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Perspectives on phage therapy for health management in aquaculture

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Abstract

Though biosecurity and best management practices (BMPs), vaccines, immunostimulants, probiotics and prebiotics are often used for health management in aquaculture, the use of chemotherapeutics/antibiotics often becomes the preferred method of choice once the infectious disease outbreak occurs. Phage-based control of bacterial pathogens (phage therapy) has recently re-emerged as an attractive therapeutic alternative due to the global emergence of antimicrobial-resistant bacterial pathogens. Target specificity with minimal disruption of natural microbiota, auto-dosing, safety, no production of toxic metabolites/residues and relatively inexpensive production are some of the distinct advantages of phages over antibiotics. In vivo, experimental studies have demonstrated the efficacy of phage therapy through immersion, oral, injection, topical application, and anal intubation routes against *Aeromonas hydrophila*, *A. salmonicida*, *Edwardsiella tarda*, *Flavobacterium columnare*, *Pseudomonas aeruginosa*, *Streptococcus iniae*, *Vibrio anguillarum*, *V. harveyi*, *V. parahaemolyticus*, etc. in aquaculture. Several factors such as phage selection, therapeutic dose, age of fish, specific targeting of the pathogen, disease condition of fish, environmental conditions, and administration route influence the efficacy of phage therapy in aquaculture. The application of a mixture of phages (phage cocktail) has also been suggested to overcome the narrow host range of phages and the development of phage resistance in bacteria. At present, a few commercial phage cocktail-based products are also available for control of *Yersinia ruckeri*, *Aeromonas* spp., *Pseudomonas* spp., and shrimp pathogenic vibrios in aquaculture. To successfully implement phage therapy, there is a need to develop region-specific/localized phage repositories with associated safety and efficacy data which could be used to quickly formulate effective phage cocktails after the identification of pathogenic bacterial strains in specific aquaculture areas.

Keywords Aquaculture · Disease outbreaks · Bacterial pathogens · Antimicrobial resistance · Phage therapy

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Introduction

Access to safe, nutritious, and affordable food for all people contributes immensely to the economic and political stability of the world. Food security is the most important step toward improving a country's socio-economic condition and combating hunger. The global human population is increasing at the rate of 75 million people per year, and it is expected to reach 9.2 billion by 2050 (Bongaarts 2009). As underdeveloped countries are still dealing with food and nutritional security issues, human nutrition inadequacies place a premium on the inclusion of animal protein in one's daily diet. The fisheries sector provides a large proportion of animal protein to people's diets all over the world. The aquatic animals are highly nutritious, and relatively cheaper protein sources, which supplement the human diet by providing essential amino acids, lipids, vitamins, minerals, and micronutrients. Total fisheries and aquaculture production reached a record high of 214 million tons worth US$ 424 billion in 2020, comprising 178 million tons of aquatic animals and 36 million tons of algae (FAO 2022). Out of 178 million tons aquatic animals, 89% were for human consumption, whereas the remaining were for non-food uses. Though still a major contributor to overall fish production, global capture fisheries production has been almost stagnant over the last decade. As the wild capture fisheries stocks are overexploited and declining, the only option is to produce more and more food from aquaculture to supplement the nutritional deficiencies of the masses in developing countries. With 87.5 million tons of aquatic animals worth about US$ 264.8 billion, the contribution of aquaculture to the total global fish production has rapidly increased from 7% in 1974 to 49.2% in 2020 (FAO 2022).

The increase in aquaculture production has not been without the challenges of serious disease outbreaks threatening the growth and sustainability of the fisheries sector. Frequent disease outbreaks are the major constraints for the growth of the aquaculture industry, which in turn impact the socio-economic development of many countries around the globe. Infectious diseases in aquaculture are often caused by bacteria, viruses, protists, helminths, fungi, and oomycetes. A big concern in aquaculture is disease outbreaks due to indigenous and non-indigenous pathogenic bacteria, particularly the multi-drug resistant ones. Globally, bacterial diseases account for > 20% of total disease outbreaks in the aquaculture sector (Karunasagar et al. 2003 ; Karunasagar and Ababouch 2012 ; Rodger 2016 ; Mishra et al. 2017). Frequent outbreaks of bacterial diseases not only lead to economic losses for the farmer but may also result in serious health consequences for the consumers, as many of these aquatic pathogens may also cause food-borne infections (Igbinosa 2016).

The need to develop strategies for the successful control of disease in aquaculture is constantly increasing, not only to improve animal welfare and increase production but also to benefit local, national, and global economies. Antibiotics are often the first port of call for the treatment of infectious diseases. However, continued use/misuse of antibiotics has resulted in the occurrence of antibiotic residues in fishery products and antimicrobial resistance in bacterial pathogens (Okocha et al. 2018). Therefore, we must be able to develop alternative therapeutic strategies for combating bacterial infections causing significant mortalities in aquaculture. The development of such techniques would benefit aquaculture, and improve food security across the globe. One such promising alternative is the use of bacterial viruses, known as bacteriophages (phages), which can infect and kill the host bacteria. Though control of bacterial pathogens by lytic phages (phage therapy) has been around for almost a century, the recent emergence of antibiotic-resistant bacteria has led to renewed interest in phage research (Lin et al. 2017). In this review, we have

summarized the currently available disease prophylactic and therapeutic strategies while extensively focusing on the use of bacteriophages (phages) for the control of bacterial pathogens (phage therapy) in aquaculture. Besides highlighting the advantages of phage therapy, we have covered the current status of phage therapy in aquaculture from laboratory research, field trials and product commercialization perspectives. Finally, we have outlined the present challenges of phage therapy in aquaculture and its future prospects.

Health Management in Aquaculture

Several disease prophylactic and therapeutic strategies are used in aquaculture.

Biosecurity and Best Management Practices

As "prevention is better than cure" is the basic underlying concept of any health management strategy, the role of biosecurity measures in aquaculture becomes very important. Farm-level best management practices (BMPs) and biosecurity measures include maintenance of ideal culture conditions, regular use of disinfectants, strict quarantine protocols, usage of certified/disease-free seed, traffic control, sanitation of hands and equipment before and after use and in between the uses, usage of clean feed and appropriate disposal of carcasses (Assefa and Abunna 2018). These practices reduce the stress on animals, thereby making them less susceptible to diseases (Scarfe and Palić 2020).

Vaccines

Vaccination is used to boost the host's immunity against a particular disease or a set of diseases. Vaccines operate by exposing an animal's immune system to an "antigen" (a portion of a pathogen or the full pathogen) and giving it time to establish a defense and a "memory/booster" that will speed up this defense during subsequent infection by the same pathogen. Normally, vaccines are given to healthy animals before disease epidemics (Sommerset et al. 2005). In 1976, the first vaccine in fish was used against enteric red mouth disease. Several types of vaccines such as inactivated/killed vaccines, attenuated vaccines, recombinant vaccines, synthetic peptide vaccines, DNA vaccines, and mucosal vaccines have been tested against various fish pathogens (Mondal and Thomas 2022). At present, 26 licensed vaccines are commercially available against fish pathogens for use in aquaculture. Most of these vaccines are formalin-inactivated vaccines made from the whole cell of pathogenic strains (Ma et al. 2019). The development of vaccines necessitates a thorough knowledge of the host, pathogen, and their interactions. Additionally, extensive financial and scientific efforts are needed for vaccine development (Sommerset et al. 2005 ; Collins et al. 2019). As reviewed previously, several factors like stress, age, sex, pre-existing diseases, degree of immune response, types of adjuvants, routes of administration, and dose can also influence the vaccine efficacy (Khati and Anita 2015 ; Shirajum Monir et al. 2020).

Immunostimulants

Immunostimulants are also regularly used in fish and shellfish farming. Immunostimulants could be prepared from a variety of biological and synthetic compounds.

Immunostimulants activate and amplify the innate and/or specific immune response by directly interacting with and stimulating the immune system cells directly, thereby boosting the body's natural defensive system and resistance to disease. They primarily enhance the activity of phagocytes. There is no production of memory components, and the immunological reaction lasts for a short time only (Wang et al. 2017a, 2017b). Compounds like peptidoglycan, chitin, chitosan, levamisole, lentinan, schizophyllan, muramyl dipeptide, oligosaccharides and glucan, vitamin, and yeast combinations as well as various animal and plant-derived products have been used as immunostimulants for the disease prevention. In fish and shrimp farming, immunostimulants have been used to successfully control pathogenic bacteria such as *Aeromonas salmonicida*, *A. hydrophila*, *Vibrio anguillarum*, *V. vulnificus*, *V. salmonicida*, *Yersinia ruckeri*, and *Streptococcus* spp., as well as the viruses that cause infectious diseases like hematopoietic necrosis, yellow head virus, and viral hemorrhage. Immunostimulants typically improve certain aspects of the non-specific immune response; although, this does not always result in a higher survival rate. Additionally, immunostimulants, administered in excessive doses or for an extended period, may have immunosuppressive effects (Mohan et al. 2019).

Probiotics and Prebiotics

Probiotics are live microbial feed supplements, which are used as biological disease control agents in aquaculture. After administration, these non-pathogenic microbes multiply, support the growth of natural microbiota and maintain the microbial equilibrium in the hosts (Martínez Cruz et al. 2012). Besides, probiotics also compete with pathogens for space and nutrients, resulting in the competitive exclusion of pathogens. Recognition of conserved microbe-associated molecular patterns (MAMPs) of probiotic bacteria by the host cells also stimulates the immune response in terms of respiratory burst, phagocytic activity, complement activity, cytokine production, serum lysozyme activity, serum protein content, and antibody production. Besides, probiotics also produce several beneficial compounds which either have antimicrobial properties or help in maintaining intestinal and immune homeostasis (Hoseinifar et al. 2018). Besides the commonly used genera *Lactobacillus* and *Bacillus*, bacteria belonging to genera *Enterococcus*, *Bifidobacterium*, *Alteromonas*, *Flavobacterium*, *Pseudomonas*, *Clostridium*, *Streptomyces*, and *Cytophaga* are also used as probiotics in the aquaculture (Sahu et al. 2008). The immunomodulatory effect of probiotics depends on various factors like source, type, dose, and duration of supplementation of probiotics (Nayak 2010).

Prebiotics are non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria (Gibson and Roberfroid 1995). Prebiotics are selectively fermented by Bifidobacteria, *Lactobacillus*, and Bacteroides. However, there is no universal prebiotic which can be fermented by all the members of gut microbiota. The ability of a bacterium to ferment specific prebiotic depends on the presence of required enzymes (Wee et al. 2022). Some of the prebiotics widely used in animal feeds include inulin and oligofructose, trans-galactooligosaccharides (TOS), lactulose, isomaltose oligosaccharides (IMO), lactosucrose, xylo-oligosaccharides (XOS), soyabean oligosaccharides, and gluco-oligosaccharides (GOS). Prebiotics have been reported to have numerous beneficial effects in fish, such as increased disease resistance (Mohammadi et al. 2022) and improved nutrient availability (Lu et al. 2019). Often probiotics and prebiotics are administered together as synbiotics to enhance their potential benefits for the host (Huynh et al. 2017).

Antibiotics

Antibiotics have been important medicines for the safety and well-being of humans and animals. Antibiotics are often used in food animals and aquaculture, and their application can be categorized as therapeutic, prophylactic, or metaphylactic. Besides, antibiotics are often used as growth-promoting agents during the production of food animals. Due to a lack of proper surveillance and monitoring systems, the exact amount of antimicrobials used worldwide is very difficult to estimate. However, estimates of antimicrobial usage in aquaculture are available. In the year 2017, 10,259 t (3163–44,727 t at 95% uncertainty interval) of antimicrobials were estimated to be used in the aquaculture sector across the globe. Majority (93.8%) of these antimicrobials were used in Asia-Pacific region with China (57.9%), India (11.3%), Indonesia (8.6%), and Vietnam (5%) accounting for the largest share. Besides, global antimicrobial consumption was estimated to increase by 33% to 13,600 tons (4193–59,295 t at 95% uncertainty interval) by the year 2030 with China (55.9%), India (11.3%), Indonesia (10.1%), and Vietnam (5.2%) again expected to emerge the largest consumers (Schar et al. 2020).

Indiscriminate use of antibiotics, without following the recommended dosage regimes and withdrawal periods, has accelerated the global emergence of antimicrobial resistance (AMR) in bacterial pathogens. The presence of AMR pathogens in various aquaculture systems has been reported across the globe (Tyagi et al. 2019 ; Reverter et al. 2020 ; Schar et al. 2021). A total of 511 research studies from 61 countries have investigated the antibiotics usage and AMR bacteria in various aquaculture environments from 1996 to 2021(Caputo et al. 2023). A systematic review of research studies conducted between the years 2000 and 2018 in Asia revealed that 33% of tested antimicrobial compounds had >50% resistance rates. Almost half of these studies were conducted on *Vibrio* spp. and *Aeromonas* spp. (Schar et al. 2021). These studies indicate the rising trends of AMR in aquaculture.

Bacteriophages

Bacteriophages, also known as phages, are viruses that infect bacteria. About 10^{31} numbers of phages, belonging to 10^{6} different species, are the most prevalent biological organisms in the biosphere. By eliminating nearly half of the bacterial population once every 48 hours, phages play a significant part in bacterial control in nature (Brussow and Hendrix 2002). The high selectivity/specificity of phages permits the targeting of specific bacteria, without affecting the desirable bacterial flora. Depending upon the life cycle, phages can be categorized as either lytic or lysogenic. After entry into the host cell, the genome of lytic phage quickly takes over the bacterial machinery to rapidly synthesize phage components. This is followed by the assembly of mature phage progenies, lysis of host bacterial cell and subsequent release of phage particles into the environment, which can go on to infect other bacterial cells. On the other hand, the genome of lysogenic or temperate phages either integrates into a host cellular replicon, such as the chromosome, a plasmid, or another phage genome or exists as a self-replicating plasmid. At this stage, the phage genome is termed a prophage. It replicates along with the host and may remain in the lysogenic life cycle for thousands of generations (Williamson et al. 2001; Clokie et al. 2011). Though discovered almost a century back, lytic phages have now emerged as attractive therapeutic alternatives due to better

understanding of phage molecular biology, and due to the rapid emergence of antibiotic resistance bacteria.

Several excellent articles and reviews cover general phage properties, biology, life cycle, morphology, and taxonomy (Brussow and Hendrix 2002 ; Nakai and Park 2002 ; Novik et al. 2017 ; Ofir and Sorek 2018 ; Kortright et al. 2019 ; Ramos-Vivas et al. 2021). To avoid redundancy, these aspects have not been covered in the present review.

Advantages of Phages over Antibiotics

Application of phage therapy has several distinct advantages over antibiotics (Fig. 1).

Specificity and Minimum Disruption of Natural Microbiota

As phages are host specific with activity against particular bacterial species, phage therapy can be applied directly to control the targeted pathogenic bacteria. Rarely, does the phage have the capacity to infect more than one closely related bacterial genus; whereas, antibiotic treatment creates collateral damage as it disrupts the microbiome (Yoon and Yoon 2018). Phage therapy is free from the short-term and long-term consequences of antibiotic-associated modification of the microbiota.

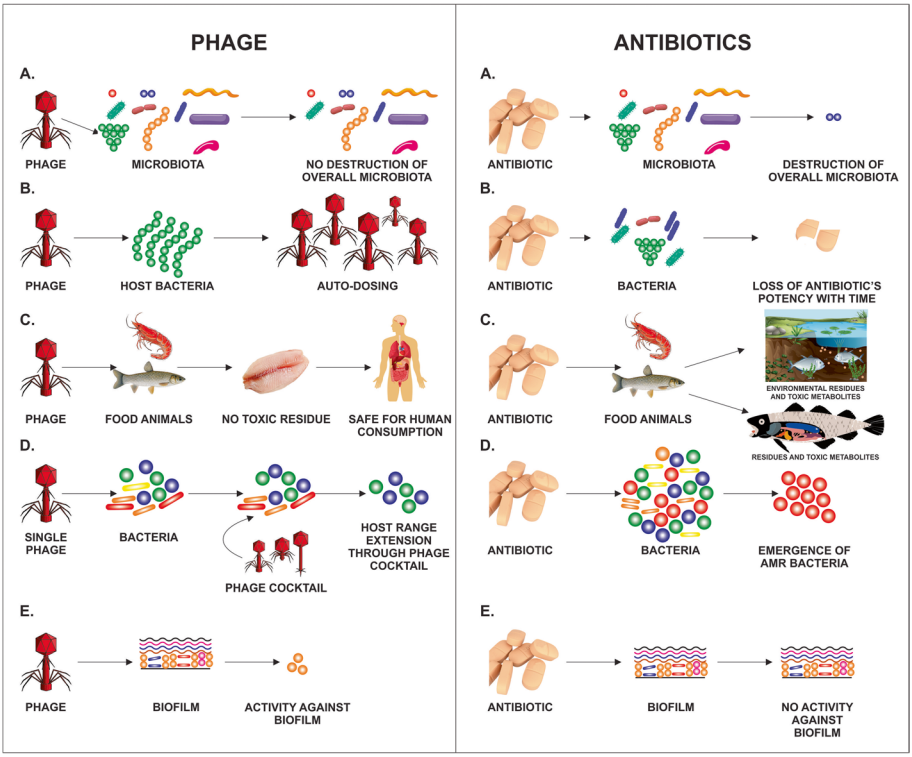

Fig. 1 Advantages of phage therapy over antibiotics

Auto-dosing and Self-limitation

As lytic phages use the host for their propagation, they increase in number when host bacteria are present. During therapeutic phage application, the presence of pathogenic host bacterium creates a unique auto-dosing effect. Due to auto-dosing, low-phage doses can be applied. Reduction of host bacterium during treatment also limits the phage propagation resulting in self-limitation of the phage population (Loc-Carrillo and Abedon 2011).

Safety

Phage are significantly safer and better tolerated, as they replicate only in the target bacterium, and cannot infect mammalian cells (Kaźmierczak and Dąbrowska 2018). However, phages may also sometimes carry potentially virulence-related or antibiotic resistance genes resulting in the phage-mediated transformation of non-pathogenic bacteria to potentially pathogenic ones. Besides, some lysogenic phages also contain genes for the integration of the phage genome into the host bacterial genome. Thus, genomes of the candidate phages should be thoroughly investigated for the presence of any harmful gene before therapeutic applications (Goodridge and Bisha 2011). With the arrival of next-generation sequencing and computational tools, it has now become possible to sequence, assemble and annotate large numbers of phage genomes at rapidly reducing costs.

No Production of Toxic Metabolites or Environmental Residues

As the phages are made up of only proteins and nucleic acids, their eventual breakdown products consist exclusively of amino acids and nucleic acids. On the other hand, toxic or carcinogenic metabolites might be produced from antibiotics. Thus, the presence of antibiotics and their metabolites in food animals and the environment is a big concern (Hassan et al. 2013).

Antibiofilm Activity

Compared to antibiotics, phages have a greater ability to destroy biofilms (Gutierrez et al. 2016). Biofilms have open structures with water-filled channels that facilitate phage access inside the biofilm. Besides, certain phages are capable of producing polysaccharide depolymerases that disrupt the biofilms (Vukotic et al. 2020)..

Reduced Potential for the Development of Resistance

Compared to antibiotics, the development of phage resistance by bacteria is less recurrent. Resistance against phages develops about ten times slower than antibiotic resistance (Schmelcher and Loessner 2014). Moreover, phages can evolve and thereby overcome bacterial resistance. Selection will favor mutant phages that are capable of infecting resistant bacteria. Besides, resistance could also cost bacteria their fitness, thereby weakening their ability to compete with their phage-sensitive counterparts (Bohannan and Lenski 2000 ; Koskella and Brockhurst 2014). Phage resistance can also impact virulence, as the receptors used by phages to attack bacteria might be virulence determinants as well (Levin and Bull 2004).

Inexpensive to Produce with Rapid Commercialization Potential

In contrast to antibiotics, phages can be easily produced on a large scale with a low cost of commercialization (Loc-Carrillo and Abedon 2011). There is a huge diversity of phages in nature. Wherever there is a host bacterium, a phage can be expected. In contrast to synthetic antibiotics, which are screened and discovered from the library of chemically altered compounds, the natural phage diversity in nature allows quick selection and characterization of pathogen-specific candidate phages. As antibiotics and their metabolites may have potentially harmful side effects, strict safety and regulatory guidelines are in place for their development and commercialization. It takes an average of 13–14 years with an investment of > 100–500 million euros from antibiotics discovery to its commercialization (Miethke et al. 2021).

Application and Formulation Versatility

Similar to antibiotics, versatile phage formulations in the forms of creams, liquids, and microencapsulated dry powders can be developed. Once developed, these formulations can be administered topically, intravenously, orally or through nebulization/inhalation. Phages targeting different species or strains of bacteria can be combined in the form of a cocktail to broaden their antibacterial activity spectrum. Besides, phages can also be combined with antibiotics to enhance their activity spectrum (Loc-Carrillo and Abedon 2011).

Phage Therapy in Aquaculture

The high incidences of disease in aquaculture, combined with concerns of antibiotic resistance and antibiotic residues, has led to a large amount of research into the potential of using phage therapy to control bacterial pathogens in aquaculture. Aquaculture farms are essentially artificial microcosms of the aquatic environment, which is naturally teaming with phages. Though conditions that are conducive to/for the growth of aquaculture species and their bacterial pathogens also appear to be conducive to the survival of phages, environmental variables may affect the therapeutic efficacy of phages in aquaculture farms. In a controlled experimental study, the variation of physicochemical parameters (pH, temperature, salinity, and organic matter) within the naturally expected range in aquaculture farms did not significantly affect phage survival. However, salinity and organic matter significantly affected the therapeutic efficacy of phage (Silva et al. 2014a, 2014b). Besides, outdoor aquaculture facilities are exposed to solar radiation which can cause decay in phage viability (Wommack et al. 1996 ; Gomes et al. 2022).

The first report of phage-based biocontrol of fish pathogens, dating back to 1981, demonstrated the efficacy of bacteriophage AH1 against *A. hydrophila* infection in Japanese weather loach (*Misgurnus anguillicaudatus*) under laboratory conditions (Wu et al. 1981). Another landmark study, leading to the development of field-level experiments on phage therapy, demonstrated the protective efficiency of phages against *Lactococcus garvieae* infection in yellowtail (*Seriola quinqueradiata*) (Nakai et al. 1999). These researchers reported that the survival rate was much higher in the yellowtail that received intraperitoneal injection of the phage after intraperitoneal challenge with *L. garvieae*, compared with that of control infected fish without phage injection (Nakai et al. 1999). Richards (2014)

reviewed the the success of phage therapy against other fish pathogenic bacteria such as *A. salmonicida*, *Edwardsiella tarda*, *Flavobacterium columnare*, *Pseudomonas aeruginosa*, *Streptococcus iniae*, *V. harveyi*, *V. parahaemolyticus*, etc. (Richards 2014).

Even after proper characterization and selection of therapeutic phages, the route of administration remains a major challenge for phage therapy in aquaculture. In the scientific literature, various phage administration methods in aquaculture have been reported: immersion, feed incorporation, intramuscular or intraperitoneal injection administration, anal intubation, topical application and direct release of phages in the culture system. Whatever method is used, the phage particles must make contact with the bacterial host, whether in water, on the surface, or inside fish (Culot et al. 2019 ; Ramos-Vivas et al. 2021). Furthermore, the combined application of two or more phages (phage cocktails) is opening new therapeutic avenues. Besides, applications of phages with other therapeutics such as antibiotics and lysozymes have also been reported (Ryan et al. 2012 ; Choudhury et al. 2019). Table 1 provides the comprehensive status of phage therapy in aquaculture from the initial landmark studies to the most recent ones. Details of the pathogen, farmed species, phages, mode of applications and significant outcomes of field trials have also been covered.

Challenges of Phage Therapy

Presence of Lysogeny, Virulence-Related, and Antibiotic Resistance-Related Genes in Phage Genomes

Sometimes, phage may also carry potentially virulent or antibiotic resistance genes resulting in the phage-mediated transformation of non-pathogenic bacteria to potentially pathogenic ones. Besides, some lysogenic phages also contain genes for the integration of their phage genome into the host bacterial genome. Thus, phage genomes should be thoroughly investigated for the presence of any harmful gene before their applications for phage therapy (Goodridge and Bisha 2011).

Development of Phage Resistance in Bacteria

As in the case of antibiotics, bacteria can develop resistance to phage, which may hamper the effectiveness of phage therapy. The first step of phage infection to bacteria is the adhesion of phage on the bacterial surface by binding to specific receptors. If the bacteria loose these specific receptors, they become resistant to phage. Bacteria may also horizontally acquire a restriction-modification system that degrades the nucleic acid of an injected phage. In addition, phage resistance may also be caused by a mutation in a gene, the product of which is essential for phage replication or assembly. The development of phage cocktails (a mixture of two or more phages) has been suggested to overcome these challenges (Carlton 1999).

Specificity and Narrow Host Range

Phage specifically infects the host bacterium. Though specificity is an important characteristic of phage therapy as it allows the targeting of specific bacterial pathogens without affecting the normal beneficial microbiota of the host, the highly specific nature of phage

Table 1 Overview of phage therapeutic studies in various aquatic animals

Farmed species	Pathogen	Mode of pathogen challenge	Phage used	Phage cocktail	Phage application route	Significant outcomes of therapeutic trials	Reference
Misgurnus anguillicaudatus (Loach)	*Aeromonas hydrophila*	Injection	AH1	No	Injection	Injection of 3-h-old *A. hydrophila* infected with phage at the multiplicity of infection (MOI) of 0.01, 0.1 and 1.0 did not cause any mortality in loach. In contrast, 60–65% mortalities in control groups injected with phage-free *A. hydrophila.*	Wu et al. (1981)

Table 1 (continued)

Farmed species	Pathogen	Mode of pathogen challenge	Phage used	Phage cocktail	Phage application route	Significant outcomes of therapeutic trials	Reference
M. anguillicaudatus	*Edwardsiella tarda*	Immersion (short duration bath)	ϕET-1	No	Immersion (short duration bath)	*E. tarda* infected with phage at MOI of 0.1 and incubated for 8 h. Afterwards, loaches were immersed in phage and host mixture for 1 h. Fish survival was 90% after 4 days. Only 5% survival in the control loach group immersed in 2 h incubated phage and *E. tarda* mixture.	Wu and Chao (1982)

Table 1 (continued)

Farmed species	Pathogen	Mode of pathogen challenge	Phage used	Phage cocktail	Phage application route	Significant outcomes of therapeutic trials	Reference
Seriola quinqueradiata (yellowtail)	*Lactococcus garvieae*	Injection, anal intubation	PlgY-16	No	Injection, oral (phage-impregnated feed)	Intraperitoneal injection of phage after 1 h of intraperitoneal injection with *L. garvieae*, improved the survival to 90% in comparison to 45% survival in control. Only 10% mortality in fish fed with phage-impregnated with feed and challenged 1 h later with *L. garvieae* by anal intubation method. In contrast, 65% mortality in infected phage-untreated control.	Nakai et al. (1999)

Table 1 (continued)

Farmed species	Pathogen	Mode of pathogen challenge	Phage used	Phage cocktail	Phage application route	Significant outcomes of therapeutic trials	Reference
Plecoglossus altivelis (Ayu fish)	*Pseudomonas plecoglossicida*	Oral feed-based	PPpW-3, PPpW-4	Yes	Oral (phage-impregnated feed)	Oral administration of phage-impregnated feed 15 min after challenge with *P. Plecoglossicida*-impregnated feed resulted in reduced (22.5%) mortality in comparison to 65% mortality in infected control receiving not phage.	Park et al. (2000)

Table 1 (continued)

Farmed species	Pathogen	Mode of pathogen challenge	Phage used	Phage cocktail	Phage application route	Significant outcomes of therapeutic trials	Reference
P. altivelis	*P. plecoglossicida*	Oral feed-based	PPpW-3, PPpW-4	Yes	Oral (phage-impregnated feed)	Oral administration of phage PPpW-3, PPpW-4, PPpW-3/W4-impregnated feeds immediately after challenge with *P. Plecoglossicida*-impregnated feed resulted in 53.3, 40 and 20% mortalities, respectively. In contrast, 93.3% mortality in the infected control group receiving no phage treatment.	Park and Nakai (2003)
Penaeus monodon (shrimp postlarvae)	*Vibrio harveyi*	Immersion (direct addition to the culture water)	*Vibrio* phage	No	Immersion (direct addition to the culture water)	Addition of phage in larval tanks for 17 days in shrimp hatchery resulted in 86% larval survival in comparison 17% survival in control tanks receiving no phage treatment.	Vinod et al. (2006)

Table 1 (continued)

Farmed species	Pathogen	Mode of pathogen challenge	Phage used	Phage cocktail	Phage application route	Significant outcomes of therapeutic trials	Reference
Salvelinus fontalis (brook trout)	*A. salmonicida*	Immersion (direct addition to the culture water)	HER 110	No	Immersion (direct addition to the culture water)	Addition of phage at MOI of 1, 24 h after immersion challenge with *A. salmonicida*, delayed onset of furunculosis by 7 days. Besides, only 10% mortality was recorded in the phage-treated group in comparison to 100% mortality in the infected control group receiving no phage treatment.	Imbeault et al. (2006)
Paralichthys olivaceus (Japanese flounder)	*Streptococcus iniae*	Injection	PSiJ31, PSiJ32, PSiJ4, PSiJ42	Yes	Injection	Injection of two or all four phages, 1 h after *S. iniae* injection, resulted in significantly lower (50%) mortalities in comparison to 100% mortalities in infected control fish receiving no phage treatment.	Matsuoka et al. (2007)

Table 1 (continued)

Farmed species	Pathogen	Mode of pathogen challenge	Phage used	Phage cocktail	Phage application route	Significant outcomes of therapeutic trials	Reference
P. monodon (shrimp postlarvae)	Luminous vibriosis	Natural *Vibrio* population	Viha8, Viha10	Yes	Immersion (direct addition to the culture water)	Addition of phages in larval tanks resulted in 86–88% survival. The survival in antibiotic (oxytetracycline or kanamycin) treated tanks was 65–68%.	Karunasagar et al. (2007)
Clarius batrachus (Magur)	*Flavobacterium columnare*	Injection	FCP1	No	Injection, Immersion (direct addition to the culture water), Oral (phage Impregnated feed)	Phage treatment through Intramuscular injection, immersion and oral routes resulted in 100% survival in experimentally infected *C. batrachus*	Prasad et al. (2011)

Table 1 (continued)

Farmed species	Pathogen	Mode of pathogen challenge	Phage used	Phage cocktail	Phage application route	Significant outcomes of therapeutic trials	Reference
Salmo salar (Atlantic salmon), *Oncorhynchus mykiss* (rainbow trout)	*F. psychrophilum*	Injection	1H, 6H	No	Injection	Mixing of pathogenic *F. psychrophilum* strains with either 1H or 6H phage, just before injection, resulted in 18–100% mortalities reduction in Atlantic salmon. Besides, 16–67% mortalities reduction in rainbow trout under similar experimental conditions.	Castillo et al. (2012)
O. mykiss	*F. psychrophilum*	Injection	FpV-9	No	Injection	The potential of phages to spread to inner organs (kidney, spleen and brain) of rainbow trout after intraperitoneal injection.	Madsen et al. (2013)

Table 1 (continued)

Farmed species	Pathogen	Mode of pathogen challenge	Phage used	Phage cocktail	Phage application route	Significant outcomes of therapeutic trials	Reference
S. salar	*V. anguillarum*	Immersion (direct addition to the culture water)	CHOED	No	Immersion (Direct addition to the culture water)	Addition of phage to tank water immediately after the immersion challenge with *V. anguillarum* protected the fish against vibriosis in laboratory and field conditions. Hundred percent fish survival in tanks receiving phage treatments at MOIs of 1 and 20. Only 7% survival in the infected control group receiving no phage treatment.	Higuera et al. (2013)

Table 1 (continued)

Farmed species	Pathogen	Mode of pathogen challenge	Phage used	Phage cocktail	Phage application route	Significant outcomes of therapeutic trials	Reference
M. anguillicaudatus	*A. hydrophila*	Injection	pAh1-C, pAh6-C	No	Injection, oral (phage impregnated feed)	During intraperitoneal phage injection to *A. hydrophila*-challenged fish, up to 43.3% and 16.7% mortalities in phage pAh1-C and pAh6-C treated groups, respectively. During oral phage administration, up to 46.7% and 26.67% mortalities in phage pAh1-C and pAh6-C treated groups, respectively. Up to 100% mortalities in infected control groups receiving no phage treatment.	Jun et al. (2013)

Table 1 (continued)

Farmed species	Pathogen	Mode of pathogen challenge	Phage used	Phage cocktail	Phage application route	Significant outcomes of therapeutic trials	Reference
Clarias gariepinus (African catfish)	*Pseudomonas aeruginosa*	Naturally infected fish with skin lesions	*P. aeruginosa* phage	No	Topical application on skin lesions	Phage therapy effectively cured the ulcerative lesions of the infected fish in 8–10 days. Phage-treated fish showed 7-fold reduced lesions than infected control fish receiving no phage treatment.	Khairnar et al. (2013)

Table 1 (continued)

Farmed species	Pathogen	Mode of pathogen challenge	Phage used	Phage cocktail	Phage application route	Significant outcomes of therapeutic trials	Reference
Artemia franciscana (brine shrimp)	*V. parahaemolyticus*	Immersion (direct addition to the culture water)	vpms1	No	Immersion (direct addition to the culture water)	Application of a single phage dose at MOI of 45, immediately after *V. parahaemolyticus* challenge in axenic brine shrimp nauplii, resulted in 91% survival. The survival in infected but phage-untreated control was only 19.4%. Even the reduction of phage dose to MOI of 0.45 did not significantly reduce their therapeutic efficacy.	Martínez-Díaz and Hipólito-Morales (2013)

Table 1 (continued)

Farmed species	Pathogen	Mode of pathogen challenge	Phage used	Phage cocktail	Phage application route	Significant outcomes of therapeutic trials	Reference
Oyster (*Ostrea plicatula*)	*V. parahaemolyticus*	Immersion (direct addition to the culture water)	VPp1	No	Immersion (direct addition to the culture water)	After infection with *V. parahaemolyticus*, the oysters were treated with phage at MOIs of 10, 1 and 0.1. During depuration at 16 °C, the MOI of 0.1 produced the best results with a reduction of *V. parahaemolyticus* counts by 2.35–2.76 log CFU/g within 36 h.	Rong et al. (2014)

Table 1 (continued)

Farmed species	Pathogen	Mode of pathogen challenge	Phage used	Phage cocktail	Phage application route	Significant outcomes of therapeutic trials	Reference
Litopenaeus vannamei (shrimp postlarvae)	*V. parahaemolyticus*	Immersion (direct addition to the culture water)	A3S, Vpms1	No	Immersion (direct addition to the culture water)	Phage application (A3S or Vpms1) at MOIs of 10, 1 and 0.1 immediately after *V. parahaemolyticus* challenge. Even at the lowest MOI of 0.1, survival was up to 75% in phage-treated groups in comparison to 53% survival in control infected groups not receiving phage treatment.	Lomelí-Ortega and Martínez-Díaz (2014)
P. monodon, (shrimp postlarvae)	*V. harveyi*	Immersion (direct addition to the culture water)	VHP6b	No	Immersion (direct addition to the culture water)	Challenge with *V. harveyi*, 30 min after addition of phage. Shrimp postlarvae survival 40–60% higher in phages treated groups in comparison to phage-free infection controls.	Raghu Patil et al. (2014)

Table 1 (continued)

Farmed species	Pathogen	Mode of pathogen challenge	Phage used	Phage cocktail	Phage application route	Significant outcomes of therapeutic trials	Reference
Danio rerio (zebrafish)	*V. anguillarum*	Immersion (direct addition to the culture water)	VP-2	No	Immersion (direct addition to the culture water)	Mortality rates of 2±3% in *V. anguillarum* challenged phage treated groups were significantly lower than mortality rates (17±3%) in *V. anguillarum* challenged phage untreated controls.	Silva et al. (2014a, 2014b)
O. mykiss	*A. salmonicida*	Injection	PAS-1	No	Injection	After intramuscular injection with *A. salmonicida*, fish were immediately injected with phage at MOI of 10,000. In phage-treated groups 26.7% survival, whereas 100% mortalities in *A. salmonicida* infected phage untreated controls.	Kim et al. (2015)

Table 1 (continued)

Farmed species	Pathogen	Mode of pathogen challenge	Phage used	Phage cocktail	Phage application route	Significant outcomes of therapeutic trials	Reference
Solea senegalensis (Senegalese sole)	*A. salmonicida*	Immersion (direct addition to the culture water)	AS-A	No	Immersion (direct addition to the culture water)	Addition of phage at MOI of 100 immediately after immersion challenge with *A. salmonicida*. No mortality in the phage-treated group, whereas 36% mortality in the infected phage-untreated control.	Silva et al. (2016)

Table 1 (continued)

Farmed species	Pathogen	Mode of pathogen challenge	Phage used	Phage cocktail	Phage application route	Significant outcomes of therapeutic trials	Reference
Apostichopus japonicas (juvenile sea cucumber)	*V. splendidus*	Immersion (prolonged bath for 2 days)	PVS-1, PVS-2, PVS-3	Yes	Oral (phage-impregnated feed)	Compared the therapeutic efficacy of individual phage treatments, phage cocktail and antibiotics. Only 18% survival in *V. splendidus* challenged control receiving no phage treatment. Survival rate of 82% each for antibiotic and phage cocktail treatments. Survival rates in the range of 50–65% for individual phage treatments. Use of phage cocktail more efficient than individual phages.	Li et al. (2016)

Table 1 (continued)

Farmed species	Pathogen	Mode of pathogen challenge	Phage used	Phage cocktail	Phage application route	Significant outcomes of therapeutic trials	Reference
A. salina	*V. alginolyticus*	Immersion (direct addition to the culture water)	φSt2, φGrn1	Yes	Immersion (direct addition to the culture water)	Application of phage φSt2 and φGrn1 cocktail in artemia culture resulted in the decline of presumptive *Vibrio* Counts by 93% after 4 h.	Kalatzis et al. (2016)
O. niloticus	*A. hydrophila*	Immersion (direct addition to the culture water)	ΦZH_1, ΦZH_2	Yes	Immersion (direct addition to the culture water)	Application of phage cocktail to *A. hydrophila*-challenged fish resulted in lower mortality of 18% in comparison to 68% mortality in *A. hydrophila*-challenged control group receiving no phage treatment	El-Araby et al. (2016)

Table 1 (continued)

Farmed species	Pathogen	Mode of pathogen challenge	Phage used	Phage cocktail	Phage application route	Significant outcomes of therapeutic trials	Reference
Haliotis laevigata (greenlip abalone)	*V. harveyi*	Immersion (prolonged bath for 4 h)	vB_VhaS-tm	No	Immersion (prolonged bath for 2 h)	After 4 h immersion challenge with *V. harveyi*, the phage treatment through immersion was applied for 2 h. Mortalities were monitored for 7 days. Total 70% survival in *V. harveyi* challenged phage-treated groups, whereas 0% survival in challenged controls receiving no phage treatment.	Wang et al. (2017a, 2017b)
D. rerio	*A. hydrophila*	Injection	pAh-1	No	Injection	Intraperitoneal phage injection at MOI of 10 after *A. hydrophila* challenge resulted in increased survival of zebrafish up to 43.3%.	Easwaran et al. (2017)

Table 1 (continued)

Farmed species	Pathogen	Mode of pathogen challenge	Phage used	Phage cocktail	Phage application route	Significant outcomes of therapeutic trials	Reference
L. vannamei, (juvenile shrimp)	*V. parahaemolyticus*	Immersion (direct addition to the culture water)	pVp-1	No	Immersion (direct addition to the culture water), oral (phage-impregnated feed	Application of phage treatment by immersion and oral methods 1 h after *V. parahaemolyticus* challenge could not protect the shrimps against disease and mortalities. Phage application by bath and oral methods, 24, 6 and 1 h before bacterial challenge, resulted in up to 50% protection against mortalities.	Jun et al. (2018)

Table 1 (continued)

Farmed species	Pathogen	Mode of pathogen challenge	Phage used	Phage cocktail	Phage application route	Significant outcomes of therapeutic trials	Reference
O. niloticus	*S. agalactiae*	Injection	HN48	No	Injection	Immediately after *S. agalactiae* challenge, fish were injected with phage at MOI of 1. Phage treatment effectively inactivated the bacteria in the kidney. Phage-treated fish had 60% survival rates, whereas 100% mortality in the infected phage-untreated control.	Luo et al. (2018)

Table 1 (continued)

Farmed species	Pathogen	Mode of pathogen challenge	Phage used	Phage cocktail	Phage application route	Significant outcomes of therapeutic trials	Reference
Pangasianodon hypophthalmus (striped catfish)	*A. hydrophila*	Injection	Φ2 and Φ5	Yes	Injection	Immediately after *A. hydrophila* challenge, fish were injected with phage cocktail at MOIs of 0.01, 1 and 100. Up to 100% survival in phage-treated groups at MOI of 100. Only 18.3% survival in infected controls receiving no phage treatment.	Le et al. (2018)
A. japonicas	*V. parahaemolyticus*	Immersion (direct addition to the culture water)	PVP1, PVP2	Yes	Oral (phage-impregnated feed)	After 60 days of oral phage cocktail feeding, the animals were challenged with a live culture of *V. parahaemolyticus*. 50-80% survival in phage-treated groups, whereas <30% survival in infected phage-untreated controls.	Ren et al. (2019)

Table 1 (continued)

Farmed species	Pathogen	Mode of pathogen challenge	Phage used	Phage cocktail	Phage application route	Significant outcomes of therapeutic trials	Reference
L. vannamei	*Vibrio* sp.	Immersion (direct addition to the culture water)	ValLY-3, VspDsh-1, VspSw-1, VpaJT-1, ValSw4-1	Yes	Immersion (direct addition to the culture water)	Phage cocktail treatment was applied 2 days after the challenge with *Vibrio* sp. The survival rate of 91.4% in the phage cocktail-treated group, whereas survival of only 20% of infected controls receiving no phage treatment.	Chen et al. (2019)
O. mykiss	*A. hydrophila*	Injection	MJG	No	Injection, immersion (short bath of 15 min), oral (phage-impregnate feed)	Phage application by injection, immersion and oral methods resulted in relative percentage survival of 100%, 66.7%, and 50%, respectively in phage-treated groups.	Cao et al. (2020)

Table 1 (continued)

Farmed species	Pathogen	Mode of pathogen challenge	Phage used	Phage cocktail	Phage application route	Significant outcomes of therapeutic trials	Reference
P. hypophthalmus	*A. hydrophila*	Immersion (prolonged bath for 24 h)	PVN02	No	Oral (phage-impregnated feed)	Oral phage administration started one day before the bacterial challenge and continued thereafter throughout the experiment. Bacterial infectious dose and phage therapeutic dose-dependent efficacy of phage therapy. Mortalities in the phage-treated group were 8.3-16.7%, whereas up to 68.3% mortality in infected phage-untreated control.	Dang et al. (2021)

Table 1 (continued)

Farmed species	Pathogen	Mode of pathogen challenge	Phage used	Phage cocktail	Phage application route	Significant outcomes of therapeutic trials	Reference
O. niloticus	*A. hydrophila*	Immersion (direct addition to the culture water)	pAh6.2TG	No	Immersion (direct addition to the culture water)	Phage and *A. hydrophila* mixtures at MOIs of 0.1 and 1 were incubated in experimental tanks for 3 h. After 3 h, fish were added to the same tanks and maintained for 14 days. The relative percent survival of the two treatments groups was 50% (MOI=0.1) and 73.3% (MOI=1).	Dien et al. (2022)
Sparus aurata (gilthead seabream larvae)	*V. harveyi*	Immersion (direct addition to the culture water)	Vibrio phage Virtus	No	Immersion (direct addition to the culture water)	Phage application at MOI of 10 after immersion challenge with *V. harveyi*. In phage-treated groups ~50% survival, whereas only 6% survival in infected phage-untreated control.	Droubogiannis and Katharios (2022)

Table 1 (continued)

Farmed species	Pathogen	Mode of pathogen challenge	Phage used	Phage cocktail	Phage application route	Significant outcomes of therapeutic trials	Reference
P. vannamei (shrimp postlarvae)	*V. harveyi*	Immersion (direct addition to the culture water)	Vibriophage-ϕLV6	No	Immersion (direct addition to the culture water)	Vibriophage-ϕLV6 reduced post-larval mortalities and luminous *Vibrio* counts in the phage-treated tank compared to the control phage-untreated tanks.	Benala et al. (2023)

with a very narrow host range could be an issue during field applications. Before the application of phage therapy, bacteria from the infection site should be tested for their sensitivity against the phage under simulated experimental conditions. In nature, polyvalent phages with activity against several strains of the same or different species do occur, and these can be used to increase the host range spectrum during phage therapy applications. Moreover, the phage cocktail approach can also be used to overcome the host range limitations of phages. However, the desire to increase coverage by adding more members to a phage cocktail must be balanced with the challenge of producing and testing well-defined multi-component mixtures for regulatory approval (Lu and Koeris 2011).

Effect of Environmental Conditions

Several physicochemical factors (pH, temperature, ionic, and bile salt concentrations) may have a significant effect on the survival and persistence of the phages. Some phages can be stored for a long period at neutral pH (6 to 8) in solution or dried form (Jończyk et al. 2011). Phage titers generally decrease slowly with pH. The proliferation of several phages is limited when the pH is lower than 4.5. One of the solutions to protect the bacteriophage at the site of infection and during its journey to the site is microencapsulation. Microencapsulation has been applied to enhance the viability of phage bacteria during processing and passage through the gastrointestinal tract (Ma et al. 2008 ; Colom et al. 2017). When applied directly in water, UV radiations can also have a detrimental effect on the phage viability (Sinton et al. 2002).

Ability of Phage to Reach the Target Bacteria

Depending upon the environmental conditions, route of administration, as well as the structure and composition of the target matrix, the ability of phage to reach the pathogenic bacteria in various tissues during systematic infection might be significantly affected. Oral feed-based administration of phage is a preferred approach in aquaculture as other routes such as immersion, intubation, injection, and topical application are almost impractical to implement in large-scale aquaculture settings (Dang et al. 2021). Though one may think that orally administered phages cannot tolerate the harsh acidic conditions of the fish gut, recent studies have shown that orally administered phages not only survive in the fish gut but also cross the intestinal barrier to reach the kidney, spleen, and other organs of fish. In juvenile rainbow trout, orally administered *Flavobacterium psychrophilum* phage FpV-9 could be continuously detected in the intestine. Besides, phages were also found in 40–50% of the sampled spleen and kidney tissues (Christiansen et al. 2014). In another study, continuous administration of *Edwarsiella tarda* phage ETP-1, bioencapsulated in *Artemia* nauplii, over 10 days led to constant detection of phage in the gut, kidney, liver, and spleen of zebrafish from the first day onwards (Nikapitiya et al. 2020). Donati and colleagues reported the presence of orally administered *F. psychrophilum* phages in all the intestine and kidney samples of rainbow trout. Besides, phages could also be detected in 20% of spleen and 40% of brain samples. In contrast to intraperitoneally injected phages, which significantly protected fish against *F. psychrophilum* infection, orally administered phages did not offer significant protection. The authors suggested that a higher phage dose during oral administration might be necessary to protect the fish against bacterial infection (Donati et al. 2021). To develop effective phage formulations for aquaculture, each

candidate phages must be specifically evaluated for its survival and distribution in the aquaculture environment/fish organs.

Rapid Clearance of Phages from Circulation and Development of Antiphage Antibodies

One of the biggest drawbacks of phage studies is the lack of any supporting pharmacokinetic data. Being a foreign object, the phage can be rapidly cleared from the circulatory system of the animal being treated (Carlton 1999). One approach to compensate for this rapid clearance is repeated administration of phages during the treatment period. Besides, the administration of phage may result in immune system activation and the development of antiphage antibodies. The production of antiphage antibodies might not be an issue during active infection. However, during chronic infection, these antiphage antibodies may neutralize a proportion of administered phages resulting in a decreased efficiency of treatment. Though not much information regarding these antiphage antibodies is available in fish, recent studies in humans have suggested that the effect of neutralizing antiphage antibodies is not significant during phage therapy (Zaczek et al. 2016). Moreover, it has also been suggested that a higher dose of phages could be administered to compensate for the activity loss due to neutralizing antiphage antibodies (Carlton 1999).

Commercial Phage Products for Use in Aquaculture

The development of commercial phage products for aquaculture use is an active area of research. Phage products for aquaculture typically involve isolating and selecting phages that are effective against specific bacterial pathogens. These phages undergo testing to assess their efficacy, safety, and stability before being formulated into commercial products. These commercial products provide a targeted and environmentally friendly approach to disease management by specifically targeting harmful bacteria while leaving beneficial bacteria unharmed. The regulatory landscape for phage products in aquaculture varies across different countries, and it can influence the availability and commercialization of phage products in particular regions. Table 2 provides an overview of phage products currently available or under development for use in aquaculture.

Conclusions

Alternative approaches to fight infectious diseases are gaining ground in the context of antibiotic resistance in bacteria and problems with drug residues in aquatic food animals. Lytic phages are the potential alternatives to antibiotics for the control of bacterial infections in aquaculture systems. Most of the studies reviewed in this paper demonstrated the efficacy of phages for controlling bacterial pathogens in various aquatic organisms. These studies also provide a positive outlook on the future benefits of phage therapy to treat aquaculture diseases. However, almost all of these studies have been carried out under controlled laboratory conditions. To better understand the effectiveness of phage therapies, research trials must be carried out under conditions that mimic the actual situations in field aquaculture settings. Before applying phage therapy against pathogenic bacteria, one must confirm the lytic activity of phages against prevalent strains of a pathogen in a particular aquaculture

Table 2 Phage-based products currently available or under-development for use in aquaculture

Product name	Targeted pathogen	Product details	Company	Availability status
CUSTUS®$_{YRS}$	*Yersinia ruckeri*	A preparation of bacteriophages with the capability to kill 99.99% of *Y. ruckeri*	ACD Pharma Lofoten Bio Centre, Storeidøya 60, N-8370 Leknes	Available in Norway
BAFADOR®	*Aeromonas* spp., *Pseudomonas* spp.	A cocktail of 5 virulent phages to control. It can be either administered orally (along with feed) or directly added to water	Protean Pharmaceuticals Ul. Tylna 3a 90-364 Łódź, Poland	Available globally
V PHAGES GROWOUT and V PHAGES HATCHERY	*Vibrio parahaemolyticus*, *V. alginolyticus*, *V. harveyi*, *V. campbellii*, other pathogenic *Vibrio* spp.	Based on the technology developed by ICAR - Central Institute of Brackishwater Aquaculture (ICAR-CIBA), Chennai, India. The phage cocktails are effective against vibriosis, running mortality syndrome and other bacterial infections in shrimp hatcheries and farms.	Salem Microbes Private Limited No: 21/10C, Bajanai Madam Street, Gugai, Salem - 636006 India.	Available in India
LUMI-NIL MBL	Luminous *Vibrio* spp.	Based on the technology developed by the College of Fisheries, Mangaluru, India. A phage preparation to reduce luminous vibrios in shrimp hatcheries	Mangalore Biotech Laboratory Embassy Plaza III Floor, Mangalore-575002 India	Available in India
aquaPHIX™	Shrimp pathogens (specific details not mentioned)	Phage-coated feeds. Prevents infectious diseases in shrimp farming including early mortality syndrome.	Fixed-Phage Ltd Kelvin Campus. 2317 Maryhill Road, Glasgow G20 0SP, UK	Under development

area. Rather than developing a universal phage formulation/cocktail to control a particular bacterium across the globe, the focus should be on developing region-specific/localized phage repositories with associated safety data. These phage repositories could be used to quickly formulate effective phage cocktails after the identification of pathogenic bacterial strains in specific aquaculture areas. The combined efficacy of phage therapy with probiotics could also be evaluated. In this approach, probiotics can promote healthy microbiota, whereas phages can selectively target specific pathogens. There is also a need to establish appropriate regulatory standards to ensure the safety and performance of commercial phage products for use in aquaculture. Additionally, it is also necessary to generate awareness about the advantages of phage-based products to increase their consumer acceptance and demand resulting in further research and development in this area.

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Declarations

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