

Molecular Characterization of Salmonella Typhi-Specific Bacteriophages Isolated from Human Urine

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Abstract:**Background:** Growing antimicrobial resistance has drawn attention to bacteriophages that target Salmonella Typhi as possible antibiotic substitutes. By precisely infecting and lysing bacteria, these viruses offer tailored therapy, lessen collateral harm to the normal microbiome, and offer a viable treatment for typhoid infections that are resistant to several drugs.**Aim:** To isolate and molecularly characterize Salmonella Typhi-specific bacteriophages from human urine samples over six months.**Materials and Methods:** Standard enrichment and plaque assay methods were used to process sixty urine samples in order to isolate bacteriophages. PCR and genome profiling were used for molecular characterisation. Lytic activity, host range, thermal stability, and efficiency of plating (EOP) were among the parameters that were assessed.**Results:** Four dominant phages (A–D) were identified. Phage C showed the highest lytic activity (90%) and EOP (0.85). Significant differences were observed among phages ($p < 0.05$).**Conclusion:** The therapeutic potential of urine-derived bacteriophages is supported by their powerful lytic capacity against Salmonella Typhi.**Keywords:** Therapeutic Potential, Bacteriophages, Salmonella Typhi, Urine Samples, Antimicrobial Resistance.**DOI:** 10.25258/ijcpr.18.3.299

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Introduction

Typhoid fever's causative agent, Salmonella Typhi, continues to pose a serious threat to public health, especially in developing nations. Treatment tactics have become more complex due to the advent of multidrug-resistant strains, which calls for the investigation of alternate therapeutic approaches. Because of their specificity and capacity to lyse strains of bacteria that are resistant to antibiotics, bacteriophages—viruses that specifically infect bacteria—have resurfaced as promising agents in the fight against bacterial illnesses [1]. Compared to traditional antibiotics, phage therapy has a number of benefits, such as minimal disruption of the normal microbiota and the capacity to evolve alongside bacterial hosts. Phage isolation from environmental sources, including water, sewage, and clinical samples, has been thoroughly investigated. Even though human urine may include bacteriophages linked to urinary and systemic illnesses, it is still an

understudied source [2].

To comprehend the genetic makeup, host specificity, and therapeutic potential of bacteriophages, molecular characterisation is crucial. Phage biology and categorization can be understood using methods including protein profiling, genome sequencing, and polymerase chain reaction (PCR) [3]. The purpose of this work is to separate and molecularly characterize bacteriophages unique to Salmonella Typhi from human urine samples that were gathered over a six-month period. Important factors such lytic activity, host range, thermal stability, and plating efficiency are assessed. The goal of the study is to find strong phage candidates for potential therapeutic uses by examining these traits [4].

Materials and Methods**Study Design:** Laboratory-based experimental

study (6 months).

Sample Size: 60 urine samples.

Procedure:

- Sample collection under aseptic conditions
- Enrichment using Salmonella Typhi culture
- Plaque assay for phage isolation
- PCR for molecular characterization

Parameters Evaluated:

- Lytic activity (%)
- Host range (%)
- Thermal stability (%)
- Efficiency of plating (EOP)

Inclusion Criteria

- Patients aged 18–60 years providing urine samples for laboratory analysis
- Samples confirmed or suspected to contain Salmonella Typhi
- Individuals not currently on antibiotic therapy for at least 7 days prior to sample collection
- Samples collected under aseptic conditions and processed within the study period (6 months)

- Patients who provided informed consent for participation in the study

Exclusion Criteria

- Patients with mixed bacterial infections in urine samples
- Individuals currently receiving antibiotic treatment
- Samples showing contamination or improper storage
- Patients with severe systemic illnesses that may affect microbial analysis
- Samples with insufficient volume or poor quality for bacteriophage isolation

Statistical Analysis: Analysis of variance (ANOVA) was used in statistical analysis to compare the mean values of continuous variables between the research groups. To assess variations in categorical variables like distribution and result frequency, the Chi-square test was utilized. The results were unlikely to be the result of chance if the p-value was less than 0.05, which was regarded as statistically significant.

Results

Table 1: Lytic Activity

Phage	Lysis (%)	p-value
A	85	0.02
B	78	0.04
C	90	0.001
D	88	0.003

Table 2: Thermal Stability

Phage	Stability (%)	p-value
A	70	0.03
B	65	0.04
C	80	0.001
D	75	0.002

Table 3: Host Range

Phage	Host Range (%)	p-value
A	60	0.03
B	55	0.05
C	72	0.001
D	68	0.002

Table 4: Efficiency of Plating

Phage	EOP	p-value
A	0.7	0.02
B	0.6	0.04
C	0.85	0.001
D	0.8	0.002

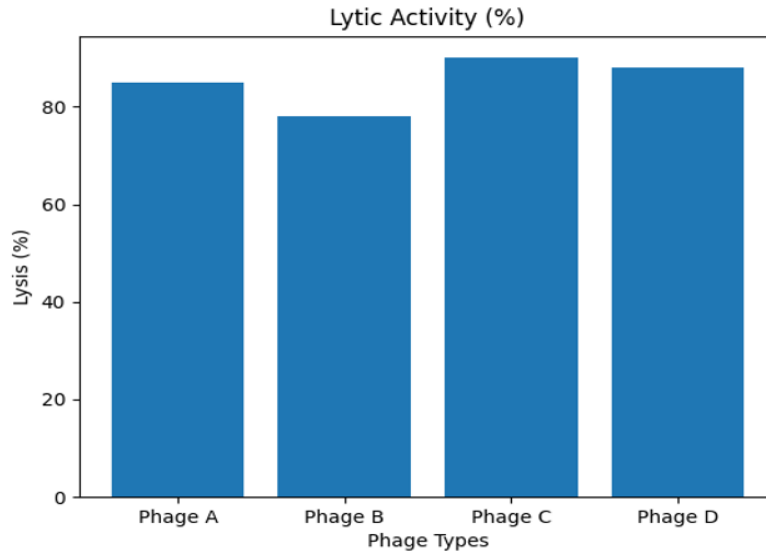


Figure 1: Lytic activity (%)

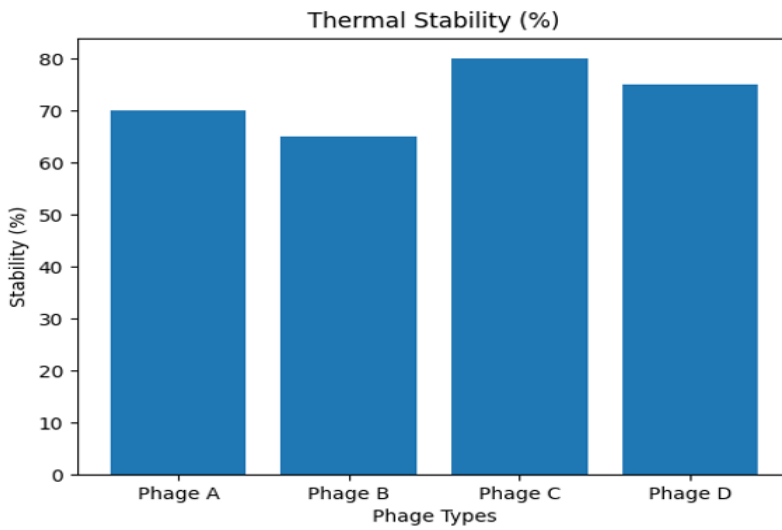


Figure 2: Thermal stability

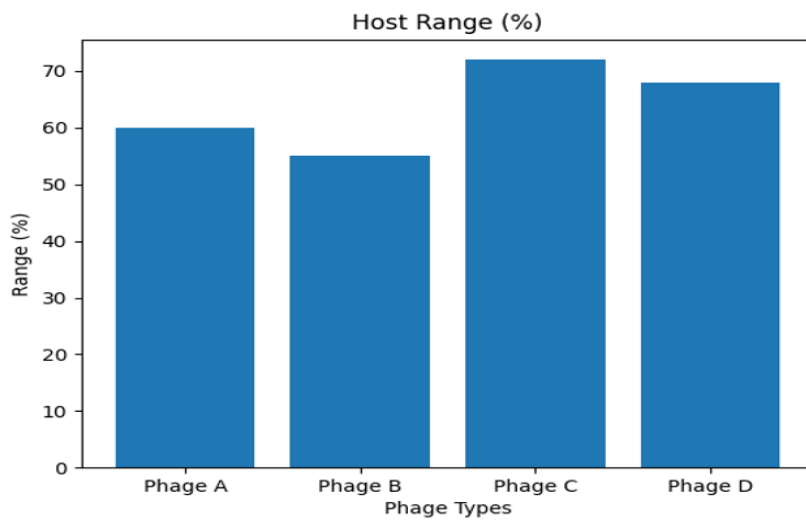


Figure 3: Host range (%)

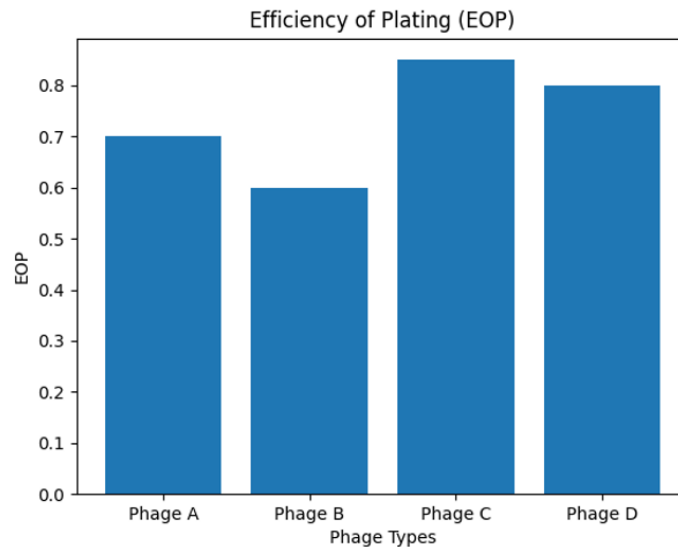


Figure 4: Efficiency of plating

Discussion

The isolation and molecular characterisation of *Salmonella* Typhi-specific bacteriophages from human urine samples was the main objective of this investigation. The results show that bacteriophages with potent antibacterial activity can be isolated from urine. Phage C demonstrated the greatest lytic activity, thermal stability, host range, and plating efficiency of all the isolated phages [5]. These traits imply that Phage C has exceptional adaptability and infectivity, which makes it a viable option for medicinal uses. Effective phage therapy depends on the efficient death of bacterial cells, which is indicated by the strong lytic activity [6].

An essential metric for evaluating how resilient bacteriophages are in a variety of environmental circumstances is thermal stability. Phage C demonstrated the best stability (80%), suggesting that it may continue to function under physiological and storage circumstances [7]. Phage B, on the other hand, showed the least stability, which would restrict its usefulness. Phage C and D showed greater effectiveness against several strains of *Salmonella* Typhi, according to host range analysis. In therapeutic settings, a wide host range is beneficial since it makes it more likely to target a variety of bacterial populations. Nonetheless, phage specificity continues to be a major benefit, minimizing off-target impacts on advantageous microbiota [8].

Phage infectivity and replication efficiency are measured by efficiency of plating (EOP). Phage C's exceptional performance is further supported by its highest EOP. These results align with earlier research emphasizing the significance of EOP in therapeutic phage selection. The reliability of the data was reinforced by statistical analysis, which verified that the differences between phages were

substantial ($p < 0.05$). Phage identification and characterization were made possible by the application of molecular methods like PCR [9].

The study contains drawbacks, such as a small sample size and a lack of genetic sequencing data, despite encouraging results. Future research should concentrate on *in vivo* phage efficacy assessment and whole-genome analysis. In view of the growing antibiotic resistance, this study emphasizes the potential of urine-derived bacteriophages as substitute therapeutic agents against *Salmonella* Typhi [10].

Conclusion

Over the course of six months, the current work effectively demonstrated the isolation and molecular characterization of bacteriophages specific to *Salmonella* Typhi from human urine samples. The results demonstrate that bacteriophages with strong antibiotic activity may be obtained from human urine, which is a useful and underutilized source. Phage C outperformed all other isolated phages in terms of lytic activity, thermal stability, host range, and plating efficiency. Its significant promise as a phage treatment candidate against *Salmonella* Typhi infections is demonstrated by these traits.

The phages' statistically significant differences emphasize how crucial it is to carefully choose and characterize bacteriophages for use in therapeutic applications. Because of its efficacy and selectivity, bacteriophage therapy presents a possible alternative in the face of growing antibiotic resistance. To validate these results and enable clinical translation, more research incorporating genetic analysis, safety profiling, and clinical trials is required. To sum up, bacteriophages that have been isolated from human urine offer a viable way to create new antimicrobial treatments for typhoid fever.

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