Detection of Salmonella Typhi bacteriophages in surface waters as a scalable approach to environmental surveillance

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As climate change drives human migration and increases water scarcity, environmental surveillance can show how these changes affect pathogen dynamics. Generally, environmental surveillance for Salmonella Typhi relies on sophisticated molecular methods, a prohibitively expensive process. In this study, we explore using low-cost plaque assay to detect bacteriophages specific to S. Typhi in water sources in Nepal. We collected 50mL samples from rivers and households of 4 regions in Nepal. We screened bacteriophages against S. Typhi. We isolated and tested phages against multiple strains to assess their host range, performed their whole genome sequencing and generated phylogenies using conserved genes. S. Typhi-specific bacteriophages were detected in 54.9% (198/361) of river water samples and 6.3% (1/16) drinking water samples from the urban Kathmandu and peri-urban Kavre. In urban Biratnagar and rural Dolakha, where typhoid incidence is low, only 6.7% (1/15, Biratnagar) and 0% (0/16, Dolakha) samples contained phages. All S. Typhi phages were unable to infect other Salmonella and non-Salmonella strains, nor a Vi-knockout S. Typhi strain. Representative strains from S. Typhi lineages were variably susceptible to the isolated phages. Phylogenetic analysis showed that S. Typhi Abstract phages belonged to *Autographiviridae* and *Siphoviridae* and clustered in three distinct groups. S. Typhi-specific bacteriophages, which required Vi-polysaccharide for infection, were highly abundant in surface waters of typhoid-endemic communities but rarely detected in low typhoid-burden communities. Screening water with a simple, low-cost assay enables the detection of S. Typhi phages and should be further evaluated as a scalable environmental surveillance tool.

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