Molecular Surveillance of Non-Typhoidal *Salmonella* from Environmental Sources in Disease Endemic Informal Settlement in Nairobi, Kenya

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Introduction

- In HIC, NTS causes self-limiting gastroenteritis.
- In LMICs, associated with severe bacteremia.
- Over 94M cases of infections, and 155,000 fatalities (Stanaway et al., 2019; WHO, 2018).

NTS disease incidence rates/100,000, by country, in 2017

Source: GBD 2017 Non-Typhoidal Salmonella Invasive Disease Collaborators
Gaps in our knowledge on environmental contamination

our understanding of the potential role of the environment as a reservoir for community transmission of NTS in disease-endemic settings is limited.
✓ To screen for NTS in various environmental samples (drinking water, effluent, soil, and raw sewers).
✓ To assess fecal contamination of the environmental samples (drinking water, effluent, soil, and raw sewers).
✓ To investigate the seasonal variations in detection of NTS in disease-endemic settings.
Mukuru Informal settlements

- Mukuru an area 5km$^2$ with over 300,000
- Poor sanitation and inadequate access to clean drinking water, creates a significant hotspot for the fast spread of NTS in slums.
Methodology

Samples
- Soil
- Effluent (Open sewer)
- Drinking water

Sample ID
- 038495_S
- 038495_E
- 038495_D

Soil Sampling

- Raw sewer (from convergence point)

Control HH

Samples
- Soil
- Effluent (Open sewer)
- Drinking water

Sample ID
- 038495_T_S
- 038495_T_E
- 038495_T_D
Sample Processing

**FIELDWORK**

Environmental Sample

**LABWORK**

Fecal contamination detection

Sample: 1mL 1:10 PBS: 9 mL
Sample: 1mL 1:1 PBS: 9 mL
Sample: 10mL 1:1 PBS: NONE

E. coli assay

**NTS detection**

Sample concentration

DNA extraction

**NTS assay**

**Specific primers** (safA- S. Ent’ and fliA-IS200 S. TM)

PCR

Metagenomic Analysis

Phase on going
### Results: Detection of Fecal contamination

<table>
<thead>
<tr>
<th>Study area</th>
<th>Sample Type</th>
<th>Fecal contamination detection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Jan-Mar</td>
</tr>
<tr>
<td>Mukuru</td>
<td>Drinking Water</td>
<td>16/30 (53.3%)</td>
</tr>
<tr>
<td></td>
<td>Effluent</td>
<td>20/22 (90.9%)</td>
</tr>
<tr>
<td></td>
<td>Soil</td>
<td>17/22 (77.3%)</td>
</tr>
<tr>
<td></td>
<td>Raw Sewer</td>
<td>4/4 (100%)</td>
</tr>
</tbody>
</table>

- An indicator of *fecal contamination*
- High fecal contamination especially in drinking water
- May indicate likelihood of environmental contamination by NTS and other coliforms
### Detection of NTS

<table>
<thead>
<tr>
<th>Study area</th>
<th>Sample Type</th>
<th>No: of Samples collected</th>
<th>Non-Typhoidal <em>Salmonella</em> (NTS) Distribution per Sample Type</th>
<th>Total NTS Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mukuru Informal Settlement</td>
<td>Drinking Water</td>
<td>86 (31.4%)</td>
<td><em>Salmonella Typhimurium</em>: 3 (3.5%)</td>
<td>12 (14%)</td>
</tr>
<tr>
<td></td>
<td>Effluent</td>
<td>86 (31.4%)</td>
<td><em>Salmonella Enteritidis</em>: 10 (11.6%)</td>
<td>21 (24.4%)</td>
</tr>
<tr>
<td></td>
<td>Soil</td>
<td>86 (31.4%)</td>
<td><em>Salmonella Enteritidis</em>: 4 (4.7%)</td>
<td>9 (10.5%)</td>
</tr>
<tr>
<td></td>
<td>Raw Sewer</td>
<td>16 (5.8%)</td>
<td><em>Salmonella Enteritidis</em>: 11 (68.8%)</td>
<td>12 (75.0%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>n=274</strong></td>
<td></td>
<td><em>Salmonella Enteritidis</em>: 28 (10.2%)</td>
<td>54 (19.7%)</td>
</tr>
</tbody>
</table>

- 29.9% detection rate of NTS from the environmental samples.
- *S.* Enteritidis was the most ubiquitous compared to *S.* Typhimurium.
- To Note: 4 NTS were isolated by culture:
  - *S.* En & STm from drinking water of cases
  - *S.* En from effluent & sewer
- This as an indicator of potential NTS dissemination from environment
Proportions of serotypes from environmental sources

- Proportionately, contamination was higher in:
  - effluent
  - raw sewer
  - drinking water
  - Soil

[Bar chart showing proportions of serotypes for Salmonella Typhimurium, Salmonella Enteritidis, Total NTS Positive in Drinking Water, Effluent, Soil, and Raw Sewer.]
The relatively high levels of NTS contamination in raw sewers and household effluent may be due to fecal contamination.

This demonstrates that fecal contamination may be the primary cause of environmental NTS contamination

NTS contamination in drinking water and soil is of concern due to their potential to facilitate uncontrolled NTS spread in the community;

- more so to children due to their indiscriminate outdoor activities that may expose them to contaminated water, open drains, and soil.
Seasonal Variation in Non-Typhoidal *Salmonella* in environmental Carriage

- Both S. Typhimurium and S. Enteritidis displayed decrease in detection in 1\textsuperscript{st} & 3\textsuperscript{rd} quarter of the year,
- Increase in detection was observed in the 2\textsuperscript{nd} & 4\textsuperscript{th} quarter, *coinciding with wet seasons*

**Normalized data. Variables represented in relation to their respective baseline values. Positive values indicate an increase in NTS detection. Precipitation included for reference.**
Conclusion and recommendation

- Establishing a comprehensive NTS surveillance (including WGS & metagenomic approach) key in:
  - Monitoring NTS genotypes and distribution at the interface between the humans and environment:
    - to gain insights into the dynamics of NTS transmission,
    - to develop effective prevention and control strategies to mitigate its spread within the community.
Acknowledgement

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Coalition Against Typhoid

Sabin Vaccine Institute