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Phage Therapy in Aquaculture: Applications, Efficacy and Challenges

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Abstract: Aquaculture and fisheries have emerged as some of the fastest-growing food sectors in recent years. However, the indiscriminate use of antibiotics in aquaculture and fisheries has led to the development and spread of antibiotic resistance. In this context, phage therapy offers an alternative, sustainable, and environmentally friendly solution for controlling pathogens that cause significant economic losses in aquaculture. Over recent years, the application of phage therapy in aquaculture has gained increasing attention. Phage therapy has shown promising results in controlling pathogens such as Vibrio, Aeromonas, and Flavobacterium. This method effectively improves fish health, reduces antibiotic usage, and preserves microbial balance due to the specificity of phages. Despite its potential, several challenges affect the efficacy and success of phage therapy. These challenges include the sensitivity of phages to environmental factors, the potential of bacteria to develop resistance against phages, difficulties in developing effective phage formulations, and scientific gaps in phage therapy research. To address these issues, biotechnological and nanotechnological methods have been employed to enhance the effectiveness of phages and increase their resilience to environmental factors. Innovative technologies such as CRISPR-Cas9 enhance the specificity of phages toward target pathogens while supporting microbial balance. Additionally, microencapsulation techniques strengthen phage stability, enabling more efficient application. However, for the large-scale implementation of phage therapy, clear regulatory frameworks and economic sustainability are required. This study provides a comprehensive evaluation of the applications and efficacy of phages, advanced techniques used in their formulation, challenges encountered in phage therapy, and existing scientific gaps in the field of aquaculture. The insights gained from this study are expected to contribute significantly to the expansion of phage therapy applications in aquaculture, raise awareness about reducing antibiotic use, and support sustainable production practices.

Keywords: Aquaculture, bacteriophages, antibiotics, resistance

Kültür Balıkçılığında Faj Terapisi Uygulamaları ve Karşılaşılan Zorluklar

Özet: Kültür balıkçılığı ve su ürünleri yetiştiriciliği son yıllarda en hızlı büyüyen gıda sektörlerinden biridir. Ancak, kültür balıkçılığı ve su ürünleri yetiştiriciliğinde antibiyotiklerin bilinçsiz kullanımı, antibiyotik direncinin gelişimine ve yayılmasına neden olmaktadır. Bu bağlamda, faj terapisi kültür balıkçılığında ekonomik kayıplara yol açan patojenlerin kontrol altına alınmasında alternatif, sürdürülebilir ve çevre dostu bir çözüm sunmaktadır. Kültür balıkçılığında faj terapisi uygulamaları, son yıllarda giderek yaygınlaşmaktadır. Faj terapisi, *Vibrio, Aeromonas* ve *Flavobacterium* gibi patojenlerin kontrol altına alınmasında umut vadeden sonuçlar göstermiştir. Bu yöntem, balık sağlığının iyileştirilmesi, antibiyotik kullanımının azaltılması ve fajların özgüllüğü sayesinde mikrobiyal dengenin korunması açısından etkili olabilmektedir. Ancak, fajların çevresel faktörlere duyarlılığı, bakterilerin fajlara direnç geliştirme potansiyeli, etkin faj formülasyonlarının oluşturulmasındaki güçlükler ve faj terapisindeki bilimsel eksiklikler, bu yöntemin etkinliği ve başarısını etkileyen önemli zorluklardır. Bu çözüm bekleyen konulardan, fajların etkinliğini artırılması ve çevresel faktörlere karşı dayanıklılığının sağlaması amacıyla biyoteknolojik ve nanoteknolojik yöntemlerden yararlanılmaktadır. CRISPR-Cas9 gibi yenilikçi teknolojiler, fajların hedef patojenlere olan spesifikliğini artırarak mikrobiyal dengeyi desteklemekte, mikroenkapsülasyon yöntemleri ise fajların stabilitesini güçlendirmektedir. Bununla birlikte, faj terapisinin geniş ölçekte uygulanabilirliğini sağlamak için yasal düzenlemelerin oluşturulması ve ekonomik sürdürülebilirliğin sağlanması gereklidir. Bu çalışmada, kültür balıkçılığında hastalıkların kontrol altına alınmasında ve çevre dostu, sürdürülebilir üretim uygulamalarında önemli potansiyele sahip olan fajların uygulamaları, etkinlikleri, formülasyonlarında kullanılan ileri düzey teknikler, faj terapisinde karşılaşılan zorluklar ve bilimsel eksiklikler ayrıntılı bir şekilde değerlendirilmiştir. Elde edilen bilgilerin, kültür balıkçılığında faj terapisi uygulamalarının artmasına, bu alanda farkındalık yaratarak antibiyotik kullanımının azalmasına ve sürdürülebilir üretimin sağlanmasına önemli katkılar sağlayacağı düşünülmektedir.

Anahtar Kelimeler: Kültür balıkçılığı, bakteriyofaj, antibiyotik, direnç

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1. Introduction

In recent years, factors such as rapid population growth, insufficient livestock and agricultural production, and inefficient use of natural resources have driven an increasing demand for food (Pereira et al., 2022). Many countries today face critical challenges, including the need for alternative food sources, sustainable food management, and high-quality protein production. In this context, aquaculture and fisheries, as the fastest-growing food sectors in recent years, present a significant opportunity. According to reports by the Food and Agriculture Organization (FAO), global fish production has increased dramatically from 3 million tons in the 1970s to 178.5 million tons in 2018 (Rocha et al., 2022).

The importance of aquaculture and fisheries in meeting the rising demand for food sustainably has grown significantly. Consequently, various aquaculture systems have been developed, with intensive production systems being widely adopted worldwide. While intensive systems aim to maximize yield per unit area, factors such as high stocking densities, improper feeding practices, and adverse environmental conditions contribute to water quality deterioration. These conditions increase the prevalence of bacterial, viral, fungal, and parasitic fish diseases (Dang et al., 2021).

The widespread occurrence of diseases in aquaculture, coupled with the indiscriminate use of antibiotics, negatively affects production performance and leads to economic losses. The excessive use of antibiotics promotes the dissemination of resistant genes within aquatic ecosystems, disrupting microbial balance and accelerating the proliferation of resistant bacteria (Kunttu et al., 2021; Liang et al., 2023). Antibiotic resistance has become a global concern in aquaculture. This resistance spreads rapidly among aquatic bacteria through genetic and biochemical pathways, threatening microbial balance and posing serious risks to human health (Ye et al., 2019; Sundberg et al., 2021). These challenges underscore the necessity for environmentally friendly and practical solutions in aquaculture.

Bacteriophages (phages) represent a promising therapeutic option. These viruses naturally target bacteria with high specificity, aiding in the preservation of microbial balance (Imbeault et al., 2006). Phages have shown efficacy against common aquaculture pathogens such as *Vibrio, Aeromonas*, and *Flavobacterium*, offering hope for disease control and the reduction of antibiotic use (Dang et al., 2021).

Recent research on phage therapy has advanced the practical application of these agents in aquaculture. Phage therapy is now recognized as an effective and environmentally sustainable treatment option in the field. This study focuses on the applications of bacteriophages in aquaculture, evaluating their impacts, benefits, and the challenges encountered during implementation.

2. Antibiotic Resistance in Aquaculture

Antibiotic resistance has emerged as an escalating threat not only in aquaculture but also across the entire ecosystem. The indiscriminate use of antibiotics in aquaculture and fisheries significantly accelerates the development and spread of antibiotic-resistant bacteria within aquatic environments. Today, many pathogens in aquaculture are reported to have developed resistance to one or more antibiotics (Pereira et al., 2022; Liang et al., 2023). Moreover, such misuse does not only induce resistance in target pathogens but also disrupts aquatic ecosystems and microbiota, causing broader ecological harm (Zhang et al., 2021).

For instance, oxytetracycline, recognized as a broad-spectrum antimicrobial agent, is widely used in aquaculture. Pathogens such as *Aeromonas salmonicida* have been reported to develop resistance to oxytetracycline and florfenicol; *Aeromonas hydrophila* to oxytetracycline, ampicillin, amoxicillin, and florfenicol; *Yersinia ruckeri* to oxytetracycline and florfenicol; *Flavobacterium columnare* to oxytetracycline; *E. tarda* to florfenicol; and *Streptococcus iniae* to sulfonamides (Scarano et al., 2018; Zhang et al., 2021; Feng et al., 2022).

Similar patterns are observed in marine fish pathogens. Prominent species, including *Vibrio parahaemolyticus, Photobacterium damselae subsp. piscicida, Tenacibaculum maritimum,* and *E. tarda*, have shown resistance to oxytetracycline. Furthermore, species such as *Vibrio, Edwardsiella*, and *Photobacterium* have exhibited resistance to florfenicol, amoxicillin, ampicillin, and oxolinic acid (Kusunur et al., 2023).

Studies conducted in Turkey in 2025 have demonstrated that aquatic isolates of *Aeromonas hydrophila* exhibit high levels of resistance to tetracyclines (doxycycline) and aminoglycosides (gentamicin) (Türe and Alp, 2016). Likewise, *Pseudomonas fluorescens* strains display widespread resistance to sulfonamides (trimethoprim–sulfamethoxazole) and β-lactams (cephalothin), and have also acquired resistance to chloramphenicol (Yılmaz and Berik, 2025). In addition, seawater–derived *Aeromonas molluscorum* isolates show reduced susceptibility to erythromycin, while *Staphylococcus haemolyticus* strains manifest phenotypic resistance to fluoroquinolones (ciprofloxacin) and glycopeptides (vancomycin) (Baytaroglu and Kucukkagnici, 2025). These data underscore the pervasive nature of antibiotic resistance among aquatic pathogens in Turkey and highlight the critical importance of phage–antibiotic combination strategies in addressing resistant infections.

In addition, certain bacterial strains in aquaculture have developed resistance to multiple antibiotics, a condition termed "multi-drug resistance," which severely limits treatment options (Kusunur et al., 2023).

Addressing this global issue necessitates the adoption of biologically based, cost-effective, sustainable, and environmentally friendly approaches. Among these, bacteriophages (phages) are highlighted as the most promising

therapeutic agents for managing pathogens in aquaculture. Phages offer targeted pathogen control, mitigating antibiotic resistance and supporting the development of sustainable aquaculture practices.

3. Bacteriophages

Bacteriophages, often referred to as phages, are viruses that specifically target and infect bacteria, serving as therapeutic agents capable of lysing bacterial cells. The term "phage" is derived from "bacteria" and the Greek word phagein, meaning "to eat." Ubiquitously distributed in nature, phages are found in environments such as oceans, soil, food, and drinking water. With an estimated population of approximately 10^{31} individuals, they represent the most abundant biological entities on Earth (Le & Kurtböke, 2019; Yıldızlı et al., 2022).

Structurally, phages are composed of key components including a capsid, tail, and tail fibers. The capsid, made of protein subunits, houses the genetic material, while the tail facilitates the transfer of this material into the host bacterium. Although phages exhibit diverse structural features, the length and morphology of their tails vary among species (Linares et al., 2020).

Phages operate through two primary life cycles: the lytic and lysogenic cycles. In the lytic cycle, phages infect bacteria, replicate within them, and ultimately cause bacterial lysis, releasing new phage particles. This cycle involves several stages: adsorption, penetration, replication, maturation, and release. Due to their ability to eliminate pathogenic bacteria, lytic phages are preferred for therapeutic applications. Conversely, in the lysogenic cycle, the phage genome integrates into the bacterial chromosome and replicates passively with the host without causing immediate harm. However, environmental stressors can trigger the switch from a lysogenic to a lytic cycle (Joy, 2021).

Phage therapy involves utilizing the antimicrobial properties of phages to treat bacterial infections. With the rising prevalence of antibiotic resistance, phage therapy has garnered renewed interest since the 2000s. Notably, lytic phages have emerged as effective biological agents against pathogenic bacteria, offering a promising alternative to traditional antibiotics sustainable aquaculture practices.

3. Phage Therapy in Aquaculture

Phage therapy has gained increasing importance in combating bacterial infections in aquaculture. Common pathogens in aquaculture include *A. hydrophila*, *A. salmonicida*, *Vibrio anguillarum*, *Vibrio harveyi*, *Vibrio vulnificus*, *V. parahaemolyticus*, *Vibrio alginolyticus*, *E. tarda*, *Edwardsiella ictaluri*, *Edwardsiella piscicida*, *Flavobacterium columnare*, *Flavobacterium psychrophilum*, *Lactococcus garvieae*, and *Yersinia ruckeri* (Sieiro et al., 2020). The use of bacteriophages as alternatives to antibiotics offers sustainable solutions. Phage therapy is a promising approach in aquaculture, and its application has been increasingly adopted in recent years.

The primary step in phage therapy is the accurate identification of the pathogen causing the infection and the subsequent isolation of phages capable of effectively infecting the host bacteria.

Literature reviews spanning 1997 to 2022 indicate a growing trend in the use of bacteriophages in aquaculture (Fig 1.). Research has focused on the genetic and morphological characterization of phages, their use in biocontrol, and their therapeutic efficacy across different life stages of aquatic organisms (e.g., eggs, larvae, juveniles, and adults) (Donati et al., 2021). Frequently studied phage families include *Myoviridae*, *Podoviridae*, and *Siphoviridae*. These phages have demonstrated effectiveness against pathogens such as *A. salmonicida*, *A. hydrophila*, *E.tarda*, *Yersinia ruckeri*, *V. harveyi*, *V. parahaemolyticus*, *V. anguillarum*, *V. alginolyticus*, *Flavobacterium columnare*, *Flavobacterium psychrophilum*, *Lactococcus garvieae*, and *Streptococcus iniae* (Donati et al., 2021). Studies conducted in recent years on aquaculture are summarized in Table 1.

Phage delivery methods in aquaculture include direct suspension, oral application, and injection. Injection has been identified as the most effective preventive approach in the literature. Additionally, the development and commercialization of phage products have gained momentum. For instance, *Intralytix* has developed phages targeting *Vibrio spp.*, while *BAFADOR*® targets *Aeromonas spp.* and *Pseudomonas spp.* Furthermore, *ACD Pharma* has produced phage solutions for *Yersinia ruckeri*, including phage pellets for aquafeed, and *LUMI-NIL MBL* has been introduced to manage shrimp pathogens (Ansari & Nagar, 2024).

In several controlled investigations, aquatic organisms were experimentally challenged with specific bacterial pathogens and subsequently treated with varying doses of bacteriophages, resulting in statistically significant improvements across multiple indices of host resistance and pathogen suppression. For instance, Droubogiannis et al. (2023) demonstrated that gilthead seabream (*Sparus aurata*) larvae infected with *Vibrio harveyi* MM46 experienced a reduction in mortality from 49 % in untreated controls to 29 % following administration of the vB_VhaS_MAG7 phage, corresponding to an approximate 20 % increase in survival. Complementary in vitro assays further revealed that vB_VhaS_MAG7 produced a 33 % inhibition of bacterial proliferation within the first five hours post-infection. Similarly, Hossain et al. (2023) reported that specific-pathogen-free (SPF) shrimp exposed to 5×10^5 CFU mL^{-1} of *Vibrio parahaemolyticus* and treated with 1.5×10^6 PFU mL^{-1} of the vB_VpS_SHB15 phage—administered both prophylactically (–24, –6, –1 h) and therapeutically (+1 h)—showed marked reductions in mortality: from 100 % in positive controls to 93 % with therapeutic treatment alone, 53 % with prophylactic feeding, 33 % with prophylactic bath

application, and only 6 % when both prophylactic and therapeutic regimens were combined. An accompanying in vitro planktonic growth assay indicated a 3-log reduction in bacterial load during the initial five-hour period following phage exposure. In a separate trial, Kumari et al. (2023) evaluated intramuscular and immersion delivery of a phage cocktail against *Aeromonas hydrophila* in *Pangasius buchanani*: fish inoculated with 8×10^5 CFU fish^{-1} exhibited 100 % mortality in the absence of phage treatment, whereas groups receiving 1×10^4 and 1×10^5 PFU fish^{-1} intramuscular injection achieved 93 % and 87 % survival, respectively. Delays of 6, 12, and 24 h in phage administration reduced survival to 83 %, 76.7 %, and 26.7 %, respectively. Conversely, simultaneous water immersion treatments at 1×10^5 and 1×10^6 PFU mL^{-1} conferred 93 % and 100 % protection, and even a 24-hour delayed immersion maintained 100 % survival. Collectively, these studies underscore the efficacy of phages characterized by high burst sizes and potent lytic activity, as well as the critical importance of optimized dosing schedules and delivery methods, in dramatically reducing both mortality and bacterial burdens in infected aquaculture species (Dang et al., 2021; Opperman et al., 2022).

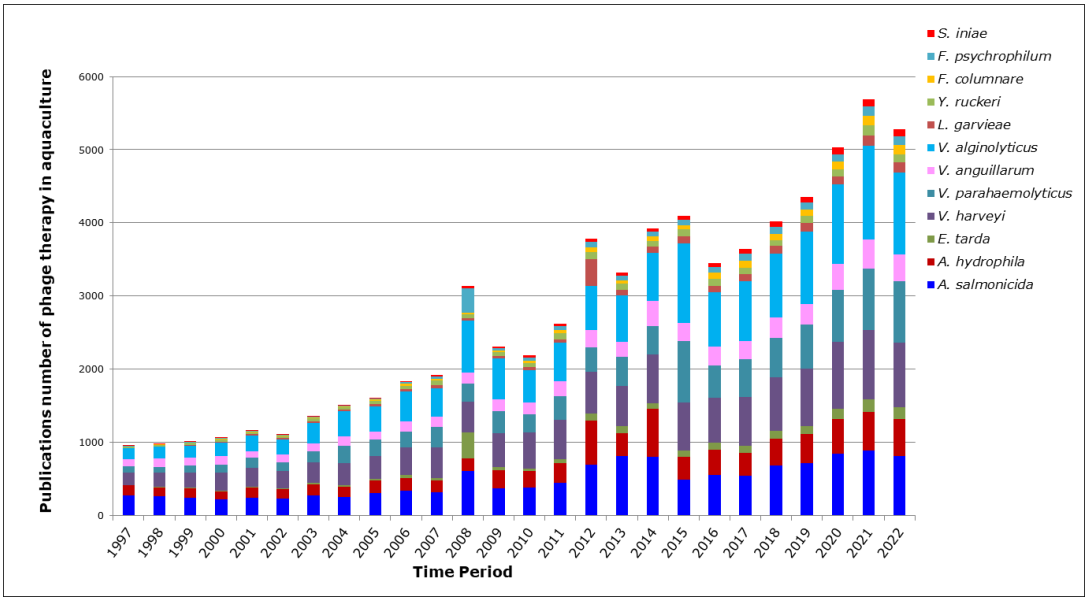

Figure 1. Literature reviews spanning 1997 to 2022 indicate a growing trend in the use of bacteriophages in aquaculture (Çağatay, 2023).

In controlled in vitro assays, *F. psychrophilum* biofilms at attachment, colonization and maturation stages were exposed to the lytic phage FPSV-D22 at varying phage-to-host ratios (PHRs), yielding >80 % inhibition of biofilm biomass with an initial PHR as low as 0.01; higher ratios further eradicated biofilm formation almost completely. Correspondingly, in an in vivo rainbow trout (*Oncorhynchus mykiss*) infection model, intraperitoneal administration of a phage cocktail (FPSV-D22 and FpV4) one day post-challenge with 8×10^7 CFU of *F. psychrophilum* reduced cumulative mortality from 67 % in untreated controls to 17 % and 13 % at PHRs of 2 and 0.02, respectively—equating to relative percentage survivals (RPS) of 76 % and 81 % (both $p < 0.001$ versus control)—whereas a PHR of 0.0002 conferred only 26 % RPS. These findings demonstrate that phages characterized by potent antibiofilm activity at low PHRs and robust in vivo efficacy can dramatically lower both biofilm biomass and fish mortality, underscoring their promise as biocontrol agents in aquaculture settings (Sundell et al., 2020). Additionally, phages targeting *F. psychrophilum* have been shown to enhance microbial diversity in aquatic ecosystems, providing protection before and after disease outbreaks (Imbeault et al., 2006).

Yersinia ruckeri and *T. maritimum* are additional pathogens that have been successfully targeted through phage therapy. Phages against *Y. ruckeri* reduced the prevalence of enteric redmouth disease by 75%, while those targeting *T. maritimum* improved the overall health of fish and prevented the spread of infections (Imbeault et al., 2006; Zhang et al., 2021).

On the other hand, some phage trials conducted in aquaculture have failed to achieve the desired outcomes due to inappropriate dosing, errors in the selection of administration methods, lack of phage standardization, the development of resistance, and unforeseen adverse factors. For example, a phage cocktail developed to control furunculosis in trout did not provide the expected protection. Despite continuous administration through feed, *Aeromonas salmonicida* infections could not be prevented, and disease symptoms and mortalities persisted within the fish population. Whether administered via feed, injection, or immersion, the phages failed to prevent fish deaths. This failure was attributed to the high contagiousness of *A. salmonicida* even at very low doses and the insufficiency of the applied phage doses to suppress the infection (Richards, 2014).

In another study targeting *Streptococcus iniae* infections, phage therapy initially appeared to reduce fish

mortalities, but complete success was not achieved. Resistant bacterial strains were isolated from fish that had been treated with phages yet still died. This highlighted the potential for phages to carry resistance genes or mediate horizontal gene transfer, raising biosecurity concerns (Richards, 2014).

Similarly, studies using phage cocktails developed against *Vibrio parahaemolyticus* in shrimp revealed comparable issues. In controlled experiments, the overall mortality rate in phage-treated shrimp was only about 4% lower than that of the control group, a difference that was not statistically significant. Only a partial effect on the disease course was observed: in phage-treated shrimp, the onset of acute mortality was delayed by a few hours, but ultimate death was not prevented. Researchers reported that this outcome might have been due to the phages remaining viable on the shrimp surfaces for extended periods and unexpectedly affecting the shrimp's immune response negatively. These findings suggest that in rapid, toxin-mediated infections such as those caused by *Vibrio parahaemolyticus*, phage therapy alone may be insufficient (González-Gómez et al., 2023).

4. Effectiveness and Challenges of Phage Therapy in Aquaculture

Phage therapy is emerging as an effective method for combating bacterial infections in aquaculture. It not only targets bacteria but also serves as a natural solution that supports environmental sustainability. As an eco-friendly approach that maintains microbial balance in aquatic ecosystems, phage therapy holds great promise. However, its success depends on the interplay of various factors, including physical and chemical parameters of the water, species-specific characteristics of fish, environmental conditions, and the pathogenic potential of bacteria (Culot et al., 2019). Understanding these complex interactions is essential for the effective and safe application of phage therapy. Additionally, the administration methods and pharmacodynamic properties of the phages used are critical considerations. In aquatic ecosystems, fish immune systems and the genetic characteristics of pathogens play a pivotal role in determining the success of phage therapies (Ly-Chatain, 2014). Therefore, optimizing phage applications in aquaculture requires careful analysis of these intricate dynamics.

The success of phage therapy is closely tied to the specificity of phages to their target bacteria and the appropriate selection of phages. This specificity is vital not only for treatment efficacy but also for safety. Studies have demonstrated that phages are specific to their target pathogens, a conclusion often verified through host range tests (Fig. 2). Failure to accurately identify the target bacterial species may result in unsuccessful therapy. Once the bacteria are correctly identified, selecting the appropriate phage(s) is critical. Proper phage selection enhances treatment efficacy and minimizes potential side effects. Isolating phage strains with high titers is a fundamental step toward achieving successful therapeutic outcomes. However, the efficacy of phages is not solely determined by their specificity and titers but also by their virulence properties (Orndorff, 2016). Rigorous research can lead to the isolation of optimal phages for application. For instance, effective phage studies have been conducted against fish pathogens such as *Flavobacterium psychrophilum* and *Vibrio* spp. (Sundell et al., 2020).

Careful phage selection and rigorous standardization processes are essential before successful phage application can be achieved. Phages included in a cocktail should have a broad host range to effectively target common aquatic pathogens such as Vibrio, Aeromonas, and Flavobacterium (Huang and Nitin, 2019). The selected phages should show strong lytic activity, avoid lysogenic properties, and maintain stability under variable environmental conditions such as salinity, pH, and temperature fluctuations (Culot et al., 2019). In addition, when formulating the phage preparation to be used, it should be decided whether it will consist of a single phage or a combination of multiple phages. In both methods, phages infect the target bacteria by binding to their specific surface receptors and then destroy the host bacteria by lysis. The most important advantage of monophage therapy is that the treatment shows extremely high specificity against the target pathogen; thus, non-target microflora is not harmed and as a matter of fact, it has been reported that a single phage application does not disrupt the balance of the intestinal microbiota in infected fish.(Platt, 2000). However, the use of a single phage has some limitations in terms of therapeutic efficacy: Its spectrum of action is narrow and the risk of the target bacteria rapidly developing resistance through mechanisms such as mutation in the relevant phage receptor is high. Phage cocktails, on the other hand, offer a broader spectrum of action by reaching a wider range of hosts thanks to the different phages they contain and can increase the effectiveness of the treatment with the synergistic interaction of phages; in addition, the use of multiple phages reduces the selection pressure in the evolution of resistance by making it more difficult for bacteria to develop resistance to all phages at once. As a matter of fact, it has been shown in the literature that a two-phage cocktail controls the bacteria and reduces mortality more effectively compared to a single phage treatment in fish infected with *Aeromonas hydrophila* (Fazzino et al., 2020). Similarly, it has been reported that the survival rate in aquatic animals applied with a phage cocktail in a Vibrio-induced disease model was significantly higher (approximately 82%) compared to a single phage application; This rate is close to that achieved with conventional antibiotic therapy. In practice, the monophage strategy is often a “tailor-made” approach that requires identifying the responsible pathogen and selecting a specific phage for each new case, whereas phage cocktails offer a significant practical advantage in the field as “ready-made” formulations that can be prepared and rapidly applied against common pathogens (Ren et al., 2019). In addition, during cocktail design, it is important to ensure that phages target different bacterial receptors to prevent the development of cross-resistance; for example, combining phages that recognize outer membrane proteins and lipopolysaccharide structures increases the robustness

of the cocktail (Mateus et al., 2014). Antagonistic interactions, where one phage interferes with the infection cycle of another, should be avoided, and phage-phage synergy should be confirmed by in vitro experiments such as spot tests and liquid culture inhibition tests (Chen et al., 2019). During the standardization phase, a multiplicity of infection (MOI) ranging from 0.01 to 1 is typically targeted to maximize bactericidal efficacy while avoiding phage overuse (Huang and Nitin, 2019). Furthermore, batch-to-batch consistency should be verified by measuring phage titers (expressed as PFU/mL), and long-term stability should be tested under appropriate storage conditions such as refrigeration or lyophilization with a shelf-life target of at least 6–12 months (Culot et al., 2019). The host range of the phage cocktail should be re-evaluated periodically, especially in open water systems where pathogen populations can rapidly evolve. Rigorous application of these selection and standardization principles is critical to the development of effective and commercially viable phage therapies to support sustainable aquaculture.

The first step of a successful phage therapy is a correct pharmacological approach. Pharmacokinetics is one of the two basic elements of pharmacology (the other is pharmacodynamics). Pharmacokinetics examines the characteristic properties and metabolic effects of the applied therapeutic agent. Therefore, the pharmacokinetic properties of phages play a key role in phage therapy (Abedon and Yin, 2009). When pharmacokinetic properties of phages are mentioned, the phage's burst size, latency period and adsorption period are understood (Castillo and Middelboe, 2016). In general, for phage therapy to be successful, it is desired that the phage first reaches the specific bacterium in a short time, lyses the bacteria in a short time after reaching it and releases a large number of virions into the environment. In this case, the phage with the shortest adsorption time, the shortest latent period and the largest burst size is always the phage that should be preferred first in phage therapy. Multiplicity of infection (MOI) guides treatment planning by defining the ratio of the applied phage dose to the bacterial load; in active treatment, kinetic properties such as burst size and latent period should be at an optimal level in order to achieve successful lysis even with low MOIs, while in passive approaches, direct bactericidal effect is achieved with high MOIs (Abedon, 2016). Host range shows the diversity of bacterial strains that the phage can infect; phages with a broad host spectrum can effectively lyse a large number of pathogen strains despite the genetic heterogeneity among clinical isolates, but specific binding property is also important in order not to harm non-target beneficial microflora. In terms of life cycle type, only obligate lytic phages are preferred; Phages with lysogenic potential can transfer toxin and resistance genes to the host bacteria due to the risk of integrating into the genome, therefore it is mandatory to meticulously analyze their entire genome for integrase, repressor and other genes associated with lysogeny (Howard-Varona et al., 2017). Genome stability ensures that the genetic structure of the therapeutic phage remains unchanged from production to application; since genetic deviations due to recombination, mutation and passage number may have negative effects on both effective lysis and safety profile, genome integrity should be monitored with long-term storage and serial passage tests (Pirnat et al., 2015). In addition, phages with “clean” genomes that do not have harmful genes (toxin and antibiotic resistance determinants) while carrying genes that may be beneficial should be used; in this direction, screening all phage candidates for virulence factors and resistance genes in databases such as VFDB and CARD by performing full genome sequencing and eliminating that phage in the presence of any risky genes is a basic security measure (Gholami et al., 2015). In this context, phages with high bursting efficiency, short latent period, rapid adsorption, effective multiplication at appropriate MOI, wide but specific host range, strict lytic cycle, robust genome stability, genome free of harmful genes and physical structure resistant to environmental conditions should be determined as the most suitable candidates before clinical application and should be tested in subsequent in vitro/in vivo models to verify the efficacy-safety balance.

Moreover, the formulation, dosage, and frequency of phage therapy are critical for effective pathogen control (Pereira et al., 2011). Developing suitable formulations tailored to fish species, age, and rearing environment will enhance therapeutic efficiency. However, formulating phages can sometimes be challenging. Among the advanced formulation techniques, microencapsulation has gained attention. Microencapsulation protects phage particles from environmental stressors and helps maintain their lytic activity over extended periods (Liang et al., 2023). In marine environments, where salinity and temperature fluctuations are common, microencapsulated phages demonstrate significantly improved stability (Dang et al., 2021). This technique also facilitates controlled release, optimizing the therapeutic process. Nanotechnology-based delivery systems represent another innovative approach that enhances the specificity and efficacy of phages. Phages combined with nanoparticles can reach target bacteria more rapidly, increasing treatment efficiency (Ye et al., 2019). For example, phages combined with nanoparticles for treating *A. hydrophila* infections have shown infection rates 30% lower than traditional methods (Imbeault et al., 2006).

Once suitable phages are characterized and formulated, scaling up their industrial production for aquaculture applications requires large-scale replication of beneficial phages. This process involves substantial costs associated with the cultivation of host bacterial strains, phage isolation, purification, and formulation development. Quality control testing must be conducted regularly to prevent contamination by unwanted bacteria or other organisms. Following production, appropriate cold chain logistics or storage conditions are necessary to deliver phages to fish farms. The rural or coastal location of most aquaculture facilities can increase transportation costs and logistical challenges. Therefore, achieving economic sustainability in the phage therapy process is essential for its widespread adoption. Regulatory procedures and customs regulations for international trade add further complexity to logistics planning, raising overall costs (Los, 2020).

Table 1. Significant Recent Bacteriophage Studies and Their Outcomes in Aquaculture.

Etiologic agent	Phage/Phages Cocktails	Fish/shellfish/ shrimp pecies	Outcomes	References
Aeromonas caviae	AC-P1, AC-P3	Tilapia, Catfish	Phages reduced mortality rates by 68% and showed significant lytic activity against antibiotic-resistant strains.	Nguyen et al., 2020
A.hydrophila	vB_AhaP_PT 2	Crucian carp	Survival rate reached 80% after phage treatment; reduced bacterial colonies in the intestine.	Liang et al., 2025
A. hydrophila	AVP3	Carp	Significant lytic activity against MDR strains; potential for biocontrol in aquaculture.	Kaur et al., 2024
A. hydrophila	AH-P10, AH-P12	Tilapia, Catfish	Phage application reduced mortality by 65% and demonstrated high efficiency against multidrug-resistant strains.	Wang et al., 2022
A. hydrophila	AHP1, AHP2	Common carp, Catfish	Phages showed strong lytic activity against multidrug-resistant *Aeromonas* strains and reduced mortality rates in infected fish populations.	Kazimierczak et al. 2018
A. salmonicida	Phage cocktail *A. salmonicida*	Salmonids	Phage therapy showed effective bacterial reduction in both in vitro and in vivo settings, providing an alternative to antibiotics for furunculosis control.	Vincent et al., 2019
Citrobacter spp.	Citrophage MRM19, Citrophage MRM57	Zebrafish (*Danio rerio*)	In vivo application in zebrafish increased survival rates by 17%-26%. The phages demonstrated high lytic activity, reducing bacterial load significantly.	Royam et al., 2020
E. tarda	ETP1, ETP5	Tilapia, Catfish	Phage cocktail effectively reduced *E. tarda* populations in aquaculture systems and improved survival rates in infected fish.	Ninawe et al. 2020
F. psychrophilum	FPSV-D22	Rainbow trout (*O. mykiss*)	Phages effectively disrupted biofilms and reduced mortalities in trout, even at low phage-to-host ratios.	Sundell et al. 2020
F. psychrophilum	FpV-1 to FpV-22,	*O. mykiss*	Phages with strong lytic potential against *F. psychrophilum* host	Stenholm et

	FpV2, FpV4, FpV7, FpV9, FpV10, FpV14, FpV18		strains thus provided the foundation for future exploration of the potential of phages in the treatment of both diseases.	al. 2008
P. damselae subsp. damselae	N4-like TEMp-D1	Marine species	Demonstrates high inhibition in PAS-treated groups; genomic characteristics support targeted therapy	Eren Eroğlu et al., 2025
Pseudomonas plecoglossicida	PP1, PP2	Ayu (*Plecoglossus altivelis*)	Phage therapy reduced bacterial loads significantly and minimized mortality rates in experimental infections.	Park and Nakai, 2003
Shewanella putrefaciens	SPX1	Shrimp	Reduced biofilm bacteria by over 98% on shrimp surfaces	Liu et al., 2025
Tenacibaculum maritimum	Prophages identified via in silico analysis	Atlantic salmon, European seabass	Key prophage elements identified for potential phage therapy; high stability and targeting efficiency	Ramírez et al., 2024
V. alginolyticus	vB_ValC_WD615	Tilapia, Catfish	Phage treatment reduced bacterial load significantly and showed stability across diverse temperature and pH conditions	Dai et al., 2024
V. alginolyticus	vB_ValC_RH2G	Grouper, Sea bream	Short latent period; efficient lysis with high specificity; genome indicates a new genus	Gao et al., 2023
V. alginolyticus	VA-2, VA-6	*Penaeus monodon* (Black tiger shrimp)	Phage application reduced mortality rates by 55% and improved water quality in aquaculture systems.	Huang et al., 2019
V. anguillarum	Lytic *Vibrio* phages	Marine fish species	Phage therapy effectively controlled *V. anguillarum* in marine aquaculture, reducing mortality rates and demonstrating its value in disease management.	Wong et al., 2024
V. anguillarum	Lytic *Vibrio* phages	*Dicentrarchus labrax*	Four lytic bacteriophages were isolated. The lytic phages inhibited the growth of their host bacteria, and TEM analysis revealed that phages belong to the *Myoviridae* and *Siphoviridae* family. One-step growth experiments showed that these lytic phages have different latent	Yıldızlı et al., 2022

			periods (30–50 minutes) and high burst sizes. Finally, Phage therapy effectively controlled *V. anguillarum.*	
Vibrio diabolicus	vB_Vc_SrVc 2	White shrimp	Delayed mortality onset by 40 hours and reduced mortality significantly	Lomelí-Ortega et al., 2024
V. harveyi	VH5, VH10, VH20	Shrimp, Grouper fish	Phage therapy reduced bacterial loads in infected shrimp and prevented disease outbreaks in aquaculture farms.	Misol et al.
Vibrio nereis	vB_VneM_N B-1	Sea cucumber (*Apostichopus japonicus*)	Reduced coelomocyte apoptosis and infection severity	Cao et al., 2025
V. parahaemolyticus	vB_Vp_PvV p04	Shrimp (*Penaeus vannamei*)	Encapsulation + freeze-drying method showed stable efficacy; 87.6% bacterial inhibition observed	Peña-Rodríguez et al., 2025
V. parahaemolyticus	vB-VpaS-SD15 (P15)	Shrimp (*Penaeus vannamei*)	Efficient lysis of 33 *V. parahaemolyticus* strains; stable across broad temperature and pH ranges	Chen et al., 2024
V. parahaemolyticus	*Vibrio*-specific phages	Shrimp	Field trials showed improved survival and health; low bacterial resistance emergence	Hossain et al., 2024
V. parahaemolyticus	vB_VpaS_P G07	Shrimp (*Penaeus vannamei*)	The phage significantly reduced shrimp mortality rates when applied after bacterial exposure, highlighting its effectiveness in AHPND management.	Ding et al., 2020
Vibrio spp.	IKEM_vK, IKEM_v5, IKEM_v14	General aquaculture species	Broad host range; stability across pH 6–11; significant biofilm inhibition	Yaşa et al., 2024

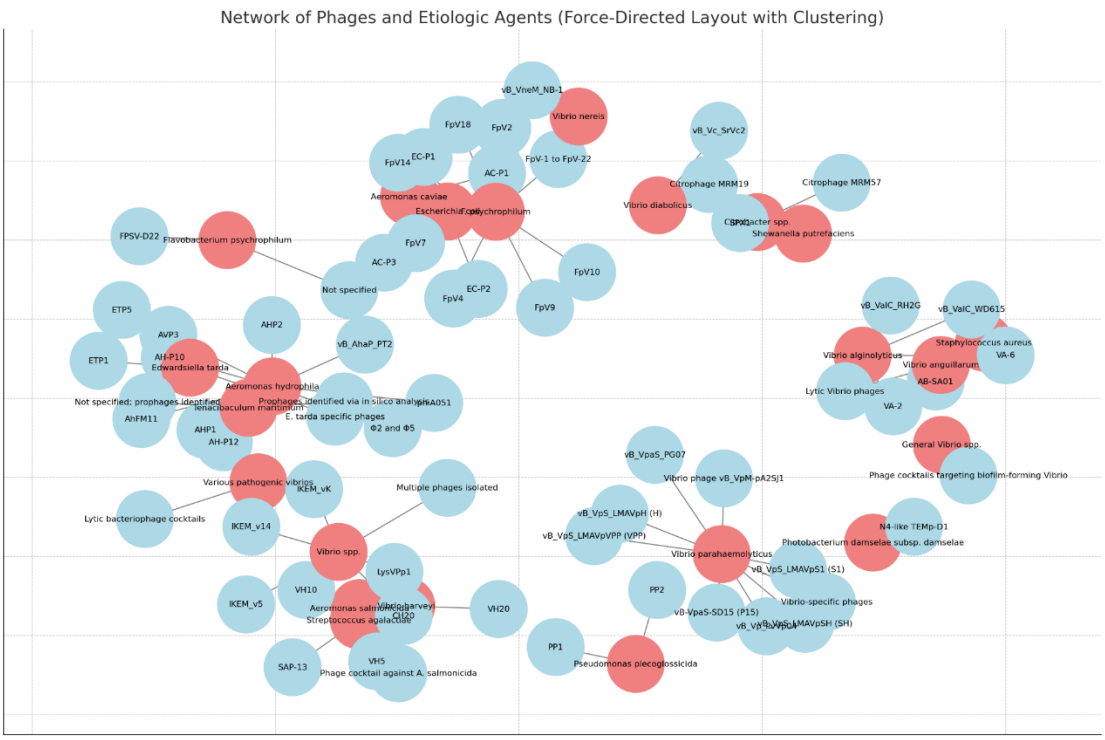

Figure 2. Key Phages Used in Phage Therapy in Aquaculture in Recent Years and Their Complex Relationships with Target Pathogens (The figure was created using Python 3.10 along with the open-source libraries networkx and matplotlib. The layout was generated using the force-directed (spring layout) algorithm.).

The method of application is another critical factor affecting the success of phage therapy. The choice of bacteriophage application route in fish farming depends on factors such as the nature of the infection, farm size, phage preparation cost, and fish species; the three commonly used methods of application in aquaculture are immersion (bath), injection, and oral administration. (Kunttu et al., 2021). Application by injection (usually intraperitoneal) allows phages to be delivered directly to the fish body and shows the highest therapeutic efficacy in systemic infections; since phages quickly enter the circulation and spread to the target organs in parenteral administration. The disadvantages of this method are the difficulty of applying it in large flocks due to the time-consuming and invasive process and the risk of stress-related mortality in fish. In fact, in one study, administration of phages by injection in trout with systemic infection caused by Flavobacterium increased survival from 56.7% to 80% compared to the control group. Oral administration (administration of phages with feed) is one of the most frequently preferred methods in aquaculture because it is practical, low-cost, and allows simultaneous treatment of large numbers of fish with minimal stress. However, phages administered orally may be adversely affected by the harsh conditions in the digestive system of the fish; stomach acidity and the presence of proteolytic enzymes can significantly reduce the infectivity of phages. To overcome this problem, methods such as adding acid neutralizers to the phage suspension or loading phages onto feed particles with acid-resistant protective coatings are used. In addition, the survival of coated phages on dry feed during storage is considered critical in terms of long-term efficacy and commercial viability. In addition, phages administered orally may have limited ability to cross the intestinal mucosa and enter the systemic circulation (Christiansen et al., 2014). Nevertheless, some studies have shown that orally administered phages can pass through the intestinal wall and be detected in internal organs when appropriate formulations are used; even successful treatment of *Pseudomonas plecoglossicida* infections in ayu fish and *F. columnare* infections in catfish has been reported with phage-coated feed. Another study showed that phages administered orally against *Vibrio alginolyticus* reduced infection rates by more than 50%, while prophylactic phages increased immune responses in fish (Liang et al., 2023). In the immersion (bath) method, fish are treated by immersing them in phage-containing water. This approach allows rapid processing of a large number of fish and contributes to the control of infections in these areas since it directly affects the external mucosal surfaces of the fish, such as the skin and gills. For example, in a disease model caused by *F. columnare*, phage bath application prevented the development of infection and provided 100% survival in zebrafish (control 0%) and ~50% survival in trout (control 8.3%). However, the effectiveness of the immersion method under field conditions may be limited due to the difficulty of maintaining sufficient phage concentration in water and the rapid dilution and inactivation of phages. For effective treatment, it is generally necessary to use high

concentrations of phages and, if necessary, to perform repeated applications; Indeed, some experimental studies have reported that phages administered only by immersion did not provide a statistically significant benefit in controlling the target infection, whereas significant improvement was achieved when administered by injection (Laanto et al., 2015).

In conclusion, phage therapy offers immense potential for combating bacterial pathogens and maintaining microbial balance in aquatic ecosystems. Addressing challenges such as phage characterization, industrial production, environmental impacts, resistance mechanisms, application methods, and regulatory frameworks will be critical to realizing its full potential. Expanding research and promoting sustainable applications can support both effective infection management and environmentally sustainable aquaculture practices.

5. Risks and Limitations of Phage Applications in Aquaculture

Phage therapy is seen as a promising alternative in combating bacterial diseases in the aquaculture sector. However, there are several barriers preventing the sustainable and widespread application of phages in aquaculture. Significant risks and limitations include the potential for bacteria to develop resistance to phages, problems related to horizontal gene transfer, and gaps in regulatory frameworks. This section addresses the risks and limitations associated with phage therapy applications in aquaculture.

Phages—particularly temperate (lysogenic) phages—can mediate horizontal gene transfer between host bacteria, potentially carrying antibiotic resistance genes or virulence factors (Geetha et al., 2020). Lysogenic phages can integrate their genome into the bacterial chromosome, enabling the transmission of genes to surrounding bacteria via a process called transduction. This presents a serious risk (Colavecchio et al., 2017). Instead of harming target pathogenic bacteria, lysogenic phages may spread undesirable genes like antibiotic resistance or virulence factors to other environmental bacteria. Therefore, the ability of lysogenic phages to mediate horizontal gene transfer represents a significant safety concern in phage therapy. The use of lysogenic phages carrying resistance or virulence genes could undermine the efficacy of phage applications and lead to major biosecurity risks. Consequently, it is recommended that only lytic (virulent) phages be used in therapy, as they reproduce by lysing the host cell and have minimal potential for gene transfer (Schulz et al., 2022). Although some studies suggest that resistance genes carried by phages may be non-functional and that data on in vivo transduction are limited, excluding high-risk phages from therapy remains an important safety precaution.

Bacterial resistance mechanisms pose a significant challenge to phage therapy, similar to antibiotics. Despite optimal phage formulations and methods, bacteria can develop resistance, threatening the efficacy of phage therapy. Bacteria can counter phages using mechanisms like the CRISPR-Cas system, which enables them to recognize and neutralize phages based on previously encountered genetic material. To mitigate resistance, strategies such as phage cocktails combining multiple phages have shown promise. This approach complicates resistance development and enhances treatment efficacy (Forti et al., 2018). Combining phages with antibiotics can also create synergistic effects, improve treatment outcomes and reduce bacterial resistance rates. The combined use of phages and antibiotics in aquaculture is an innovative and promising strategy. Depending on the application conditions, these combinations can show synergistic effects such as filamentation, depolymerase, temperature phase synergy (tPAS), modulation of surface receptors, evolutionary trade-offs, desensitization of persister cells, use of phase-induced lysozymes (endolysin/lysin), and efflux pump disruption (Jo et al., 2016). Among these synergy mechanisms, filamentation and depolymerase have been reported as the most frequently encountered synergy mechanisms. Some antibiotics can trigger filamentous cell extension (filamentation) in bacteria, increasing the effectiveness of phage infection, and enzymes such as depolymerase secreted by phages can facilitate the penetration of antibiotics into these protected structures by breaking down the biofilm matrix. These interactions play an effective role in controlling pathogenic bacteria and provide significant benefits by increasing treatment efficacy and especially in controlling persistent infections caused by biofilms (Möller et al., 2013). On the other hand, there are also cases where antagonistic interactions are observed: for example, some bacteriostatic antibiotics inhibit bacterial protein synthesis and prevent phage proliferation, and when applied together, the total antimicrobial effect may be lower than expected (Torres-Barceló et al., 2018). Therefore, in order for these combinations to be successful in aquaculture applications, the selection of appropriate phage and antibiotic species and the dose and sequence to be applied must be carefully determined. When planned appropriately, this approach can reduce antibiotic use in aquaculture and stand out as a valuable tool in combating resistant pathogens and biofilms.

Furthermore, the uncontrolled release of phages into aquatic ecosystems may impact not only the target pathogen but also indirectly affect natural microbial communities (Álvarez & Biosca, 2025). Phages play a key role in shaping bacterial population dynamics in natural environments; in marine water, they are reported to eliminate up to 40% of bacterial biomass daily. Introducing high concentrations of foreign phages into aquaculture systems may cause unexpected ecological shifts. For example, studies in agricultural

environments suggest that intense phage addition could lead to widespread resistance selection and the emergence of bacterial populations unresponsive to future phage therapies (Oliveira et al., 2012). Additionally, the normally balanced microbial community structure could be disrupted. Although phages are more targeted than antibiotics, introducing external phages into an ecosystem can disturb the existing bacterial equilibrium. In one study, applying phages to the microbial community inside marine sponges led to the disappearance of certain low-abundance bacterial species while opportunistic bacteria like *Vibrio* proliferated dramatically, significantly altering community structure (Hossain et al., 2024). Such microbial imbalances can impact overall ecosystem health and increase the risk of new, unexpected infections. Moreover, once phages are released into the environment, they cannot be selectively retrieved; as long as suitable hosts are available, they continue to propagate. Therefore, it is critical to assess the environmental impacts of phage applications through small-scale preliminary trials and carefully monitor unintended ecological consequences.

Phages generally exhibit high host specificity. While this specificity protects beneficial microbiota by sparing non-target bacteria—a significant advantage—it also poses practical limitations. A phage may not effectively control multiple pathogens or variant strains present in a complex aquatic environment. Thus, the use of phage cocktails is often necessary (Liu et al., 2022). Combining different phages expands the host range and enables simultaneous targeting of multiple bacterial threats in aquaculture. For example, a phage narrowly targeting *Vibrio* species alone may be ineffective against other pathogens, whereas a cocktail can offer broader protection (Aziz et al., 2024). Using well-characterized phages minimizes off-target effects; however, if a phage's host range is poorly understood or if a benign environmental bacterium shares similar antigenic structures with a target pathogen, unintended targeting could occur. Therefore, careful determination of phage host ranges and strict selection criteria are essential for safe and effective therapy. In short, phage specificity is a double-edged sword: it minimizes collateral damage when used correctly but limits the breadth of application, requiring a tailored phage for each pathogen.

Environmental stability—the durability and activity duration of phages in aquaculture settings—is another key factor influencing therapeutic success. Parameters such as water pH, salinity, temperature fluctuations, and organic load can affect phage survival and infectivity. For instance, phages administered orally via feed face harsh conditions in the fish gastrointestinal tract, such as acidic pH and digestive enzymes that may inactivate phage particles. Studies show that phage suspensions rapidly lose viability due to stomach acid and proteases, indicating a need for protective formulations like encapsulation or enteric coating for oral delivery (Islam et al., 2017). Additionally, ensuring the survival of phages on dry feed pellets during high-temperature pelleting and long-term storage presents challenges. While some phages show broad stability across pH (6–11) and temperature (4–50°C) ranges, others are more fragile (Rai et al., 2023). Ultraviolet (UV) light from sunlight is another destabilizing factor for phages in open systems, as it can damage phage DNA and inactivate them, leading to preferred application in shaded or evening conditions. Dilution of phages in large water bodies may also reduce their effectiveness; achieving the necessary infective dose in vast ponds or sea cages is practically challenging. Effective biocontrol often requires a high phage-to-bacteria ratio, which is difficult to maintain under real-world aquaculture conditions (Oliveira et al., 2012). Therefore, phages in the application field often necessitate repeated dosing or specialized formulations such as microencapsulation or protective additives.

In recent years, regulatory frameworks for the therapeutic use of phages have remained unclear compared to those for conventional veterinary drugs. In many countries, phages have not been fully categorized as either vaccines or drugs, complicating approval and licensing procedures. Current regulations often require the registration of a single phage species as a product, whereas in practice, effective treatment usually requires phage cocktails. Registering a cocktail demands individual approval and evaluation of each phage, making the process bureaucratically burdensome and time-consuming (Sieiro et al., 2020). Globally, only a few commercial phage products have been approved, and none have been developed specifically for aquaculture. Although agencies such as the European Medicines Agency (EMA) emphasize the need for faster adoption of phage therapy in veterinary fields, existing frameworks are poorly adapted to the unique biological nature of phages, treating each application almost as an exceptional case. Regulatory agencies remain cautious, requiring extensive data on environmental impacts and horizontal gene transfer risks, particularly for phage cocktails (Culot et al., 2019). For instance, proving the safety of multi-phage preparations for fish and the environment involves more complex assessments than single-compound drugs. Additionally, regulatory ambiguity exists regarding whether phages are classified as prophylactic or therapeutic agents, which influences the applicable regulations. In the United States, some phage preparations have been approved under the "Generally Recognized As Safe" (GRAS) status for food safety applications (e.g., anti-*Listeria* preparations in food processing), but a similar pathway has yet to be established for veterinary use in aquaculture (Aquaculture and Aquaculture Drugs Basics, 2020). Overall, regulatory uncertainty remains one of the biggest barriers to the commercialization of phage therapies. Clear guidelines and the development of specific frameworks for phages (e.g., under EU Regulation 2019/6) are critical for wider adoption in the industry. Phage therapy products have been classified as 'novel therapies' under Regulation (EU) 2019/6 as of 1 January

2022, and the special provisions added to Annex II by Regulation (EU) 2021/805 envisage a flexible, risk-based approach to the quality, safety and efficacy requirements for phage therapy VMPs. In this context, EMA's 13 October 2023 guideline mandates that marketing-authorization dossiers for monophage or polyphage cocktails systematically address critical quality attributes (CQAs), monitor process parameters (CPPs), provide genomic and phenotypic characterization, confirm the lytic lifecycle and demonstrate absence of toxin/resistance genes in their quality documentation. The guideline further details the structure of Post-Approval Change Management Protocols (PACMPs), the procedures for adaptive variation applications, and the comparability assessment of monophage components in accordance with ICH Q5E principles, thereby enabling compositional updates in response to geographic or resistance-profile variations while ensuring that each change remains controllable in terms of quality, safety and efficacy. This approach simultaneously upholds transparency, predictability and openness to innovation throughout both pre- and post-authorization phases of phage therapy VMPs, enhancing regulatory flexibility and patient access (European Medicines Agency, 2022).

Finally, scaling up laboratory-scale successful phage applications to industrial-scale use presents practical challenges. Commercial production requires standardized processes to produce sufficient quantities of high-quality phages. Since phages are propagated using host bacteria, residual bacterial contaminants must be carefully removed to avoid the presence of endotoxins or exotoxins in the final product (Hietala et al., 2019). Although clinical-grade phage preparations undergo sterility and endotoxin testing, concerns persist regarding possible unwanted elements like pathogenicity islands or toxic proteins (Rai et al., 2023). Therefore, achieving contamination-free phage products according to Good Manufacturing Practices (GMP) remains a significant hurdle. Consistency between production batches is also critical; phage concentration and efficacy must not vary (Jassim & Limoges, 2014). Additionally, storage and shelf-life stability must be ensured: liquid phages require cold chain logistics, while lyophilized forms must retain stability during storage (Muramatsu et al., 2022). These technical aspects are not yet as mature or cost-effective as antibiotic production.

In conclusion, although phages hold significant potential for pathogen control in aquaculture, unresolved issues such as horizontal gene transfer risks, uncontrolled ecological impacts, resistance development, and gaps in regulatory frameworks remain major barriers and limitations to their widespread adoption. Once these challenges are carefully addressed and solutions are developed, the use of phages in aquaculture is expected to become much more widespread.

6. Phage Application Strategies, Biotechnological Advances and Genetic Modifications

In recent years, genetic engineering techniques have been employed to enhance the therapeutic efficacy of phages. Genetically modified phages have been found to exhibit higher specificity against pathogens and greater resilience to environmental conditions. Additionally, phages equipped with CRISPR-Cas9 technologies not only target pathogens but also support natural microbial communities (Ye et al., 2019). These innovations aim to establish lasting therapeutic success for phages while offering a complementary approach to existing methods.

Phage therapy, as a rapidly advancing field of next-generation biological agents for pathogen control, is being significantly expanded by genetic modification techniques. Genetic engineering plays a vital role in increasing phage specificity, preventing resistance development, and creating more environmentally resilient phages. Moreover, innovative technologies such as synthetic biology and CRISPR-Cas have introduced groundbreaking applications that enhance the therapeutic efficacy and flexibility of phages. By optimizing the characteristics of phages, genetic engineering seeks to increase their therapeutic potential. Modified phages are particularly effective against pathogens that have developed antibiotic resistance (Sundell et al., 2020). For example, genetically engineered phages can target antibiotic resistance genes, deactivate them, and directly inhibit the development of resistance. Another key application is the optimization of the life cycle. Enhanced lytic phages can replicate more rapidly and effectively in infected areas, significantly reducing pathogen loads. Studies have demonstrated that genetically modified phages effectively reduce infection rates caused by common aquaculture pathogens such as *Flavobacterium psychrophilum* and *Vibrio* spp. (Imbeault et al., 2006;).

CRISPR-Cas systems represent an innovative technology enabling the genetic engineering of phages for increased specificity. CRISPR-equipped phages selectively target pathogenic bacteria while maintaining the balance of microbial communities in aquatic ecosystems. For instance, studies utilizing CRISPR-phage combinations against antibiotic-resistant bacteria like *Aeromonas hydrophila* have reported effective pathogen elimination (Sundberg et al., 2021). In another study, targeted genome editing was performed on the phage TT4P2 derived from *Vibrio natriegens* using CRISPR–Cas9 technology. The *orf6* gene of the phage was excised and replaced with a gene encoding lysozyme, thereby enhancing bacterial cell lysis. This high-efficiency modification, achieved through a dual-plasmid system, enables the development of genetically engineered, effective, and customized phages for use in aquaculture. (Zhang et a., 2022). In another study, a combination of the natural lytic phage CH20 and its recombinant endolysin (LysVPp1) was evaluated as a

preventive strategy against *Vibrio*-induced infections during the larval stage in aquaculture. The lytic phage CH20 was isolated from *Vibrio alginolyticus*, and the gene encoding the endolysin was cloned via synthetic biology into an *E. coli* expression system, where it was purified as a His-tagged recombinant protein. The lytic activity of this protein was tested against logarithmic-phase cultures of *V. alginolyticus*, *V. parahaemolyticus*, and *V. splendidus*, and its efficacy was confirmed through optical density reduction. In combination therapy trials administered to live feed (rotifers) and gilthead sea bream (*Sparus aurata*) larvae, both *Vibrio* load and larval mortality rates were monitored. The results demonstrated that the phage–endolysin combination significantly reduced bacterial load and improved larval survival compared to treatments with the phage or endolysin alone. These findings highlight the potential of engineering-based phage therapies as effective biocontrol strategies for early life stages of fish in aquaculture (Romeo et al., 2024). Choudhury et al. (2019) developed a recombinant lysozyme (r-lysozyme)-supported approach to enhance the therapeutic efficacy of phage applications targeting *Vibrio harveyi* infections. In their study, a lytic phage specific to *V. harveyi* was first isolated and characterized. Subsequently, a recombinant shrimp lysozyme gene was cloned into an expression vector and produced in an *E. coli* system. The purified lysozyme protein was co-applied with the phage to *V. harveyi* cultures under various environmental conditions (pH 5–9, salinity 5–35 ppt) in vitro, and bacterial lysis was monitored by changes in optical density (OD600). In parallel, microcosm models simulating brackish water environments similar to shrimp aquaculture systems were established to assess the effects of the phage, lysozyme, and their combination on bacterial load. The results revealed that the addition of r-lysozyme significantly enhanced the lytic effect by promoting phage adsorption and cell wall degradation. This bioengineering-based strategy is particularly promising against *Vibrio* infections in shrimp farming (Choundry et al., 2019). Furthermore, CRISPR-Cas systems enhance the genetic durability of phages, making them more stable against environmental factors (Ye et al., 2019). As a result, controlling resistant bacteria in aquatic environments becomes more efficient and sustainable.

Recent advancements in biotechnological methods and artificial intelligence applications have facilitated the design of synthetic phages. These phages can be genetically modified to target specific pathogens and are produced more controllably than natural phages. For instance, synthetic phages designed against *Vibrio parahaemolyticus* have shown a broader spectrum of activity and greater stability under environmental conditions compared to natural phages (Dang et al., 2021; Liang et al., 2023). This technology is expected to revolutionize the aquaculture industry. Additionally, genetic engineering enables phages to acquire new functions, such as antibiotic production, toxin neutralization, or enhancing immune responses. For example, phages carrying genes to boost fish immune responses have been shown to reduce disease incidence and improve growth rates (Kunttu et al., 2021). These multifunctional phages provide an innovative solution that combines treatment and protection applications in aquaculture.

On the other hand, preventing resistance development is critical for the long-term success of phage therapy. Genetic engineering allows phages to be modified for continuous evolution against bacteria. Moreover, phage cocktails targeting multiple pathogens simultaneously have proven to be an effective strategy in reducing resistance development (Sundell et al., 2020). This approach significantly slows resistance acquisition as pathogens encounter multiple defense mechanisms concurrently.

The successful implementation of phage therapies depends on integrating innovative technologies and developing appropriate application strategies. Techniques such as microencapsulation, nanotechnology, and genetic engineering enhance the efficacy of phages, paving the way for broader adoption in the aquaculture sector. These advancements not only support environmental sustainability by reducing antibiotic usage but also help mitigate economic losses in aquaculture.

7. Conclusion

Aquaculture has become an increasingly vital sector in meeting the global demand for animal protein. However, the intensification of farming practices has been accompanied by a rise in infectious diseases, prompting the widespread use of antibiotics. This in turn has led to the emergence of antibiotic-resistant bacterial strains, posing a critical challenge for both aquatic animal health and environmental sustainability. In light of this, bacteriophage therapy has attracted increasing interest as an alternative.

Phage therapy offers the unique advantage of targeting specific bacterial pathogens without disturbing the beneficial microbiota of the aquatic environment. Nonetheless, several limitations impede its widespread application, such as phage-host specificity and the genetic diversity of pathogenic bacteria. These challenges necessitate integrated strategies combining traditional approaches with emerging biotechnological tools.

Recent advances in molecular biology and genetic engineering have opened new avenues to enhance the efficacy of phage therapy. Notably, CRISPR-Cas systems have been employed to edit phage genomes, allowing the construction of recombinant phages with broader host ranges, improved LYTIC activity, or engineered payloads such as antimicrobial peptides and lysins. For instance, CRISPR-mediated deletion or insertion of genes into phage genomes has enabled the design of phages with enhanced antibacterial

capabilities against aquaculture-relevant pathogens such as *Vibrio spp.* and *Aeromonas spp.* Furthermore, the co-application of recombinant lysins and phages has demonstrated synergistic effects in reducing pathogen loads in larval rearing systems, improving survival rates without resorting to antibiotics.

In conclusion, the integration of phage therapy with genetic engineering technologies such as CRISPR represents a transformative approach for disease control in aquaculture. While traditional phage therapy alone faces limitations in field conditions, the development of engineered phages tailored for enhanced stability, spectrum, and efficacy offers a promising path forward. These innovations not only respond to the urgent need to reduce antibiotic dependency but also align with the goals of sustainable aquaculture production. Moving forward, interdisciplinary research that bridges microbiology, aquaculture, and biotechnology will be essential to develop robust, scalable, and regulatory-compliant phage-based solutions for the industry.

8. Compliance with Ethical Standard

a) Author Contributions
Single author.

b) Conflict of Interests
The authors declared that they have no conflict of interest.

c) Statement on the Welfare of Animals
Not relevant

d) Statement of Human Rights
There are no human subjects in this study.

e) Funding
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