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REVIEWS IN Aquaculture

REVIEW

Review of alternatives to antibiotic use in aquaculture

Melba G. Bondad-Reantaso[1] | Brett MacKinnon[2] | Iddya Karunasagar[3] | Sophie Fridman[1] | Victoria Alday-Sanz[4] | Edgar Brun[5] | Marc Le Groumellec[6] | Aihua Li[7] | Win Surachetpong[8] | Indrani Karunasagar[3] | Bin Hao[1] | Andrea Dall'Occo[1] | Ruggero Urbani[9] | Andrea Caputo[10]

[1]Fisheries and Aquaculture Division, Food and Agriculture Organization of the United Nations (FAO), Rome, Italy

[2]Jockey Club College of Veterinary Medicine and Life Sciences, City University of Hong Kong, Hong Kong SAR, China

[3]Nitte University, Medical Sciences Complex, Mangaluru, Karnataka, India

[4]National Aquaculture Group (NAQUA), Al Lith, Kingdom of Saudi Arabia

[5]Norwegian Veterinary Institute, Ås, Norway

[6]Direction of Domestication and Genetics, Pathology and Biosecurity, Aqualma/Unima Group, Majunga, Madagascar

[7]Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China

[8]Department of Veterinary Microbiology and Immunology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand

[9]Veterinary Department Prevention, Rome, Italy

[10]ReAct - Action on Antibiotic Resistance, Uppsala University, Uppsala, Sweden

Correspondence

Melba G. Bondad-Reantaso, NFIMF: Food Safety, Nutrition and Health, Fisheries and Aquaculture Division (NFID), Food and Agriculture Organization of the United Nations (FAO), Viale delle Terme di Caracalla, 00153 Rome, Italy.
Email: melba.reantaso@fao.org

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Abstract

With the rapid growth of the aquaculture production since the 1980s, there has been a concomitant increase in disease outbreaks. The injudicious and/or incorrect use of antimicrobial agents against diseases of farmed aquatic species poses a considerable threat to the development and growth of a successful and sustainable aquaculture industry. An increase in antimicrobial resistance (AMR) is an important consequence, resulting to the difficulty in treating common bacterial diseases in populations of aquatic organisms, combined with the presence of antibiotic residues in food fish and their products, leading to import refusals and negative impacts on international trade. To reduce the frequency of AMR, good aquaculture and effective biosecurity practices should include the prudent and responsible use of antibiotics and also consider the use of alternatives to antibiotics, in addition to disease prevention management. This article reviews the literature discussing the scope of the problem pertaining to antibiotic use, the emergence of AMR in aquaculture and to consider and discuss viable alternatives (e.g., vaccination, bacteriophages, quorum quenching, probiotics and prebiotics, chicken egg yolk antibody and medicinal plant derivative). We also discuss lessons learnt, from specific case studies such as the vaccination of farmed salmon in Norway and the use of 'specific pathogen-free' seed—as primary and essential part of a biosecurity strategy.

KEYWORDS

alternatives to antimicrobials, AMR, antibiotics, aquaculture, microbiome, vaccination

1 | INTRODUCTION

Aquaculture, or the rearing of aquatic animals and plants for food, is complex and covers a wide range of variables.[1] Aquaculture systems may vary in their environment, that is, fresh-, brackish- and sea-water; coastal, riverine and land-based; tropical to temperate regions; in type, that is, farmed species may include seaweeds, molluscs, crustaceans and finfish species; in scale, that is, extensive, semi-intensive and intensive farming; relatively low numbers of high-value finfish to large numbers of low-value invertebrates; in input, that is, natural and artificial diets; and wild harvested stocks to cultured progeny from eggs to adults. Variations in the applicability of technologies to control the aquaculture environment depend on national and commercial economies and infrastructures, as well as the species under culture. Consequently, aquaculture varies from large, international high technology farming of high-value species, to labour-intensive, low-technology subsistence farming in earthen ponds. To meet the growing demands for aquatic food production, aquaculture has expanded rapidly since the 1980s to become the world's fastest-growing food production sector,[2] particularly in Asian countries that supply 89% of the global aquaculture production.[3] The rapid development, intensification and globalisation of the sector have led to many challenges, including the emergence and spread of diseases, resulting to reliance on antimicrobials to improve aquaculture production.

Aquatic organisms live among an array of microbes, some of which are potential pathogens, depending on a variety of factors specific to the host, pathogen and environment. Most bacterial pathogens in aquatic animals are aerobic, gram-negative rods and, for this reason, most antibiotics used in aquaculture are effective against gram-negative bacteria.[4] In fact, a survey conducted by the Food and Agriculture Organisation of the United Nations (FAO) in 2012 reported oxytetracycline, florfenicol and trimethoprim/sulfadiazine as the most commonly used antibiotics for controlling diseases on farms.[5] The availability and use of antibiotics in aquaculture vary widely and is controlled in Europe,[6] North America and Japan, but not in many developing countries, that dominate aquaculture production.[7] For example, Norway and Scotland use ∼0.02–0.39 g of antibiotics per metric tonne (MT) of harvested salmon, compared to ∼660 g per MT in Chile.[7] It is not practical to treat individual animals in aquaculture; therefore, metaphylactic use of antibiotics to treat entire populations is common practice.[8]

All exposure to antimicrobials, either during treatment or chronic and sub-therapeutic level exposure would select resistant mutants that may emerge spontaneously. This is classic evidence of evolution. Once a bacterial strain is resistant, this resistance can be transferred to other bacterial species and strains via horizontal gene transfer.[9] Common bacterial diseases occurring in aquaculture, such as furunculosis (*Aeromonas salmonicida*) and edwardsiellosis (*Edwardsiella tarda*), are becoming harder to treat due to an increase in antimicrobial resistance (AMR).[10] The situation in human medicine has now progressed to the stage where diseases such as pneumonia, tuberculosis, septicaemia, gonorrhoea and salmonellosis can be difficult to treat due to resistance to commonly used antibiotics. This has been attributed to inappropriate or excessive use of antibiotics in human medicine. Indeed, while there is evidence that sub-therapeutic levels of antibiotics found in aquaculture environments can have human origins from wastewater,[11,12] it should be noted that most of the antibiotics in aquaculture environments come from direct use in this activity.[8] Wastewater treatment is currently developing technologies to remove these molecules prior to release into the environment.[13–15] Studies also report the association between the development of AMR in agriculture or aquaculture environments contributing to the resistance of human pathogens to antibiotics.[16,17]

Though closely related genetic factors contributing to AMR have been found in animal and human pathogens, there is no conclusive evidence to show the direction of gene flow. A systematic review concluded that though some studies suggested that transmission of AMR from food animals to humans may occur, robust conclusions on the directionality of transmission cannot be drawn due to limitations in study methodologies.[18]

Zoonotic pathogens, *such as Streptococcus iniae*, *Aeromonas hydrophila*, *Vibrio vulnificus*, *Photobacterium damselae* and *Mycobacterium marinum* carry extended-spectrum beta-lactamases (ESBL) and other AMR genes (ARGs) that spread through food web.[19] People can contract zoonotic bacteria through contact with aquatic animals, which would, of course, prove that antimicrobial-resistant bacteria and ARGs from aquaculture can be transmitted to humans.[20,21]

Antimicrobial residues in food have received widespread attention, and their presence in animal products constitutes a socio-economic challenge to food safety and public health. The major public health implications of antimicrobial residues include the development of AMR, allergies (penicillin), carcinogenicity (sulfamethazine, oxytetracycline and furazolidone), anaphylactic shock, nephropathy (gentamicin), mutagenicity, teratogenicity, bone marrow depression and disruption of normal intestinal flora.[22,23] The indiscriminate use of antimicrobial agents in aquaculture results in residues in aquaculture products and associated adverse effects on human health, and therefore control measures are needed to reduce the use of antibiotics in aquaculture, to ensure consumer protection.

The FAO/OIE/WHO Report of a joint FAO/OIE/WHO expert consultation on antimicrobial use in aquaculture and antimicrobial resistance held in Seoul, Republic of Korea, 13–16 June 2006,[10] summarised that the hazards associated with antimicrobial use in aquaculture are: (a) antimicrobial residues associated with products of aquaculture and (b) selection and spread of AMR. It was concluded that of these two potential hazards, the second one is more serious since AMR does not respect phylogenetic or geographical borders and can spread between aquatic bacteria, animal and human pathogens and the gene flow can occur in any direction. For example, selection of resistance may happen in pathogens of aquatic animals making the treatment of fish diseases ineffective or resistance may be transferred from aquatic bacteria to pathogens of animals or humans making treatment in these sectors difficult. Another problem with use of antimicrobials in aquaculture is that unlike in the terrestrial environment, where individual animals can be treated or antimicrobials delivered by injection, treatment of aquatic animals is predominantly through feed (Figure 1). Sick animals may have reduced feed

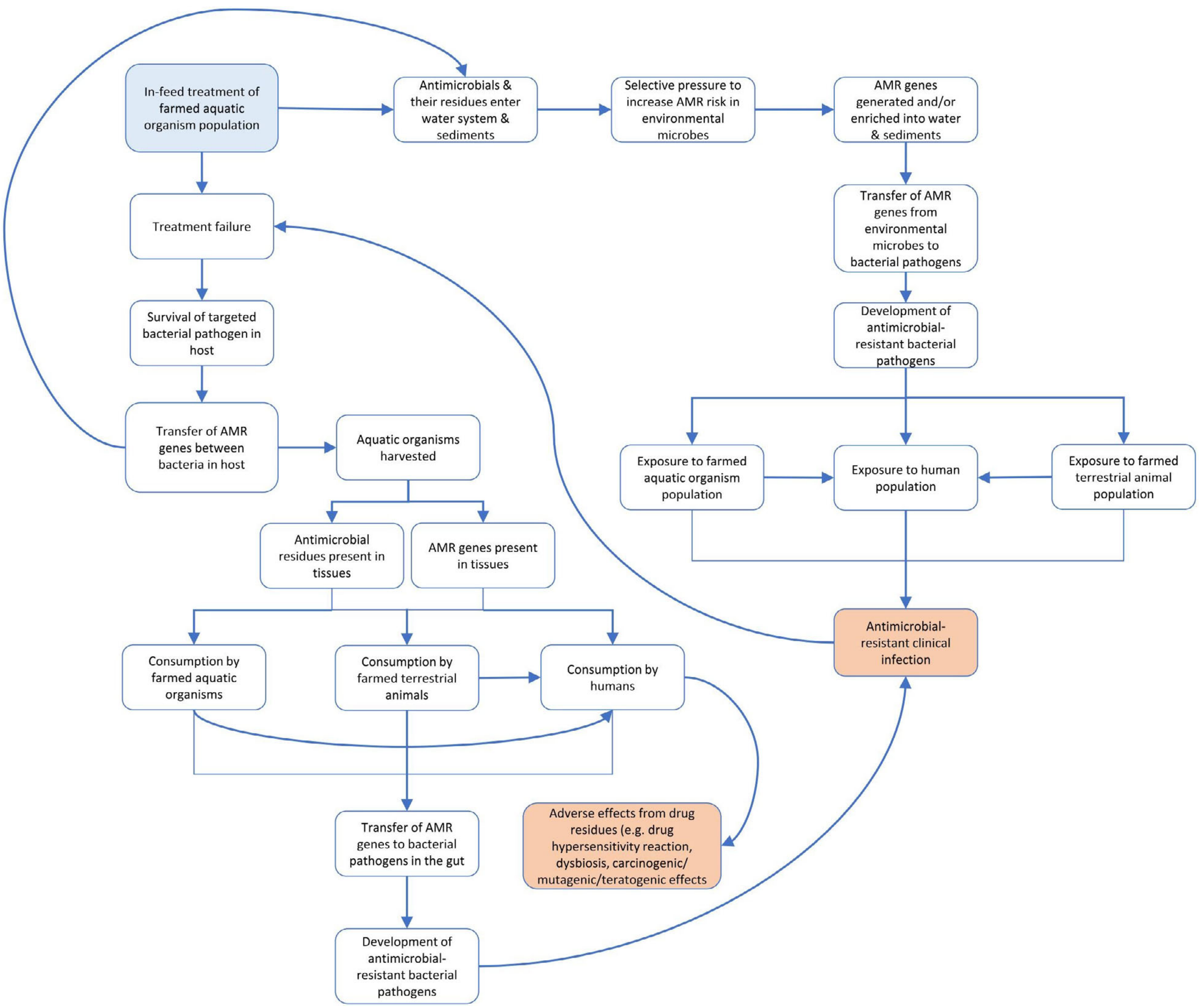

FIGURE 1 Potential negative consequences of antimicrobial resistance (AMR) in aquaculture through medicated feed treatments on farms (Figure credit: Brett MacKinnon, Hao Bin, Andrea Dall'Occo)

intake, further impacting the treatment efficiency. Unutilized medicated feed may end up in sediments (Figure 1), where selection of resistant bacteria could occur. This would contribute to enhancing the pool of resistance in the aquatic environment. In view of these, alternatives to antibiotics for treatment of fish diseases are essential for improving the sustainability of the aquaculture sector.

Antibiotic residues, or metabolites found in trace amounts in any edible portion of the animal product after the administration of the antibiotics, represent a serious threat to human health. Indeed, the presence of antibiotic residues in fish and shellfish is one of the most common causes of detentions at the borders of the largest fish markets of the European Union (EU), the United States of America (USA) and Japan. This often leads to the destruction of the products concerned, with substantial negative economic consequences for the exporting countries. Residue monitoring in most of the aquaculture producing countries is driven by international market requirements. As a single trading block, the EU accounts for over 60% of imports, and the regulations in EU member countries are consistent and uniform.[24] Therefore, many aquaculture-producing countries strive to comply with EU requirements. For chemicals banned for use in aquaculture, the EU follows the approach of using the most sensitive method available for detection and the regulations establish the minimum required performance limit for the method to be used. Most aquaculture producing countries have adopted these methods and the laboratories performing residue monitoring are accredited to ISO 17025. There are some antibiotics, for example, tetracyclines and parasiticides, permitted for use in food fish in the EU[25]; however, there is no uniformity in drugs permitted for aquaculture in many producing countries and there have been some instances of differences in maximum residue limits and methodology used for determining their levels. There are also some NGOs that recommend against consumption of fish raised with excessive amounts of antimicrobials. Overall, there has been a drastic reduction in import refusals and rapid alerts for veterinary drugs in aquaculture products.[26,27]

Antimicrobial resistance (AMR) in environmental bacteria is a natural phenomenon. Even in environments where exposure to antimicrobial agents is negligible, resistant bacteria have been found, for example, in over 500 km offshore areas and in deep sea.[28] AMR may arise due to naturally occurring mutations or through horizontal transfer of genes from resistant bacteria though phenomenon such as transformation (transfer of cell-free DNA to bacteria that are receptive or competent) or transduction (bacteriophage-mediated gene transfer) or conjugation (transfer of mobile genetic elements like plasmids through cell to cell contact). Resistant bacteria may be selected and proliferate when subjected to selective pressure in environments where antimicrobials are used. Dissemination of AMR may occur through aquatic environments (effluents from hospitals and farms reaching lakes, rivers) and use of water for irrigation, in animal farms or in aquaculture. Further, wild animals and birds like seagulls, which travel long distances are known to disseminate resistant bacteria to different environments.[29]

The World Health Organization (WHO), the World Organisation for Animal Health (WOAH, formerly OIE) and FAO, in collaboration with relevant public and private organisations, launched a global response to the threat of AMR. The international context of veterinary medicines in aquaculture, their usage and benefits, as well as concerns on their mis- or over-usage and how to address AMR, were extensively discussed by FAO,[30] highlighting the need to promote good aquaculture practices for health management. It included the prudent and responsible use of antibiotics in aquaculture and the reduction in bacterial antibiotic resistance on a global scale, as well as alternative strategies to improve the immunity of aquatic organisms to bacterial diseases or to mitigate pathogen virulence.

The objective of this article is to review available literature that has discussed the scope of the problem pertaining to drug use, the emergence of AMR in aquaculture and to consider vaccination, bacteriophages, quorum quenching, probiotics and prebiotics, chicken egg yolk antibody and medicinal plant derivatives as alternatives to antibiotics. We also discuss lessons learnt, from the vaccination of farmed salmon in Norway and the use of 'specific pathogen-free' (SPF) seed—as a primary and essential part of a biosecurity strategy.

2 | SCOPE OF THE PROBLEM

The spread of diseases in aquaculture may be due to inadequate management and poor environmental conditions, including feeding levels, removal and restocking and inadequate nutrition.[31] These situations may lead to secondary bacterial infections and therefore the use of antimicrobial agents in aquaculture is required for the treatment and prevention of infectious diseases. Antibiotics are commonly used in aquaculture as therapeutic, prophylactic or metaphylactic agents.[31,32] The most commonly used antibiotics in aquaculture worldwide are tetracycline, oxytetracycline (tetracyclines), oxolinic acid, flumequine, sarafloxacin, enrofloxacin (quinolones), amoxicillin (β-lactams), erythromycin (macrolides), sulfadimethoxine (sulfonamides), ormetoprim (diaminopyrimidines) and florfenicol (amphenicols).[30] Each country has its own legislation regarding the approval of antibiotics, usage practices and residue limits in aquaculture products.

As a result of this increased antibiotic use and misuse, mutations in bacterial DNA and horizontal gene acquisition have led to survival and establishment of bacteria resistant to those specific antibiotics.[33] The genetic elements and genes involved in the generation and dissemination of ARGs in aquatic bacteria are similar to those previously characterised in terrestrial bacteria.[34–39] The resistance genes (Table 1) are spread via horizontal gene transfer between bacterial species and genera[40] via DNA plasmids or other mobile genetic elements.[40,41–47] Some bacteria may become multidrug-resistant by acquiring genes from multiple sources.[45,48–56] Multi-drug resistance is affected by vertical and horizontal gene flow across different food webs; however, it may be controlled by bacteriophages.[57]

Approximately 80% of antimicrobials used in aquaculture enter the environment with their activity intact.[7] The commonality of the mobilome between aquatic and terrestrial bacteria and the presence of residual antimicrobials, biofilms and high concentrations of bacteriophages in an aquatic environment that is also contaminated with human and animal pathogens, can result in horizontal gene transfer between aquatic and terrestrial bacteria.[8] Antibiotic residues may persist in sediments,[41,58–61] water[62,63] or host tissues,[64–68] and are considered a risk to human health, requiring a withholding period after treatment.[56,69] Of particular concern is the prophylactic use of antibiotics,[52,55,58,70] often in the ornamental fish trade.[71] This not only leads to emergence of resistant strains, but moves them, and their resistance genes, globally.

On a global scale, the active ingredients used in aquaculture are often the same as those used in antibiotic therapies for terrestrial animals (livestock and pets) in the veterinary sector. One common example is that of the sulfonamides and quinolones that seem to be irreplaceable in aquaculture, which are also widely used within the poultry sector. Several cases of inter-species transference of antibiotic residues in animal production have been reported, with negative effects of bacterial resistance on both species involved.[72,73] It should also be recognised that the by-products of poultry farming are often used in the production of aquaculture feed.[74]

This decade, resistance to all antibiotic groups have been reported from aquaculture globally. A brief description of these antibiotic groups, their modes of action and examples of literature reporting the use of such drugs in aquaculture are provided below:

- *Tetracyclines*: Tetracyclines are among the most common bacteriostatic drugs used in aquaculture. Naturally, derived tetracyclines have been available since the 1950s and several semi-synthetic derivatives have been produced over the following decades.[75] Tetracyclines inhibit bacterial protein synthesis by binding to the ribosomal 30S subunit of the cell. Oxytetracycline (OTC) and chlortetracycline have been used in aquaculture due to their broad-spectrum activity, wide availability and low cost. OTC is approved for use in food fish in the major importing countries, including the European Union, USA and Canada.[25,76,77] The excessive usage of OTC on farms has lead to resistance of many bacterial pathogens to tetracycline antibiotics in general.[78] OTC is commonly used to treat bacterial diseases of fish, such as ulcer

TABLE 1 Antibiotics and their resistance genes discovered in aquatic pathogens and aquaculture effluent

Antibiotic	Target microbe/source	Resistance genes	Reference
Tetracyclines			
Tetracycline	*Piscirickettsia salmonis*	*tetA* and *tetG*	Shah et al. (2014)
Tetracycline	*Edwardsiella tarda*	*tetA* and *tetM*	Lo et al. (2014)
Amoxicillin	*Edwardsiella tarda*	*blaTEM*	Algammal et al. (2022)
Tetracycline	*Edwardsiella tarda*	*tetA*	Algammal et al. (2022)
Tetracycline	Korean fish farm effluents	*tetA*, *tetB*, *tetD*, *tetE*, *tetG*, *tetH*, *tetM*, *tetQ*, *tetX*, *tetZ*, *tetBP*	Jang et al. (2018)
β-Lactams			
Amoxicillin	*Piscirickettsia salmonis*	*blaTEM*	Shah et al. (2014)
β-Lactams	Korean fish farm effluents	*blaTEM*, *blaCTX*, *blaSHV*	Jang et al. (2018)
Aminoglycosides	*Piscirickettsia salmonis*	*sat1* and *aadA1*	Saavedra et al. (2018)
Trimethoprim			
Trimethoprim	*Piscirickettsia salmonis*	*dfrA1*, *dfrA5* and *dfrA12*	Shah et al. (2014)
Trimethoprim	*Edwardsiella tarda*	*sul1*	Algammal et al. (2022)
Amphenicols			
Chloramphenicol	*Piscirickettsia salmonis*	*cat2*	Saavedra et al. (2018)
Florfenicol	Korean fish farm effluents	*floR*	Jang et al. (2018)
Quinolones and fluoroquinolones			
Quinolones	Korean fish farm effluents	*qnrD*, *qnrS*, *aac(6′)-Ib-cr*	Jang et al. (2018)
Quinolones	*Flavobacterium columnare*	*parC* and *gyrA*	Mata et al. (2018)
Sulfonamides			
Sulfamethizole	*Piscirickettsia salmonis*	*sul1* and *sul2*	Shah et al. (2014)

disease (*Hemophilus piscium*), tenacibaculosis (*Tenacibaculum maritimum*) and furunculosis (*Aeromonas salmonicida*).[79,80] Tetracycline and doxycycline are semi-synthetic derivatives used to a limited extent in aquaculture. The following are recent papers reporting the use of tetracyclines in aquaculture in various countries and regions: Brazil,[48] Finland,[58] Chile,[45,59,81] Taiwan Province of China,[43] Vietnam,[40] China,[51,60,82] Bangladesh,[83] Korea,[55,71] South Africa,[84,85] Tunisia[86] and Portugal.[87]

- *β-Lactams*: β-lactams are antibiotics with a wide range of therapeutic activities and minimal side effects. This class of antibiotics interfere with peptidoglycan synthesis, which is a major component of bacterial cell walls[88] and destroys the integrity of the cell walls, causing lysis of the cell. Common β-lactam antibiotics used in aquaculture include amoxicillin, cephalosporins, penicillin, ampicillin, cephalexin, cefradine and cefotaxime.[89] The following are some recent reports on the use of β-lactams in the aquaculture industry: Brazil,[48] Italy,[90] Turkey,[42] Chile,[59] China,[50,91] Vietnam,[40] Korea[55,71,92] and South Africa.[84,85]
- *Aminoglycosides*: Aminoglycosides are bactericidal, broad-spectrum antibiotics that bind to the 30S subunit of ribosomes, inhibiting the protein synthesis of bacteria.[93] Natural or semi-synthetic derivatives exist.[93] Neomycin, gentamycin S, kanamycin and apramycin have been reported as the most widely used aminoglycosides among the major 15 aquaculture-producing countries from 2008 to 2018.[89] Aminoglycosides are highly soluble; however, limited information is available on their presence in the environment, making it difficult to determine their role in the development of AMR. Recent reports of the use of aminoglycosides in the aquaculture sector include: Italy,[90] Turkey,[42] China,[51] Korea,[71,92] South Africa,[84,85] Chile[45] and Portugal.[87]
- *Amphenicols*: Amphenicols are a class of broad-spectrum antibiotics that inhibit microbial protein synthesis via binding with the peptidyl transferase enzyme at the 50S subunit of the 70S bacterial ribosome, resulting in bacteriostatic effects.[94] Despite having been banned in the EU and many other countries, chloramphenicol is a widely used drug to treat fish, particularly in developing countries.[89] Chloramphenicol has been commonly used in human medicine until an irreversible, non-dose-related aplastic anaemia resulting from the use of this drug became apparent in the early 1960s.[95] However, it is still widely used in developing countries in human medicine. Thiamphenicol and florfenicol, amphenicols that do not have this side effect in humans, are widely used in veterinary medicine around the world. These two derivatives vary from chloramphenicol in their chemical structure, in which a *p*-methylsulphophenyl group is present instead of the *p*-nitrophenyl group found in chloramphenicol.[95] Florfenicol is approved for use in all the major aquaculture-producing countries and its use in aquaculture has been reported in many countries, including Turkey,[42] China,[50,51] Viet Nam,[40] Chile,[45] Korea[55] and Portugal.[87]

- *Quinolones and fluoroquinolones*: Quinolones are broad-spectrum bactericidal antibiotics that have a bicyclic core structure related to 4-quinolone.[96] Fluoroquinolones are the most common quinolones used in veterinary medicine and contain a fluorine atom in their chemical structure. Quinolones inhibit the activity of enzymes required for DNA replication in bacteria.[96] They are the most commonly used class of antibiotics in aquaculture worldwide,[97] with oxolinic acid, enrofloxacin, ciprofloxacin, norfloxacin, nalidixic acid, ofloxacin, levofloxacin, enoxacin, sarafloxacin and flumequine having the highest usage in the major aquaculture-producing countries between 2008 and 2018.[89] In particular, oxolinic acid is widely used in aquaculture, administered in feed.[98] This antibiotic has a low bioavailability in fish (15% in *Sparidae*, 25% in salmonids), but is rapidly absorbed and eliminated. Oxolinic acid was once widely used throughout Asia to treat vibriosis in farmed shrimp, however, AMR has limited its usefulness.[99] Flumequine is a synthetic fluoroquinolone effective against gram-negative bacteria in aquaculture.[100] This drug is cost-effective and widely used to treat various fresh and seawater-farmed fish species, at low and high temperatures. The treatment of aquatic food animals with quinolones has been reported in several aquaculture-producing countries, and include China,[50,51] Vietnam,[40] Korea,[55,71] Portugal[87] and Thailand.[101] Quinolones are still essential antimicrobials for the treatment of human infections and as such should not be first-line drugs used in veterinary medicine.
- *Nitrofurans*: Nitrofurans—such as furazolidone, nitrofurantoin, nitrofurazone and furaltadone—are synthetic broad-spectrum antibacterial drugs with a 5-nitro structure that interfere with several bacterial enzymes.[102] They were commonly used for the treatment of protozoan and bacterial infections in veterinary medicine, however, since the 1990s, these drugs have been banned from use in food animals in many countries due to their public health risk.[103] Nitrofurans are still used legally or illegally in farmed animals in some countries, which has led to rejections of exported consignments with detections of these antibiotics. Recent reports of nitrofuran use in aquaculture are as follows: China,[50,51] Vietnam,[40] Korea[71] and Portugal.[87]
- *Rifamycins*: Rifamycins are broad-spectrum, semi-synthetic antibiotics that inhibit DNA-dependent RNA polymerase activity in bacteria.[104] They are particularly effective against mycobacteriosis. Rifamycins most often used in aquaculture include rifampicin and rimamycin.[89] However, their effectiveness in fish and shellfish is declining, even when used in combination with tetracyclines, due to the development of resistant bacterial strains.[105] Rifamycin use in aquaculture has been reported in the following countries: China,[50,51] the Philippines and Vietnam.[89]
- *Sulphonamides potentiated with diaminopyrimidines (e.g., trimethoprim or ormetoprim)*: Sulfonamides are a class of synthetic bacteriostatic antibiotics that interfere with folic acid, purine and DNA synthesis in bacteria.[94] Commonly used sulfonamides in the major aquaculture-producing countries that include sulphadiazine, sulphamethoxazole and sulphadimethoxine.[89] Potentiated sulfonamides are combinations of a sulfonamide and a diaminopyrimidine, such as trimethoprim or ometoprim, which increases the antibacterial potency. For example, two potentiated sulfonamides (sulphadiamethoxine-ormetoprim and trimethoprim-sulfadiazine) are approved for use in Canada to control bacterial diseases in salmonids.[76] Sulfonamides are used in aquaculture around the world, with recent reports including Chile,[45,59] Israel,[52] China[60] and Korea.[55]

3 | ALTERNATIVES TO ANTIBIOTICS

With the rapid global expansion and intensification of the aquaculture industry in recent years, there has been a concomitant increase in aquatic disease outbreaks, challenging sustainability of production. In view of the threat posed by injudicious and/or incorrect use of antimicrobial agents that can lead to the development of ARGs,[106] we review a number of alternatives to antimicrobials in aquaculture. These include vaccination strategies, phage therapy, quorum quenching, probiotics, prebiotics, chicken egg yolk antibody (IgY) and plant therapy (Figure 2). The use of 'clean seed' or specific pathogen free (SPF) stocks as a primary and essential part of a biosecurity strategy is also discussed.

3.1 | Vaccines

Vaccines are preparations made of pathogenic microorganisms, for example, bacteria, viruses and so forth and their metabolites, which are artificially attenuated, inactivated or genetically modified to prevent infectious diseases.[107] They are recognised as critical tools for the prevention and control of fish diseases and are considered an essential route to the reduction in antibiotic usage within the aquaculture industry.[108,109] This is particularly apparent in the Norwegian salmon farming industry; in 1987, approximately 50,000 kg of antibiotics were used annually, however, by 1997, following the introduction of preventive vaccination strategies, the quantity of antibiotics used annually dropped to less than 1000–2000 kg.[110,111]

The fish vaccination programme was initiated in 1942 with the first commercially available vaccine against the bacterium *Aeromonas salmonicida* in Cutthroat trout (*Oncorhynchus clarkii*)[112] and, since that time, advances in biotechnology and immunology have led to the development and commercialisation of many fish vaccines. Vaccination is currently used for protection against a range of bacterial and viral diseases in aquaculture (Tables 2 and 3).[113–120]

Most licensed vaccines have traditionally used microorganisms that have been inactivated or killed either through physical, chemical or radiation processes,[121] formulated with or without adjuvants[122,123] and delivered by either immersion or injection routes.[105] Whole-cell inactivated vaccines are most effective against extracellular bacteria, evoking a humoral antibody response, but intracellular bacteria evade antibodies[124] and are destroyed by cell-mediated immunity (CMI) for which CMI vaccines are required.[125,126] A stronger antibody response and cellular memory can be achieved with the use of live vaccines, delivered by oral or immersion routes, due to their ability to

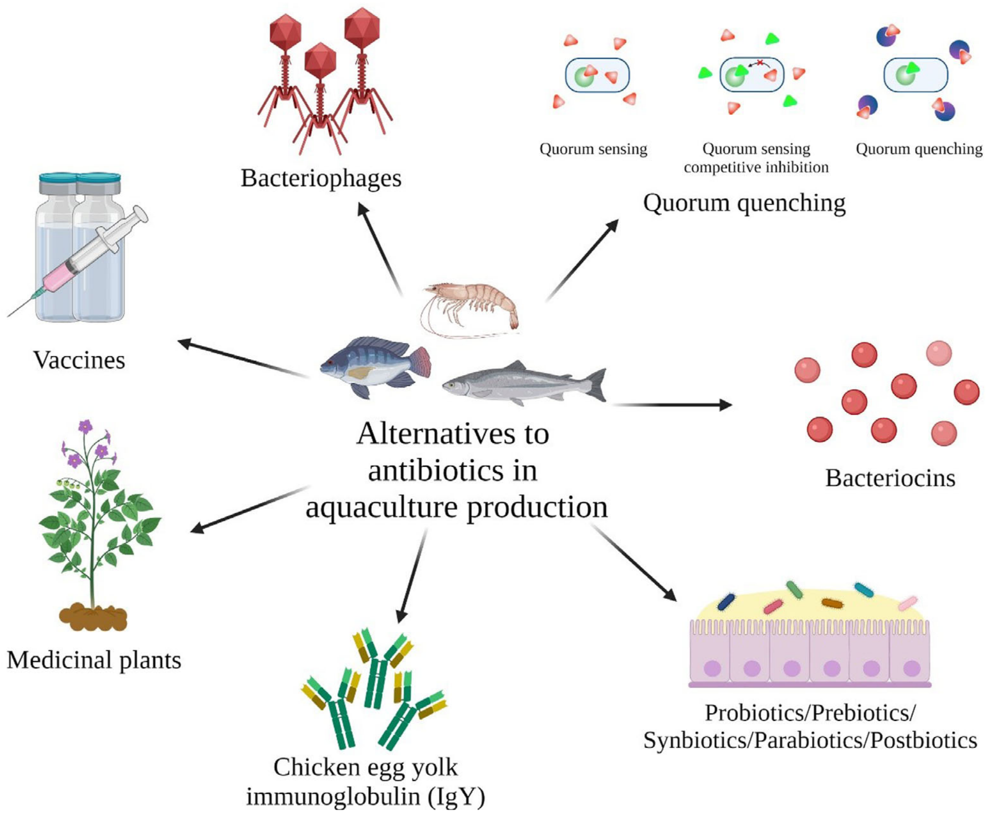

FIGURE 2 Alternative approaches to reduce the use of antimicrobials in aquaculture, for example, vaccines, bacteriophages, quorum quenching, bacteriocins, chicken egg yolk immunoglobulin, medicinal plants and microbiomes.

proliferate or enter the host, eliciting both innate and adaptive immunity[127] and which can reduce the number of required booster immunizations.[105] Modified live vaccines are prepared from viruses or bacteria that display attenuated virulence, achieved by physical or chemical processes, serial passage in culture or culture under abnormal conditions or natural low virulence towards the target species.[105,128,129] Molecular manipulations to produce genetically modified mutants that lack virulence has also been used to induce attenuation in vaccine candidates and this approach has been used successfully for large DNA viruses such as herpesviruses like koi herpesvirus and also for bacteria, for example, *Streptococcus* spp. and *Edwardsiella* spp.[105,130]

The use of polyvalent or multivalent injectable vaccines that contain adjuvant and multiple antigens to protect against different diseases are currently used in large-scale commercial aquaculture operations, especially those focused on high-value species such as Atlantic salmon (*Salmo salar*; Tables 2 and 3).[110] In addition, autogenous vaccines, created from site-specific, isolated pathogens of interest, offer cost-effectiveness and more flexibility in production, speed of delivery and implementation in the face of a disease outbreak.[119]

Modern, alternative technological approaches to vaccine manufacture that target specific pathogen components, that is, subunit, recombinant technology or DNA/RNA particle vaccines, appear to induce an even greater level of immunity. Subunit vaccines use only the antigenic component for vaccination, thus removing the risk of replication in the host, non-target host or environment.[131] Immunogenic components can be isolated and purified directly from the target pathogen, or specific immunogenic proteins can be manufactured using recombinant expression vectors, for example, an *Escherichia coli* expression system is used to produce plasmids carrying genes that encode specific protective antigens, and has been used successfully against infectious pancreatic necrosis (IPN) in salmonids in Norway.[105] They can be freeze-dried, allowing for non-refrigerated transport and storage,[131,132] however, due to their limited number of antigenic components, they can stimulate a weaker immune response,[105] require effective adjuvants and multiple booster immunizations,[132] and are expensive to produce.[133] Virus-like particles (VLP) are components of advance subunit vaccines and are formed from the self-assembly of viral capsid proteins into particles that mimic the natural structure of the virus.[134] They can potentiate both adaptive and innate immune responses and offer the advantage of lacking genomic material, thus preventing replication in the host.[135,136] Interest in this technology has increased over the past decade and VLP vaccines have been shown to work experimentally against certain fish diseases.

In recent years, several nucleic acid vaccines have been developed for use in aquaculture and appear to elicit a strong cellular and humoral immunity. They consist of DNA or RNA encoding antigen(s) of interest and are relatively easy to manufacture and safe to administer and are cost competitive.[105,137] DNA vaccines can be produced in

TABLE 2 Commercially available vaccines against major infectious bacterial diseases of finfish

Target disease	Target pathogen	Target fish species	Type of vaccine	Product name	Route of administration
Monovalent					
Bacterial kidney disease (BKD)	*Renibacterium salmoninarum*	Salmonids	*Arthrobacter davidanieli*, live culture	Elanco: Renogen	Injection
Edwardsiellosis/ Enteric septicaemia of catfish (ESC)	*Edwardsiella ictaluri*	Catfish spp., that is, channel catfish, freshwater catfish, striped catfish, brown bullhead, *Danio* spp.	*Edwardsiella ictaluri*, avirulent live culture	MSD Animal Health: AquaVac-ESC™	Immersion
	E. ictaluri	Pangasius	*Edwardsiella ictaluri*, inactivated live culture	Pharmaq: ALPHAJECT Panga 1 and 2	Injection
Flavobacteriosis/ rainbow trout fry syndrome/ Columnaris disease	*Flavobacterium columnare*	Cyprinids, salmonids, catfish carp, trout, perch, tilapia	*Flavobacterium columnare*, attenuated bacterin	FryVacc1 and 2	Immersion
	F. columnare	Catfish, largemouth bass	*Flavobacterium columnare*, avirulent, live culture	MSD Animal Health: AquaVac-Col™	Immersion
Furunculosis	*Aeromonas salmonicida*	Salmonids, flounder, turbot, carp, tilapia, sole	*Aeromonas salmonicida*, inactivated bacterin	Elanco: Furogen Dip	Injection
	A. salmonicida	Salmonids	Iron-regulated outer membrane protein (IROMP) antigens of 2 strains of *Aeromonas salmonicida*, non-mineral oil based	MSD Animal Health: AquaVac® FNM	Injection
	A. salmonicida	Salmonids	*Aeromonas salmonicida*	Pharmaq: AlphaJect 1200	Injection
Lactococcosis	*Lactococcus garvieae*	Salmonids, European sea bass, gilthead sea bream, *Seriola* spp., yellowtail, (hiramasa), amberjack	*Lactococcus garvieae*, inactivated	MSD Animal Health: Amalin™ Rensa	Oral
	L. garvieae	Rainbow trout	*Lactococcus trucha*, inactivated	Hipra: ICTHIOVAC® LG	Injection
Pasteurellosis	*Photobacterium. damselae* spp. *piscicida*	European sea bass and gilthead seabream	*Photobacterium piscida*, inactivated	MSD Animal Health: AquaVac Photobac Prime™	Immersion
	P. damselae spp. *piscicida*	Gilthead seabream	*Photobacterium piscida*, inactivated	Hipra: ICTHIOVAC® PD	Immersion
Streptococcosis	*Streptococcus agalactiae*	Grouper, salmonids, turbot, flounder, sturgeon,	*Streptococcus agalactiae* biotype 2 bacterin, inactivated, oil adjuvant	MSD Animal Health: AquaVac® Strep Sa; AquaVac® Strep Sa1	Injection
		Amberjack, yellow tail, red porgy, barramundi, rabbitfish, seabass, seabream, hybrid striped bass, catfish, mullet, pomfret, tilapia, koi, carp			
	S. iniae	Warm-water marine and freshwater finfish	*Streptococcus iniae*, inactivated	MSD Animal Health: AquaVac® Strep Si	Injection
	S. iniae	Turbot	*Streptococcus iniae*, inactivated	Hipra: ICTHIOVAC® STR	Injection
Vibriosis	*Vibrio anguillarum*, *V. ordalii*	rainbow trout, European seabass	(*Listonella*) *Vibrio anguillarum* (biotype I and II), *V. ordalii*, inactivated	MSD Animal Health; AquaVAC® Vibrio	Injection
	V. anguillarum, *V. ordalii*	Rainbow trout, European seabass	*V. anguillarum 01* and *02a* (*V. ordalii*), inactivated	MSD Animal Health: AQUAVAC® Vibrio Oral	Oral

TABLE 2 (Continued)

Target disease	Target pathogen	Target fish species	Type of vaccine	Product name	Route of administration
Yersiniosis/Enteric redmouth (ERM)	*Yersinia ruckeri*	Rainbow trout	*Yersinia ruckeri* (Hagerman strain), inactivated	MSD Animal Health: AquaVac® ER; AquaVac® ERM Oral	Immersion/oral
	Y. ruckeri	Rainbow trout	*Y. ruckeri* biotype 1 and biotype 2 (Hagerman type 1 and EX5 biogroup), inactivated	MSD Animal Health: AQUAVAC® RELERA™	Immersion/oral
Tenacibaculosis	*Tenacibaculum maritimum*	Turbot	*T. maritimum*, inactivated	Hipra: ICTHIOVAC®TM	Injection
Multivalent					
Vibriosis and Pasteurellosis	*Photobacterium damselae* subs. *piscicida*, *Listonella anguillarum* serotype O1, *L. anguillarum* serotype O2a, *L. anguillarum* serotype O2b	European seabass	*Photobacterium damselae* subs. *piscicida*, *Listonella anguillarum* serotype O1, *L. anguillarum* serotype O2a and *L. anguillarum* serotype O2b, inactivated	Hipra: ICTHIOVAC VR®/PD	Injection
Lactococcosis, Pseudotuberculosis and Vibriosis	*Lactococcus garviae*, *Photobacterium damselae sp. Piscicida*, *Vibrio anguillarum*	Yellowtail, amberjack	*Lactococcus garviae*, *Photobacterium damselae sp. piscicida* and *Vibrio anguillarum*, inactivated oil adjuvant	MSD Animal Health: NORVAX® PLV 3-way Oil	Injection
Vibriosis and Pasteurellosis	*Photobacterium damselae* subsp. *piscicida*	European seabass	*Listonella anguillarum* (01) and *Photobacterium damselae* subsp. *piscicida*, inactivated	Pharmaq Fishteq: ALPHA JECT 2000	Injection
Pasteurellosis, Streptococcosis	*Photobacterium damselae*, *Lactococcus garviae*	Yellowtail, amberjack	*Photobacterium damselae* and *Lactococcus garviae*, inactivated oil adjuvant	MSD Animal Health: NORVAX® Ruiketsu Rensa Oil	Injection
Furunculosis, classical Vibriosis, cold-water Vibriosis, wound or winter ulcer disease and infectious pancreatic necrosis (IPN)	*Listonella* (*Vibrio*) *anguillarum*, *Aeromonas salmonicida* subsp., *Salmonicida*, *Vibrio salmonicida*, *Moritella viscosa*, Infectious pancreatic necrosis virus (IPNV)	Atlantic salmon	*Listonella* (*Vibrio*) *anguillarum* serovar O1, *L.* (*Vibrio*) *anguillarum* serovar O2, *Aeromonas salmonicida* subsp *salmonicida*, *Vibrio salmonicida*, *Moritella viscosa* and surface protein from IPN virus serotype spp., inactivated	MSD Animal Health: Norvax® Minova 6	Injection
Vibriosis, Pasteurellosis	*Photobacterium damselae*, *Vibrio anguillarum*, *V. ordalii*	European seabass	*Vibrio anguillarum* (biotype I and II), *V. ordalii* and *Photobacterium damselae* (subsp *piscicida*), inactivated	MSD Animal Health: QUAVAC® Vibrio Pasteurella	Injection
Infectious salmon anaemia (ISA), Furunculosis, Vibriosis	*Aeromonas salmonicida*, *Vibrio anguillarum*, *V. ordalii*	Salmonids	*Aeromonas salmonicida*, *Vibrio anguillarum* serotypes I and II, *V. ordalii* and *V. salmonicida* serotypes I and II, inactivated	Forte V II	Injection

(Continues)

TABLE 2 (Continued)

Target disease	Target pathogen	Target fish species	Type of vaccine	Product name	Route of administration
Vibriosis, ISA, Wound disease	*Vibrio anguillarum*, *V. salmonicida*, *Aeromonas salmonicida* subsp. *salmonicida*	Salmonids	*Vibrio anguillarum*, serotypes O1 and O2α, *V. salmonicida* and *Aeromonas salmonicida* subsp. *salmonicida*, inactivated	Pharmaq: AlphaJect 5200	Injection

bacterial cells that contain an expression plasmid that carries a specific gene coding for a selected antigenic protein and multivalent vaccines can be produced providing cross-protection by the use of gene coding for multiple antigens in the plasmid design.[138,139] A DNA vaccine against infectious haematopoietic necrosis virus (IHNV) is licensed and commercialised in Canada (Apex-IHN; Table 3). RNA-based vaccines can be either conventional, non-amplifying mRNA or self-amplifying mRNA and offer much promise in both humans and animals,[140,141] having demonstrated efficacy in stimulating antigen-specific immune responses in a broad range of host cells when compared to conventional plasmid DNA vaccines.[142]

3.2 | Bacteriophages

Bacteriophages or phages are bacterial viruses that invade bacterial cells and, in the case of lytic phages, disrupt bacterial metabolism and cause the bacterium to lyse.[143] With their initial discovery by Twort and d'Herelle in the early 1900s,[144] bacteriophages were seen as the solution to controlling or eradicating bacterial diseases, but interest declined following the discovery of antibiotics. With the emergence of genetic resistance to antibiotics, phage therapy is gaining interest again.[145,146] Phages are globally the most abundant microorganisms,[147,148] particularly in marine and freshwater environments,[149,150] in which they can survive more than 5–7 months and several weeks, respectively. Marine species occur at near surface to deep benthic environments, down to the deep-sea floor with their distribution in the water column matching that of their hosts.[151,152] Despite the abundance of phages in marine environments, genetically identical phages occur over vast distances,[153] such as between Europe, Chile and the USA,[153–156] for decades,[157] although there may be some regional differences.[155]

In general, phage survival is not affected by pH, salinity, temperature or organic matter concentration,[150] although *E. coli* phages may be affected by a combination of salinity and organic matter.[158] Bacteriophages can also exist as prophages integrated into the DNA of the host or as replicons, such as *Vibrio* spp.[151,154,156] Prophages may or may not be associated with lysogeny,[156,159] which may vary according to geographic regions,[155] and with depth of marine species.[152] Phages infect many species of bacterial pathogens of fish (Table 4).[160]

Phage therapy has been successfully used to control bacterial infections in aquatic animals,[161–173] but multiple phage therapy has proven to be more successful than single phage therapy.[149,168,174–179] There are many reports of phage therapy used against the bacterial genera *Vibrionaceae*, which are abundant in the aquatic environment and are the most common bacterial genera that cause disease in aquatic organisms.[70,180–182] Phages may be used to control the most destructive bacteria; for example, *Vibrio harveyi*,[159,178,183–186] *V. parahaemolyticus*,[164,178,187] *V. anguillarum*,[151,156,157,166,172,178,181,188,189] *V. alginolyticus*[168,178] and *V. splendidus* which infects molluscs, crustaceans, echinoderms and fish.[176,190]

However, the interaction of phages with their hosts is complex,[191–195] with both existing as strains of varying virulence with gene transfer between them,[181,191,192] affecting both their genetic traits and the host:phage relationship.[8,174,181] Bacterial hosts may contain prophage encoded virulence factors,[151,181] which alter the virulence of the host, either increasing or diminishing virulence,[151,159,196,197] thus allowing phages to be used for anti-virulence therapy.[198] The development of resistance to phage infection,[147,148,153,191,199–201] may result in the development of bacterial-resistant strains.[148,199] Consequently, phages diversify genetically to overcome bacterial defences, such as adsorption inhibition, restriction-modification, CRISPR-Cas (clustered regularly interspaced short palindromic repeats-CRISPR-associated proteins) systems, abortive infection and increased phage infectivity and host range, which are also associated with expansion of phage genome size.[147,191,194,202] Co-evolution may be common in host:phage relationships.[174,194] Bacterial resistance to one phage may result in susceptibility to others,[153] but some phages are broadly pathogenic.[172,203] Some phages and their hosts may have a mutualistic relationship, perhaps explaining their global distribution.[156] Therapies with phages in aquaculture do represent an alternative to the traditional pharmacology treatment and there are already some commercial phage-based products available, in particular, to target *Vibrio* spp., however, this treatment modality will require further research before common use in aquaculture.[204]

3.3 | Quorum quenching

Quorum quenching (QQ) relates to all processes involved in the disturbance of quorum sensing (QS) which refers to the capacity of bacteria to monitor their population density and regulate gene expression accordingly.[205] Numerous bacteria can use QS signals to coordinate and synchronise several behaviours under differing environments, including microbe–microbe and host–microbe interactions. Quorum quenching encompasses very diverse phenomena and mechanisms, and QQ molecular actors are also diverse in nature, that is, enzymes,

TABLE 3 Commercially available vaccines against major infectious viral diseases of finfish

Target disease	Target pathogen	Target fish species	Type of vaccine	Product name	Route of administration
Monovalent					
Infectious haematopoietic necrosis (IHN)	Infectious haematopoietic necrosis virus (IHNV) *Rhabdovirus*	Salmonids	DNA vaccine	Elanco: Apex-IHN (Canada)	Injection
Infectious pancreatic necrosis (IPN)	Infectious pancreatic necrosis virus (IPNV) *Birnavirus*	Atlantic salmon	VP2 and VP3 subunit proteins	MSD Animal Health: AQUAVAC® IPN Oral	Oral
Pancreatic disease (PD) virus/Salmonid alphavirus (SAV)/ Salmon pancreas disease	SAV *alphaviruses*	Salmonids	Inactivated SAV F93-125	MSD Animal Health: Norvax® Compact PD	Injection
	SAV *alphaviruses*	Salmonids	Inactivated strain AL V405	MSD Animal Health: Alpha Ject Micro 1 Pd	Injection
Koi herpesvirus (KHV) disease	KHV *Herpesvirus*	Koi carp	Live, attenuated viral vaccine	Kovax Ltd., Israel: KV-3	Immersion or injection
Infectious spleen and kidney necrosis (ISKNV)	ISKNV *Iridovirus*	Asian seabass, grouper, pompano Japanese yellowtail	Inactivated ISKNV	MSD Animal Health: AQUAVAC®IridoV	Injection
Viral Nervous Necrosis (VNN)	*Betanodavirus*	European sea bass	Inactivated Betanodavirus strain	Hipra: ICTHIOVAC® VNN	Injection
Multivalent					
Infectious Salmon Anaemia (ISA), Furunculosis, Vibriosis	*Infectious salmon* anaemia *virus* (ISAV), *Aeromonas salmonicida, Vibrio anguillarum, V. ordalii*	Salmonids	Infectious Salmon Anaemia virus (ISAV), *Aeromonas salmonicida, Vibrio anguillarum* serotypes I and II, *V. ordalii* and *V. salmonicida* serotypes I and II, inactivated	Forte V II	Injection
Furunculosis, classical Vibriosis, cold-water Vibriosis, wound or winter ulcer disease and infectious pancreatic necrosis (IPN)	*Listonella* (*Vibrio*) *anguillarum, Aeromonas salmonicida* subsp *salmonicida, Vibrio salmonicida, Moritella viscosa, Infectious pancreatic necrosis virus* (IPNV)	Salmonids	*Listonella* (*Vibrio*) *Anguillarum* serovar O1, *Listonella* (*Vibrio*) *anguillarum* serovar O2, *Aeromonas salmonicida* subsp *salm onicida, Vibrio salmonicida, Moritella viscosa* and surface protein from IPN virus serotype spp., inactivated	MSD Animal Health: Norvax® Minova 6	Injection

chemical compounds, mode of action, that is, QS-signal cleavage, competitive inhibition and so forth. All the main steps of the QS pathway, including synthesis, diffusion, accumulation and perception of the QS signals, may be affected. Hence, QS disruption is a field that is being developed and used for biocontrol of bacterial diseases in some fields such as aquaculture, crop production and anti-biofouling.[206]

Bacteria attached to a surface may proliferate and exist as biofilms, embedded in a hydrogel matrix,[9,188,207,208] in which they are more resistant to antibiotics than conspecific planktonic forms.[208,209] Within biofilms, bacteria communicate by QS, a method of communication-related to cell density and species composition, using small diffusible signalling molecules, called autoinducers, which activate genes controlling several functions, including biofilm formation, virulence, bioluminescence, invasion and spread.[189,198,200,210–214] Autoinducers include acyl-homoserine lactones (AHLs), auto-inducing oligo-peptides (AIPs) and autoinducer 2.[211,212,215] Certain compounds can inhibit AHL synthesis, degrade AHLs or inhibit AHL/receptor interaction and as a consequence, prevent pathogenic bacteria from producing virulence factors, forming biofilms and reducing virulence.[211] The understanding that blocking QS would stop the gene expression controlling virulence, disease and the microbial environment has led to research into blocking QS, commonly termed as QQ.[209–211,216–222]

3.4 | Bacteriocins

Bacteriocins, bioactive compounds produced by bacteria, have been proposed as a sustainable and promising alternative strategy to the use of antibiotics in the aquaculture industry.[223] They are ribosome

TABLE 4 Bacteriophages, their bacterial hosts and source related to aquatic pathogens

Bacteriophage	Bacterial host	Isolated from	Reference
V1G, V1P1 and V1P2	*Vibrio* spp. CV1	Shrimp	Barbosa et al. (2013)
VOB	Infective in *Vibrio harveyi* and *V. campbellii*	*V. owensii*	Busico-Salcedo and Owens (2013)
Bacteriophage YC (*Myoviridae*)	*Vibrio coralliilyticus* P1 (LMG23696)	Coral	Cohen et al. (2013)
Bacteriophage (*Myoviridae* and *Siphoviridae*)	*Vibrio* harveyi, *V. campbellii*, *V. rotiferianus* and *V. parahaemolyticus*	Shrimp farm effluent	Crothers-Stomps et al. (2006)
Vibrio phage vB_VorS-PVo5 (*Siphoviridae*)	*Vibrio ordalii*	Purple mussel (*Perumytilus purpuratus*)	Echeverria-Vega et al. (2016)
WP-1, WWP-2 and SP-2 (Podoviridae)	*Lactococcus garvieae*	Unknown	Ghasemi et al. (2011)
Three types	*Vibrio anguillarum* and *V. ordalii* (not *V. parahaemolyticus*)	Atlantic salmon (*Salmo salar*)	Higuera et al. (2013)
Vibriophage, KVP40	78 Vibrio and 1 *Photobacterium* sp.	Unknown	Inoue et al. (1995)
Bacteriophage (phage), pVp-1	*Vibrio parahaemolyticus*	Oysters	Jun et al. (2014)
Bacteriophage pAh6-C	*Aeromonas hydrophila*	Korean river water	Jun et al. (2015)
Bacteriophages φSt2 and φGrn1	*Vibrio alginolyticus*	Gilt-head bream (*Sparus aurata*)	Kalatzis et al. (2016)
Isolation as a cocktail	*Pseudomonas* spp., *Vibrio harveyi* and *V. parahaemolyticus*	Green sea turtle (*Chelonia mydas*)	Delli et al. (2017)
VR1, VR2 and VR3 variable regions	*Vibrio anguillarum*	Aquaculture and environment, vast geographical sites	Kalatzis et al. (2017)
4 bacteriophages, 2 *Siphoviridae*	*Vibrio harveyi*	Oysters, shrimp hatchery water	Karunasagar et al. (2007)
vB_VspP_pVa5, N4-like lytic bacteriophage	*Vibrio splendidus*	Aquaculture farm	Katharios et al. (2017)
Aeromonas phage PAS-1	*Aeromonas salmonicida*	Rainbow trout (*Oncorhynchus mykiss*)	Kim et al. (2015)
VhKM4 (*Myoviridae*)	*Vibrio harveyi* and *V. parahaemolyticus*	Tropical fish aquaculture	Lai et al. (2017)
VpKK5 (*Siphoviridae*)	*Vibrio parahaemolyticus*	Unknown	Lal et al. (2016)
vB_VspS_VS-ABTNL-1 (PVS-1), vB_VspS_VS-ABTNL-2 (PVS-2), vB_VspS_VS-ABTNL-3 (PVS-3)	*Vibrio splendidus*	Sea cucumber (*Apostichopus japonicus*)	Li et al. (2016a)
vB_VcyS_Vc1 (*Vibrio* phage Vc1)	*Vibro cyclitrophicus*	Sea cucumber	Li et al. (2016b)
A3S and Vpms1	*Penaeus vannamei*	Unknown	Lomelí-Ortega et al. (2014)
VP-1, VP-2 and VP-3	*Vibrio parahaemolyticus*	Unknown	Mateus et al. (2014)
Vibriophage KVP40	*Vibrio parahaemolyticus*, 8 Vibrio and 1 *Photobacterium* sp.	Seawater	Matsuzaki et al. (1992)
Phi S(M) and Phi S(T)	*Cellulophaga baltica* MM#3	Unknown	Nilsson et al. (2020)
VHML (*Myoviridae*)	*Vibrio harveyi*	Moribund farmed whiteleg shrimp (*Penaeus. vannamei*)	Oakey and Owens (2000)
PPp-W4 (*Podoviridae*), PPpW-3 (*Myoviridae*)	*Pseudomonas plecoglossicida*	Ayu (*Plecoglossus altivelis*)	Park and Nakai (2003)
VHP6b *Siphoviridae*	*Vibrio harveyi*	Oysters and clams	Raghu Patil et al. (2014)
VPp1	*Vibrio parahaemolyticus*	Oysters under depuration	Rong et al. (2014)
Vibriophage KVP40	*Vibrio anguillarum*	Atlantic cod (*Gadus morhua*), Turbot (*Scophthalmus maximus*)	Rørbo et al. (2018)
VHM1 and VHM2 (*Myoviridae*), VHS1 (*Siphoviridae*)	*Vibrio harveyi* (growth inhibition), *V. parahaemolyticus* and *V. alginolyticus*	Aquaculture environments	Stalin and Srinivasan (2017)
ΦH20 (*Siphoviridae*) and KVP40 (*Myoviridae*)	*Vibrio anguillarum* BA35 and *V. anguillarum* PF430-3	In vitro	Tan et al. (2015a)

TABLE 4 (Continued)

Bacteriophage	Bacterial host	Isolated from	Reference
KVP40	*Vibrio* anguillarum PF430-3	In vitro	Tan et al. (2015b)
11 vibriophages	24 *V. anguillarum* strains and 13 *Vibrio* spp.	In vitro	Tan et al. (2014)
PLgW-1, PLgY-16, PLgY-30 (*Siphoviridae*)	*Lactococcus garvieae*	Marine fish	Hoai et al. (2018)
Phi S(M), Phi S(T)	*Cellulophaga baltica MM#3*	Unknown	Middelboe et al. (2009)

synthesised, low molecular weight bactericidal peptides, encoded either in chromosome or extrachromosomal elements, usually 20–60 amino acids in length.[224,225] They have antimicrobial properties due to their ability to inhibit or kill both closely or distantly related microorganisms.[226–228] Their benefits include being eco-friendly, biodegradable, non-lethal to host or environment while still being antagonistic to harmful gut pathogens and promoting beneficial bacteria.[40,229–232] Studies on the gut microbiota of vertebrates have identified beneficial bacteriocins,[233–235] including those in fish.[229,231,232,236–246]

3.5 | Probiotics and prebiotics, synbiotics, parabiotics and postbiotics

In recent years, some publications have pointed out the importance of maintaining a healthy and stable gut microbiome in fish and shellfish to reduce the risks of disease occurrence.[247] This is essential to optimise nutrient digestion and minimise stress in rearing conditions. A disturbed microbiome has frequently been related to a disease condition, and is considered by some scientists as an interesting biomarker to detect a pathological problem.[248] Some bacterial species are found dominant in healthy animals, while in infected animals, occurrence of other species increase drastically, suggesting that diseased animals have difficulties to control their digestive microbiota, which then becomes more influenced by environmental factors and stress. For example, *Faecalibacterium prausnitzii* and *Pantoea agglomerans*, were found in healthy cultured shrimp, while diseased shrimp had different bacterial communities, including *Aeromonas taiwanensis*, *Simiduia agarivorans* and *Photobacterium angustum*,[249] therefore, confirming previous observations.[248] Similarly, some farmed fish species might succumb to infection due to poor quality of microbiome in their gut system.[250] It was also reported that while a beneficial gut microbiome does not cause any diseases or disorders in host organisms, a disturbance in the balance of microbial community can induce a higher prevalence of harmful pathogens, which can trigger infections and diseases.[251–254] For example, it was found that the population density of *Aeromonas* bacteria was higher in abundance in diseased affected fish samples when compared to healthy individuals, which indicates that in healthy fish the pathogenic expression of the *Aeromonas* was totally prevented due to the presence of healthy microbiome.[176] Microbiomes can be influenced by diets, for example, proportions of fishmeal, protein, lipid and energy levels,[255] and by specific nutrients,[256] or by medicinal plant extracts, which can notably display anti-bacterial or immunostimulant activities.[257,258]

Probiotics are the most commonly and commercially available way used worldwide to positively influence microbiomes. They are live, non-pathogenic microorganisms administered to improve microbial balance, particularly in the gastrointestinal tract. They consist of various microorganisms, notably yeast or bacteria, such as *Lactobacillus* and *Bifidobacterium* species, and are administered as dietary supplements in foods.[259] Probiotics have demonstrated efficacy in preventing and treating various medical conditions, particularly those involving the gut. Probiotics exert their beneficial effects through various mechanisms. They usually promote health conditions by inhibiting harmful bacteria. Basic probiotic modes of action in the aquatic animal gut include inhibition of pathogen adhesion; production of antimicrobial components, including bacteriocins and defensins; competitive exclusion of pathogenic microorganisms; enhancement of barrier function; reduction in luminal pH; and modulation of the immune system. For example, lowering intestinal pH induces a decreasing colonisation and invasion by pathogenic organisms and is modifying the host immune response.[260]

Probiotics can also be beneficial to aquatic animals by synthesising and providing essential nutrients, regardless of their location, either in the digestive tract, in the water column or sediments. These include polyunsaturated fatty acids[261] and also some vitamins such as vitamin B12.[262] Other probiotics, in particular those belonging to Bacillus genus, are used to improve the rearing environment, in particular, by assimilating organic pollutants (ammonia, nitrites, etc.), which might otherwise accumulate and induce stress and toxicity to farmed aquatic animals. Moreover, by competing with opportunistic pathogens for access to these nutrients, those probiotics which occupy the same ecosystem as these bacterial pathogens, consequently prevent them from reaching critical levels above which they can become harmful for aquatic animals, as this is the case for several species of *Vibrio* spp.

Particularly beneficial probiotics promoting disease resistance in aquatic animals include:

- Lactic acid bacteria (LAB),[233,240,243,263,264] such as *Lactobacillus* spp.[264–284]
- *Phaeobacter* spp.[285–287]
- *Bacillus* spp.[277,280,288–306]

Besides resistance to diseases,[277,280–282,298–305,307–311] some probiotics improve digestion,[277] water quality[277] and growth in fish.[269,277,280–282,300,302,303,307,309,311]

Prebiotics are non-viable food ingredients, usually oligosaccharides, a family of carbohydrates non-digestible to the host, but which are digestible to specific bacterial populations residing in the gut, and therefore act as selective substrates for bacterial fermentation to only promote beneficial intestinal bacteria.[312] This modification of the microbiome then induces specific changes, both in the composition and/or activity in the intestinal microflora, that confers benefits upon host well-being and health.[313] Microflora of the gut can be optimised through dietary modulation by prebiotics that stimulates the number and/or activity of bifidobacteria and lactobacilli, which can increase host resistance to pathogenic bacteria and stimulation of the immune response.[314]

The beneficial effects of probiotic bacteria may be increased by the use of prebiotics, and synbiotics, which are a combination of probiotics and prebiotics.[233,315] They include indigestible fibre that enhances beneficial commensal gut bacteria.[236,246,281,316–319] Their beneficial effects are due to by-products derived from the fermentation of intestinal commensal bacteria and include modulation of the immune system and its ability to stimulate systemic and local immunity[278] through the action of immunosaccharides on the innate immune system of fish and shellfish.[316]

New evidence revealed that parabiotics (i.e., dead cells of probiotics, also named as ghost probiotics) and postbiotics (i.e., supernatants from probiotic cultures, containing soluble factors or metabolic by-products secreted by bacteria) also have an important impact on microbiome and disease occurrence.[312] Moreover, new metagenomic techniques, notably next-generation sequencing (NGS) technology gives opportunity to identify many more bacterial species in the microbiomes, including non-culturable species, which were previously totally undetected.[247] These discoveries open whole new fields of research to better understand the factors influencing microbiomes,[320] giving more opportunities to find credible alternatives to antibiotics, better control and stabilise microbiomes and thus improve health of aquatic organisms.

3.6 | Chicken egg yolk immunoglobulin

Chicken egg yolk immunoglobulin (IgY) is a useful antibody for passive immunisation due to the fact that high titers of pathogen-specific IgY are produced after immunisation of hens and simple methods have been developed for IgY extraction from egg yolk. Chicken egg yolk immunoglobulin has been successfully used in humans, livestock animals and aquatic animals. One of the major characteristics of IgY is that, compared with immunoglobulin G (IgG), it is more stable, less expensive to make in high yields and exhibits minimal conformational changes, hence is more cost-effective for use for a diverse range of purposes.[321,322]

Chicken egg yolk immunoglobulin has been found to have effective therapeutic value in controlling various bacterial and viral pathogens in fish and other aquatic animals,[323] for example, IgY has been used for the treatment of diseases like White Spot Disease (WSD), a viral disease of shrimps and crayfish; *Vibrio harveyi* infection in Indian white shrimp (*Fenneropenaeus indicus*)[324]; *V. anguillarum* and *Yersinia ruckeri* in rainbow trout (*Oncorhynchus mykiss*); *V. splendens* in sea cucumber (*Apostichopus japonicas*)[177,325]; *Aeromonas hydrophila* in polyploid gibel carp (*Carassius auratus gibelio*) and Wuchang bream (*Megalobrama amblycephala*)[326]; *A. salmonicida* in koi carp (*Cyprinus carpio koi*)[327]; and Edwardsiellosis in Japanese eel (*Anguilla japonica*)[328] and small abalone (*Haliotis diversicolor supertexta*).[329]

Chicken egg yolk immunoglobulin can be administered in several forms including purified egg yolk IgY,[330] one-step aqueous extract of egg yolk[331] or whole egg yolk powder[327] from vaccinated chickens. However, the most studied form is the purified egg yolk IgY. It can be administered through a variety of different routes, that is, intraperitoneal injection, immersion or oral administration and can provide protection for fish against diseases through passive immunisation. Efficacy in conferring protection was confirmed in rainbow trout following a single intraperitoneal injection of anti-*V. anguillarum* IgY[331] and protective effects of IgY were achieved in sea cucumber by intraperitoneal injection of anti-*V. splendidus* IgY antibodies or immersing the sea cucumber (*A. japonicas*) in aqueous IgY.[177] The application of IgY against *V. parahaemolyticus* is reported to improve the survival rate of whiteleg shrimp (*Penaeus vannamei*) without affecting the water quality and consecutive immersions of fish into rearing water containing specific IgY antibodies, completely prevented ulcer disease outbreaks caused by *A. salmonicida* in koi carp during a cohabitation infection challenge.[327] These indicate the therapeutic value of IgY antibodies by immersion treatment in the prevention of diseases caused by pathogens that invade the skin and gills in aquaculture animals. In addition, oral IgY antibodies offer promising potential for passive immunisation strategies. The oral application of specific egg yolk antibody powders (encapsulated) provided protection against vibriosis in whiteleg shrimp (*P. vannamei*) at different developmental stages.[332] In another study, fish that received IgY in their diet had substantial IgY levels in the serum, and feeding of specific anti-*V. anguillarum* IgY enhanced resistance of rainbow trout to vibriosis.[331] This indicated that IgY can be absorbed into the blood system through the gastrointestinal tract of rainbow trout. It has also been reported that IgY was significantly absorbed in agastric carp after feeding, while plasma IgY concentration of gastric rainbow trout could not be detected.[330]

3.7 | Medicinal plants

In recent years, medicinal plants and their derivatives have received considerable attention as alternatives to antibiotics,[333,334] immunoprophylactics or immunostimulants.[258,335] There is considerable interest in their application due to their ease of preparation, low cost, lower risk of side effects and environmental impacts, as reflected in the current wealth of available scientific literature concerning the development and application of medicinal plants in aquaculture (see review of Tadese 2021[335]).

Medicinal plants may include herbs, spices, seaweeds, herbal extracted compounds, traditional Chinese medicines and commercial plant-derived products[258] and their active ingredients include secondary metabolites, for example, phenolics, essential oils, pigments, alkaloids, terpenoids, tannins, polypeptides and polysaccharides, steroids and flavonoids.[336] Herbal plants contain antimicrobial substances that can fight a wide range of bacteria responsible for aquatic animal diseases.[337–354]

In addition, many plant-derived products are also effective at stimulating both the innate or specific immune response and the non-specific immune response in aquatic animal hosts to increase resistance to pathogens.[257,355–365] Many immunostimulants are composed of microbial cell wall or outer membrane with molecular patterns that are recognised by the innate immune system of the host (i.e., glucans, lipopolysaccharides, chitin, chitosan, peptidoglycans). The innate immune response involves a cascade of reactions that activates cells to identify and remove microbial pathogens in the host. The majority of commercial immunostimulants contain β-glucans (β-1,3 and β-1,6), alginates and polysaccharides produced from yeast and seaweeds, respectively[366]; these immunostimulants are typically delivered via feed or bath immersion for larval stages and via feed for grow-out stages.

4 | DISCUSSION

A number of factors can determine the best alternatives to antibiotics to be used within an aquaculture system.[367,368] It is well acknowledged that vaccination strategies are an integral part of fish health management programmes. However, while advances in vaccine development have been promising, actual implementation has been limited due to the practical and logistical challenges of mass vaccination in a commercial setting as well as cost-effectiveness and, generally, only high-value finfish species are vaccinated.[115]

World aquaculture production of farmed aquatic animals has grown, on average, by 5.3% per year in the period 2001–2018[2] and aquaculture is currently the fastest growing of the animal food-producing sectors. Currently, Southeast Asia is considered to be the hub of aquaculture due to its suitability for productive inland and coastal aquaculture; between 2015 and 2019, Southeast Asia's total production from aquaculture steadily increased by about 1.1% per year and in 2019 the region's total production from aquaculture accounted for about 54.0% of the region's total fishery production in terms of volume.[369] However, vaccination against commercially important aquaculture pathogens in Asia is rare, due possibly to the cost-effectiveness of use for farmed low-value freshwater finfish species (e.g., tilapia, rohu, common carp, hybrid and striped catfish), and also the lack of knowledge regarding epidemiology of diseases, pathogen characterisation and pathogenic mechanisms.[370] In addition, challenges exist in the implementation of the use of commercially available vaccines against commonly occurring diseases due to variations in vaccine registration processes within Asian countries.[370] In this case, the use of ‘rapid’ autogenous vaccines could potentially provide a solution, as could be the trend towards the use of efficacious and inexpensive immersion vaccines, which can facilitate mass vaccination in the field for low value species.

Existing delivery routes of the vaccine include immersion, parenteral, that is, intra-peritoneal (i.p.) and oral. Immersion vaccines, where the antigens are taken up by the skin, gills or gut, are suitable for mass vaccination of fish that are too small for parenteral vaccine. Although this method is less costly and time-consuming, uptake and efficacy, however, can vary depending on the age or size of the fish, vaccine dose and duration, adjuvant performance, temperature and so forth. The oral route, less stressful than parenteral delivery, potentially offers the best approach to fish immunisation due to its ease of administration, and can be used with both small and larger-sized fish. However, there are few commercial oral vaccines currently available due mainly to lack of efficacy and also the logistic and cost-associated challenges related to the production of the required large quantities of antigen.[371] In addition, a lack of knowledge on the impact of the stomach environment on antigen presentation is also a constraint[372] and future research should focus on increasing understanding of sites of immune induction within the intestinal tract.

Encapsulation or the incorporation of material into small capsules is an interesting approach for antigen delivery via the oral route, protecting against degradation in the stomach. Alginate particles have shown promising results for DNA plasmids, for example, chitosan for oral delivery of a DNA vaccine against *Vibrio anguillarum*[373] and *V. parahaemolyticus*,[374] both in Asian sea bass (*Lates calcarifer*); however, unknowns regarding the biological impact of nanoparticles on cell function currently causes some concern.[375,376] More recently there has been some interest in the use of plants as antigen production systems, that is, the use of microalgae,[377] whole plants or in vitro cultured plant cells/tissues, due to the advantages such as ease of scaling up, reduced production costs and good safety margins.[358,378]

Commercial vaccines are only available for bacterial or viral infections and the challenge of vaccine development against important parasites, for example, myxozoans, protozoans, crustaceans, amoebae, monogeneans and helminths, still exists. The annual global loss of juvenile fish on account of parasitic infections was estimated to vary from 107.31 to 134.14 million USD and loss of marketable size fish from 945.00 million to 9.45 billion USD, the total estimate being 1.05 billion to 9.58 billion USD,[379] and in recent years the incidence of parasitic disease outbreaks globally appears to be increasing. Indeed, global annual direct and indirect losses in salmonid aquaculture due to infestations with sea lice (*Lepeophtheirus salmonis*) has been estimated to be 500 million to 1 billion USD.[380] A greater understanding of host–parasite interaction and parasite biology and life-cycle as well as the immunobiology of pathogenic parasites is vital in order to progress. To these ends, ‘omics’ studies or the high-throughput analysis of cellular macromolecules, which include genomics, transcriptomics and proteomics, offer powerful methods for developing vaccines. Potential vaccine candidates and successful vaccines, with the possibility of the development of multivalent vaccines which offers a combination of several antigens, potentially overcome the challenge of the diverse antigenic profile of various developmental stages and strains of parasites.[381]

Phage therapy does not damage the gut microbiota or surrounding microbial communities and can safely be used within microbial environments, such as earthen ponds, as well as within more developed aquaculture operations. In the latter case, where there are solid surfaces, disrupting QS and biofilm formation by pathogenic bacteria is theoretically promising for the future, but how it can be implemented without affecting other surrounding microbial populations is unclear. However, as phage therapy and probiotics use living organisms, they are susceptible to point mutations and genetic drift, making both therapies less effective unless new phage/bacteria combinations or probiotic bacteria are identified. The existence of phage and bacterial strains, and their differences in relatively short geographical distances due to environmental variations, makes it necessary to develop solutions for each region.

Gene-editing could potentially allow more specific targeting of pathogens by manipulation of the virus and/or bacterial genomes; genetic modification of phages, bacteria and hosts may provide scientific solutions but may not be acceptable to the general public. For example, a gene from the skin of toads, magainin 1, which was inserted into the oyster (*Ostrea edulis*) genome,[382] successfully protected oysters from the protozoan pathogen *Bonamia ostreae*, however, the resistant oysters were not marketed because of perceived public antipathy.

Pro- and pre-biotics play an important role in providing resistance to disease through conferring immune benefits, improving epithelial barrier integrity and providing beneficial microbes in the host gut and surrounding environment, thus offering an alternative to the use of antibiotics. However, there is a lack of knowledge concerning the exact mechanisms of action and more information is required on host/microbe interaction in vivo. Further research is also required to identify optimal strains, doses, as well as application routes and the possibility of acquisition of genes encoding the virulence and antimicrobial drug resistance traits from pathogens to probiotics through horizontal transfer of genes is a cause for concern.[383–385]

In addition to the enhancement of biosecurity measures and improvement of water quality on farms, mathematical and statistical modelling may provide guidance for reducing the likelihood of antibiotic resistance where other solutions are not possible.[386–389] Good husbandry practices such as determining optimum stocking densities[390] and fallowing periods[391] may minimise bacterial outbreaks in aquaculture. Controls must also continue to be put in place to minimise the likelihood of the development of resistance genes. This is particularly relevant where currently such measures are difficult to implement, for example in aquaculture in some developing countries,[392] and in the ornamental fish trade, which uses prophylactic antibiotic treatment indiscriminately, and which can translocate resistance genes of human and animal importance, as well as aquatic diseases, over intercontinental distances.

Of the various alternatives to antimicrobials presented above, vaccination (Figure 3) stands out as presenting a high likelihood of being a proactive solution to disease prevention in finfish.

Major salmon aquaculture-producing countries are Norway, Chile, Canada and Scotland. The Norwegian experience of minimising antimicrobial use through effective vaccination is often cited. On the other hand, Chilean salmon aquaculture industry used 530 g of antibiotic per tonne of salmon harvested. The difference is due to the availability of vaccines against disease problems faced by Norway and Chile in salmon aquaculture. In Norway, the major disease problems are vibriosis and furunculosis against which effective vaccines are available; while in Chile, the major disease problem is due to *Piscirickettsia salmonis* against which effective vaccines are currently not available.[81,393]

We discuss in the following section lessons learnt, both from the vaccination of farmed salmon in Norway and the use of SPF seed—both are essential elements of a proactive biosecurity strategy.

4.1 | Lessons from the vaccination of farmed salmon in Norway—a case study

The Norwegian salmon industry, during the 1980s and early 1990s, was heavily affected by bacterial infections in their cultured stocks. Additionally, in the mid-1980s, Norway experienced the first-ever outbreak of a new viral disease, infectious salmon anaemia (ISA), affecting salmonids.[394,395] These problems underlined the urgent need to develop a national biosecurity programme in cooperation with national authorities, the industry and research institutions. The implemented programme throughout the early 1990s managed to alleviate the burden of these infections.

One important factor in this biosecurity programme was the development and endorsement of efficient vaccines. It is fair to say that vaccination, since the early 1990s, has been the single most important measure to control bacterial diseases in the Norwegian salmonid industry. The introduction of efficient vaccines against furunculosis and *Vibrio* infections, especially cold-water vibriosis, dramatically reduced the use of antibiotics on farms. From a total use of almost 50 metric tonnes (MT) and a production of 200,000 MT in the early 90s, the annual use of antibiotics prescribed for salmonids in the Norwegian salmon industry since 1996 has varied between 500 kg and 1500 kg.[7]

The Norwegian Food Safety Authority has the mandate to enforce vaccination as a tool to control an infection in special situations, as well as to illegalize vaccination against specific infections as vaccines may hide a true infection situation. In Norway, the production of juvenile Atlantic salmon (*Salmo salar*) for grow-out in seawater was approximately 400 million in 2019 and the industry routinely vaccinates all smolts against one or more pathogenic agents prior to sea transfer, according to the various needs of the salmon producing companies in relation to the geographical and epidemiological situation. According to the Norwegian Medicines Agency (NoMA), Norway has 19 vaccines approved for salmonids (*S. salar* and *O. mykiss*)[396] against bacterial and viral infections, from multivalent seven-antigen component vaccines to vaccines consisting of just one antigen. Most vaccines consist of inactivated agents combined with an adjuvant for intraperitoneal administration (Tables 2 and 3). Recently, one DNA vaccine has been made commercially available.[116] Vaccination is

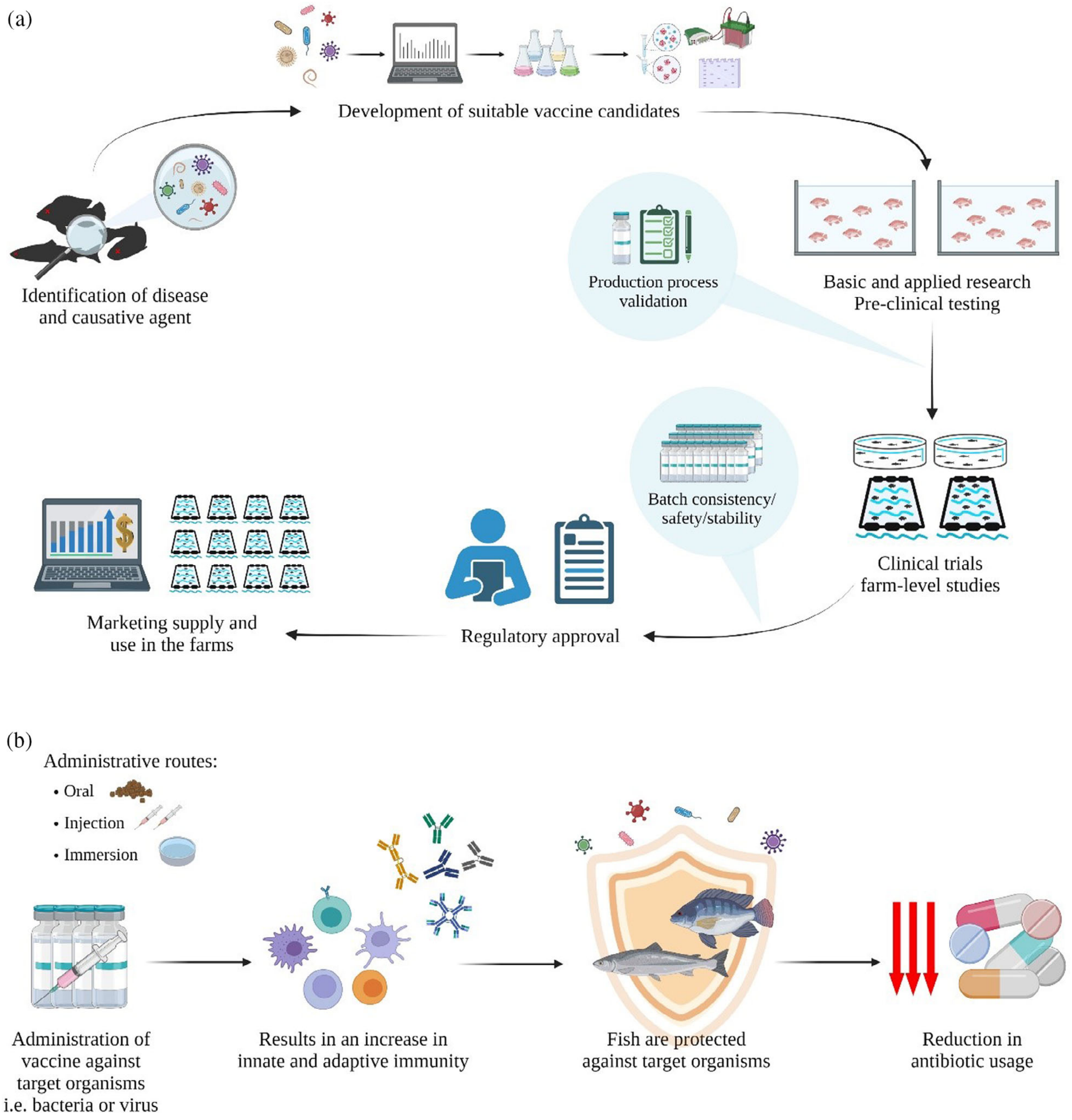

FIGURE 3 Vaccination is a key tool to ensure sustainable aquaculture production. (a) Vaccine developmental stages from identification of disease and causative agent to research, production process validation, clinical trials and farm-level studies, to regulatory approval, marketing and application. (b) Vaccine provides protection against target organisms through increasing innate and adaptive immunity leading to reduction in antibiotic usage

routinely carried out by injection according to strict vaccination and quality protocols. Generally, more than 400 degree-days is required to develop a proper immune response, implying the vaccination should occur at the latest 6–10 weeks prior to sea transfer, depending on the water temperature in the hatchery. Juveniles are vaccinated at a size greater than 20 g in order to produce immunocompetency.

Salmon production began in Norway in the late 1960s as a diversification of small-scale farmers supported by the government, with little or no regulation.[396] In 1973, the first law on concessions in salmon aquaculture was introduced, with permissions required to set up a fish farm,[397] and, in 1985, the first specific aquaculture-related law was issued. In the late 1980s and early 1990s, the industry experienced great challenges due to furunculosis, vibriosis and cold water vibriosis causing high consumption of antibiotics. Based on the Norwegian aquaculture law from –85 and the availability of efficient vaccines, biosecurity measures were implemented in combination with compulsory vaccination against these three bacterial infections.

These measures created a dramatic reduction in disease occurrence and antibiotic use. When implementing EU directive 66/88 in Norway in 2009/2010, the vaccination mandate was lifted as the three bacterial diseases were no longer listed. However, the industry continued the vaccination routines on a voluntary basis.

In 2005, key environmental issues were addressed, with new regulations focussing on the sustainable production and growth of an already significant and environmentally impactful industry, which included goals to reduce the impact of disease on cultured stock. Indeed, the Norwegian salmon farming has seen exponential growth over the last 50 years, and is continuing to grow; it has historically and continues to rank first among the major global salmonid producers, accounting for 1.49 million MT live weight in 2020[2] and constitutes almost 71% of total seafood export value from Norway in 2021, thus by far surpassing the traditional fisheries (Norsk sjømat 2022; https://nokkeltall.seafood.no or https://en.seafood.no/).

4.2 | The importance of SPF seed—A case study

Specific pathogen-free (SPF) animals refer to stocks coming from a population that have (1) tested negative for specific pathogens for at least two consecutive years; (2) been raised in high biosecurity facilities under stringent biosecurity measures; (3) been fed with biosecure feeds; and have (4) a surveillance program in place, including testing with molecular and histopathological methods.[396] Reducing the impact of diseases must begin at the origin of the production line, with the use of pathogen-free seed or fry. Vertical transmission of pathogens, occurring through infected eggs, milk or gonadal fluids, is a common and very efficient pathway. The use of healthy broodstock is essential to produce clean seed and avoid the spread of diseases as disinfection of eggs is not always possible and vertically transmitted pathogens may spread to fry if infected broodstock are used.

The strategy used in aquaculture was adapted from the SPF strategy developed in the 1950s for the poultry industry, upon the realisation that poultry research was dependent on the use of animals that were free of diseases. The value of SPF stocks was subsequently proven valuable also for industrial-scale production. SPF has shown to be fundamental for selection and expression of genetic gains and laboratory-based studies, such as disease challenges and other nutritional and biochemical studies. In an aquaculture context, SPF status is part of a biosecurity strategy to prevent the introduction of infected animals into the production system.[396] Even if the same level of biosecurity cannot be maintained during the grow-out phase, using SPF fry will decrease the chances of infection and hence reduce the prevalence and the impact of diseases. It should be understood that SPF only refers to the health status of the stocks, not their degree of tolerance or resistance to a particular disease.

One of the arguments against the development and use of SPF broodstock is the high investment and maintenance costs involved. It is in fact a centralised investment, for example, requiring a high technical level of staff, know-how, facilities and so forth and should be considered a relatively small financial outlay when compared to the very significant and widespread cost of disease impacts. For shrimp diseases alone, a recent study[379] estimated the economic losses in Thailand due to acute hepatopancreatic necrosis disease during the period 2010–2016 at USD 7.38 billion, with a further USD 4.2 billion in lost exports. Furthermore, losses in Thailand due to *Enterocytozoon hepatopenaei* could be up to USD 180 million per year. According to the China Fisheries Statistical Yearbook, in 2018, disease outbreaks affecting Chinese aquaculture resulted in a direct production loss of 205,000 MT, worth USD 401 million (National Bureau of Statistics of China, 2018). These two pathogens were introduced into the aquaculture industry through the feeding of infected fresh/live feeds to broodstock, therefore breaching the conditions of SPF status of the animals (to be fed with biosecure feeds). The 2018 Census of Aquaculture survey conducted by the United States Department of Agriculture reported diseases as the leading cause of production losses on farms.[397] The use of SPF stocks not only reduces the impact of diseases, but at the same time reduces the use of antimicrobials; as healthier animals are stocked and raised, fewer disease events are faced by the farmer.

While the use of SPF shrimp stocks varies greatly between regions and farming practices, evidence is increasingly showing that they have reduced the introduction of pathogens and disease expression in farms and provided a means for the safe introduction of both *P. vannamei* around the world—the species of choice and the dominant species in shrimp farming[2,396] and *P. monodon*. The SPF strategy is also applied in the salmon industry and is increasingly permeating other aquaculture species.

5 | CONCLUSION

The Interagency Coordination Group (IACG) on AMR recommends that Member States support the accessibility of cost-effective alternatives to antimicrobials, particularly in low- and middle-income countries.[398] The alternatives to antibiotics that have been reviewed in this paper have great potential; some have proven benefits while others are still in the experimental stage. Nonetheless, they should be carefully considered based on factors related to the needs of the country, the aquaculture system and species, targeted pathogen, ease of administration, economics (cost–benefit), risks and public perception. Research funding should therefore be targeted to promote the development of innovative and sustainable alternatives to antimicrobial usage.

Dealing with diseases in cultured aquatic populations requires a good understanding of the environment, the host and the pathogen and their interactions,[399] in order that prevention strategies can be put in place that may reduce the need for the use of antimicrobials, especially antibiotics. Controlling the aquatic environment demands an awareness of the potential source of stressors that predispose aquatic populations to infectious diseases. Managing and optimising the varying parameters of the aquatic environment, that is, salinity, temperature, oxygen, pH, heavy metals, metabolites, eutrophication and organic loading and monitoring the entry of potential pathogens through biosecurity measures, is vital in preventing the risk of infection.

The host's immune or nutritional status, genetics and presence of concurrent infection(s) or existing lesions or wounds can all influence their susceptibility to bacterial infection. Managing and optimising the host's ability to withstand disease is critical and vaccination programmes are useful tools for the prevention and control of infection. The minimisation of husbandry stressors by good husbandry methods further enhances the innate immunity of the cultured animals. The use of immunostimulants to enhance innate immunity such as prebiotics and probiotics, phage therapy via feeds, chicken egg yolk immunoglobin (IgY) and medicinal plants and all other alternatives to antibiotics discussed in this review are all proving useful approaches. However, more research is needed on nutrition as some apparent disease resistance associated with probiotics, prebiotics and plant feeds may be due to antibacterial substances, or simply due to better nutrition improving host health. In addition, more knowledge and research are needed in order to better understand the successes and failures, cost implications, efficacy, risks, practicality (especially for smallholders), adverse effects on the farm environment and how such alternatives improve health and enhance host immunity.

Good aquaculture and biosecurity practices, including the prudent and responsible use of antibiotics and use of alternatives to antibiotics, underpins the basic actions that may reduce the likelihood of AMR. Having a biosecurity plan, part of a national strategy on health management of aquatic species, in place can reduce the introduction and spread of infectious agents into defined locations or facilities and their transmission to other areas. Other strategies include avoiding the entry of pathogens through the use of SPF seed.

To conclude, there should be provision for increased resources for research in the aquaculture sector that should focus on the health of aquatic organisms, with an emphasis on disease prevention, that should include increasing knowledge of aquatic diseases, of the efficacy and safety of veterinary medicines in different environmental conditions, of the environmental impacts of and alternatives to the use of antimicrobial agents and methodologies for active and passive surveillance on withdrawal times, effluent treatments, residues, AMU and AMR. In addition, in order to promote a better control on the use of antibiotics, their sales should be regulated and their usage managed under the supervision of trained aquatic health professional/personnel to eliminate or mitigate their impacts on the environment and on food safety.

Last but not least, we wish to emphasise the importance of addressing the AMR issue from a One Health perspective, and the central role of aquatic food systems through aquaculture as an interface between food security, the environment and human health.

AUTHOR CONTRIBUTIONS

Melba G. Bondad-Reantaso: Conceptualization; formal analysis; funding acquisition; investigation; methodology; project administration; resources; software; supervision; validation; visualization; writing – original draft; writing – review and editing. **Brett MacKinnon:** Conceptualization; data curation; formal analysis; investigation; validation; writing – original draft; writing – review and editing. **Iddya Karunasagar:** Data curation; formal analysis; validation; writing – original draft; writing – review and editing. **Sophie Fridman:** Data curation; formal analysis; investigation; methodology; validation; writing – original draft; writing – review and editing. **Victoria Alday-Sanz:** Data curation; formal analysis; investigation; validation; writing – original draft; writing – review and editing. **Edgar Brun:** Data curation; formal analysis; investigation; validation; writing – original draft; writing – review and editing. **Marc Le Groumellec:** Data curation; investigation; validation; writing – original draft; writing – review and editing. **Aihua Li:** Data curation; investigation; validation; writing – original draft; writing – review and editing. **Win Surachetpong:** Data curation; investigation; methodology; visualization; writing – review and editing. **Indrani Karunasagar:** Data curation; investigation; validation; writing – original draft; writing – review and editing. **Bin Hao:** Data curation; investigation; validation; writing – original draft; writing – review and editing. **Andrea Dallocco:** Investigation; writing – original draft; writing – review and editing. **Ruggero Urbani:** Data curation; investigation; validation; writing – original draft; writing – review and editing. **Andrea Caputo:** Data curation; writing – review and editing.

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CONFLICT OF INTEREST

None declared.

DATA AVAILABILITY STATEMENT

Date sharing not applicable to this article as not datasets were generated or analysed during the current study. Existing literature was used, as well as Open and Restricted Access papers, peer review journals, relevant reports and so forth.

ORCID

Melba G. Bondad-Reantaso https://orcid.org/0000-0002-2380-3549
Brett MacKinnon https://orcid.org/0000-0001-7475-1542
Iddya Karunasagar https://orcid.org/0000-0001-8783-1269
Sophie Fridman https://orcid.org/0000-0002-0159-0474
Victoria Alday-Sanz https://orcid.org/0000-0001-6923-2393
Marc Le Groumellec https://orcid.org/0000-0001-9842-663X
Aihua Li https://orcid.org/0000-0003-0867-9823
Win Surachetpong https://orcid.org/0000-0002-5707-3476
Indrani Karunasagar https://orcid.org/0000-0003-0690-5129
Bin Hao https://orcid.org/0000-0002-5284-7946
Andrea Dall'Occo https://orcid.org/0000-0003-1334-8092
Ruggero Urbani https://orcid.org/0000-0002-7053-6244
Andrea Caputo https://orcid.org/0000-0002-7353-9168

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