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Effectivity of bacteriophage therapy against *Streptococcus agalactiae* in Tilapia: A systematic review

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ABSTRACT

Streptococcus agalactiae infection remains one of the most significant bacterial diseases affecting tilapia, leading to substantial economic losses in global aquaculture. Increasing antibiotic resistance has prompted the need for sustainable and effective alternatives such as bacteriophage therapy. This study aims to systematically review and synthesise the current evidence regarding the efficacy of bacteriophage therapy in controlling *Streptococcus agalactiae* infections in tilapia. Following the PRISMA 2020 guidelines, a comprehensive literature search was performed across PubMed, Scopus, ScienceDirect, and Google Scholar to identify studies published between 2001 and May 2025. Eligible studies were selected according to predefined inclusion and exclusion criteria based on the PICO framework. Relevant data were extracted on phage morphology, host specificity, growth kinetics, physicochemical stability, and therapeutic efficacy. The methodological quality and potential risk of bias of the included studies were evaluated using the SYRCLE Risk of Bias tool. A total of 6 studies matched with our framework were obtained from 2013 to 2024, describing 10 bacteriophages with potential as therapeutic agents against *Streptococcus agalactiae* infections in tilapia. All identified phages belonged to the class *Caudoviricetes*, comprising 7 *Siphoviridae*, 2 *Myoviridae*, and 1 unclassified phage. Phages were isolated from several sources, including infected tilapia (3 isolates), rearing pond water (6 isolates), and milk from a mastitis-infected cow (1 isolate). Reported growth kinetics showed burst sizes ranging from 20 to 1,236 PFU per cell, with multiplicity of infection (MOI) values between 0.01 and 0.1. The phages were generally stable below 30°C and at pH around 8. These characteristics and combined with their high host specificity indicate promising potential for phage therapy in tilapia combating *Streptococcus agalactiae*. The included studies demonstrated a low risk of bias in reporting and attrition domains. However, it remained unclear in selection, performance, and detection domains due to limited methodological details. Bacteriophages from *Caudoviricetes* reported in this study show promising potential as environmentally friendly options for controlling *S. agalactiae* infections in tilapia. Future work should focus on standardizing experimental procedures, improving reporting quality, and increasing in vivo testing under real aquaculture conditions.

Introduction

Aquaculture is one of the promising sectors that plays a key role in meeting the global protein demand driven by the growing world population (FAO, 2024, 2025; UNDESA, 2022). In 2023, Nile tilapia (*Oreochromis niloticus*) ranked as the second most-produced finfish species worldwide, with an estimated production of 5 million tonnes valued at approximately USD 11 billion, of which around 90% originated from aquaculture (FAO, 2025). Despite this remarkable productivity, tilapia aquaculture continues to face several challenges that constrain its full potential. Streptococcosis, a septicemic disease caused by Gram-positive bacteria of the family Streptococcaceae, is considered as a worldwide fish health challenge that has caused economic loss around USD 250 million annually (Klesius *et al*., 2008). The susceptibility of *O. niloticus* to this disease is well documented, with *Streptococcus agalactiae* and *Streptococcus iniae* identified as the primary etiology (Abdallah *et al*., 2024; Jantrakajorn *et al*., 2014; Mishra *et al*., 2018).

To control bacterial infections, farm practitioners have traditionally relied on antibiotic administration. However, the long-term application of antibiotics promotes antimicrobial resistance (AMR) that has wide adverse implications from failed disease control in farms to human health and environmental risks (Li *et al*., 2022; Lulijwa *et al*., 2020; Schar *et al*., 2021). According to the World Health Organization (2023), antibiotic-resistant infections were responsible for approximately 4.95 million deaths globally between 2019 to 2023. A recent analysis further projected that this number could exceed 39 million by 2050 (Naddaf, 2024). Therefore, it is essential to explore alternative strategies that are not only effective but also safe for both farming operations and public health.

Bacteriophages, viruses that infect bacteria, naturally occur and are abundant in various environments. These viruses undergo a lytic infection cycle in which they inject their genetic material into bacterial hosts, replicate within them, and subsequently assemble and release new viral particles that lyse and kill the host cells. The therapeutic approach that harnesses this lytic cycle to eliminate pathogenic bacteria is referred to as phage therapy (Kortright *et al*., 2019). Unlike antibiotics, which require repeated administration to animals, phage therapy focuses on proliferation and amplification of phage which is not limited by bacterial resistance. Moreover, whereas antibiotics exhibit broad-spectrum activity that can eliminate both harmful and beneficial bacteria, bacteriophages display a high degree of host specificity, targeting only particular bacterial species without disrupting the commensal microbiota of the treated animals (Ganeshan & Hosseinidoust, 2019). Therefore, bacteriophages have the potential to be a viable, eco-friendly, and precise biocontrol agent as an alternative to antibiotics in aquaculture. Recent reports suggest that bacteriophages have been effective in combating outbreaks of infectious diseases caused by various bacterial infections in aquaculture, such as *Aeromonas hydrophila*, *Vibrio parahaemolyticus*, and *Vibrio harveyi* (Cui *et al*., 2021; Dien *et al*., 2022; Ren *et al*., 2019).

Efforts to mitigate streptococcosis infections in aquaculture through bacteriophage therapy remain scarce and predominantly restricted to *in vitro* characterization studies, with *Streptococcus agalactiae* representing the most extensively explored target for phage isolation (Luo *et al*., 2018;

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Preenanka & Safeena, 2023; Abdel–Razek *et al*., 2025). Although bacteriophages have gained increasing attention as sustainable and highly specific biocontrol agents against bacterial pathogens, their application within aquaculture systems has progressed slowly compared to advances in human and terrestrial animal health. This research gap, coupled with the absence of standardized methodologies and large-scale in vivo validation, continues to hinder the translation of laboratory findings into practical disease management strategies for farmed fish. To date, no comprehensive synthesis has been published that consolidates current evidence on the therapeutic efficacy of phage therapy against *S. agalactiae* infections in tilapia. Thus, this study aimed to systematically review and synthesize the available evidence on the efficacy of bacteriophage therapy for controlling *Streptococcus agalactiae* infections in tilapia.

Materials and methods

Protocol and registration

A systematic literature review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines (Page *et al*., 2021). The protocol for this systematic review was prospectively registered with the Open Science Framework (OSF) [https://osf.io/wapc6/overview], the review was conducted in full accordance with the registered protocol without any deviations.

Eligibility criteria

This systematic review considers all original journal articles published from 2001 to May 2025 in English. The inclusion and exclusion criteria for this study were determined based on the PICO framework as follows; Population: *Streptococcus agalactiae* from Tilapia, Intervention: therapy of *Streptococcus agalactiae* phage, Comparison: Group without *Streptococcus agalactiae* phage therapy, Outcome: phage morphology, one-step growth curve, and burst size.

Search strategy

A comprehensive search of the literature was conducted using a combination of keywords such as "phage", "bacteriophage", "Nile Tilapia","*Oreochromis niloticus*", and "*Streptococcus agalactiae*" from four international databases (PubMed, Scopus, ScienceDirect, and Google Scholar). The literature identified through database searches was collated and uploaded into Rayyan-Intelligent Systematic Review (https://www.rayyan.ai/) for initial screening process (Ouzzani *et al*., 2016).

Study selection

The retrieved studies were independently screened by two authors (HW and EIR) to determine their eligibility based on the established inclusion and exclusion criteria. The criteria of included studies were experimental study, original article or conference proceeding, using *S. agalactiae* from Tilapia, consisting of data effectivity of *S. agalactiae* phage therapy, and in English. The exclusion criteria were as follows: cross sectional study or cohort study, review article, news, theses, and any other type of article, using others bacterial phage therapy, and *S. agalactiae* from other fish or animals.

Data extraction, quality assessment and analysis

Data from the included studies were extracted into Microsoft Excel 2016 (Microsoft, Redmond, WA, USA). The following characteristics were extracted from each study: author (s), phage ordo and family, name of phage, phage and bacterial source, latent period at one-step growth curve, burst size, multiplicity of infection (MOI) of phage, optimum temperature and pH for phage stability. The studies that fulfilled the inclusion criteria were summarised in Table 1.

Risk of Bias (RoB) Assessment

Risk of bias (RoB) assessments were performed independently for *in vitro* and in vivo research to guarantee methodological rigor. For in vivo experiments, we utilized the initial iteration of the SYRCLE (Systematic Review Centre for Laboratory Animal Experimentation) Risk of Bias tool, specifically developed for animal research (Hooijmans *et al*., 2014). This instrument assesses potential bias across ten critical domains: selection bias (encompassing randomization in allocation, similarity in baseline characteristics, and allocation concealment), performance bias (blinding of researchers administering interventions and caring for animals), detection bias (blinding of researchers assessing outcomes), attrition bias (management of incomplete outcome data), and reporting bias (selective reporting).

For *in vitro* experiments, we employed a customized version of the SYRCLE tool tailored for the laboratory research situation. This revised version preserves the original 10-domain framework, yet the inquiries have been tailored to confront particular biases in *in vitro* experiments: selection bias (randomness in the selection of bacterial/phage isolates, consistency of baseline conditions, and concealment of sample allocation), performance bias (blinding of researchers conducting experiments and maintaining cultures), detection bias (blinding of those measuring outcomes), attrition bias (completeness of data from initial experiments), and reporting bias (completeness of result reporting and indications of selective reporting). Each domain will be evaluated as 'Low', 'Unclear', or 'High'. We utilized the robvis web tool (McGuinness & Higgins, 2021) to illustrate the outcomes of the risk of bias evaluation. The results were interpreted qualitatively.

Results

A comprehensive systematic search across the four databases resulted in 499 articles, with 42 duplicates removed during the screening process. After removing duplicates, 457 records were screened, resulting in 15 records being retrieved. Among the retrieved articles, 7 reports were excluded because they were literature reviews, leaving 8 for full text screening. Following the assessment of eligibility, a total of 6 studies met all the inclusion criteria and were included in the systematic review as shown in Figure 1. All the studies were conducted from 2013 to 2024. There were 7 subjects in this systematic review, comprising 6 *S. agalactiae* collected from infected Tilapia, and 1 *S. agalactiae* collected from bovine. The *S. agalactiae* strains used in across studies, such as symh01, symh02, KSA/01, CMFRI/SA-01, and SS130920.

Phage characteristics and sources

A total of six studies investigating the use of bacteriophages against *Streptococcus agalactiae* in tilapia were reviewed. From those studies, 9 bacteriophages were identified as Caudovirales. Among them, 6 phages were categorized in the *Siphoviridae*/*Caudioviricetes* family including vB_Sags-UPM1, phage SAP-13, phage *JX01*, *Phage*-15F, Phage-12P and Phage-16E. The other 2 phages, namely Phage-20D and Phage-1A, were categorized as *Myoviridae*/*Caudioviricetes*. While 1 bacteriophage, HN48, was yet identified at family level. Distinct morphological head and tail structures were observed, *Siphoviridae* has an icosahedral head at diameter of 50 nm to 83.34 nm, and possess non-contractile tails at length between 110 to 220 nm. *Myoviridae* exhibits isometric head at diameter of 79.88 to 101.52 nm, and possess contractile tail structures with length around 62.60 to 98.26 nm. While, HN48 has a hexagonal head with diameter of 98 nm and long tail around 200 nm. The bacteriophages were isolated from diverse aquatic and infection-related environments, including

Table 1. Summary of studies included.

Study No.	Phage ordo	Phage family	Name of phage	Phage source	Bacteria	Bacteria source	One-step growth curve	Burst size	MOI	Phage stability		Authors
										Temperature	pH	
1	Caudovirales	*Siphoviridae*	vB_Sags-UPM1	Infected tilapia from outbreaks pond	*Streptococcus agalactiae* symh01	Infected tilapia from outbreaks pond	latent period : 30 min	356.53±38.53 PFU/cell	NR	NR	NR	(Khair *et al*., 2023).
	Caudovirales	*Siphoviridae*	vB_Sags-UPM1	Infected tilapia from outbreaks pond	*Streptococcus agalactiae* symh02	Infected tilapia from outbreaks pond	latent period : 40 min	99.48±14.44 PFU/cell	NR	NR	NR	
2	Caudovirales	*Siphoviridae*	SAP-13	Water from aquaculture farms	*Streptococcus agalactiae* KSA/01	Commercial tilapia farms which reported streptococcosis	latent period : 30 min	610 PFU/cell	0.01	30°C	8	(Preenanka & Safeena, 2024).
3	Caudovirales	*Siphoviridae*	*JX01*	Milk from mastitis-affected cows	*Streptococcus agalactiae*	Bovine and infected tilapia	latent period : 30 min	20 PFU/cell	NR	<50°C	11-Mar	(Bai *et al*., 2013).
4	Caudovirales	*Siphoviridae*	Phage-15F	Water from aquaculture farms	*Streptococcus agalactiae* CMFRI/SA-01	Infected tilapia	latent period : 20 min	1,236 PFU/cell	0.01	<30°C	8	(Preenanka & Safeena, 2023).
	Caudovirales	*Myoviridae*	Phage-20D	Water from aquaculture farms	*Streptococcus agalactiae* CMFRI/SA-01	Infected tilapia	latent period : 30 min	640 PFU/cell	0.01	<30°C	8	
	Caudovirales	*Siphoviridae*	Phage-12P	Water from aquaculture farms	*Streptococcus agalactiae* CMFRI/SA-01	Infected tilapia	latent period : 50 min	790 PFU/cell	0.01	<30°C	8	
	Caudovirales	*Siphoviridae*	Phage-16E	Water from aquaculture farms	*Streptococcus agalactiae* CMFRI/SA-01	Infected tilapia	latent period : 30 min	729 PFU/cell	0.01	<30°C	8	
5	Caudovirales	*Myoviridae*	Phage-1A	Infected tilapia	*Streptococcus agalactiae* CMFRI/SA-01	Infected tilapia	latent period : 20 min	119 PFU/cell	0.01	4-37 °C	8	(Preenanka *et al*., 2023).
6	Caudovirales	NR	HN48	Tilapia pond which reported streptococcosis	*Streptococcus agalactiae* SS130920	Infected tilapia	latent period : 25 min	200 PFU/cell	0.1	<60 °C	10-Jun	(Luo *et al*., 2018).

NR = not reported

water from aquaculture farms, infected tilapia ponds, and, in one case, milk from mastitis-affected cows.

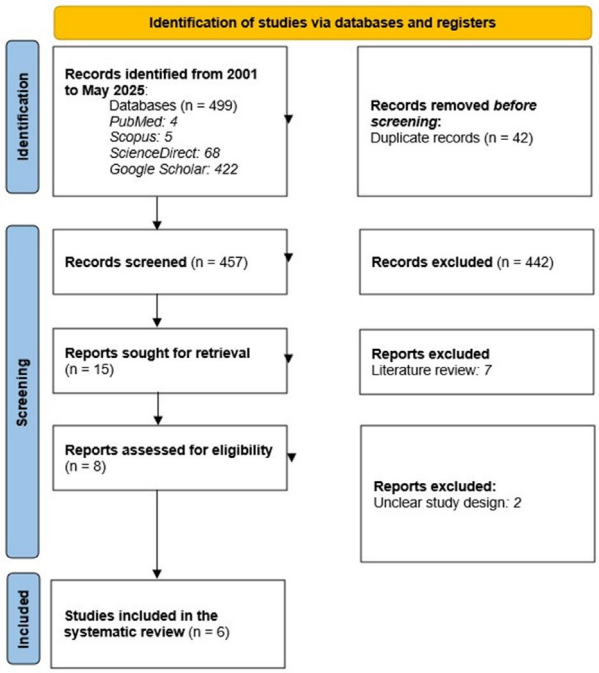

Figure. 1. The systematic review and meta-analysis PRISMA flow diagram for this study.

Growth kinetics of phages

Phage growth characteristics were assessed using one-step growth curve assays, revealing latent periods between 20 and 50 minutes. Burst size varied significantly, from 20 PFU/cell (*JX01*) to as high as 1236 PFU/cell (Phage-15F), indicating differences in replication potential. The multiplicity of infection (MOI) was reported in some studies and ranged around 0.01 to 0.1, which is considered efficient for *in vitro* phage proliferation.

Physico-chemical stability of phages

Thermal and pH stability testing showed that most phages remained stable below 30°C and across a pH range centered around 8. One phage (Phage-1A) demonstrated stability from 4°C to 37°C, suggesting its resilience in variable environmental conditions. However, data on phage stability were not consistently reported across all studies.

Risk of Bias (RoB) Assessment

The results of the Risk of Bias Assessment analysis for all studies can be seen in Figure 2.

Discussion

This systematic review summarized the studies on the application of bacteriophages targeting *Streptococcus agalactiae* in tilapia aquaculture. A total of six studies published between 2013 and 2024 were identified, indicating a growing but still limited research focus on phage therapy as an alternative strategy to control this pathogen. Several promising candidates were identified, including SAP-13 and Phage-1A that exhibit strong lytic potential and environmental resilience (Preenanka *et al*., 2023; Preenanka & Safeena, 2024), and also vB_Sags-UPM1 stands out for possessing an endolysin gene (Lys60) that may strengthen its antibacterial activity (Khair *et al*., 2023). All identified phages in the reviewed studies were historically classified under the order Caudovirales, primarily within the *Siphoviridae* family, with a smaller representation from *Myoviridae*. However, taxonomic revisions made in 2022 by the International Committee on Taxonomy of Viruses (ICTV) have redefined bacteriophage classified Caudovirales and its morphological families (Podoviridae, *Siphoviridae*, and *Myoviridae*) into a new class called *Caudoviricetes*. This class includes all tailed double-stranded DNA viruses with icosahedral capsids (Turner *et al*., 2023).

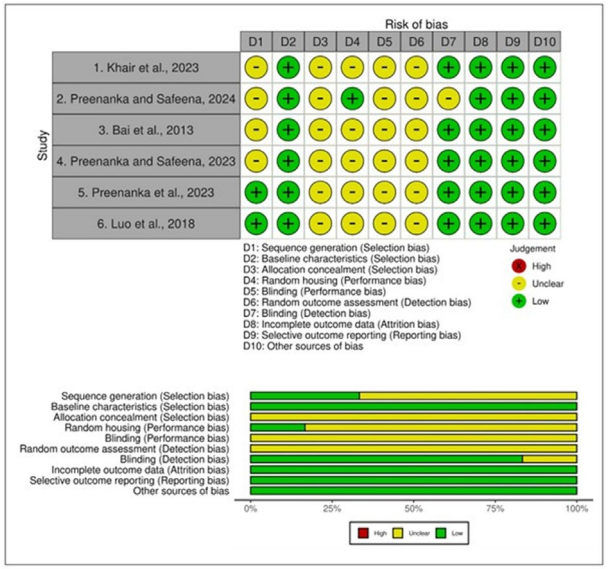

Figure 2. Results of the Risk of bias assessment analysis using the SYRCLE tools approach for all included studies.

As of September 2025, *Caudoviricetes* consists of 11 orders, 105 families, 132 subfamilies, 1,679 genera, and 5,798 species. Members of this class represent about 95% of all known bacteriophages and have been widely applied in both animal and environmental health studies (Żbikowska *et al*., 2020). Their complex structure, characterized by an icosahedral head and tail fibers, facilitates efficient host recognition, rapid adsorption, and effective bacterial lysis. Lytic *Caudoviricetes* phages also lack lysogenic genes, reducing the risk of horizontal gene transfer, and have shown safety across multiple routes of administration, including oral, topical, intranasal, and intravenous (Loponte *et al*., 2021; Mehmood Khan *et al*., 2023). For these reasons, *Caudoviricetes* phages are considered promising candidates for developing robust, scalable, and biosafe phage therapeutics in aquaculture. The frequent detection of *Siphoviridae*-like morphotypes among *Streptococcus agalactiae* phages suggests variation in tail structures and head sizes that may influence these phages' attachment to host, environmental survivability, and host specificity. These parameters should be explored in future studies.

Most of the phages described in the reviewed studies were isolated from infected tilapia and aquaculture pond water, supporting the idea that phages are abundant in environments where their bacterial hosts exist. Studies by Preenanka *et al.* (2023) and Preenanka & Safeena. (2023, 2024) consistently showed that *Streptococcus* phages tend to have a narrow host range, infecting about 75–80% of different *S. agalactiae* strains, while maintaining high specificity (100%) for the intended pathogen. This host restriction can be beneficial for therapy as it reduces unwanted effects on commensal or beneficial microbiota which is an important biosafety concern for aquaculture applications (Scarascia *et al*., 2018). Interestingly, a report by Bai *et al.* (2013) demonstrated a different case where a *Streptococcus*-phage from mastitis-infected cow's milk showed broad activity against bovine *S. agalactiae* but limited infectivity toward tilapia isolates. This finding highlights the ecological specificity of phage-host interactions, although some phages can cross ecological boundaries (Bignaud *et al*., 2025). Such ecological confinement is both an advantage and a limitation as it improves the therapeutic precision and environmental safety, but it needs local isolation and characterization of phages that are suited to specific aquaculture pathogens and environments.

Distinct variation was found among studies in phage growth kinetics and stability. Most studies used a one-step growth curve to estimate burst size and latent period by monitoring a single round of infection.

The latent period ranged from 20 to 50 minutes, while burst size varied from 20 to 1,236 PFU per cell. The latent period is the time needed for a phage to complete its lytic cycle, from the initial attachment to the host bacterium to the release of new viral particles after cell lysis. For many bacteriophages, this period usually lasts between 10 minutes and one hour (Dominguez-Mirazo *et al*., 2024). The burst size, which indicates the average number of phages released from each infected cell, depends on the duration of the intracellular phase, during which new virions are assembled (Kannoly *et al*., 2023). Both burst size and latent period are related to factors such as host strain, growth medium, temperature, and the host's growth rate (Sun *et al*., 2012).

The multiplicity of infection (MOI) was reported inconsistently, making cross-study comparison of *S. agalactiae* phages difficult and highlighting both biological variation and methodological differences. MOI represents the ratio between infecting phage particles and bacterial host cells. A low MOI (<0.1) often promotes single infections and clearer plaque formation (Abedon, 2023) while infection by a single phage typically results in lysis, whereas a higher MOI results in lysogeny (Nguyen *et al*., 2023). Stability results also varied widely, and several studies did not report them in detail, limiting conclusions about which phages have the best environmental tolerance. Future research should include systematic testing of temperature and pH stability to identify phages that can survive under the changing conditions typical of aquaculture environments.

A careful risk of bias assessment was carried out to evaluate the internal validity of the included studies. This step is important in any systematic review because it helps identify possible errors in study results caused by biases such as selection, performance, detection, attrition, or reporting (Hopp, 2015; Reitsma *et al*., 2023; Seo *et al*., 2023). Risk of bias (RoB) analysis of four *in vitro* studies: Khair *et al*. (2023), Preenanka & Safeena. (2024), Bai *et al*. (2013) and Preenanka & Safeena. (2023) showed a consistent risk profile. Although these studies had low risk of bias in the reporting and attrition domains due to complete and transparent data reporting, they had an unclear risk of bias in the selection (randomization and allocation concealment), performance, and detection (blinding) domains. This unclear condition was mainly due to the lack of specific reports on bias mitigation measures in the methods section of the paper. In contrast, two in vivo studies Preenanka *et al*. (2023) and Luo *et al*. (2018) showed a different RoB profile. Both studies had a low risk of bias in the domains of selection (randomization of allocation and similarity of baseline characteristics) as well as attrition and reporting. However, these studies also had an unclear risk of bias in the domains of performance and detection because there was no information about blinding for researchers conducting the intervention or outcome measurements. Most *in vitro* studies showed a good quality of methodology and adequate data reporting. However, some aspects are lacking, such as randomization, allocation concealment, and blinding that were often not clearly explained. Similarly, in vivo studies in general showed low risk in selection and reporting areas, but they often had unclear risks in performance and detection due to lack of information about investigator or assessor blinding. These gaps reduce confidence in the results and should be interpreted carefully. Assessing risk of bias is closely related to internal validity and remains essential to ensure the reliability and clarity of the overall findings (Marshall *et al*., 2015; Viswanathan *et al*., 2018; Yang *et al*., 2017). Therefore, future studies should implement stronger experimental designs with clear randomization, blinding, and standardized reporting to improve the reproducibility and credibility of phage therapy research in aquaculture.

This systematic review has several limitations that need to be acknowledged. First, the number of studies investigating bacteriophage therapy against *S. agalactiae* in tilapia is limited with wide variation in experimental design, sample size, and outcome measures. These differences make it difficult to combine results or directly compare findings across studies. Second, most of the included research was conducted under *in vitro* conditions and only a small number used in vivo experiments. Thus, how well the results can be applied to real aquaculture settings remains unclear. Third, the results of risk of bias assessment showed a lack standardized method which may have affected the reported results and makes comparison between studies more challenging. Overall, these limitations show the need for more comprehensive and standardized research before clear conclusions can be drawn about the reliability of bacteriophage therapy for tilapia health management.

Conclusion

Bacteriophages, especially members of *Caudoviricetes* class, show promising potential as an environmentally friendly option for controlling *S. agalactiae* infections in tilapia (*Oreochromis niloticus*). The reports support bacteriophage's potential following its high host specificity, safety, and strong lytic effects. However, the number of studies is limited and lack consistent methods that reduce the reproducibility and credibility of the results. Therefore, future work should focus on standardizing experimental procedures, improving reporting quality, and increasing in vivo testing under real aquaculture conditions. As antimicrobial resistance continues to challenge global aquaculture, bacteriophage therapy offers a practical, sustainable, and much-needed alternative for maintaining fish health and production.

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Conflict of interest

The authors declare that they have no conflict of interest.

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