Environmental surveillance for *Salmonella*, and AMR genes in Tamil Nadu, India

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Salmonella Typhi in the environment

- Few studies have detected S. Typhi in environmental samples (drinking water, sewage, food)
- S. Typhi detected in 11% of irrigation water samples using Moore swab
- Few recent studies have detected S. Typhi in drinking water samples using molecular methods, but not culture
Issues with culture of S. Typhi

- Entry into a VBNC state of S. typhi was shown in all microcosms

- S. typhi survived longer in groundwater than in pond water as a VBNC state

- Ability of salmonellae to become internalized and to survive and replicate in free living amoebae
Environmental surveillance (ES) in Vellore

- Vellore city, located on the banks of the Palar River in the north-eastern part of Tamil Nadu, India

- Area- 87.915 km²

- Population- about 500,000 (2011 Government of India census)

- Semi-arid climate with high temperatures throughout the year and relatively low rainfall

- Three seasons: summer (March-July, temperatures >40° C), rainy (August-November) and winter (December-February, low of 15°C)
Summary of ES results for S. Typhi in Vellore for 2017

- A total of 99 blood culture typhoid cases during 2017
- Marked seasonality during rainy season
- Sewage samples from 10 households positive for S. Typhi by qPCR (no positives by culture)
- No drinking water samples positive for S. Typhi
Proposed project 2018-2019
Objectives

• Standardization of for detection of S. Typhi, and antimicrobial resistance (AMR) genes in sewage

• Surveillance for S. Typhi, and AMR genes in sewage, food, and community drinking water in Vellore

• Application of detection methods in Vellore town over a period of 1 year to explore spatial relationships within households, with potential hot-spots and within the community
ES- sampling locations in Vellore city

- **Generic locations** – convergence of sewage lines in the network in areas where typhoid disease burden estimation is carried out

- No big industries in the locality that could dump waste water which may contain chemicals

- Higher chances of obtaining fecal matter from children

- Relatively higher population density within a specified radius around the point

- **Specific locations** - Transit points, sewage treatment plant

- Timing of sampling – between 8 am to 10 am
Sample collection and testing flowchart (BMFS): detection of all targets using direct molecular methods, culture not used.
Molecular assays for detection of S. Typhi and other *Salmonella* spp.

- Multiplex qPCR for detection of
  - Pan *Salmonella*
  - S. Typhi,
  - S. Enteridis,
  - S. Typhimurium,
  - S. Paratyphi A

- In environmental samples (sewage, drinking water)

- In addition, singleplex qPCR protocol (modified Nga et al, 2010) used for detection of S. Typhi. This assay was also used in the Kathmandu study for detection of S. Typhi in drinking water (Karkey A, et al)
Proportion of community sewage samples positive for *Salmonella* (February, 2018- February, 2019)

• Volume collected: 5-6 litres (depending on site) using BMFS (between 8 AM- 10 AM)

• Samples collected after 2 NIDs in 2018 (28\(^{th}\) January, 11\(^{th}\) March), as samples were also tested for Sabin polioviruses (1 and 3)

• *Salmonella* Typhi singleplex qPCR: 11% (23/210)

• *Salmonella* multiplex qPCR:
  • Pan *Salmonella*: 71.4% (150/210)
  • S. Typhi: 6.7% (14/210)
  • S. Typhimurium: 33.8% (71/210)
  • S. Enteritidis: 0%
  • S. Paratyphi A: 0%
### S. Typhi singleplex qPCR (Nga et al, 2010)

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**NC- not collected**
Prevalence of AMR genes in Vellore sewage (February, 2018- January, 2019)

**Gram positive resistance genes**
- MEC A: 0.0
- VAN B: 0.0
- VAN A: 0.0

**Quinolone resistance genes**
- QNR S: 89.9
- QNR B: 59.1
- QNR A: 27.8

**Carbenem resistance genes**
- KPC: 91.9
- OXA-48 LIKE: 61.1
- VIM: 97.0
- NDM: 85.9

**CTX-M genes**
- CTX-M25: 4.0
- CTX-M9: 31.3
- CTX-M8: 0.5
- CTX-M2: 81.8
- CTX-M1: 98.5
Detection of *Salmonella* and AMR genes in hospital sewage, food, and community drinking water in Vellore
Salmonella & AMR surveillance

- **Hospital sewage** (upstream & downstream where selected health care settings meet the community sewage network), 10 samples/month (1 upstream, 1 downstream for 5 hospitals)

- Sampling for animal and vegetable matter collected as per *SaniPath* protocol

- **Animal and vegetable samples**: 25 samples every 3 months (total of 100 samples/year)

- **Community drinking water**: 15 samples every 3 months (total of 60 samples/year)
Community Drinking water

**Transport & filtration**
- 2.5 litre (Whirl-Pak bags), and 200ml in Dippas™
- Transported under cold conditions to lab
- 2.5 litres filtered (0.45u filters)

**Enrichment & qPCR**
- Enrichment by inoculating filters in 10ml Selenite F broth overnight at 37°C
- After overnight incubation, DNA extraction from broth and qPCR (singleplex and multiplex qPCR assays)

**Culture**
- After enrichment, culture using Xylose Lysine Deoxycholate (XLD) agar (100μl/ plate, overnight incubation at 37°C)
- Suspected colonies (pink colonies with black centres) inoculated in Triple Sugar Iron (TSI)
- If biochemical reaction in TSI suggestive of S. Typhi-subculture on nutrient agar followed by agglutination with poly-O and O9 antisera

**Coliform count**
- 100ml filtered in 1 filter, cultured on MacConkey agar
Food (animal and vegetable matter)

- **Transport, processing, and filtration**
  - Food collected in Whirl-Pak bag
  - Samples transported to the lab within 4h of collection under cold conditions
  - In lab, 500 mL of PBST buffer added to the bag. Bag sealed, shaken well, incubated for 30 minutes at 37°C
  - Food removed from bag by squeezing out
  - PBST wash solution (1/10 dilution) filtered through 0.45μm filter

- **Enrichment & qPCR**
  - Enrichment by inoculating filters in 10ml Selenite F broth overnight incubation at 37°C
  - After overnight incubation, DNA extraction from broth and qPCR (singleplex and multiplex qPCR assays)

- **Culture**
  - After enrichment, culture using Xylose Lysine Deoxycholate (XLD) agar (100ul/ plate, overnight incubation at 37°C)
  - Suspected colonies (pink colonies with black centres) inoculated in Triple Sugar Iron (TSI)
  - If biochemical reaction in TSI suggestive of S. Typhi-subculture on nutrient agar followed by agglutination with poly-O and O9 antisera
Food & Community drinking water results for *Salmonella*

- Food matter collected every 3 months from food outlets in Vellore

- Results available for *July, October, 2018* and *January, 2019* (25 food and 15 community drinking water for every collection)

- Food (75 samples: chicken, mutton, coriander, green leaf, cucumber, tomato, milk)
  - Pan *Salmonella*: 29.3% (22/75)
  - *S. Typhimurium*: 6.7% (5/75)
  - No *S. Typhi*, *S. Paratyphi A*, *S. Enteritidis*

- Community drinking water (45 samples: public tap, sintex tank, overhead tank)
  - Pan *Salmonella*: 40% (18/45)
  - *S. Typhimurium*: 4.4% (2/45)
  - No *S. Typhi*, *S. Paratyphi A*, *S. Enteritidis*
Hospital sewage results for *Salmonella*

- 5 hospitals from Vellore included in the study

- Upstream & downstream/ pre-treatment & post-treatment (whichever available) sewage samples collected & tested every month (From *July, 2018*)

- Samples tested (*July, 2018- February, 2019*): 96 samples
  - Pan *Salmonella*: 83.3% (80/96)
  - *S. Typhi*: **19.8%** (19/96; singleplex and/or multiplex qPCR)
  - *S. Typhimurium*: 29.2% (28/96)
  - *S. Enteritidis*: 3.1% (3/96)
  - *S. Paratyphi A*: 0%
Typhoid case control study
• **Study period:** April, 2018- March, 2019
  • Number of cases: 31
  • Number of case-control pairs recruited: 31 pairs
  • Household samples collected:
    • Household sewage (Moore swab, BMFS if possible)
      • Moore swab tied to household sewage for 48-72 hours
      • Incubated in Selenite F broth (200ml) at 37°C overnight
      • After incubation, broth used for culture and qPCR
    • Floor swab (EnviroMax swab)
      • In lab, 10ml selenite F broth inoculated & incubated at 37°C overnight
      • After incubation, broth used for culture and qPCR
    • Drinking water
      • 2.5 litres for culture and qPCR, 100ml for coliform count
    • Mother hand wash
      • 500ml (PBS) for filtration, enrichment, culture and qPCR
    • Child (case) hand wash
      • 500ml (PBS) for filtration, enrichment, culture and qPCR
• Results available-
  • Drinking water- 30 pairs
  • Mother hand wash- 30 pairs
  • Child hand wash- 30 pairs
  • Floor swab- 30 pairs
  • Moore swab- 27 cases, 30 controls
  • BMFS (household sewage)- 14 cases, 15 controls (collected started from end of July, 2018)
S. Enteritidis
---
Case: 3.3

S. Paratyphi A
---
Case: 10.0

S. Typhimurium
---
Case: 23.3

Pan Salmonella
---
Case: 23.3

S. Typhi
---
Case: 53.3

---

S. Enteritidis
---
Control: 3.3

S. Paratyphi A
---
Control: 3.3

S. Typhimurium
---
Control: 3.3

Pan Salmonella
---
Control: 16.7

S. Typhi
---
Control: 73.3

Legend:
- Child hand wash
- Mother hand wash
- Drinking water
- Floor swab
- Household Sewage
To summarize........

- *Salmonella* (S. Typhi & other *Salmonella* serotypes) were detected in the main sewage channels and hospital sewage in Vellore using qPCR

- No *S. Typhi* found in food and community drinking water, although other common *Salmonella* serotypes (*S. Typhimurium*) were detected

- *S. Typhi* detected in sewage from households of typhoid cases, compared to none from control households

- High burden of AMR genes in the main sewage channels of Vellore
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