Development and Application of Pilot *Salmonella* Typhi Environmental Surveillance Program in Kolkata, India

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Outline

• Kolkata background
• Sampling strategy
• Lab methods
• Environmental surveillance pilot studies
• Next steps
Collaborating Institutions

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Goutam Chowdhury

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University of Brighton
Huw Taylor
James Ebdon
Kolkata background

- Size of city: Area ~ 80 mi², population 4.5 million, divided into 144 administrative wards

- Previous estimates of incidence of:
  - Typhoid - 235/100,000 person years
  - Paratyphoid - 105/100,000 person years

- Active surveillance since November 2017
  - Location: Wards 58 & 59
  - Low income – both horizontal and “vertical”, high population density, intermittent municipal water supply, shared pour-flush toilets, seasonal flooding
  - Number of clinical cases detected by active surveillance (till Jan 2019): 55 (50 typhoid, 5 paratyphoid fever)
Modelling for Adaptive Sampling Site Allocation

Mathematical model to simulate fecal shedding dynamics and pathogen fate in the sewage network – Please see presentation by Yuke Wang

160,000 People
3,000 toilets
1 Pumping Station

Sampling Sites:
1. Pumping Station
2. Toilet
3. Primary Sampling Unit (PSU), pooled sample from shared toilets
# Focused on Two Types of Sampling Locations

<table>
<thead>
<tr>
<th>Sample Location</th>
<th>Strengths</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sewage Pumping Stations</td>
<td>• Captures feces from more people</td>
<td>• Feces are more dilute</td>
</tr>
<tr>
<td></td>
<td>• Large volume sample ~40 L has higher probability of capturing pathogen target (e.g., greater sensitivity)</td>
<td>• Greater distance and travel time from fecal source – more opportunity for pathogen die-off</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Large volume sample is more challenging to collect and process</td>
</tr>
<tr>
<td>Shared toilets (&quot;Primary sampling unit&quot; PSU)</td>
<td>• Closer to fecal source so less opportunity for pathogen die-off</td>
<td>• Captures feces from fewer people</td>
</tr>
<tr>
<td></td>
<td>• Less dilution of feces</td>
<td>• Smaller sample volume decreases probability of capturing pathogen target</td>
</tr>
<tr>
<td></td>
<td>• Smaller sample volume (500ml) is easier to collect and process</td>
<td></td>
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</tbody>
</table>
Decision tree for selecting type of sampling location based on physical and epidemiological attributes of the target pathogen and detection methods

Which characteristics are true for S. Typhi?

Polio ES →
How can we ensure that the environmental surveillance system is capturing excretion from high-risk populations?

Additional questions for selecting sampling locations

1) Where do feces from young children go – into toilets? into solid waste? or elsewhere?
2) Where do feces from the poorest populations (without access to toilets) go?
Sampling Strategies for Typhoid Environmental Surveillance

- **Grab samples** from selected sampling sites
  - Pros: Ability to collect different sample volumes; allows quantitative detection
  - Cons: Cumbersome to collect and process large volumes; longer sample processing time; comparatively expensive

- **“Trigger” samples** from households with clinical cases
  - Pros: allow correlation b/w clinical and environmental surveillance
  - Cons: Requires clinical surveillance system; Does not capture whole city or asymptomatic cases

- **Trap samples** (Moore swab) from selected sampling sites
  - Pros: comparatively easier sample collection and processing; less expensive; allows sampling from more sites
  - Cons: only presence-absence detection
Pilot ES Study in Two Wards in Kolkata, India

Collection of grab samples from selected sites

<table>
<thead>
<tr>
<th>Time period</th>
<th>Aug- Dec 2018</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location</td>
<td>Wards 58 &amp; 59</td>
</tr>
</tbody>
</table>
| Sampling sites  | • 3 Sewage Pumping Stations  
                  • 6 PSUs (shared toilets) (3/ward) |
| Sampling schedule | Weekly      |
| Sample volume   | • Pumping station- 40 L  
                  • PSU- 500 mL |
Study Sites (Wards) in Kolkata

- Shared Toilet
- Pumping station
# Environmental Surveillance Informed by Clinical Surveillance

Collection of “trigger” samples from HH of clinical cases identified during active clinical surveillance

<table>
<thead>
<tr>
<th><strong>Time period</strong></th>
<th>Nov 2018- present</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Location</strong></td>
<td>Wards 58 &amp; 59</td>
</tr>
</tbody>
</table>
| **Sample types**| Pooled sewage from shared toilets  
                  Piped drinking water |
| **Sampling schedule** | Samples collected at 3-day interval over a period of two weeks after case was identified |
| **Sample size** | 12 samples/case  
                  • 6 sewage samples  
                  • 6 drinking water samples |
| **Sample volume** | • Sewage from shared toilets – 500 mL  
                       • Drinking water- 40 L from nearest public tap |
Methods: Sample Processing and Testing

• Microbial Source tracking (MST)- Screening assay for human feces using *Bacteroides* phage
• Membrane filtration for *E. coli*
• *Salmonella* Typhi and Paratyphi A
  • Ultrafiltration (UF) and/or PEG precipitation- Sample concentration
  • Quantitative real time PCR (qRTPCR)- detection of *S. Typhi* and *S. Paratyphi A*
  • Lab methods developed and validated using seeded sewage samples in Atlanta and Kolkata. Limit of detection $\sim 10^3$ cells for UF. Limit of detection for Moore Swab with enrichment $\sim 10$ cells
Methods: Phage for human-specific bacterial host (*Bacteroides fragilis* GB-124) and somatic coliphage

**Sample Processing and Testing**

1 mL test sample (Sewage)  
1 mL host (GB-124/WG-5)  
3 mL ss BPRMA (GB-124) / ssMSA (WG-5)

Pour on BPRM/ MSA agar plate  
Incubate anaerobically (BPRM)/ aerobically (MSA) at 37°C for 18-24 hr
Methods: Sewage/Water Sample Concentration

Please see poster by Dr. Renuka Kapoor

Sewage from shared toilets

Environmental sample

Sewage from Pumping station, Piped drinking water

Methods:

Sewage/Water Sample Concentration

PEG precipitation

Total DNA extraction

qRTPCR (Baker’s method)

Ultra-filtration

PEG precipitation

Total DNA extraction

qRTPCR (Baker’s method)
## Results: August 2018-February 2019

<table>
<thead>
<tr>
<th>Sample type</th>
<th>No. tested</th>
<th>PCR Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pumping station</td>
<td>31</td>
<td>No detection</td>
</tr>
<tr>
<td>Pooled sewage from shared toilets - routine samples</td>
<td>56</td>
<td>No detection</td>
</tr>
<tr>
<td>Pooled sewage from shared toilets - Trigger samples</td>
<td>18</td>
<td>3 positive Confirmed S. Typhi by sequencing</td>
</tr>
<tr>
<td>Piped drinking water - Trigger samples</td>
<td>38</td>
<td>No detection</td>
</tr>
</tbody>
</table>

Please see poster by Dr. Goutam Chowdhury
Next Steps: City-Wide Surveillance + SaniPath Exposure Assessment

Proposed Timeline

Mar 2019
Moore swab validation

Apr-June 2019:
Pilot Moore Swab study

July 2019- June 2020
City-wide surveillance

July 2019- October 2019
SaniPath-Typhoid Exposure Assessment
Next Steps: Moore Swab Pilot April 2019-June 2019

Sampling sites

• 5 polio environmental surveillance sites
• 25 pumping stations
• Shared latrines in Wards 58 & 59 to complement active clinical surveillance
• Weekly samples
Next Steps: Test Collection and Analyses of Moore Swabs as a Screening Method

Moore Swabs (pumping stations & shared toilets) → Enrichment culture → DNA extraction → PCR (Baker’s method)

Negative: Drop sampling site and add new sampling site

Positive: Collect large volume grab sample from same site for concentration & qRT-PCR analysis
Next Steps:
City-Wide Surveillance July 2019 - June 2020

Sampling Sites:
• 5 polio environmental surveillance sites
• 25 sewage pumping stations across city
• 20? shared latrines in Wards 58 & 59 to complement active clinical surveillance

Sampling Frequency:
• Weekly samples?
• Moore swabs followed by large volume samples?

Lab Methods:
• qRT-PCR with Baker primers or other primers?
• Confirm % of presumptive positives by sequencing
Urban environments have multiple risks....

Which exposures pose the greatest risk for typhoid transmission?
Estimating Exposure to Fecal Contamination

Tool uses Bayesian analysis to estimate the distribution of environmental contamination and frequency of exposure.

Other parameters: intake volumes, duration of exposure, etc.

The mean dose and proportion of the population exposed are summarized from simulated distributions and displayed in risk profiles.

Results are presented in a normalized and comparable unit – Dose as MPN E. coli ingested per month.

Please see SaniPath.org
SaniPath-Typhoid Exposure Assessment

• Conduct structured observations, focus group discussions, GPS tracking of peak typhoid age groups to get more detailed behavior information
  
  eg. Street food consumption, surface water contact

• Collect relevant environmental samples and test for *E. coli*, phage markers for human feces, and *S. Typhi* and *S. paratyphi A*

• Bayesian modeling to develop city-level risk profiles for typhoid and paratyphoid and identify key transmission pathways

Dhaka, March 2017
Thank You

For more information visit SaniPath.org

@SaniPath

This study is made possible through the generous support of the Bill & Melinda Gates Foundation. Special thanks to Megan Carey.
Moving forward...

1. Continue to develop and test lab methods in Atlanta using environmental samples seeded with known amounts of S. Typhi and S. Paratyphi A
2. Test lab methods in Kolkata using seeded environmental samples
3. Pilot environmental surveillance using best lab methods on field samples in Kolkata. Attempt to sequence presumptive positives.
4. Examine field results while simultaneously working to improve lab methods
5. Repeat steps 2-4