

Research Article

Sediments as Hotspots for Bacteria and Bacteriophages in Freshwater Fish Ponds in Lapai, Niger State, Nigeria

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Abstract

Bacteriophages are key regulators of bacterial populations in aquatic ecosystems and are increasingly recognized for their potential application as biocontrol agents in aquaculture. This study investigated the occurrence, distribution, and preliminary characterization of bacteriophages in freshwater fish ponds in Lapai, Niger State, Nigeria. Water and sediment samples were collected from two semi-urban aquaculture sites (Daudu Maza Road and Federal Low-Cost) and analyzed for bacterial loads and phage presence. Bacterial enumeration on selective media revealed significantly higher counts of *Pseudomonas* spp. and *Escherichia coli* in sediments compared to surface water, with the highest loads recorded in Federal Low-Cost Pond sediments. Bacteriophages were isolated using enrichment and double-layer agar techniques with *Pseudomonas* spp. and *E. coli* as host bacteria. Lytic phages were predominantly associated with *Pseudomonas* spp., exhibiting higher titers in sediment samples (up to 4.6×10^6 PFU g⁻¹), whereas *E. coli*-specific phages were detected only at low abundance in one water sample. Distinct plaque morphologies suggested potential diversity among the isolated phages. The results demonstrate that fish pond sediments serve as critical reservoirs for both bacteria and bacteriophages, with phage distribution closely reflecting host availability. This study provides baseline data on bacteriophage occurrence in Nigerian aquaculture systems and highlights their ecological relevance and potential role in sustainable pathogen management as alternatives to antibiotics.

Keywords

Sediments, Hotspots, Aquaculture, Bacteriophages, Fish Pond

1. Introduction

Bacteriophages (phages) are ubiquitous and diverse viruses that exclusively infect bacteria by lysing bacterial cells and injecting their genetic material, taking over the host's cellular machinery to replicate themselves. Bacteriophages are incredibly diverse, with different types infecting specific bacterial species or strains [1, 2]. They play a central role in modulating microbial community dynamics in aquatic ecosystems [3, 4]. Through host-specific lysis, phages influence bacterial population structure, nutrient cycling,

and horizontal gene flow, making them important ecological agents in freshwater and aquaculture environments [5]. Fish ponds are nutrient-rich systems with high bacterial diversity due to inputs such as fish excreta, uneaten feed, and external runoff, creating conditions conducive to bacteriophage abundance and activity.

Aquaculture is the practice of cultivating aquatic plants and animals, such as fish, shellfish, and seaweed, in controlled environments like ponds, tanks, or ocean enclosures.

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It has emerged as one of the fastest-growing sectors in global food production, providing nearly half of the world's aquatic animal supply [6]. Aquaculture environments frequently harbor bacteria of significant economic and health concern, including genera such as *Aeromonas*, *Vibrio*, *Pseudomonas*, and *Flavobacterium*, which are associated with diseases that reduce fish productivity and increase mortality [6, 7]. The continued reliance on antibiotics to manage these pathogens has contributed to the emergence of antimicrobial resistance and residual contamination in aquatic environments, prompting exploration of alternative control strategies [8, 9]. Bacteriophages offer several advantages as biocontrol agents—including high specificity for target bacteria, minimal disruption to non-target microbiota, and self-replication in the presence of host bacteria, highlighting their potential in sustainable aquaculture management [8, 10].

Isolation and identification of bacteriophages from fish pond environments are essential steps toward developing phage-based therapeutics by identifying phages that target specific pathogenic bacteria prevalent in fish ponds [11]. Generally, involve enrichment of pond samples with suitable bacterial hosts, followed by detection of lytic activity through plaque assays such as the double-layer agar method. Subsequent characterization includes analysis of plaque morphology, host range specificity, and environmental stability, with advanced identification achieved through morphological and molecular techniques [12]. Recent studies have isolated lytic phages from aquaculture ponds and characterized their host specificity, environmental tolerance, and genomic features to assess their suitability for biocontrol applications (e.g., phages targeting *Streptococcus agalactiae* in tilapia ponds) [13]. Such research contributes to understanding phage diversity and ecology in aquaculture systems and provides a foundation for developing phage-based interventions that may help mitigate bacterial diseases and reduce dependency on antibiotics.

The aquaculture industry in Nigeria has been significantly hampered by bacterial infections, leading to substantial economic losses and threatening food security. Notably, *Edwardsiella tarda* has emerged as a significant pathogen, causing high morbidity and mortality across various fish species and posing zoonotic risks to humans [14]. Traditional reliance on antibiotics for disease management has led to the emergence of multidrug-resistant bacterial strains, rendering treatments less effective and raising public health concerns [14]. Despite the critical need for alternative antimicrobial strategies, there is a paucity of research on the presence and application of bacteriophage viruses that specifically infect and lyse bacteria in Nigerian aquaculture settings. This gap in knowledge hinders the development of phage-based interventions that could serve as sustainable and effective measures against bacterial pathogens in fish ponds. Despite extensive studies on bacteriophages in marine environments, limited research has been conducted on their prevalence and diversity in artificial freshwater systems such as fish ponds in Nigeria,

particularly in Lapai. This study aims to isolate and identify bacteriophages from fish ponds in Lapai, providing baseline data on their existence and potential applications. By identifying bacteriophages present in fish ponds, this research will contribute to the broader field of aquatic microbiology, laying the groundwork for potential applications in disease control and water quality management in aquaculture.

2. Materials and Methods

2.1. Study Area

This study was carried out in Lapai Local Government Area of Niger State, Nigeria, focusing on fish ponds located at two specific semi-urban environments: Daudu Maza Road and Federal Low Cost. These areas are notable for their blend of artisanal and small-scale commercial fish farming activities. Each site was selected to represent diverse pond management practices within the same geographical setting, providing a practical scope for isolating and identifying bacteriophages in aquaculture systems. Daudu Maza Road, positioned at 9°02'45.000" N and 6°34'30.000" E, features ponds that benefit from easy road access and frequent interactions with human activities, including runoff from nearby roads and settlements. The Federal Low-Cost area, situated at approximately 9°02'30.000" N and 6°34'00.000" E, hosts community-based ponds that are often influenced by domestic waste and are mainly used for subsistence aquaculture.

2.2. Sample Collection

Water samples were collected from two man-made fish ponds across the study area: One pond along Daudu Maza Road and another pond at Federal Low-Cost. A composite sampling method was employed to ensure representativeness. From each pond, 500 mL of water was collected in sterile plastic bottles from two points (water and sediment) at a depth of 15–20 cm due to high organic matter and low oxygen level, which favours anaerobic bacteria [15, 16]. Samples were transported on ice to the microbiology laboratory within 4 hours of collection for immediate processing.

2.3. Isolation of Bacteriophages

Bacteriophages were isolated using the enrichment and double-layer agar method as described by [17], with slight modifications from [18, 19]. 100 mL of each water sample was filtered using a 0.22 µm syringe filter to remove debris and bacteria. The filtrates were incubated with 10 mL of exponentially growing host bacterial culture (e.g., *Escherichia coli*, *Pseudomonas aeruginosa*, or *Aeromonas hydrophila*) in Tryptic Soy Broth at 37 °C for 18–24 hours to promote phage amplification [19]. After incubation, the mixtures were centrifuged at 10,000 rpm for 10 minutes to remove bacterial cells. The supernatants were filtered again and used as the phage-

containing lysate. 100 µL of phage lysates were mixed with 100 µL of fresh bacterial culture and 3 mL of soft agar (0.7% agar), then poured onto a solidified nutrient agar plate. After overnight incubation at 37 °C, the formation of plaques (clear zones) will indicate the presence of bacteriophages [17, 18].

2.4. Identification of Bacteriophages

Preliminary identification of isolated bacteriophages was performed based on plaque morphology, including plaque size, shape, margin, and degree of clarity on double-layer agar plates. These characteristics provided an initial indication of phage lytic activity and potential phage diversity [20].

2.5. Data Analysis

Data were entered into Microsoft Excel and analyzed using SPSS v25. Descriptive statistics were summarized, including bacteriophage prevalence and bacterial diversity. Inferential

statistics (ANOVA, Chi-square) determine significant differences between locations at $p < 0.05$.

3. Results

3.1. Quantitative Analysis of Bacterial Load in Fish Pond Samples on Selective Media

Bacteriological analysis of water and sediment samples from Daudu Maza Road (DM) and Federal Low-Cost (FL) fish ponds revealed marked spatial variation in bacterial loads (Table 1). Counts on *Pseudomonas* Agar were consistently higher in sediment samples than in surface water across both sites, indicating greater microbial accumulation in pond sediments. The lowest *Pseudomonas* count was recorded in DM surface water ($3.2 \pm 0.4 \times 10^3$ CFU mL⁻¹), while the highest was observed in FL sediment samples ($9.2 \pm 1.1 \times 10^5$ CFU g⁻¹).

Table 1. Mean bacterial counts of *Pseudomonas* spp. and *Escherichia coli* in water and sediment samples from selected fish ponds.

Sample Code	Mean CFU on <i>Pseudomonas</i> Agar (CFU mL ⁻¹ / g ⁻¹)	Mean CFU on EMB Agar (CFU mL ⁻¹ / g ⁻¹)	Observation
DM-W	$3.2 \pm 0.4 \times 10^3$	$4.5 \pm 1.1 \times 10^1$	<i>Pseudomonas</i> spp. present; <i>E. coli</i> confirmed by green metallic sheen
DM-S	$5.8 \pm 0.7 \times 10^5$	$1.2 \pm 0.3 \times 10^4$	High abundance of <i>Pseudomonas</i> spp. and <i>E. coli</i>
FL-W	$4.1 \pm 0.5 \times 10^3$	ND	<i>Pseudomonas</i> spp. present; <i>E. coli</i> not detected
FL-S	$9.2 \pm 1.1 \times 10^5$	$3.8 \pm 0.6 \times 10^4$	Highest bacterial and coliform load observed

Key:
DM-W = Daudu Maza Road fish pond surface water;
DM-S = Daudu Maza Road fish pond sediment;
FL-W = Federal Low-Cost fish pond surface water;
FL-S = Federal Low-Cost fish pond sediment;
ND = Not detected.

Eosin Methylene Blue (EMB) Agar analysis confirmed the presence of *Escherichia coli*, identified by the characteristic green metallic sheen. *E. coli* counts were substantially higher in sediment samples than in corresponding water samples, with FL-S showing the highest coliform load ($3.8 \pm 0.6 \times 10^4$ CFU g⁻¹). Notably, no coliforms were detected in FL surface water (FL-W), suggesting comparatively lower fecal contamination at the water surface of this pond at the time of sampling.

Overall, the data indicate that pond sediments serve as major reservoirs for both *Pseudomonas* spp. and coliform bacteria, while surface water generally exhibits lower microbial loads, particularly in the Federal Low-Cost fish pond.

3.2. Isolation of Bacteriophages from Fish Pond Samples

Bacteriophages specific to *Pseudomonas* spp. and *Escherichia coli* were isolated from fish pond water and sediment samples using the double-layer agar technique (Table 2). Plaque formation with distinct morphologies confirmed the presence of lytic bacteriophages in several samples.

Phages infecting *Pseudomonas* spp. were more frequently detected and exhibited higher titers compared to *E. coli*-specific phages. The highest *Pseudomonas* phage titers were recorded in sediment samples from Federal Low-Cost (FL-S; $4.6 \pm 0.5 \times 10^6$ PFU g⁻¹) and Daudu Maza Road (DM-S; $3.0 \pm 0.4 \times 10^6$ PFU g⁻¹). Plaque morphology varied across samples, ranging from large, clear plaques with halos (3–5 mm) to

small, turbid plaques (1–2 mm), suggesting differences in phage–host interactions and possible phage diversity. These findings indicate that pond sediments serve as more favorable reservoirs for bacteriophages, likely due to higher bacterial densities previously observed in sediment samples.

In contrast, *Pseudomonas* phages were not detected in the Federal Low-Cost water sample (FL-W), indicating either absence or phage concentrations below the detection limit.

E. coli-specific bacteriophages were comparatively rare. Detectable plaques were observed only in the Daudu Maza Road water sample (DM-W), with a titer of $1.0 \pm 0.2 \times 10^4$

PFU mL⁻¹. These plaques were pinpoint and clear, measuring approximately 0.5–1 mm in diameter. No *E. coli* phages were detected in the remaining samples (DM-S, FL-W, and FL-S). This suggests a lower abundance or uneven distribution of *E. coli*-infecting phages in the pond environment, despite the confirmed presence of coliform bacteria in most samples.

Aeromonas hydrophila was not isolated, which may be due to high antibiotic levels in the fish ponds and possible outgrowth by the bacteria isolated, and an incubation period of 37 °C that was employed, because the bacteria's optimum temperature ranges from 25 °C–30 °C.

Table 2. Isolation and Characterization of Bacteriophages from Fish Pond Samples.

Sample Code	Target Host Bacteria	Phage Titer (PFU mL ⁻¹ or PFU g ⁻¹)	Plaque Morphology on Host Lawn
DM-W	<i>Pseudomonas</i> spp.	$2.0 \pm 0.3 \times 10^5$	Small, clear plaques (1–2 mm diameter)
DM-S	<i>Pseudomonas</i> spp.	$3.0 \pm 0.4 \times 10^6$	Large, clear plaques with halos (3–5 mm)
FL-W	<i>Pseudomonas</i> spp.	ND	No plaques observed
FL-S	<i>Pseudomonas</i> spp.	$4.6 \pm 0.5 \times 10^6$	Small, turbid plaques (1–2 mm)
DM-W	<i>E. coli</i>	$1.0 \pm 0.2 \times 10^4$	Pinpoint, clear plaques (0.5–1 mm)
DM-S	<i>E. coli</i>	ND	No plaques observed
FL-W	<i>E. coli</i>	ND	No plaques observed
FL-S	<i>E. coli</i>	ND	No plaques observed

Key:

DM-W = Daudu Maza Road fish pond (water, upper layer);

DM-S = Daudu Maza Road fish pond (sediment);

FL-W = Federal Low-Cost fish pond (water);

FL-S = Federal Low-Cost fish pond (sediment);

ND = Not detected.

Note: Phage titers were calculated from countable plates at 10⁻² or 10⁻³ dilutions using the formula:

PFU = (number of plaques × dilution factor) / volume plated.

Values are expressed as mean ± standard deviation of technical replicates.

4. Discussion

The quantitative bacteriological analysis of fish pond samples revealed significant spatial variation in microbial loads between surface water and sediments, consistent with patterns observed in other aquaculture systems. In this study, sediments exhibited markedly higher counts of both *Pseudomonas* spp. and *Escherichia coli* compared to surface water, except in FL-W where *E. coli* was not detected (Table 1), suggesting sediment as a key reservoir for bacterial communities. This finding aligns with recent high-throughput sequencing studies demonstrating that pathogenic and overall bacterial abundances tend to be higher in pond sediments than in water columns, driven by nutrient gradients and organic matter accumulation in sediments (e.g., nitrogen and phosphorus), which

support bacterial colonization and persistence [18, 19, 21].

The elevated bacterial loads in sediments likely reflect the accumulation of organic matter, fecal material, and feed residues, which provide substrates for microbial growth, a trend widely reported in aquaculture pond ecosystems. For example, Akter et al. observed that bacterial loads were greater in bottom waters and sediments compared to surface water, partly influenced by temperature and decomposed materials settling from the water column [8]. This supports the notion that sediment microbial communities are more stable and enriched relative to fluctuating surface waters.

Isolation of bacteriophages demonstrated greater phage presence associated with *Pseudomonas* spp. than with *E. coli*, and phage titers were generally higher in sediment samples. This is ecologically plausible since phage abundance is typically linked to host density. Phages often mirror the distribution of their bacterial hosts in aquatic environments. Indeed,

bacteriophages represent one of the most abundant biological entities in water bodies, and their population dynamics are tightly coupled with bacterial community structure [22, 23]. Our observation that *Pseudomonas* phages were frequently isolated from sediments suggests that sediments may act as hotspots for phage–host interactions due to the higher bacterial densities.

Conversely, *E. coli*-specific phages were comparatively rare, detected only in one water sample. This likely reflects the lower overall *E. coli* densities in water observed in this study, consistent with established ecological principles where phage prevalence is constrained by host availability [23]. Moreover, the variability in plaque morphology among phage isolates, from large, clear plaques with halos to smaller, turbid plaques, points to possible diversity in phage populations and differences in phage host interaction dynamics.

Spatial differences in microbial and phage abundances between ponds may also be influenced by pond management practices such as feeding intensity, water exchange, oxygen levels, and nutrient loading. Studies in aquaculture have shown that physicochemical and management factors significantly shape bacterial community composition and pathogen abundance, often resulting in increased bacterial diversity and potential pathogens when nutrient inputs are high or water quality is poor [21]. In this context, DM (Daudu Maza Road) and FL (Federal Low-Cost) ponds may differ in nutrient dynamics or organic inputs, influencing microbial patterns.

Taken together, the data suggest that sediment by virtue of its higher bacterial densities, nutrient richness, and lower disturbance relative to surface water, is a principal reservoir for both bacteria and bacteriophages in these pond ecosystems.

5. Conclusion

This study underscores key ecological principles governing microbial dynamics in aquaculture ponds:

- 1) Sediments act as microbial reservoirs: The significantly higher bacterial loads in sediments compared to surface water indicate sediments serve as repositories for heterotrophic and potentially pathogenic bacteria in aquaculture systems. This is consistent with recent microbiome studies showing greater pathogenic and overall bacterial abundances in pond sediments.
- 2) Phage distribution mirrors host availability: Bacteriophages specific to *Pseudomonas* were more abundant and diverse than those targeting *E. coli*, reflecting host density patterns. Phages localized predominantly in sediments emphasize the role of host-rich environments as key niches for phage persistence and ecological interactions.
- 3) Ecological implications for pond health: The presence of high bacterial and phage densities in sediments suggests that microbial interactions — including bacterial proliferation and viral lysis — are significant regulators of mi-

crobial community dynamics, with potential implications for nutrient cycling and pathogen control. Bacteriophages may naturally modulate bacterial populations, hinting at ecological balance mechanisms that could be harnessed for sustainable aquaculture pathogen management.

- 4) Management relevance: Understanding spatial variations in microbial and phage communities can inform pond management strategies, such as optimizing feeding, aeration, and sediment management, to mitigate pathogen proliferation and support water quality.

6. Recommendations

Fish pond sediments represent critical hotspots for both bacterial growth and bacteriophage activity. Future research should include molecular-based community profiling (e.g., 16S/viral metagenomics) and assessments of environmental drivers, including nutrient dynamics and management practices, to deepen understanding of microbial ecology in aquaculture settings and to explore phage-based approaches for targeted pathogen control.

Abbreviations

DM	Daudu Maza Road
FL	Federal Low-Cost
PFU	Plaque-forming Unit
EMB	Eosin Methylene Blue
CFU	Colony Forming Unit

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Conflicts of Interest

I hereby report that there were no conflicts of interest.

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