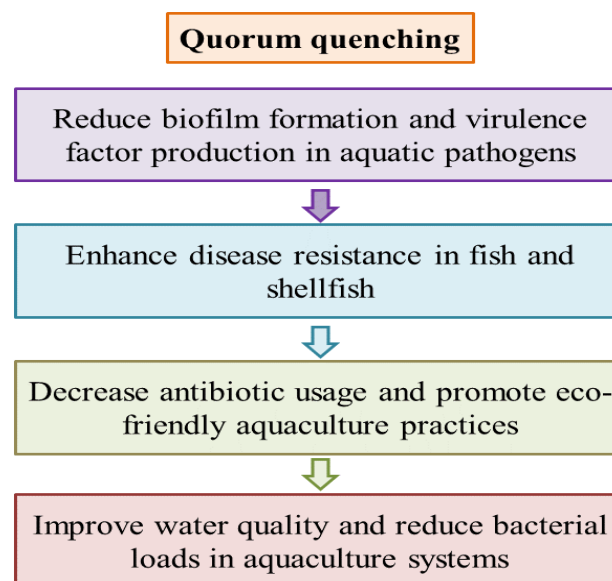


Fig. 7 Quorum quenching in combating disease resistance (Bondad-Reantaso et al. 2023)



discovery of phages in 1915 (Dhama et al. 2013; Tiwari et al. 2014). In aquaculture, phages offer a viable treatment option for bacterial infections due to their specificity, rapid generation time, and minimal environmental impact (Rong et al. 2014; Jamal et al. 2015).

The advantages of phage therapy include its effectiveness against mucosal and topical infections, specificity to particular bacterial populations, and excellent tolerance in therapy. The presence of phages in the human microbiome further contributes to their therapeutic potential. Phage technology has emerged as a promising substitute for antibiotics in medicine, agriculture, and related fields (Weber-Dąbrowska et al. 2016).

### Quorum quenching

Quorum quenching (QQ) disrupts the quorum sensing (QS) system, which enables bacteria to synchronize behaviors such as biofilm production, pathogenicity, and light emission (Dong et al. 2001). QS relies on autoinducers, including Acyl-Homoserine Lactones (AHLs), to activate genes in response to population density and species composition (Bondad-Reantaso et al. 2023).

Certain substances can disrupt AHL-mediated QS by degrading AHLs, inhibiting their synthesis, or blocking their interaction with receptors. This disruption prevents harmful bacteria from forming biofilms, producing virulence factors, and reduces their pathogenicity. The potential of QQ to halt gene expression regulating disease progression, virulence, and microbial interactions has attracted considerable research attention (Reen et al. 2018; Figure 7).

QQ has significant potential in aquaculture to prevent bacterial infections and promote sustainable practices. SShaheer et al. (2021) explored the use of AHL-degrading *Bacillus* spp. as a viable substitute for antibiotics in preventing luminescent vibriosis in shrimp hatcheries. This innovative bio-therapeutic approach offers a promising solution for disease management in shrimp aquaculture.

### Conclusion

In conclusion, a multifaceted approach is necessary to address antibiotic resistance, incorporating therapies to minimize antibiotic use. A combination of general and specific strategies is necessary to prevent and treat infections. Effective alternatives include immunotherapeutics, vaccinations, probiotics, and herbal plant-based interventions. Recent biotechnology innovations have enabled the development of novel therapies against aquaculture pathogens. Bio-based and immunoprophylaxis approaches, such as phytotherapeutics and phage therapy, show promise in preventing and treating diseases in aquaculture. To promote the ethical and sustainable implementation of these therapies, further research is necessary to evaluate their efficacy, cost implications, and environmental impact. Collaboration among scientists, researchers, and regulatory bodies is crucial for refining these alternative approaches and promoting a healthier and more sustainable aquaculture industry.

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**Conflict of interest** The authors report no conflict of interest.

**Data availability statement** Data will be made available on request.

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