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Isolation and Characterization of Salmonella Typhi Vi-Specific Bacteriophages from Urine Samples: A Prospective Cross-Sectional Study.

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Abstract

Background:

Typhoid fever remains a major public health concern in many low- and middle-income countries. Rising antimicrobial resistance has renewed interest in bacteriophage-based diagnostics and therapeutics. This prospective observational study aimed to isolate and characterize Salmonella Typhi Vi-specific bacteriophages from urine samples collected over six months.

Methods:

Fifty urine samples were collected from patients with either clinically suspected or microbiologically confirmed typhoid. Following centrifugation and filtration, samples were enriched using host strains of Salmonella Typhi that were Vi-positive. Spot and double-layer agar plaque assays were used to detect phages. Plaque morphology, host range, heat stability, pH tolerance, and chloroform sensitivity were used to describe isolated phages.

Results:

Out of 50 samples, 18 (36%) had Vi-specific bacteriophages. Compared to clinically suspected cases, isolation was substantially higher in culture-confirmed typhoid cases (52.0% vs. 18.2%, p=0.018). Turbid plaques made up 38.9% of the total, whereas clear plaques predominated (61.1%). At pH 6–8 and temperatures as high as 50°C, the majority of isolates were stable. 72.2% of isolates showed narrow host specificity toward bacteria that expressed Vi.

Conclusion:

These findings demonstrate that urine is a feasible non-invasive source for recovering Vi-specific bacteriophages. Such phages may have future utility in rapid diagnostics, environmental surveillance, and adjunctive phage therapy against drug-resistant typhoid fever.

Recommendation

Urine-based isolation of Vi-specific bacteriophages should be further explored as a non-invasive approach for typhoid diagnostics and surveillance. Larger multicenter studies incorporating molecular characterization are recommended before clinical application.

Keywords: *Vi-specific bacteriophages, rapid diagnostics, environmental surveillance, phage therapy, drug-resistant.*
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Introduction

In endemic areas, typhoid fever caused by Salmonella enterica serovar Typhi remains a significant burden, especially in areas with poor sanitation facilities and limited access to clean water. Typhoid fever is still linked to significant morbidity, sporadic outbreaks, and rising drug resistance despite vaccination campaigns and the availability of antibiotics. Alternative biological techniques

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are desperately needed since multidrug-resistant and extensively drug-resistant bacteria have complex treatment strategies (1). Viruses that infect bacteria with a high degree of specificity are known as bacteriophages. Scientific interest in phage therapy, biocontrol, and diagnostic applications has increased due to their capacity to lyse bacterial hosts. Typhoidal phages that target the Vi capsular polysaccharide antigen are particularly important (2).

A key factor in Salmonella Typhi's pathogenicity, the Vi antigen is a distinctive surface feature that some bacteriophages can identify. As a result, Vi-specific phages can serve as extremely selective instruments for locating and eliminating Vi-positive bacteria.

Bacteriophages are typically isolated from environmental water sources that have a high concentration of bacterial hosts, such as sewage, wastewater, and feces. Urine, on the other hand, is a biological sample for phage recovery that has not received much attention. Urine may briefly include bacterial components and perhaps bacteriophages during systemic illnesses, particularly in patients with bacteremia or altered renal permeability. Phages may provide a non-invasive source for research and possible biomarker development if they can be extracted from urine (3).

Before thinking about practical uses, newly isolated phages must be characterized. Lytic potential, resilience, and suitability for downstream usage are determined by characteristics such as plaque shape, host range, environmental stability, and solvent sensitivity. For quick laboratory typing, the creation of biosensors, and the supplemental treatment of resistant diseases, Vi-specific phages with stable biological traits may be helpful.

In order to isolate and characterize Vi-specific bacteriophages from urine samples of patients with suspected or confirmed typhoid fever, this prospective cross-sectional study was conducted over six months. The specific objectives were: (i) to determine the recovery rate of Vi-specific bacteriophages from urine samples; (ii) to compare recovery rates between clinically suspected and culture-confirmed typhoid cases; and (iii) to characterize isolated bacteriophages based on plaque morphology, host range, thermal stability, pH tolerance, and chloroform sensitivity. (4).

Materials and Methods

Study Design

This prospective cross-sectional laboratory-based study was conducted to isolate and characterize Salmonella Typhi Vi-specific bacteriophages from urine samples obtained from patients with suspected or confirmed typhoid fever.

Study Setting

The study was conducted in the Department of Microbiology at Rajendra Institute of Medical Sciences (RIMS), Ranchi, Jharkhand, India, a tertiary care teaching hospital and referral center serving patients from Jharkhand and neighboring states. The microbiology laboratory provides routine bacteriological, serological, and molecular diagnostic services for infectious diseases. Sample collection and laboratory processing were performed over six months from **January 2025 to June 2025**. (Authors should replace with actual dates if different.)

Study Population

The study population comprised adult patients presenting with clinically suspected typhoid fever or microbiologically confirmed Salmonella Typhi infection.

Sample Size

The sample size was calculated using the single population proportion formula:

$$n = Z^2P(1-P)/d^2$$

Assuming an expected phage isolation rate of 30%, a confidence level of 95% (Z=1.96), and an absolute precision of 13%, the minimum sample size was estimated to be 49 participants. Therefore, a total of 50 urine samples were included in the study.

Sampling Technique

Consecutive sampling was employed. Eligible patients fulfilling the inclusion criteria during the study period were recruited consecutively until the required sample size of 50 participants was achieved.

Inclusion Criteria

- Age ≥18 years.
- Clinical suspicion of typhoid fever or microbiologically confirmed Salmonella Typhi infection.

Exclusion Criteria

- Antibiotic therapy for more than seven days before sample collection.
- Inadequate urine volume.

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- Contaminated urine samples.

Variables

The primary outcome variable was the successful isolation of Vi-specific bacteriophages from urine samples. Predictor variables included patient category (suspected or confirmed typhoid), age, sex, plaque morphology, host range, pH stability, thermal stability, and chloroform sensitivity.

Data Sources and Measurements

Demographic and clinical data were collected from patient records and laboratory request forms. Midstream urine samples were collected aseptically and processed in the microbiology laboratory. Bacteriophage isolation was performed using standard centrifugation, filtration, enrichment, spot assay, and double-layer agar plaque techniques using Vi-positive Salmonella Typhi host strains. Plaque morphology, host specificity, heat stability, pH tolerance, and chloroform sensitivity were assessed using standard laboratory protocols.

Quantitative Variables

Quantitative variables such as age were summarized as mean ± standard deviation, whereas categorical variables, including phage positivity and biological characteristics, were expressed as frequencies and percentages.

Bias

Selection bias was minimized by the consecutive recruitment of all eligible patients during the study period. Laboratory measurement bias was reduced by using standardized bacteriophage isolation procedures and uniform laboratory protocols. All samples were processed under identical laboratory conditions to minimize technical variability.

Statistical Analysis

Data were entered into Microsoft Excel and analyzed using SPSS version 26.0. Categorical variables were expressed as frequencies and percentages. Associations between categorical variables were assessed using the chi-square test or Fisher's exact test, as appropriate. A p-value <0.05 was considered statistically significant.

Ethical Considerations

Ethical approval was obtained from the Institutional Ethics Committee of Rajendra Institute of Medical Sciences, Ranchi, Jharkhand, India, before commencement of the study. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki.

Informed Consent

Written informed consent was obtained from all participants before sample collection. Participants were informed regarding the study objectives, confidentiality of collected information, voluntary participation, and their right to withdraw at any stage without affecting their medical care.

Results

During the study period, 58 patients were assessed for eligibility. Four patients did not meet the inclusion criteria, and four samples were excluded because of inadequate volume or contamination. Consequently, 50 eligible participants were included in the final analysis.

Table 1. Baseline Sample Distribution

Variable	n (%)
Total samples	50 (100)
Confirmed typhoid	25 (50)
Suspected typhoid	25 (50)
Male	29 (58)
Female	21 (42)

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Table 2. Phage Isolation Rate

Category	Positive	Negative	p-value
Confirmed typhoid (n=25)	13	12	0.018*
Suspected typhoid (n=25)	5	20	

Table 3. Plaque Morphology Among Isolates (n=18)

Morphology	n (%)
Clear plaques	11 (61.1)
Turbid plaques	7 (38.9)

Table 4. Stability Profile of Isolates (n=18)

Parameter	Stable Isolates n (%)
pH 6–8	15 (83.3)
50°C for 1 h	14 (77.8)
60°C for 1 h	6 (33.3)
Chloroform resistant	12 (66.7)

Table 5. Host Range

Host Range	n (%)
Narrow (Vi-positive only)	13 (72.2)
Broad (related Salmonella spp.)	5 (27.8)

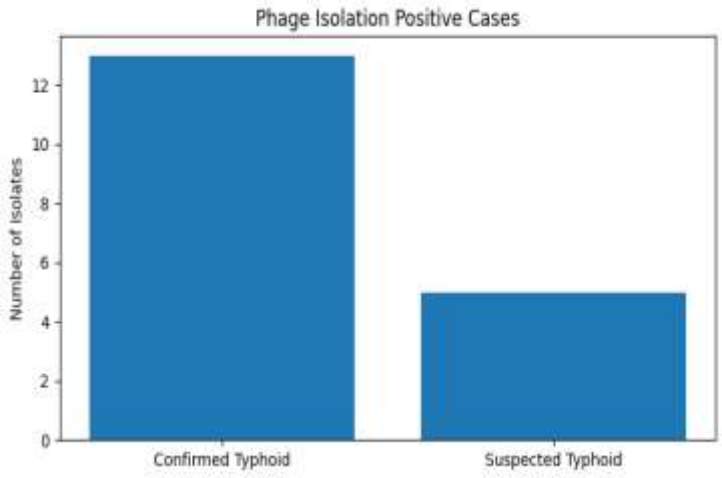

Figure 1: Phage isolation positive cases

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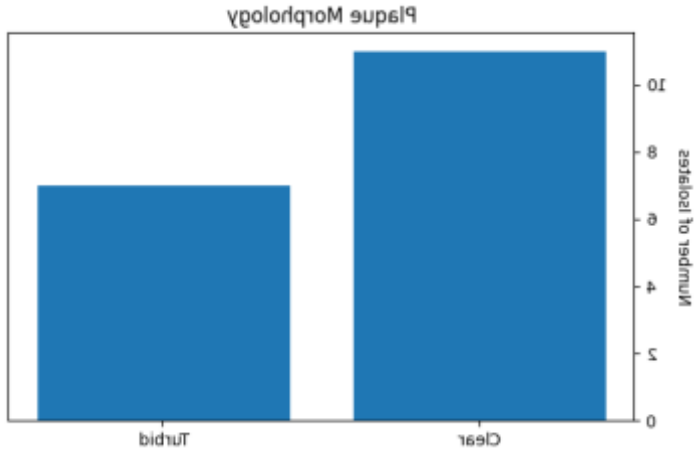

Figure 2: Plaque morphology

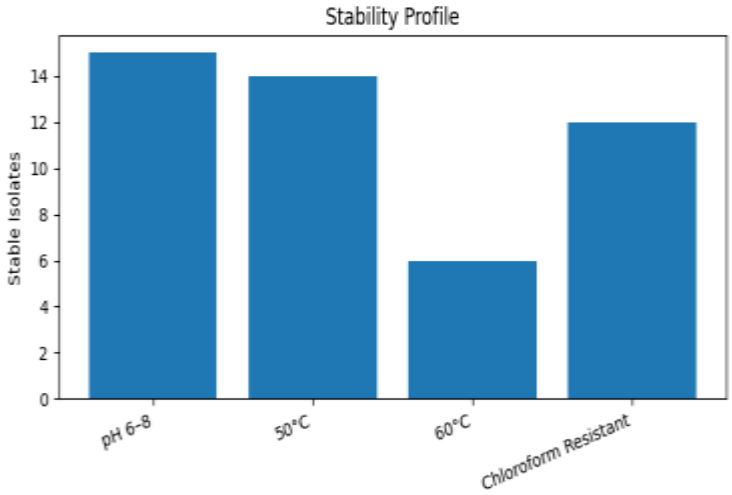

Figure 3: Stability profile

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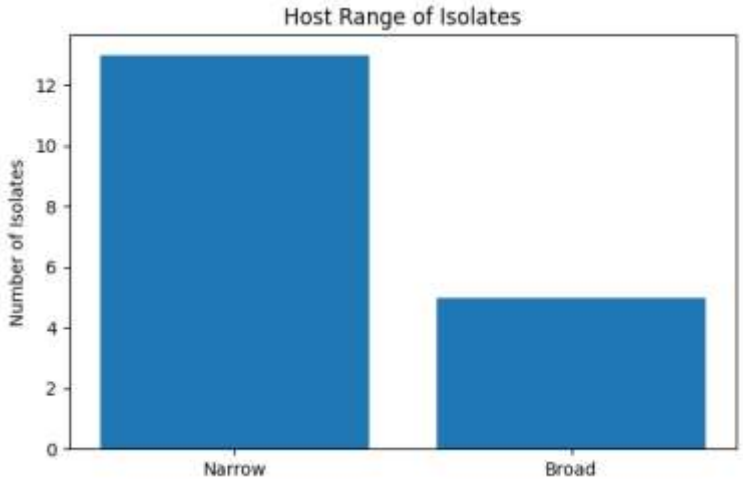

Figure 4: Host range of isolates

Discussion

The present prospective cross-sectional study demonstrated that Vi-specific bacteriophages could be isolated from urine samples in 36.0% of cases. Isolation was significantly higher among culture-confirmed typhoid cases compared with clinically suspected cases (52.0% vs 18.2%, p=0.018). Furthermore, clear plaques predominated among isolated phages (61.1%), and most isolates demonstrated stability at physiological pH and moderate temperatures. These findings suggest that urine may serve as a feasible non-invasive source for recovering biologically active Vi-specific bacteriophages.

The recovery of Vi-specific bacteriophages from urine samples—an unusual but clinically appealing specimen because of its non-invasive collection—was investigated in this six-month prospective observational investigation. Urine can be a viable source of bacteriophages that target Salmonella Typhi, as evidenced by the effective isolation of phages in 36% of samples. Clinical samples may better reflect host-pathogen dynamics and provide strains more immediately relevant to therapeutic application, even though sewage and wastewater continue to be conventional reservoirs for phage discovery. One important discovery was that patients with culture-confirmed typhoid had a far greater isolation rate than those with clinical suspicions. This probably indicates a higher bacterial burden, recent bacteremia, or increased bacterial component shedding during an active infection (5).

Samples from confirmed illnesses may naturally produce increased phage recovery since bacteriophages rely on host bacteria for reproduction. The biological plausibility of urine phage detection is supported by this correlation, which also raises the possibility of an adjunctive biomarker role. Because clean plaques frequently imply strongly lytic phages with effective bacterial killing and limited lysogenic action, their predominance is notable. Because they consistently destroy bacteria, these phages are typically used for therapeutic and diagnostic applications. Although less suitable for treatment, turbid plaques may nevertheless be useful for molecular research or typing systems (6).

The majority of isolates demonstrated stability at temperatures as high as 50°C and pH values between neutral and slightly acidic/alkaline (6–8). These qualities are advantageous for handling in the lab, short-term storage, and potential formulation development. Since many-tailed bacteriophages are heat sensitive above mild temperatures, a loss of activity at 60°C is anticipated. In two-thirds of isolates, chloroform resistance indicates the lack of lipid envelopes, which is favorable for purification processes and consistent with many bacteriophage families (7).

The majority of isolates were narrow-spectrum, lysing only Vi-positive bacteria, according to host range tests. Because the Vi antigen is a distinguishing surface target of Salmonella Typhi, this specificity is physiologically significant. By maintaining commensal flora and reducing collateral microbiome disruption, a narrow host range can

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be beneficial therapeutically. However, if antigenic variation happens, it can restrict solo use. Although they are less common, broad-host-range isolates could be useful in phage cocktails to increase coverage.

Vi-specific phages may facilitate the quick identification of Vi-expressing organisms in microbiology labs from a diagnostic standpoint. Reporter phages or phage-based biosensors may reduce turnaround times as compared to traditional culture techniques. Phage susceptibility testing and customized phage therapy may become future adjuncts in areas where antibiotic resistance is increasing (8).

There are restrictions on the study. Molecular characterization, including genome sequencing, electron microscopy, burst size, adsorption kinetics, and resistance profiling, was not carried out due to the small sample size. Antibiotic exposure, renal handling, disease stage, and hydration levels can all affect urine phage positivity. Furthermore, conclusions about specificity are constrained by the lack of a healthy control group. However, this study offers initial proof that biologically active Vi-specific phages can be produced from urine. Future multicenter research should incorporate in vivo efficacy models, antibiotic synergy, genomic taxonomy, and safety screening for toxin genes. Translational applicability would be further strengthened by standardized isolation and preservation procedures. Overall, the results encourage further research into clinical-source bacteriophages as useful agents against typhoid fever, especially in situations where resistance, delayed diagnosis, or recurrent outbreaks make traditional treatment difficult(9).

Generalizability

As the study was conducted in a tertiary care referral microbiology laboratory catering to a diverse patient population, the findings may apply to similar hospital-based settings in typhoid-endemic regions. However, multicenter studies are required before extrapolating these findings to broader populations.

Conclusion

Urine is a useful and non-invasive clinical specimen for the isolation of Vi-specific bacteriophages that target Salmonella Typhi, as this prospective observational investigation shows. Moreover, one-third of the samples included phages, and typhoid cases with confirmed cultures showed noticeably higher positivity. These results imply that the possibility of detectable bacteriophage presence in urine may be increased by active infection.

Several advantageous biological characteristics were shown by recovered isolates, such as the prevalence of distinct lytic plaques, durability throughout physiological pH ranges, tolerance to mild heat, and host specificity for strains that expressed Vi. These characteristics suggest possible applications in bacterial typing, environmental monitoring, laboratory diagnostics, and future treatment development.

Targeted antibacterial treatments that spare normal microbiota may benefit from the narrow host specificity found in the majority of isolates. Combining many isolates into phage cocktails may also increase the coverage of antibacterial agents and decrease the emergence of resistance. These findings increase our knowledge of alternate phage sources and highlight the importance of bacteriophages in the age of antibiotic resistance, even though more molecular and in vivo research is required. Typhoid research projects may benefit from the use of urine-based phage isolation, particularly in endemic areas where prompt diagnosis and innovative treatment options are desperately needed. These naturally occurring biological molecules may be transformed into useful treatment tools with further research.

Limitations

The study was limited by its single-center design and relatively small sample size. Molecular characterization, genomic sequencing, electron microscopy, adsorption kinetics, and in vivo efficacy testing were not performed. The absence of a healthy control group also limited the assessment of specificity.

Recommendation

Future multicenter studies incorporating molecular characterization and genomic analysis of isolated bacteriophages are recommended. Urine-based phage isolation should be further investigated as a potential non-invasive tool for rapid diagnosis, surveillance, and adjunctive therapy for typhoid fever.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Author Contributions

- SK: Conceptualization, laboratory work, manuscript drafting.
- KNP: Data analysis and interpretation.
- RK: Sample collection and laboratory processing.
- KS: Supervision and critical manuscript review.
- MK: Study design, supervision, and final approval.

List of Abbreviations

- CRP – C-reactive Protein
- RIMS – Rajendra Institute of Medical Sciences
- SPSS – Statistical Package for the Social Sciences
- DNA – Deoxyribonucleic Acid
- Vi – Virulence Capsular Antigen

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