Turk. J. Fish.& Aquat. Sci. 25(3), TRJFAS26235
https://doi.org/10.4194/TRJFAS26235

RESEARCH PAPER

Turkish Journal of FISHERIES and AQUATIC SCIENCES

Effects of Oral Application of Bacteriophage Cocktails to Treat Aeromoniasis Caused by *Aeromonas hydrophila*

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How to Cite

Ture, M., Aygur, E., Calıskan, N. K., Cebeci, A., Polat, E. K., Altinok, I. (2025). Effects of Oral Application of Bacteriophage Cocktails to Treat Aeromoniasis Caused by *Aeromonas hydrophila*. *Turkish Journal of Fisheries and Aquatic Sciences*, *25(3)*, *TRJFAS26235.* https://doi.org/10.4194/TRJFAS26235

Article History
Received 12 June 2024
Accepted 13 Novomber 2024
First Online 29 November 2024

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Keywords
Phage therapy
Phage cocktails
Oral administration
Aeromonas hydrophila
Rainbow trout

Abstract

Aeromonas hydrophila is a prominent pathogen of freshwater fish. Antimicrobials can be used to treat motile Aeromonas septicemia (MAS) caused by *A. hydrophila*. However, bacteria may become resistant to these drugs, and antimicrobials could pollute water. Innovative, eco-friendly approaches must be developed to avoid and address MAS. The present study used bacteriophage cocktails to treat rainbow trout infected with MAS. Fish were administered an oral cocktail of *Aquaneticvirus* APT65, AP-Y28, AP-T5, and AP-ATCC phages to evaluate the therapeutic efficacy of phage therapy against *A. hydrophila-induced* fish mortality. The survival of *Aquaneticvirus* APT65 phage in fish organs was also evaluated over an 8-day study period. *Aquaneticvirus* APT65 phage was found in fish internal organs, demonstrating that the phage may cross the intestinal barrier. In challenge trials with the LD_{70} dose of *A. hydrophila,* phage cocktail doses of lx10^8 PFU/g feed reduced mortality in rainbow trout by 32-44.8%. Phage treatment prior to infection significantly increased fish survival compared to treatment after one day of infection. Relative percent survival results showed that oral phage cocktails protected fish against *A. hydrophila* mortality in a time-dependent way. This study is valuable for farmer-level application because it includes simple, practical procedures for phage cocktail formulation, medicated feed preparation, and oral administration, as well as data on phage survival and protection data.

Introduction

Aquaculture is the world's fastest-growing food industry, significantly fulfilling rising global seafood demand (Stevens et al., 2018). Global aquaculture output reached 122 million metric tons, with an initial sales value of nearly US$281 billion (FAO, 2022). However, intensive aquaculture production has created serious hurdles in the form of infectious diseases, preventing sustainable aquaculture growth and resulting in significant economic losses. Aeromonas spp. are the most common bacteria in freshwater environments and are associated with serious diseases in farmed fish species (Jun et al., 2013). *Aeromonas hydrophila,* a Gram-negative opportunistic bacterium, causes motile Aeromonas septicemia (MAS) in many fish species, including channel catfish (*Ictalurus punctatus*), Atlantic salmon (*Salmo solar*), and rainbow trout (*Oncorhynchus mykiss*) (Austin & Austin 2016).

Fish farmers have used a variety of approaches to prevention and treatment of bacterial fish diseases, including vaccination, antimicrobial treatment, and

chemotherapy. (Ozturk and Altinok). Despite the discovery of numerous vaccines to prevent *A. hydrophila* infection, development of a commercial vaccine against A. *hydrophila* remains challenging due to strain diversity, lack of cross-protection between heterologous strains, and economic feasibility (Mzula et al., 2019).

Furthermore, the widespread use of antibiotics to treat *A. hydrophila* infections often leads to multidrug resistance. *A. hydrophila* has an enhanced ability to transmit antibiotic resistance genes or use the antibiotic-resistant characteristics of its own outer membrane proteins, resulting in antibiotic-resistant isolates (Bhat and Altinok, 2023). Several antibiotic-resistant *A. hydrophila* strains have been identified in several countries (Vivekanandhan et al., 2002; Thi, 2014; Nhinh et al., 2021). *A. hydrophila* strains were recovered from fish and shrimp in southern India; all strains were resistant to methicillin, rifampicin, bacitracin, and novobiocin (Vivekanandhan et al., 2002). Another study found that *A. hydrophila* isolates were extremely resistant to tetracycline and florfenicol, two widely used antibiotics in aquaculture (Thi, 2014). Therefore, there is an urgent need for new, ecologically safe methods to manage *A. hydrophila* infections in aquaculture. Phage treatment is a potential new alternative method for combating bacterial resistance to antibiotics in aquaculture (Rai et al., 2024). Phages are bacterial viruses that infect bacteria and are the most common creatures in nature (Clokie et al., 2011). Because lytic phages infect and kill bacteria by a different mechanism than antibiotics, phage treatment is an effective method for eliminating antibiotic-resistant bacteria (Bhat and Altinok, 2023). Phages, due to their high selectivity, cause minimal harm to native fish and have a low environmental impact. Phages can be developed quickly and inexpensively and are easy to use and store (Tan et al., 2016).

Several *A. hydrophila* phages have been identified and characterized, but only a handful have been evaluated in vivo for the treatment of fish diseases (Rai et al., 2024; Kaur et al., 2024). Injection and immersion phage treatment of crayfish (*Procambarus clarkii*) provided 66% and 20% protection against *A. hydrophila,* respectively (Huo et al., 2021). According to Zhang et al. (2021), the *A. hydrophila* phage PZL-Ahl effectively protects crucian carp against *A. hydrophila.* Rainbow trout treated with *A. hydrophila* phage by immersion or injection were completely protected against *A. hydrophila* infection, while oral treatment provided substantial protection. (Cao et al., 2020). Aeromonas phages often have limited host ranges, making them less effective for biocontrol applications (Pereira et al., 2022). Alternatively, phage cocktails may be more effective if they target many species and/or strains (Rai et al., 2024). However, few phages or combinations of thereof have been tested in live fish to treat *A. hydrophila* infections in farmed trout (Yazdanpanah-Goharrizi et al., 2020; Cao et al., 2020). The purpose of this study was to evaluate the protective effects of previously identified and described Aquaneticvirus APT65, AP-T5, AP-Y28, and AP-ATCC phages (Ture et al., 2022a) when administrated orally against *A. hydrophila* T65 infection in rainbow trout in vivo.

Materials and Methods

Antimicrobial Resistance of *A. hydrophila*

The antibiotic sensitivities of the four *Aeromonas hydrophila* strains T-65, T-5, Y-28, and ATCC, used as hosts for bacteriophage production, were determined using the disk diffusion method (Kirby-Bauer). Commercial antibiotic disks were used for this purpose, including penicillin (P-10), amoxicillin (AX-20), oxalinic acid (OA-2), flumequine (FLM-30), erythromycin (E-15), florfenicol (FFC-30), oxytetracycline (OT-30), and enrofloxacin (ENR-15). The test was performed and interpreted according to the Clinical and Laboratory Standards Institute (CLSI, 2015) guidelines. The antibiotic susceptibilities of the host bacteria were determined, and whether they were multi-drug resistant was assessed (Ture et al. 2018).

Bacteria and Phage Cocktail

The DNA gyrase gene regions of the bacteria were previously amplified, and sequence analysis and phylogenetic analysis were conducted (Ture et al., 2022a). The host bacteria *Aeromonas hydrophila* T65, *A. hydrophila* Y-28, *A. hydrophila* T5, and *A. hydrophila* ATCC were used for the propagation of the *Aquaneticvirus* APT65 phage, AP-Y28, AP-T5, and AP-ATCC phages, respectively. Bacteria were first taken from the stock (-80°C), inoculated onto tryptic soy agar (TSA**,** Merck), and cultured for 48 hours to ensure purity. A single colony from each bacterial plate was inoculated into tryptic soy broth (TSB) (Merck) and kept in an incubator at 15°C for daily analysis.

Preparation of Bacteria and Phage Cocktail

To propagate the phage, 1 mL of phage suspension was combined with 1 mL of the pertinent bacterial suspension grown overnight and 8 ml of TSB. The mixture was kept overnight at 25°C in a shaking water bath. The next day, the mixture was centrifuged at 5000 x g for 15 minutes and filtered through a 0.22 µm syringe filter. Each phage was propagated independently, and the titer of phages was evaluated using Double Layer Agar (DLA) procedures, including the spot test (Wintachai et al., 2019; Ture et al., 2022a). The titer of four bacteriophages was set at $1x10^9$ plaque-forming units (PFU)/ml. Phage cocktails containing four different phages were used to combat potential phage resistance. Just prior to treatment, a phage cocktail was prepared with equal doses of each phage ($1x10^9$ PFU/ml). The treatment with the phage was carried out in accordance with the infection protocol (Richards, 2014).

Infection Protocol

Fish were acclimated to laboratory conditions for three weeks, during which no cases of disease or mortality were recorded. To determine the LD_{50} value of *A. hydrophila,* the *A. hydrophila* T65 strain was selected, as the lytic effect of four phages on this strain has been previously established (Ture et al., 2022a).

The research was carried out at the Fish Diseases Research Center, which is part of the Central Fisheries Research Institute in Trabzon, Türkiye. The Institutional Animal Care and Use Committee of the Central Fisheries Research Institute approved all of the studies reported in this paper, with approval number 324.04.02-8. The experiment was conducted in 30-liter fiberglass tanks with a running water system (200-300 mL/min). The average daily water change in the tanks was 15-20 times. Throughout the experiment, the water temperature in the tank was between 15 and 16°C. Hypochlorite was used to neutralize the effluent. Cumulative mortality was recorded daily throughout the experiment, and the no-recovery rate was determined. Fish were anesthetized with benzocaine (30-40 mg/L) before being injected with *A. hydrophila* or PBS.

Determination of LD_{50}

Prior to the experiment, fish weighing 6-7 g in the acclimation tanks were sampled for screening of bacteria and parasites in internal organs or on skin, fins, and gills (Kayis et al., 2009; Ture et al., 2018). The *A. hydrophila* T65 strain was selected as the host cell for the experiment, based on previous knowledge of the lytic effect of four phages on this strain (Ture et al., 2022a). Prior to the experiment, the *A. hydrophila* T65 strain was used to infect trout by intraperitoneal injection, and the pathogens were isolated. This method ensures that the isolate can induce aeromoniasis with greater efficiency. A series of 30-liter aquariums containing ten fish each were used to estimate the LD_{50}/10-day value of *A. hydrophilia*. Experiments were performed in triplicate. The fish received 0.1 mL of bacteria at concentrations of 0, $8x10^2$, $8x10^4$, $8x10^6$, and $8x10^8$ CFU/mL. The control group received 0.1 mL of PBS. Fish were monitored for 10 days, and the LD_{50} value of the bacteria was calculated using probit analysis (SPSS).

Preparation of Phage-containing Feed

Four phages (AP-T65, AP-Y28, AP-T5, and AP-ATCC) were mixed equally (25 µl per gram of feed at a concentration of $1x10^9$ PFU/mL for each phage) and gently absorbed into a relevant amount of pellet feed within a sterile Petri dish using a pipette tip. To prevent premature dispersion of the phage particles in the water, fish oil (0.05 ml per gram of feed) was sprayed onto the mixture, which was then left to dry for thirty minutes in a sterile cabinet at room temperature. The daily feed intake was calculated to contain an average phage concentration of 1x10^8 PFU/g. Fish were fed with commercial trout feed (Sürsan A.S., Türkiye) at a rate of 3% of their total body weight.

In vivo Experimental Design

The LD_{50} value of the *A. hydrophila* T65 strain was calculated as $8x10^6$ CFU/mL and the LD_{70} of the *A. hydrophila* T65 strain ($1.3x10^7$ CFU/mL concentration) was used for intraperitoneal infection of fish. A total of 300 rainbow trout with an average weight of 6.0±0.6 g were used. The fish were randomly divided into five groups (3 replicates x 20 fish/tank per group), and the experiment was designed with three replicates. The first group of fish were fed with a diet containing phage cocktail for 3 d prior to infection with *A. hydrophila* T-65 strain ($1.3x10^7$ CFU/0.1 ml), and the diet containing phage cocktail was continued until the end of the experiment. (Table 1). The second group of fish was fed a control diet containing no phages before infection with *A. hydrophila* and continued on the same diet until clinical signs, such as mortality, swimming disorders, and hemorrhage at the base of the fins, were observed. (Table 1). After clinical signs were observed, the fish were fed with a diet containing a phage cocktail until the end of the experiment. The third group of fish was fed with a phage-free diet, serving as a positive control to assess exclusively the impact of *A. hydrophila* T65. (Table 1). The fourth group of fish was fed with phage cocktails containing diet to determine any toxic or side effects of the phage cocktails, representing the negative control (Table 1). The fifth group was fed a control diet, and the second negative control group was used to monitor the effects of the PBS injection. Throughout the experiments, the fish were fed twice a day at 3% of their body weight. (Table 1). The research was conducted for 15 days. Any fish that died were immediately removed and recorded.

The protection rate of the phage cocktail was calculated using the relative percentage survival (RPS) method:

RPS = 1— (% mortality of phage-treated group /% mortality in control group) X 100

Persistence of *Aeromonas hydrophila* and Aquaneticvirus APT65 Phage in Fish Tissues

Since *A. hydrophila* T65 strain is a host of the *Aquaneticvirus* APT65 phage (Cebeci et al., 2023), only these bacteria and phage were selected as a model for the persistence experiment. A new experimental design was carried out to determine the number of *A. hydrophila* T65 strain and *Aquaneticvirus* APT65 phage in fish tissue, as described in the in vivo experimental design section. The first samples were taken just before the infection with *A. hydrophila* T65 (day 0). Therefore, bacterial treatment was considered as day 0, and three

Table 1. Experimental procedure of the challenge test to determine protection effects of the bacteriophage cocktail efficiency.

Treatments	Groups				
	1	2	3	4	5
Fish were fed phage cocktail-containing feed prior to 3 days of *A. hydrophila* infection and fed the same feed at the end of the experiment.	Yes	No	No	No	No
Fish were fed phage cocktail-containing feed after one day of post-infection with *A. hydrophila* and fed the same feed at the end of the experiment.	No	Yes	No	No	No
Fish were fed control feed, which contained no phage (positive control) and was infected with *A. hydrophila*.	No	No	Yes	No	No
Throughout the experiment, fish were fed phage cocktail-containing feed, but they were not infected with *A. hydrophila* (1. negative control).	No	No	No	Yes	No
Throughout the experiment, fish were fed control feed without *A. hydrophila* infection but only PBS injection (2. negative control).	No	No	No	No	Yes

fish from each group were randomly sampled on days 0, 2, 4, 6, and 8. For this purpose, liver, kidney, and spleen tissues were collected in a sterile bag, pooled and homogenized with PBS. Bacterial counts were determined by inoculating the homogenate onto TSA agar in 10-fold serial dilutions. In addition, after centrifugation and filtration of the homogenate, the phage titer was determined using the DLA method. The titer of AP-T65 phage in the homogenates was determined using the T-65 strain as an indicator bacterium. Each assay consisted of three replicates (Cao et al., 2020; Ture et al., 2022b).

Water Quality Parameters

During the in vivo and LD_{50} tests, daily measurements of dissolved oxygen (8.30±1.1 mg/L), pH (7.7±0.2), temperature (16±1.3), ammonia (0.01±0.01 mg/L), and nitrite (0.014±0.01 mg/L) were taken in the fish tanks. Water quality parameters were within the predicted range due to constant aeration and frequent water exchange.

Histopathology

To investigate the potential tissue damage caused by phages, a new experiment was designed for groups 4 and 5, with 10 fish in each group. Two weeks after the start of experiment, gills, spleens, and liver samples were collected from five fish in each group, preserved in Bouin's solution, and embedded in paraffin following standard tissue processing. Tissue sections of 5 1.1 µm were stained with hematoxylin-eosin and examined under a light microscope (Altinok & Capkin, 2007).

Statistical Test

Probit analysis (SPSS 2002, SPSS Chicago, IL, USA) was used to determine the LD50 values of the *A. hydrophila* T65 strain on fish. The Kaplan-Meier survival analysis test was used to compare mortality rates of fish treated with phage cocktails and *A. hydrophila* T65 with those of control fish. The Cox-Mantel test (Statistica, Statsoft, Tulsa, OK, USA) was used to analyze the means when significant differences between groups were found (Altinok et al., 2016).

Results

Antimicrobial Resistance

The antibiotic sensitivities of four *Aeromonas hydrophila* strains used for phage isolation were tested against eight antibiotics using the disk diffusion method. The results showed that all strains were resistant to penicillin, amoxicillin, and erythromycin, while they were sensitive to oxalinic acid, florfenicol, oxytetracycline, and enrofloxacin. These findings indicate that the bacterial strains were multidrug resistant.

Protection of Phage Cocktails

In the first and second groups, mortality occurred on the second day after infection with *A. hydrophila* T65, and in the third group, death occurred on the first day. No mortality occurred after the seventh day of the experiment. During the experiment, swimming disorders, reduced feed intake, dorsal darkening, exophthalmos, and skin redness were observed. In the first, second, and third groups, 23.3%, 28.3%, and 41.6% of the fish died, respectively (Figure 1). No mortality was observed in groups 4 and 5. Fish pre-treated with bacteriophage cocktails prior to 3 days of infection had an RPS of 44.8%, but after clinical signs were observed, the RPS decreased to 32%. On the other hand, treatment of fish with bacteriophage cocktails after clinical signs were observed protected the fish by 32%. Phage treatment significantly ($p< 0.001$) reduced fish mortality due to *A. hydrophila* infection.

Persistence

The in vivo kinetics of *A. hydrophila* T65 and *Aquaneticvirus* APT65 were determined using pooled head kidney, liver, and spleen. Bacterial and phage recovery occurred on days 2, 4, 6, and 8 after a single-dose of bacteria. The number of phages in the first and second groups did not change over the 8-day sampling period, whereas the CFU of *A. hydrophila* T65 decreased dramatically with sampling time (Figures 2 and 3). The PFU of the phage cocktails and CFU of *A. hydrophila* T65 in groups 3 and 4 were decreased with increasing sampling time (Figures 4 and 5). Compared to groups 1 and 2, the bacterial CFU was significantly higher in group 3. The decrease in bacteriophage PFU in the only phage-treated group suggests that the nonspecific immune system of the fish detects and phagocytoses phages. Histopathological examination of fish organs showed that the phage cocktails used in the experiment had no adverse effects on the fish.

Discussion

The protection rates, or efficiency, of phages depend on the method of application. Injection is the most successful method, although it has some disadvantages. Fish are stressed during the injection, which is labor-intensive and only administered once. Another approach is immersion, which is simple but requires a large volume and concentration of phages and can result in low phage uptake. While oral phage administration is the preferred method, it is critical to assess the pH tolerance of the phage before using it as a treatment. The physical and biochemical properties of the phages used in this study had been reported in a previous study, and their pH stability, even at low pH, was very high (Ture et al., 2022a). Considering the environments in which aquaculture activities are conducted, fish populations, and culture conditions, it can be confidently stated that the most easily applicable method is the administration of phages incorporated in feed. For that reason, only oral phage administration method was used in the present study. Antibiotics are the most commonly used treatment for bacterial diseases. Bacterial infections often acquire antibiotic resistance as a result of prolonged exposure (Boran et al., 2013; Capkin et al., 2017; Ture et al., 2018). As a result, there is a need for alternative eco-friendly treatments that can be used alone or in combination with presently known therapeutic approaches to avoid bacterial infections in aquaculture. Phages typically exhibit lytic activity against only a few strains of host bacterial species due to their limited host range. Furthermore, if bacteria develop resistance to the phage, it may become useless. Phage cocktails containing multiple phages have emerged as a promising technique to combat phage resistance and prolong the efficacy of phage treatment (Pires et al., 2020; Ture et al., 2022a). In the present study, a cocktail of Aquaneticvirus APT65, AP-Y28, AP-T5, and AP-ATCC phages was used to eliminate any phage resistance. Combining four different phages can abolish phage resistance. If bacteria develop resistance to one or two phages, the remaining phages will infect and kill them. As a result, bacteria may not develop resistance to bacteriophages.

Other important factors to be considered involve the optimization of the dosing regimen, timing of administration, and environmental conditions. Moreover, the legislative framework concerning the application of bacteriophages in aquaculture is still in its evolution process. It will be necessary to standardize the production process and perform safety and efficiency tests in order to obtain a license for the practice of

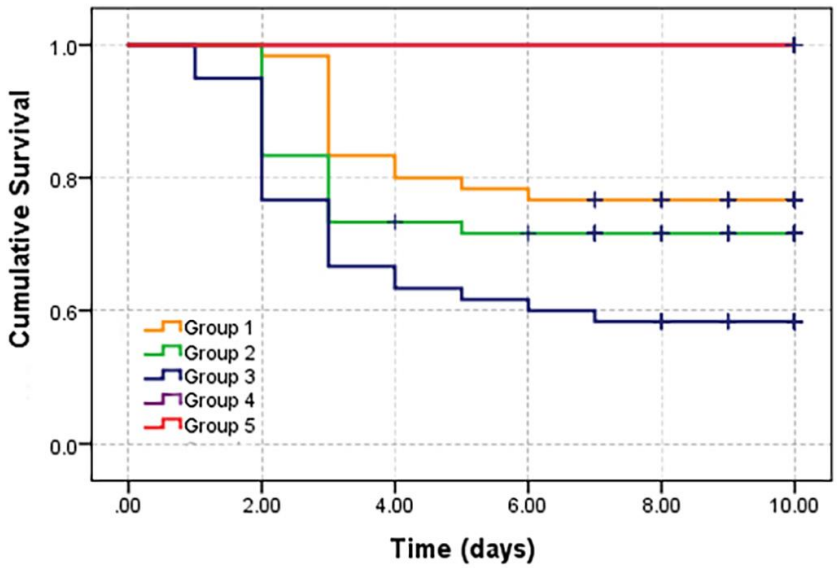

Figure 1. Cumulative mortality rates in the groups. It is noteworthy that the cumulative mortality rates for groups 4 and 5 are identical and overlap with each other.

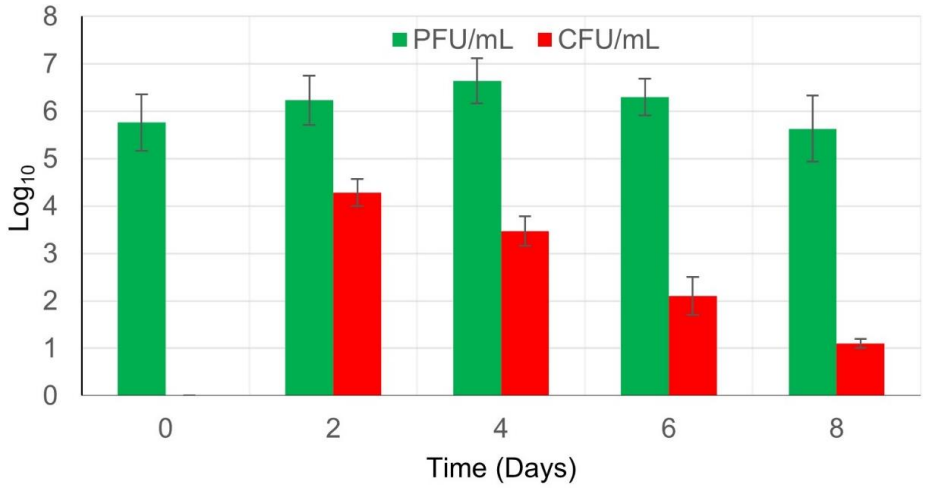

Figure 2. The first group was fed phage-supplemented feed for the first three days and followed by a bacterial injection on the fourth day. Phage-supplemented feeding continued until the end of the experiment.

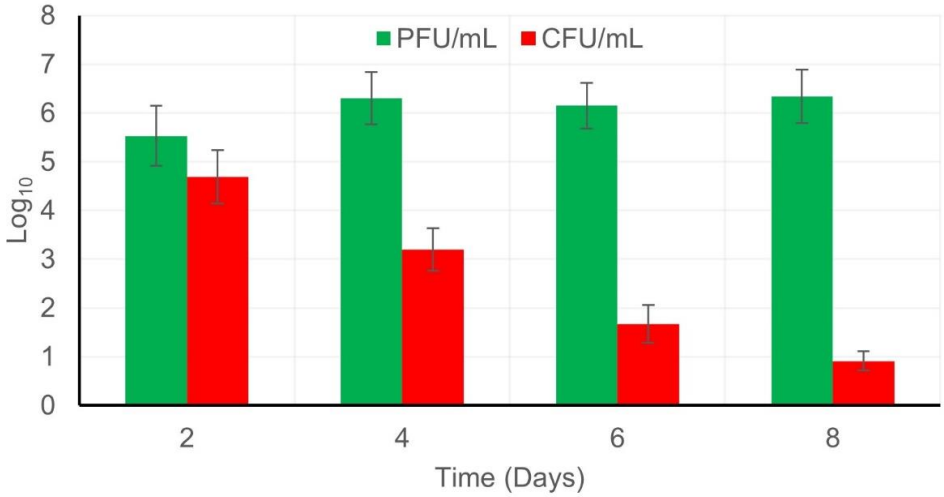

Figure 3. The second group, which began receiving phage-supplemented feed the first day after the bacterial injection, continued this feeding until the end of the experiment.

aquaculture. It is vitally important that active liaison between researchers, aquaculture producers, and regulatory authorities produce protocols for ensuring the safe and efficient application of bacteriophage therapy.

From the farmer`s point of view, the most practical method is the oral administration of therapeutics in combination with feed. Other treatment techniques, such as immersion in pond water, injection, or topical application to the fish body, are impractical on a large scale. In order to assess the therapeutic efficacy of the oral delivery strategy, the survival and distribution of each putative therapeutic phage in fish organs must be properly evaluated. In the present study, Aquaneticvirus APT65 was found in the internal organs of fish fed with phage-containing feed. The presence of phage in fish internal organs, demonstrating that the phage can breach the intestinal barrier. Phages were also found in internal organs of fish, demonstrating that the phage can breach the intestinal barrier. In the present study, only fish that were treated with a phage cocktail were able to recover phage concentrations, which were significantly reduced from day 2 (lx10^7 PFU/ml) day 8 (1x10^2 PFU/ml), despite being fed a phage-containing feed. Phages can be cleared from internal organs of fish as a result of a response by the fish`s immune system; it is possible that the first few days of exposure to phages stimulate the fish`s immune system before the non-specific immune system phagocytoses the phages. However, when both phage cocktails and host *Aeromonas hydrophila* were present in fish, the amount of *Aeromonas hydrophila* in the fish's internal organs decreased significantly with sampling time, but the number of phages remained constant. As a result, the phages infected and killed *Aeromonas hydrophila* inside the fish. *Aeromonas hydrophila* phages were found in the intestines and kidneys of Labeo rohita after 7 days of feeding on phage-containing feed. After the oral

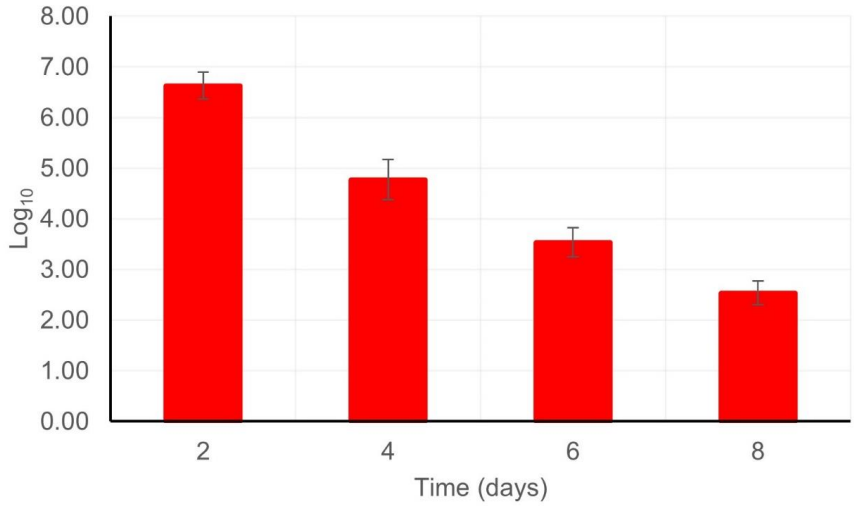

Figure 4. The third group received only a single dose of bacteria.

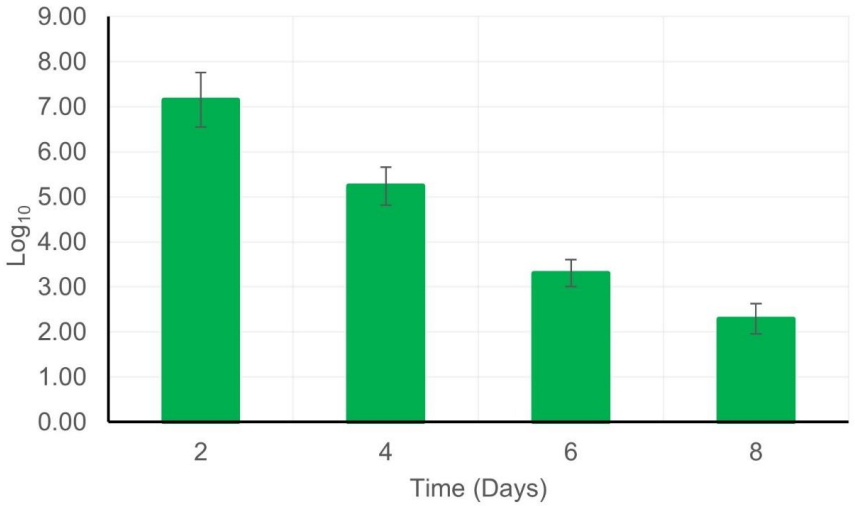

Figure 5. The fourth group received only phage-supplemented food. Titers in all experiments represent the average of the experiments performed in three replicates, and error bars represent the standard deviation.

phage administration was discontinued, both phages disappeared from the fish intestine and kidney within 1-3 days (Rai et al., 2024). It is possible that the fish's immune system or its effective excretion and filtration system are responsible for the rapid removal of phages from the intestines and kidney (Rai et al., 2024). Previous research has also shown the survival and distribution of orally administered phage in numerous fish organs. When *F. psychrophihnn* phage was administered to juvenile rainbow trout via food, it was shown to persist in their gastrointestinal tract for an extended period of time. Phages were rapidly removed from all organs once the phage-containing feed supply was discontinued (Christiansen et al., 2014). In another work, *Edwarsiella tarda* phage was bio encapsulated in Anemia's nauplii and given to zebrafish. Throughout the 10-day study, phages were found in intestines, kidney, liver, and spleen tissue samples, beginning on day one. Phage might remain in fish organs for less than a day after oral delivery was discontinued (Nikapitiya et al., 2020). The clearance of phages in the fish may be due to the development of the immune system of the fish against the phage.

Administering a phage cocktail with feed may effectively safeguard fish against fatal outcomes caused by systemic infection with *A. hydrophila*. Fish mortalities under the *A. hydrophila* LD_{70} challenge were considerably decreased when the phage cocktail was administered orally via feed. Based on the mortality and RPS data, it was clear that the effectiveness of the phage mixture in providing protection depended on the timing of phage administration. For instance, administering phage therapy before infection greatly enhanced fish survival compared to administering it one day after

infection. While previous research has indicated that administering phages through oral feed can safeguard fish from harmful bacteria (Prasad et al., 2011; Li et al., 2016; Cao et al., 2020), no study has yet provided a thorough examination of a phage cocktail-based method involving phage-coated feed, the viability of orally administered phages in fish organs, and the assessment of the phage cocktail's effectiveness against *A. hydrophila* infection.

Phages have started to be used in aquaculture as an alternative method for treating or preventing bacterial infections in fish (Ture et al., 2022a). Phage isolation and kinetic property determination, however, are quite challenging and require both a microbiology specialist and a typical bacteriology laboratory. When aquaculture farmers wish to employ phages to treat or prevent bacterial fish diseases, they must acquire and use laboratory-prepared phages that have been stored under the proper conditions. Phages are more advantageous when they are unique to certain species and, in some cases, even specific to certain strains. Bacteriophages are highly specific to their hosts, and even phages targeting the same bacterial species may not be universally effective against all strains of that species. This specificity can be a challenge, but it is also an advantage for precision targeting. To increase the range of the bacteriophage in a species of bacteria, phage cocktails were used to increase the strain range and eliminate phage resistance. Farmers may simply mix the ready-made phage into their regular feed in single or multiple doses.

Bacteriophages are not detrimental to fish and are unable to persist in fish or water without host bacteria. Bacteriophages primarily target bacteria in the digestive tract. An essential characteristic that sets them apart from antibiotics is their specificity to bacterial species, which means they do not impact the normal microflora. Due to their distinct method of action, bacteriophages are capable of effectively targeting bacteria that exhibit resistance to several drugs. As a result, whichever bacterial infections are most prevalent in fish can be treated once a week using bacteriophages unique to that disease agent. As a result, fish may be protected against bacterial infections.

The use of bacteriophage cocktails presents a groundbreaking approach to treating aeromoniasis caused by Aeromonas hydrophila. This method provides a targeted, efficient, and environmentally friendly alternative to conventional treatments, addressing a crucial need in the aquaculture sector. As research progresses and regulatory frameworks evolve, phage therapy is set to become a vital tool for managing bacterial diseases in aquaculture, contributing to the industry's sustainability and productivity. Treatment with oral bacteriophages thus appears to be a promising alternative for the management of *A. hydrophila* infections in rainbow trout, whether as a reduction in the use of antibiotics or for an improved fish health outcome. Further studies optimizing phage preparations and verifying their use over time in aquaculture systems will be necessary. Addressing the existing challenges, bacteriophage therapy could be one of the valued tools in the sustainable management of fish diseases, making aquaculture more resilient to emerging bacterial pathogens.

Ethical Statement

All studies reported in this paper were approved by the Institutional Animal Care and Use Committee of the Central Fisheries Research Institute, Trabzon, Türkiye under authorization number 324.04.02-8.

Funding Information

This project was funded by the Scientific and Technological Research Council of Türkiye (TUBITAK-123Z627).

Author Contribution

Mustafa Türe: Funding acquisition, Investigation, Methodology, Writing-original draft. Elif Aygür: Investigation. Nihal Kutlu Çalışkan: Investigation. Ayşe Cebeci: Investigation. Esen Kulaç Polat: Investigation. Ilhan Altinok: Conceptualization, Supervision, Methodology, Funding acquisition, Investigation, Writing-review editing.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

Acknowledgements

This project was funded by the Scientific and Technological Research Council of Türkiye (TUBITAK-123Z627).

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