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Invited Review Article

Alternatives to antibiotics for combating the antimicrobial resistance in aquaculture

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Abstract

Aquaculture is evolving into a highly concentrated industry with much intensive farming. Infectious diseases are becoming more prevalent, resulting in significant economic losses, and their treatment demands the use of antimicrobial agents. However, the use of antimicrobials in aquaculture has resulted in the emergence and spread of antibiotic-resistant bacteria and genes, as well as the presence of residues in aquaculture products and the environment. Also, the use of antibiotics as disease control agents and growth promoters in aquaculture has recently sparked significant concerns in many countries due to the potential risks to human health. As this has become a global concern, alternative therapies for bacterial pathogens in animal production, particularly in aquaculture, are required. This review article aims to present the most recent information on antibiotic use in aquaculture systems. In addition, several new alternative control measures are briefly elaborated in this review.

Key words: Antimicrobials, Alternative, Antibiotic, Antibiotic resistant bacteria, Antibiotic resistant genes

Highlights

- This article provides information on the current state of antibiotic use in aquaculture.
- Elaborates on the negative implications of antibiotic resistance.
- Potential alternatives to antibiotic alternatives for combating antibiotic resistance in aquaculture.
- Enlisted the commercially available immunostimulants in aquaculture.
- Antimicrobial peptides as therapeutic agents

INTRODUCTION

Aquaculture plays a crucial role in global food security, but the continued use of antibiotics and chemicals ensures a problem for international food buyers. Antibiotics are frequently used to prevent and control bacterial diseases in aquaculture (Santos and Ramos, 2018). The policy framework for using antibiotics in aquaculture is not so strict and varies considerably among countries, and many of the fish producing countries have little to no enforcement on usage of antibiotics (Pruden *et al.*, 2013). A significant amount of antibiotic residues had accumulated in the environment as a consequence of the increasing use of antibiotics, having detrimental effects on public health (Liang *et al.*, 2013). Antimicrobial-resistant bacteria (AMRB) are developed as a result of the unrestrained use of antibiotics and promote the mechanisms for the emergence of antibiotic resistance (Pruden *et al.*, 2013), such as lowering intracellular drug concentrations, using different or safer target sites, and directly rendering antibiotics inactive, which will lead to multidrug resistance (MDR) (Scarano *et al.*, 2014; Letchumanan *et al.*, 2015; He *et al.*, 2016). Nakayama *et al.* (2006) discovered that a steady increase in oxytetracycline concentration could result in the emergence of *Vibrio harveyi* which is resistant to the drug. Control measures have been implemented over the years due to the wide variety of chemicals currently used in aquaculture production. The development and spread of antimicrobial-

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resistant bacteria and resistance genes, as well as the presence of antimicrobial residues in aquaculture products and the environment, are all public health risks associated with antimicrobial use in aquaculture (Romero *et al.*, 2012). The purpose of this chapter is to present current information on antibiotic use in aquaculture systems. Alternatives for limiting the use of antimicrobial agents in aquaculture systems will also be discussed here.

Antimicrobial resistance in aquaculture

The excessive and indiscriminate use of antibiotics develops a growing number of resistant microorganisms, making the treatment of infectious diseases more difficult. The global community's attention has been drawn to the emergence and spread of antimicrobial-resistant genes (Bhat *et al.*, 2020). Antimicrobials have the potential to cause the advent of antimicrobial resistant microorganisms and further promote the spreading of resistant bacteria and resistance genes (Liu *et al.*, 2017). The accumulation of antibiotic residues in sediments, according to several authors, may cause changes in microbial communities via selection for antibiotic-resistant species (Yang *et al.*, 2010; Luo *et al.*, 2011; Thuy and Loan, 2011; Samuelsen *et al.*, 2014). Tamminen *et al.* (2011) reported that genes that cause resistance to antibiotics continue to exist in the environment years after the use of the drugs. Long-term antibiotic use in aquaculture at levels well below the minimum inhibitory concentration of the susceptible wild-type population puts pressure on microbes and also leads to horizontal gene transfer between human and fish pathogens (Gullberg *et al.*, 2011). Since antibiotics are non-biodegradable and are comparatively stable, they pose a risk to public health by leaving residues in commercially produced fish and shellfish products for a longer period of time (Cabello, 2006). Done and Halden (2015) found traces of the antibiotic tetracycline macrolide and sulphonamide in samples of farmed salmon, tilapia, and trout from 11 nations, including the United States, China, Mexico, Thailand, Scotland, and Canada. Similarly, fluoroquinolone, tetracycline, beta-lactam, macrolide, sulfonamide, and phenicol antibiotic residues were found in overall 52% of the samples (40-91% of the finfish sample and 17% of the shrimp sample) collected across Shanghai city for 20 common antibiotics (Wang *et al.*, 2017). Lulijwa *et al.* (2020) reported about 10.0 - 100.0 µg/kg antibiotic residues of sulphamethoxypyridine, sulphadimethoxine and sulphamethazine from *Penaeus monodon* in Indian shrimp farms.

Alongside causing the emergence of antibiotic-resistant bacteria, the associated antimicrobial residues have the potential to bioaccumulate in fish and shellfish tissues, which could harm trade (Okocha *et al.*, 2018). Through eating and being eaten process, these residues can disrupt the normal intestinal flora of humans and animals and can be carcinogenic and teratogenic. Studies conducted in India (Swapna *et al.*, 2012), Bangladesh (Hassan *et al.*, 2013), Nigeria (Olatoye and Basiru, 2013), and Iran (Mahmoudi *et al.*, 2014) have demonstrated that aquaculture products contain antimicrobial residues. According to FAO (2020), about 28% and 20% of the EU and US rejections of aquaculture imports are attributed to antibiotic residues. Vietnam, China, Bangladesh, and Thailand's aquaculture products are frequently rejected because they contain antimicrobial residues that exceed the permissible limit (FAO, 2020). Karunasagar *et al.* (1994) reported mass mortality due to Vibrio, resistant to antibiotics in the shrimp hatcheries. The mobile genetic factors like transposons, plasmids and integrons carrying AMR genes have been identified in *Aeromonas* spp. from specific aquaculture sites of the world (Piotrowska and Popowska, 2015). Because of the health risks associated with antibiotic use in animal production, there is a growing recognition that antibiotics should be used with greater caution (Defoirdt *et al.*, 2011). There is also a need for alternative therapies for pathogens in animal production, particularly

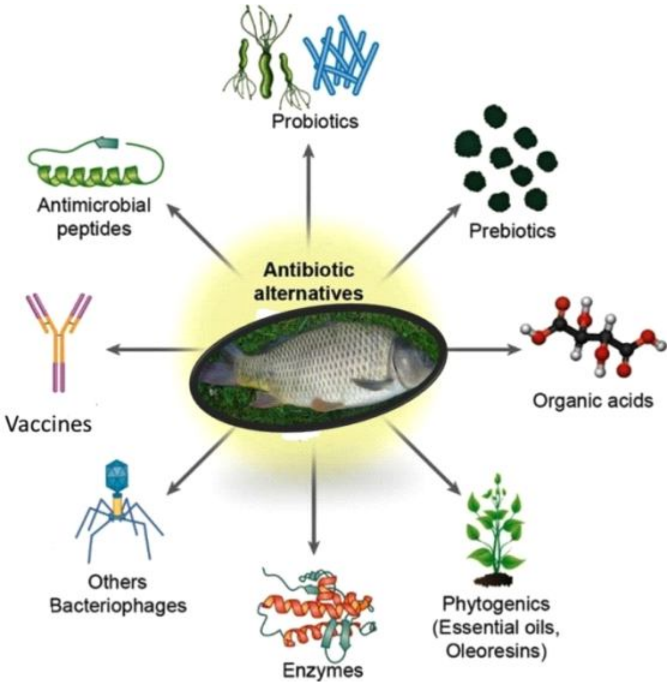

Fig. 1. Potential alternatives to antibiotics for combating antimicrobial resistance in aquaculture

in aquaculture. Several antibiotic-free alternatives have been used successfully in aquaculture (Rasul and Majumdar, 2017) and are discussed in this chapter (Fig. 1)

The Indian Council of Agricultural Research (ICAR), New Delhi, has launched a "Network Programme on Antimicrobial Resistance (AMR) in Aquaculture & Fisheries" in response to the threats posed by antimicrobial resistance in global aquaculture at the ICAR-NBFGR, Lucknow on 24th March 2018. Considering the impact of antimicrobial resistance in aquaculture, the following sections briefly describe about the various antibiotic alternatives used in aquaculture.

Antimicrobial peptides

Antimicrobial peptides (AMPs) are endogenous peptides that are short, cationic, amphipathic, and germline-encoded, with a molecular weight of not more than 13 kDa. These crucial components of the innate immune system can serve as the first line of defence against a broad range of microbial pathogens without having a great level of specificity or memory (Ravichandran *et al.*, 2010). Their existence in insects, plants, animals, and humans has been affirmed, and they are renowned for protecting living organisms from microbial invasion due to their evolutionary conservation as defence system components (Hancock and Scott, 2000). Although the sequences and structures of these endogenous peptides are incredibly diverse, they all share similar characteristics like positive charge, hydrophobicity, and amphipathic structure (Hancock and Lehrer, 1998; Hancock and Scott, 2000; Hancock and Rozek, 2002). Cationic AMPs typically have 20 to 50 amino acid residues in length and are abundant in basic amino acid (lysine & arginine) residues, with 50% of the amino acids being hydrophobic (Hancock, 2001). Disulphide bridges keep the hydrophilic and positively charged domains well separated from the hydrophobic domains, maintaining the three-dimensional amphiphilic structures (Hancock, 2001). The highly conserved N-terminus and hypervariable C-terminal domain make up the AMPs domain architecture (Vanhoye *et al.*, 2003). The wide antimicrobial spectrum of AMPs is provided by the variety in sequence, quantity, and nature of cationic residues in the C-terminal domain (Vanhoye *et al.*, 2003). Defensins and cathelicidins are among the many AMPs found in mammals that have been thoroughly characterized (Cederlund *et al.*, 2011). In addition to their antimicrobial properties, AMPs are known to play a role in biological processes like immunomodulatory functions, demonstrating their versatility (Mayer *et al.*, 2010; Steinstraesser *et al.*, 2011).

Antimicrobial peptides (AMPs) play a crucial role in fish's innate immune system and are extremely vital for eggs and early larval stages. AMPs are more environmentally friendly, effective against a larger range of pathogens, such as viruses, fungi, and parasites, and do not appear to cause any bacterial resistance (Bhat *et al.*, 2020; Chaturvedi *et al.*, 2020; Bhat *et al.*, 2022a; Bhat *et al.*, 2022b). Cheng *et al.* (2017) reported that antimicrobial peptides (AMPs) produced by *Bacillus subtilis* E20-fermented soybean meal (FSBM) have significant antimicrobial action against *V. alginolyticus* and *V. parahaemolyticus* in shrimp aquaculture. Several synthetic peptides used as antimicrobial agents, including frog Caerin1.1, European sea bass Dicentracin (Dic), NK-lysin peptides (NKLPs), and sole NKLP27, were tested against a variety of fish viruses and bacteria. The results showed these peptides to have excellent antiviral and antibacterial activity (Leon *et al.*, 2020).

Immunostimulants

A relatively new and still-emerging field of research is the use of phytotherapy in the field of fish and fisheries (Raman, 2017). As an alternative to using synthetic drugs for the treatment of many fish diseases, phytotherapy and other immunostimulatory agents are gradually becoming accepted (Eirna-Liza *et al.*, 2018). Since they are affordable and eco-friendly, phytotherapy-based techniques and compounds were preferred by the aquaculture industry for building resistance in cultured organisms against a variety of aquatic pathogens (Chong *et al.*, 2020; Jeyavani *et al.*, 2022). To effectively manage fish health, the herbal remedies are applied as decoctions (each one separately), concoctions (each one together), or in combination with other medications (Harikrishnan, 2003). The main idea behind the application of phytotherapy in fish farming is (Fig. 2) (i) it can help reduce the need for chemotherapeutic parasiticides with reduced environmental risks, as well as the ability to treat fish diseases and thereby prevent mortality and consequently economic losses (ii) use of phytotherapeutics makes fish less likely to develop disease resistance (Kulkarni *et al.*, 2013) as a result of their mode of action affecting multiple targets simultaneously (Bakkali *et al.*, 2008). Plants produce a wide range of bioactive substances to withstand the habitat, unfavourable weather, and pathogenic infections and these are often referred to as secondary metabolites (SMs) alkaloids, essential oils, phenolics, saponins, terpene and carbohydrates (Varijakzhan *et al.*, 2020). Secondary metabolites have the ability to influence the recognition, binding, catalytic activity, and turnover of protein-protein interactions (Hoseinifar *et al.*, 2020). Many plant derived compounds have been found to trigger the non-specific immunostimulatory response in animals which is more evaluated in shellfish and finfish (Sharma *et al.*, 2010). Herbal immunostimulants such as Bemisiatabaci (Srinivasan *et al.*, 2001), Neemazal-T/S (El-Shazly and El-Sharnoubi, 2000), 1-cysteine, nemicidine, nemol and vimicidine (derived from neem plant) and Curcumin (derived from turmeric) (Gangopadhyay, 1994; Tandel *et al.*, 2021), Croton tiglium (Babu, 1965), Mahua oil cake (Bhatia, 1970), act as immunostimulants. The following table lists some of the commercially available immunostimulants (Table 1). The various herbal-based therapeutic measures adopted for the treatment and control of different diseases of finfish and shellfish and fish health management can be categorized as follows.

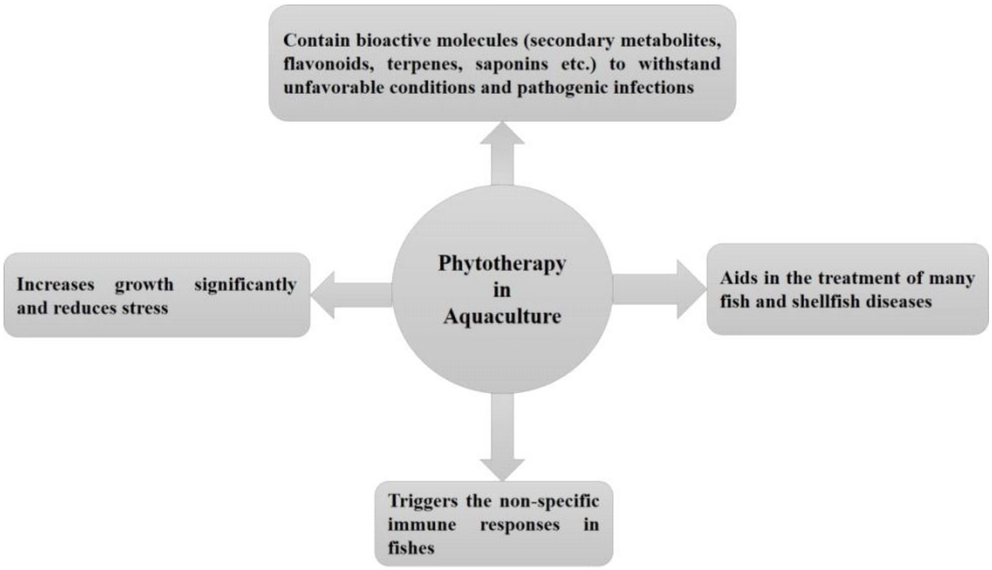

Fig. 2. Application of phytotherapy in aquaculture

Table 1. Commercially available immunostimulants

Company	Product(s)
Aqua-in-Tech	Immunostimulants (β-glucans, peptidoglycans, nucleotides) and probiotics
Cenzone Tech	Immunostimulant (Aqua Gold) and probiotic (Aqua-Start)
Citura	Immunostimulant derived from chicory root (Bonuline)
Dinatec	Immunostimulant containing β-glucans (Dinamune)
EWOS	Nucleic acid feed immunostimulant (EWOS boost)
ImmuDyne	β - glucan (AquaStim ®)
Intervet International/Schering Plough Animal Health	Algine - based immunostimulant (AquaVacErgosan)
Inve Aquaculture	Proprietary immunostimulants (Sanoguard ®)
Levapan SA	Yeast feed additives
Park Tonks	Immunostimulant composed of plant extracts and vitamins (Aquamune XL)
Pharmaq AS	β - glucan (Vetregard)
Zeigler Bros	Proprietary immunostimulant (Vpak ™ [Vitality pak])

Herbal products also have an effect on the growth and survival of fishes. Artemia nauplii enriched with herbal products like stressol-I and stressol- II increased the growth and efficiencies significantly and reduced the osmotic stress in *P.indicus* postarvae (PL 10–20) (Chitra, 1995). The herbal extracts of thyme (*Thymus vulgaris*), rosemary (*Rosmarinus officinalis*) and fenugreek (*Trigonella foenum graecum*) as feed additives to enhance growth performance were reported in European sea bass *Dicentrarchus labrax* (Yilmaz *et al.*, 2006). The effect of dietary doses of *Achyranthes aspera* extract as a natural antibiotic on the growth performance of *Labeo rohita* suggests that the dietary herbal extract act as immunostimulants and growth promoter (Hasan-Uj-Jaman *et al.*, 2017). The effect of a phytogenic feed additive on the growth performance and susceptibility of *Oreochromis niloticus* to *Aeromonas hydrophila* were studied (Abo State *et al.*, 2017). The report revealed that optimum growth performance, feed utilization and resistance against A. *hydrophila* were obtained at 0.5 g phytogenic feed additive (Silaacid® Encapsulated) kg^{-1} diet. In addition to herbs, other immunostimulants, like chitin, chitosan, levamisole, and B-glucan, have a significant role to play in aquaculture. Chitin and chitosan are known to be effective in the short term as non-specific immunostimulators. (Anderson and Siwicki, 1994; Kamilya and Khan, 2020). Maqsood *et al.* (2010) also demonstrated that, as compared to the control, chitosan fed common carp (2 and 5%) had higher phagocytic index, phagocytic ratio, and serum bactericidal activity. According to Esteban *et al.* (2000), the administration of a chitin diet at a rate of 25 or 50 g/kg boosts immune function in seabream by non-specific modulation of haemolytic complement activity, leukocyte respiratory burst activity, and cytotoxicity. Additionally, it has been demonstrated that levamisole, the synthetic phenylimidathiazole, can enhance non-specific immune responses in carps, rainbow trout, and gilthead sea bream (Kajita *et al.*, 1990). Furthermore, it has been established that beta-glucan is superior to all immunostimulants used in aquaculture. Commercially available glucan products like macrogard and vitastim are used to augment fish feed (Maqsood *et al.*, 2010).

Probiotics

The term probiotic, meaning 'for life', is derived from the Greek word 'pro' and 'bios. Lilly and Stillwell (1965) coined the phrase for the first time to refer to "substances secreted by one microorganism that stimulates the growth of another". With the demand for environmentally friendly aquaculture, research into probiotics for aquatic animals is expanding (Gatesoupe, 1999). A probiotic is a cultured product or live microbial feed supplement that influences the host favourably by improving its intestinal (microbial) balance (Fuller, 1989). Probiotics have been proven to be effective in promoting the growth, survival, and health of aquatic animals (Hai, 2015). Bacteria (gram-positive and gram-negative), bacteriophages, yeasts, and unicellular algae have all been included in the probiotics that have been examined for use in aquaculture (Irianto and Austin, 2002). A wide variety of gram-positive bacteria are frequently used as probiotics all over the world. The wide application belongs to endospore-forming *Bacillus* genera members, of which *Bacillus subtilis* is frequently used in aquaculture (Hong *et al.*, 2005; Hai, 2015). The probiotic potential of the *Bacillus* bacteria has been studied, and it is reported to enhance the water quality by modifying the composition of waterborne microbial populations and further lowering the number of pathogens in the fish environment. Consequently, the bacilli are believed to antagonize potential pathogens in the aquatic environment. (Irianto and Austin, 2002). Probiotic administration may have some advantages that have already been mentioned, including (Fig. 3) (i) exclusion of pathogenic bacteria through competition (Moriarty, 1997; Balcazar, 2003; Balcazar *et al.*, 2004) (ii) source of nutrients and enzymes that aids in digestion (Prieur *et al.*, 1990) (iii) direct bacterial-mediated uptake of dissolved organic material (Garriques, 1995; Moriarty, 1997) (iv) increased larval survival, reduced pathogen growth, strengthened immunity, accelerated growth, and increased stress tolerance (Gatesoupe, 1999; Balcázar *et al.*, 2006; Dimitroglou *et al.*, 2011). Also, it has been demonstrated that probiotic strains can alter innate immune responses in their hosts, thereby facilitating the exclusion of potential pathogens (Pérez Sánchez *et al.*, 2014). Probiotics are set to become an essential component of fish farming. They are predicted to have benefits for the environment in addition to operational and economic ones. (Iribarren *et al.*, 2012).

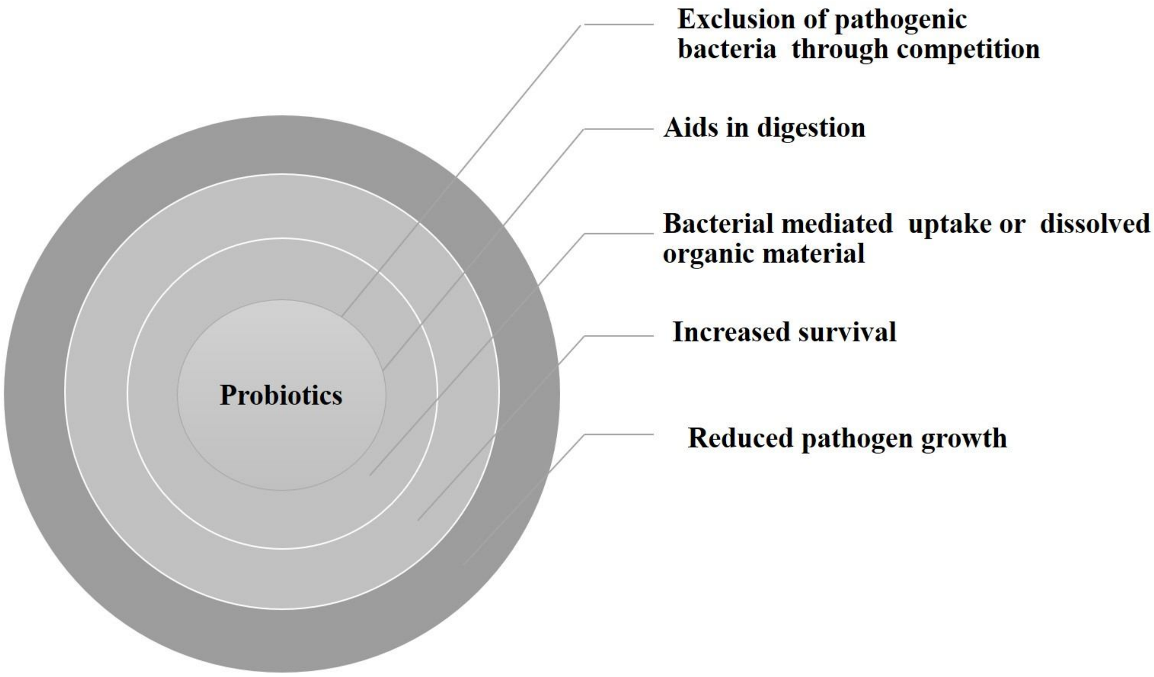

Fig. 3. Mechanism of action of probiotics

It has been demonstrated that the oral administration of *Clostridium butyricum* to rainbow trout can increase their resistance to vibriosis by boosting the phagocytic activity of leucocytes (Sakai *et al.*, 1995). Studies done on the role of *Bacillus* in aquaculture have found that it has great potential to contribute to the sustainability of fish farming by maintaining the overall well-being of cultured fish, including improvements in growth, feed utilisation, immune response, protection against diseases, infections, particularly against bacterial infections, as well as improvements in water quality (Kuebutornye *et al.*, 2019). *Litopenaeus vannamei,* has also shown better survival against vibriosis when exposed to the probiotic *B. subtilis* UTM 126 (Balcázar and Rojas-Luna, 2007). Additionally, Soltani *et al.* (2019) discuss the presence of *Bacillus* spp. in the digestive systems of fish and shellfish, as well as their capacity to produce enzymes and antibacterial compounds as well as their effectiveness and potency as probiotics in aquaculture (Soltani *et al.*, 2019). Kim *et al.* (2022) suggests that two bacterial species, *Salipiger thiooxidans* and *Exiguobacterium aestuarii*, extracted from the Saemangeum reservoir can improve the quality of aquaculture water, and *E. aestuarii* in particular may be useful as a probiotic for *L. vannamei*. The impact of nutritional supplementation with probiotic bacterial strains *B. coagulans* (MTCC 9872), *B. licheniformis* (MTCC 6824), and *Paenibacillus polymyxa* (MTCC 122) on common carp, *Cyprinus carpio* fry growth performance, non-specific immunity, and protection against *A. hydrophila* infection was assessed in an experiment (Gupta *et al.*, 2014). It has been demonstrated that *P. polymyxa* is a potentially beneficial probiotic that can be utilised in aquaculture to enhance common carp fry growth, feed consumption, non-specific immune responses, and disease resistance. Furthermore, the application of probiotic *Lactobacillus* spp. improved immune responses and resistance against *Pseudomonas fluorescens* and *Streptococcus iniae* in Nile tilapia (Hoseinifar *et al.*, 2018)

Bacteriophages

Bacteriophages, also known as "phages (ϕ)", are man's ally to fight bacterial infections because they are bacteria's natural enemies. Phage therapy is currently regarded as a viable alternative to antibiotics for the treatment of bacterial infections in aquaculture systems (Rao and Lalitha, 2015). The first phage was isolated from the marine environment (Spencer, 1955), and the vast majority of ocean viruses are believed to be phages (Breitbart, 2012). Myoviridae, Siphoviridae, and Podoviridae are the three families of bacteriophages that are most prevalent in the aquatic environment (Paul and Sullivan, 2005). Bacteriophages provide a significant advantage over antibiotics because the amplification persists at the site of infection as long as the hosts are present due to their self-replicating and self-limiting nature. Bacteriophages are an effective alternative method for treating bacterial infections in aquaculture due to their exceptional qualities for treating zoonotic pathogens, their quick generation time, non-toxicity, high specificity to the bacterial host, and the fact that they do not negatively impact the viability of other flora in the environment (Hudson *et al.*, 2013; Rong *et al.*, 2014; Jamal *et al.*, 2015). One major benefit of phage therapy is the ubiquitous nature of these viruses. In the early stages of farmed species like eyed and fry, which lack a fully developed immune system and therefore cannot benefit from vaccines, phage therapy would prove particularly helpful. Phages appear to be one of the most promising alternatives to antibiotics for aquaculture, which explains the growing interest in both private and public research. Gon Choudhury *et al.* (2017) summarised the primary host/phage associations currently being investigated while reviewing recent developments in bacteriophage research for aquaculture.

Vibrio phage is one of the studied bacteriophages in aquaculture used to combat vibriosis infection, which causes significant financial losses in shrimp farms (Chatterjee and Haldar, 2012). The second most targeted bacterial genus is *Flavobacterium*, specifically *F. psychrophilum*, which infects salmonids but is currently incurable by vaccines, and *F. columnare*, which infects tilapia, salmonids, eels, and catfishes (Declercq *et al.*, 2013; Long *et al.*, 2014). *Aeromonas* sp., which infects salmonids, *Pseudomonas* sp., which targets ayu, and *Lactococcus* sp., which primarily infects trout, tilapia, and yellowtail, are the three species that have received the least amount of research done (Nishimori *et al.*, 2000; Austin and Austin, 2016; Fernández-Álvarez *et al.*, 2016). Due to their higher proportions of prioritised patents, China, the United States, and Korea can be regarded as the top nations conducting industrial research on phage used in the breeding industry. Several private enterprises have expressed an interest to develop phage-based aquaculture solutions, however, there aren't many commercially available products at the moment. Only a few are commercially released:

- To combat *V. tubiashii* and *V. coralliitycis* infections in oyster aquaculture, Intralytix is creating a phage cocktail (Intralytix, Inc., 2018).
- Phage Biotech Ltd. is developing a phage-based treatment against *V. harveyi* in shrimp hatcheries (Phage Biotech, 2017).
- In 2017, Proteon Pharmaceutical patented the BAFADOR® product targeting *Pseudomonas* spp. and *Aeromonas* spp. via immersion in commercial aquaculture (Grzelak, 2017).
- ACD Pharma states that it is creating phage-based treatments for a number of aquaculture pathogens and is conducting extensive testing on a product against Atlantic salmon yersiniosis (ACD Pharma, 2021).
- A technology to bind phages to various surfaces, such as aquaculture feed pellets, is being developed by Fixed Phage Ltd (Mattey, 2020).

For potential biocontrol applications, several groups in India are working to isolate and characterise phages against *Vibrio* species (Shivu *et al.*, 2007; Bhowmick *et al.*, 2008; Chrisolite *et al.*, 2008; Alagappan *et al.*, 2010; Surekhamol *et al.*, 2014; Stalin and Srinivasan, 2017; Dubey *et al.*, 2021). Surprisingly, all researchers were able to isolate and characterise the bacteriophages from the salty waters to prevent Vibriosis infection in the Indian shrimp farms. From a shrimp pond, two broad-spectrum lytic tailed bacteriophages of the Myoviridae family against *V. parahaemolyticus* were isolated and their stability and infectivity were assessed (Stalin and Srinivasan, 2016). On shrimp larvae infected with *V. parahaemolyticus*, the biocontrol effectiveness of the VVP1 phage was assessed. A single treatment with 2×10^{10} PFU/mL of phage resulted in 80% survival, a treatment regimen of 4 doses at 24 h intervals resulted in >90% survival, and a 3 log reduction of the pathogen, whereas the survival rate was only 30% in the untreated control (Stalin and Srinivasan, 2016; Stalin and Srinivasan, 2017)

The LUMI-NIL MBL product, created by Mangalore Biotech Laboratory, is intended to biocontrol luminous bacteria in shrimp hatcheries (Mangalore Biotech Laboratory, 2019). In 2021 ICAR-CIBA and M/S Salem Microbes signed a memorandum of understanding (MOU) for the technology transfer of "phage therapy" for the management of bacterial disease in shrimp hatchery rearing systems. Insights gained from these studies may hasten the development of phage therapy in Indian aquaculture. Several reports on the isolation of phages against fish pathogens of commercial importance exist; one of these reports describes a novel phage that targets *Pseudomonas aeruginosa*, a bacterium that infects catfish and is resistant to carbapenem (Khairnar *et al.*, 2013). For biocontrol, the authors employed a *Pseudomonas* phage that was isolated from wastewater and concentrated using the bituminous coal method. Phage therapy cured the catfish from the drug-resistant *pseudomonas* infection and reduced ulcerative skin lesions by seven times in just 8 to 10 days. Recently, Rai *et al.* (2019) isolated, characterised the lytic bacteriophage against *A. hydrophila*.

Vaccines

In order to maintain sustainable aquaculture, both economically and environmentally, disease prevention and control are essential, and immunoprophylaxis or vaccination strategies have become an important part of it (Gudding and Van Muiswinkel, 2013; Dadar *et al.*, 2017). The use of antibiotics has decreased as a result of vaccines, which have also been proven to be cost-effective (Markestad and Grave, 1997). Vaccines induce a strong immune response in an animal which acts as a defense mechanism against pathogens to prevent the host from being infected by these pathogens (Shah and Mraz, 2020). Antigens from a disease-causing agent that has been rendered non-pathogenic in various ways are extracted to create vaccines (Strugnell *et al.*, 2011). Fish vaccines are generally divided into three categories based on how they are prepared: inactivated or killed vaccine, live or attenuated and genetically engineered vaccine (Su *et al.*, 2021). Inactivated vaccines are inactivated pathogenic microorganisms that remain immunogenic and have the ability to induce specific resistance in aquatic animals after inoculation (Su *et al.*, 2021). Because inactivated vaccines don't persist within the environment or in fish that have been vaccinated, so they are usually found safe (Baxter, 2007). Attenuated vaccines are not inactivated but weakened genetically or chemically so that they live and induce an immune response in the host for a short period of time, not causing disease or mortality (Adams *et al.*, 2008). Because the virus's residual virulence in targeted species could spread to non-target species, the practical administration of the virus attenuated or avirulent forms may not be desirable (Shao, 2001; Salgado-Miranda *et al.*, 2013). To combat this disadvantage of attenuated live vaccines, subunit vaccines were developed. Subunit vaccines comprise only a unit of pathogen containing its antigenic component (Moyle *et al.*, 2013). These are the vaccines which are extraction or synthesis of special protein structures of bacteria, that is, vaccines made of epitopes (Liljeqvist and Ståhl, 1999). The protective effect of subunit vaccines on bacteria is increased by preparing multivalent subunit vaccines i.e., by combining various immunogenic antigens (Xing *et al.*, 2018). The main disadvantage of subunits is that they produce a weaker immune response as compared to killer and live attenuated vaccines, which is because of the limited number of components present and generally exhibit shorter half lives *in vivo* (Xiang *et al.*, 2006). The most reliable and effective ways of vaccination in fisheries is either oral administration or by injection (Shah and Mraz, 2020).

Polish researchers Snieszko *et al.* (1938) worked on protective immunity in carp immunised with *A. punctata* is likely the first report of disease prevention using vaccines. But unfortunately, the paper was written in Polish. Later, English Researcher Duff published the first report in English in 1942, demonstrating the protection against *A. salmonicida* in trout immunized by parenteral inoculation and by oral administration (Duff, 1942). The first vaccine for aquaculture was approved in the USA in 1976 for the prevention of yersiniosis in salmonid fish (Tebbit *et al.*, 1980). With time there has been an advancement in the vaccination process and in the vaccines as well. The most effective commercial vaccines on the market right now are those for bacterial infections, yersiniosis, and vibriosis (Mondal and Thomas, 2022). Other vaccines which are experimentally designed and used nowadays are: Recombinant vaccines and DNA vaccines. Apart from these vaccines, which include a part or whole of a pathogen, scientists have made a different kind of vaccine using a molecule called messenger RNA (mRNA). Messenger RNA is a type of RNA which necessary for protein formation. Many years back, RNA was conceptualized as a wrong choice for vaccine platform because of its poor half-life *in vi*vo and its immunogenicity. Eventually it was found that the mRNA vaccines are safer and more superior as compared to DNA or viral vector vaccines.

Initially, developing mRNA vaccine as prophylactic was unsure because of the issues related to RNA instability and its manufacturing on a very large scale; since then, DNA has become the epicenter of nucleic acid vaccine research. Nevertheless, these issues are no longer regarded as a hurdle due to technological innovations in RNA biology and chemurgy (Ulmer *et al.*, 2015). The future of mRNA vaccines is very bright as they have great potential and are highly advantageous over killed, live attenuated, subunit and nucleic acid DNA vaccines. Some of their favourable aspects are (i) safety – non-infectious, no potential risk of infection (ii) efficacy - can be administered repeatedly, anti-vector immunity can be avoided, efficient *in vivo* delivery (iii) production - rapid production, inexpensive production (Pardi *et al.*, 2018). Also, the ease of manufacturing and easy design contributes to its future success (Cai *et al.*, 2021).

mRNA vaccines have also been subjected to many preclinical and clinical trials as prophylaxis and therapeutics. The results revealed that they showed promising results for preventing infectious diseases, treating tumours, and being safe (Zhang *et al.*, 2019). The advancement in recognizing and bringing down the innate immune sensing of mRNA has helped in active vaccination and passive immunization for many infectious diseases and tumours (Pardi *et al.*, 2018). In addition, the mRNA vaccine will be gaining rise due to its efficiency in different delivery strategies, safety and efficacy and its scalable production (Wang *et al.*, 2021). mRNA vaccine is known to bring about more effective immune responses than conventional fish vaccines and has shown prospective for the future of vaccines in aquaculture providing a substantial deal of economic potential for farmers (Ma *et al.*, 2019). Since mRNA has proved to be a boon in Covid 19 vaccination process, it has great potential and scope to be used in other fields also.

Organic acids

Recently, there has been a lot of research and commercial interest in the use of dietary organic acids in the culture of aquatic animals (Ng and Koh, 2017). Aquaculture researchers have been paying more attention to short-chain organic acids (C1-C7) and their salts or mixtures, also known as acidifiers, as promising alternatives to antibiotic growth promotors (AGP) (Lückstädt, 2008; Ng and Koh, 2011). It has been reported that some organic acids have potent

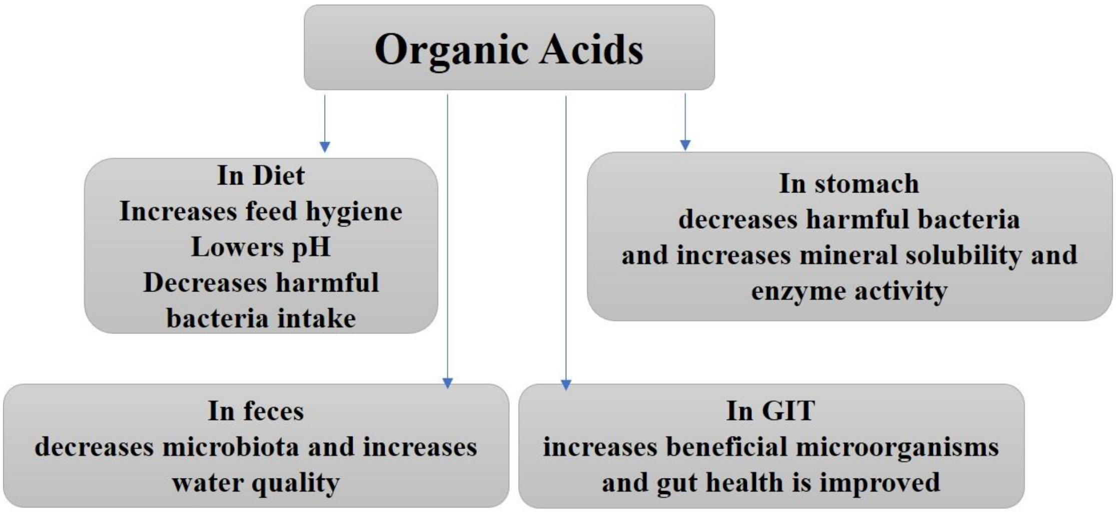

Fig. 4. Functions of organic acid

antibacterial effects against significant foodborne pathogens like *Escherichia coli* and *Salmonella* spp. (Van Immerseel *et al.*, 2003; Skøivanová *et al.*, 2006). Additionally, it is well known that organic acids have effective antimicrobial qualities. By directly lowering the pH of the environment by releasing hydrogen ions, organic acids are able to exert their antimicrobial activity on microbes while preventing or hindering the growth and spread of acid-sensitive bacteria (Stratford and Eklund, 2003). The ability of organic acids to cross the semi-permeable membrane of bacteria and to dissociate in the pH neutral cytoplasm is now widely acknowledged as the primary cause of their antimicrobial efficacy (both bacteriostatic and bactericidal) (Cherrington *et al.*, 1991; Booth and Stratford, 2003). Aquafeeds containing organic acids, their salts, and/or mixtures of these can affect the bacterial population of aquatic animals' gastrointestinal tracts. In olive flounder (Park *et al.*, 2011) and Pacific white shrimp (da Silva *et al.*, 2016) dietary organic acids' antimicrobial efficacy on the gut microbial community was documented (Fig. 4)

Conclusion

With the increasing demand for fish meat, the increased use of antimicrobials, and the potential public health risks, regular monitoring of antimicrobial resistance in aquaculture is essential. Furthermore, persistence of the high proportions of antibiotic-resistant bacteria in sediments and farm environments may pose a threat to fish farms. Adapting rearing techniques that minimise stress on the fish and reduce the likelihood of infections requiring antibiotic treatment is one approach to lowering antibiotic use in aquaculture. Other strategies include the use of AMPs, immunostimulants, probiotics, bacteriophages and organic acids. Some scientists also suggested that regulatory control by the government may be required in veterinary prescriptions on antimicrobial therapy in aquaculture. It is necessary to fill up research gaps on the contribution of antibiotic usage in aquaculture and its presence in surface water, the potential risks to the environment and human health, and the presence and spread of antibiotic resistance genes in aquaculture.

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Author's contribution: RAHB: Writing, conceptualization and design; RST: Writing, compilation; PKP: Manuscript checking.

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