Development of a Low-Cost Environmental Surveillance Method for Effective Typhoid Fever Control

Shuborno Islam
Child Health Research Foundation (CHRF)
Bangladesh  বাংলাদেশ
Typhoid is a big problem
Typhoid is a big problem

- 913 per 100,000 person per year in Dhaka
  - 242 per 100,000 person-years were hospitalized
- Highest incidence was among 2-4 years old children
To utilize the vaccines effectively, we need accurate data

Blood Culture surveillance

- Sensitivity is ~ 60%
- Can be costly for majority of the patients
- Requires human enrollment

Considering *Salmonella* Typhi is a human restricted pathogen, shed in stool and transmitted by contaminated water

**Might environmental surveillance be a supplementary method to estimate burden of *Salmonella* Typhi?**
Test environmental water samples for S. Typhi DNA

Typhoid cases shed S. Typhi in stool
Presence of S. Typhi DNA correlates with Typhoid burden

Evaluating PCR-Based Detection of Salmonella Typhi and Paratyphi A in the Environment as an Enteric Fever Surveillance Tool

Senuqi Saha,†1 Arif M. Tamnoy,†1‡, Jason R. Andrewes,‡ Mohammad S. I. Sajib,† Alexander T. Yu,‡ Stephen Baker,§ Stephen P. Luby,†‡ and Sarmi K. Saha†1‡
1Child Health Research Foundation, Department of Microbiology, Dhaka Shitali Hospital, Dhaka, Bangladesh; 2Department of Medical Microbiology and Infectious Diseases, Erasmus Medical Centre, Rotterdam, The Netherlands; 3Division of Infectious Diseases and Geographic Medicine, Department of Medicine, Stanford University, Stanford, California; 4Emerging Infections, Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam; 5Bangladesh Institute of Child Health, Dhaka Shitali Hospital, Dhaka, Bangladesh

Abstract With prequalification of a typhoid conjugate vaccine by the World Health Organization, countries are deciding whether and at what geographic scale to provide the vaccine. Optimal local data to clarify typhoid risk are expensive and often unavailable. To determine whether quantitative polymerase chain reaction (qPCR) can be used as a tool to detect typhoidal Salmonella DNA in the environment and approximate the burden of enteric fever, we tested water samples from urban Dhaka, where enteric fever burden is high, and rural Mirzapur, where enteric fever burden is low and sporadic. Sixty-six percent (36/59) of the water sources of Dhaka were contaminated with typhoidal Salmonella DNA, in contrast to none of 33 samples of Mirzapur. If these results can be replicated in larger scale in Bangladesh and other enteric fever endemic areas, drinking water testing could become a low-cost approach to determine the presence of typhoidal Salmonella in the environment that can, in turn, guide informed-design of blood culture-based surveillance and thus assist policy decisions on investing to control typhoid.

Enteric fever, caused by infection with Salmonella Typhi or Paratyphi A, B, or C (typhoidal Salmonella), is among the most common bacterial causes of morbidity worldwide, with the greatest burden occurring in low- and middle-income countries (LMICs). However, estimates of enteric fever incidence suffer from coarse, geographical and temporal resolution, because of a lack of surveillance systems for these diseases. This paucity of incidence data is in part because traditional surveillance requires population-based surveillance, which is resource intensive, requiring both robust laboratory infrastructure and population-based clinical data collection with substantial numbers of participants. Consequently, very few environmental, water-based surveillance strategy could help fill this knowledge gap by leveraging the important role that water has in Salmonella Typhi/Paratyphi A transmission. Finally, areas with high detectable levels of typhoid in the water supply overlap with areas of typhoid disease, then sampling water could be utilized as a preliminary surveillance proxy that can guide informed selection of geographical locations for blood culture surveillance and assist policy decisions on investing to control typhoid.

There are no established methods to reliably isolate Salmonella Typhi/Paratyphi A from water. Recently, a quantitative real-time PCR (qPCR)-based method was used to demonstrate the presence of S. Typhi DNA in environmental samples from Bangladesh and Indonesia. The aim of this study was to assess the potential of qPCR to detect environmental Salmonella Typhi DNA in Dhaka and Mirzapur, Bangladesh.
Limitations behind PCR-based molecular diagnostics

- Do not prove if viable bacteria is present
- No information on AMR
- Large amounts of water is needed
  - Filtration of large amounts of water is cumbersome, hard to scale
- Requires molecular set up
  - PCR, maintenance, technical skills, etc
Environmental Surveillance

Test environmental water samples for *S. Typhi* DNA

Typhoid cases shed *S. Typhi* in stool
Environmental Surveillance

Dr. Jason Andrews
Stanford University, USA

Typhoid cases shed S. Typhi in stool

Test environmental water samples for S. Typhi Phages

Funded by BMGF
Sample collection & phage detection

Collect environmental water (Sewage, lakes, rivers, ponds, stagnant water)

Filtration to remove bacteria and large particles

Incubation with BRD948 for phage enrichment

Spin to remove debris

Dilute to isolate phages (check plaque morphology)

Overnight incubation

DLA method to lawn

* S. Typhi BRD948 = laboratory strain (Dr. Andrews Lab)
<table>
<thead>
<tr>
<th></th>
<th>Dhaka</th>
<th>Mirzapur</th>
<th>Chittagong</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td>67</td>
<td>4</td>
<td>23</td>
<td>94</td>
</tr>
<tr>
<td><strong>Total Collection</strong></td>
<td>212</td>
<td>316</td>
<td>275</td>
<td>803</td>
</tr>
<tr>
<td><strong>Phages</strong></td>
<td>83</td>
<td>5</td>
<td>25</td>
<td>113</td>
</tr>
</tbody>
</table>

Isolation of phages
Typhi phages correlated with typhoid burden

Blood culture +% = 0.6%  
(n = 2,789)  
Phage +% = 8.4%  
(n = 275)

Blood culture +% = 4.7%  
(n = 4,620)  
Phage +% = 31.8%  
(n = 212)

Blood culture +% = 0.1%  
(n = 3,788)  
Phage +% = 1.3%  
(n = 316)

Blood culture +% = 0.6%  
(n = 2,789)  
Phage +% = 8.4%  
(n = 275)

Bacteriophage detected
Bacteriophage not detected
Phages in neighboring countries

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


Shrestha & Da Silva et al MedRxiv 2023
Phages have diverse killing spectra
Takeaway

• Good correlation between phage positivity vs clinical cases in an area

• Cost effective
  Less than 10 mL of sewage water required
  Less resources required

• Phages can be a rapid and low-cost surveillance tool
Future directions

• Expand the surveillance throughout Bangladesh

• Studying Typhi Dynamics in the environment

• Study the role of phages in the spread of drug-resistant S. Typhi
Mapping typhoid fever in Bangladesh using environmental surveillance
Al Amin, Abstract: 07

Exploring Diversity and Environmental Dynamics of *Salmonella* Typhi and its Bacteriophages
Rathindranath Kabiraj, Abstract: 48

Environmental surveillance to unravel the spatiotemporal dynamics of *Salmonella* Typhi bacteriophages in Dhaka
Sadnane Hussain Pranto, Abstract: 93
Thank You!!