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

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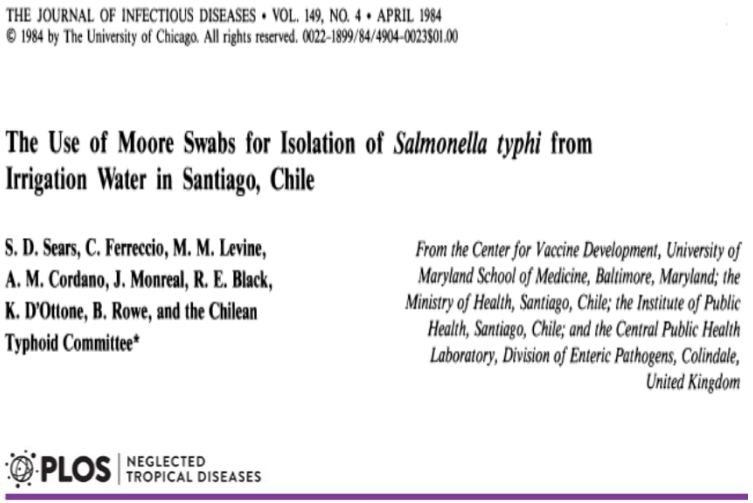
Hanoi, 26th March, 2019

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



THE JOURNAL OF INFECTIOUS DISEASES • VOL. 149, NO. 4 • APRIL 1984
 0022-1899/84/4904-0023$01.00

The Use of Moore Swabs for Isolation of *Salmonella typhi* from Irrigation Water in Santiago, Chile

S. D. Sears, C. Ferreccio, M. M. Levine, A. M. Cordano, J. Monreal, R. E. Black, K. D'Ottone, B. Rowe, and the Chilean Typhoid Committee*

From the Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, Maryland; the Ministry of Health, Santiago, Chile; the Institute of Public Health, Santiago, Chile; and the Central Public Health Laboratory, Division of Enteric Pathogens, Colindale, United Kingdom

- Few recent studies have detected *S*. Typhi in drinking water samples using molecular methods, but *not culture*

PLOS | NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

The Ecological Dynamics of Fecal Contamination and *Salmonella* Typhi and *Salmonella* Paratyphi A in Municipal Kathmandu Drinking Water

Abhilasha Karkey[1☯], Thibaut Jombart[2☯], Alan W. Walker[3,4], Corinne N. Thompson[5,6], Andres Torres[7], Sabina Dongol[1], Nga Tran Vu Thieu[5], Duy Pham Thanh[5], Dung Tran Thi Ngoc[5], Phat Voong Vinh[5], Andrew C. Singer[8], Julian Parkhill[3], Guy Thwaites[5,6], Buddha Basnyat[1], Neil Ferguson[2], Stephen Baker[5,6,9*]

AMERICAN SOCIETY FOR MICROBIOLOGY | Applied and Environmental Microbiology®

Environmental Survey of Drinking Water Sources in Kampala, Uganda, during a Typhoid Fever Outbreak

J. L. Murphy,[a] A. M. Kahler,[a] I. Nansubuga,[b] E. M. Nanyunja,[c] B. Kaplan,[d] N. Jothikumar,[a] J. Routh,[a] G. A. Gómez,[e] E. D. Mintz,[a] V. R. Hill[a]

Issues with culture of *S*. Typhi

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

ELSEVIER

FEMS Microbiology Letters 170 (1999) 257–264

FEMS MICROBIOLOGY LETTERS

Viable, but non-culturable, state of a green fluorescence protein-tagged environmental isolate of *Salmonella typhi* in groundwater and pond water

Jang-Cheon Cho, Sang-Jong Kim *

Department of Microbiology, College of Natural Sciences, and Research Center for Molecular Microbiology, Seoul National University, Seoul 151-742, South Korea

- 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

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, June 2004, p. 3706–3714 Vol. 70
0099-2240/04/$08.00+0 DOI: 10.1128/AEM.70.6.3706–3714.2004

Uptake and Replication of *Salmonella enterica* in *Acanthamoeba rhysodes*

Dilek Tezcan-Merdol,[1] Marianne Ljungström,[2] Jadwiga Winiecka-Krusnell,[2] Ewert Linder,[1,2] Lars Engstrand,[1,2] and Mikael Rhen[1,2]*

Microbiology and Tumor Biology Center, Karolinska Institute, 171 77 Stockholm,[1] and Swedish Institute for Infectious Disease Control, 171 82 Solna,[2] Sweden

Environmental surveillance (ES) in Vellore

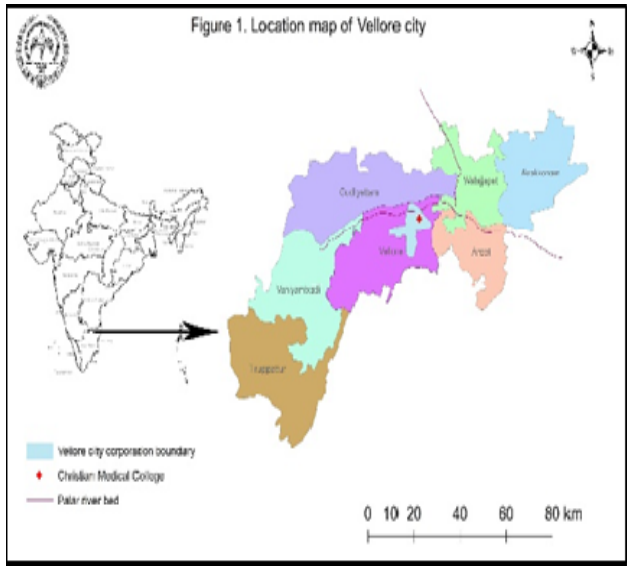
- Vellore city, located on the banks of the Palar River in the north-eastern part of Tamil Nadu, India

- Area- 87.915 km^2

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

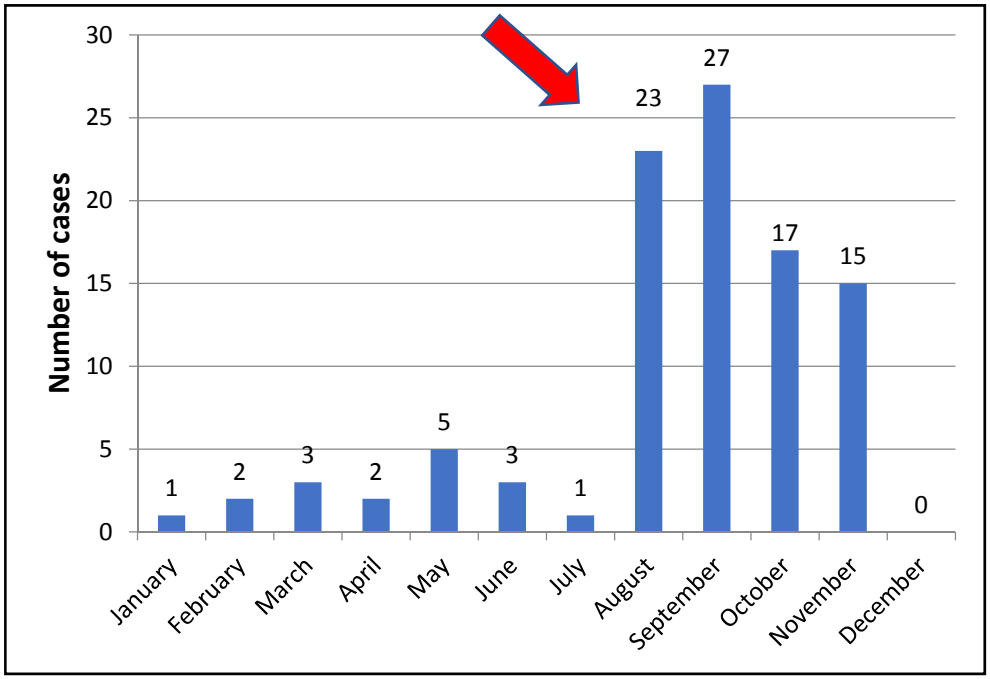
- Semi-arid climate with high temperatures throughout the year and relatively low rainfall

- 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

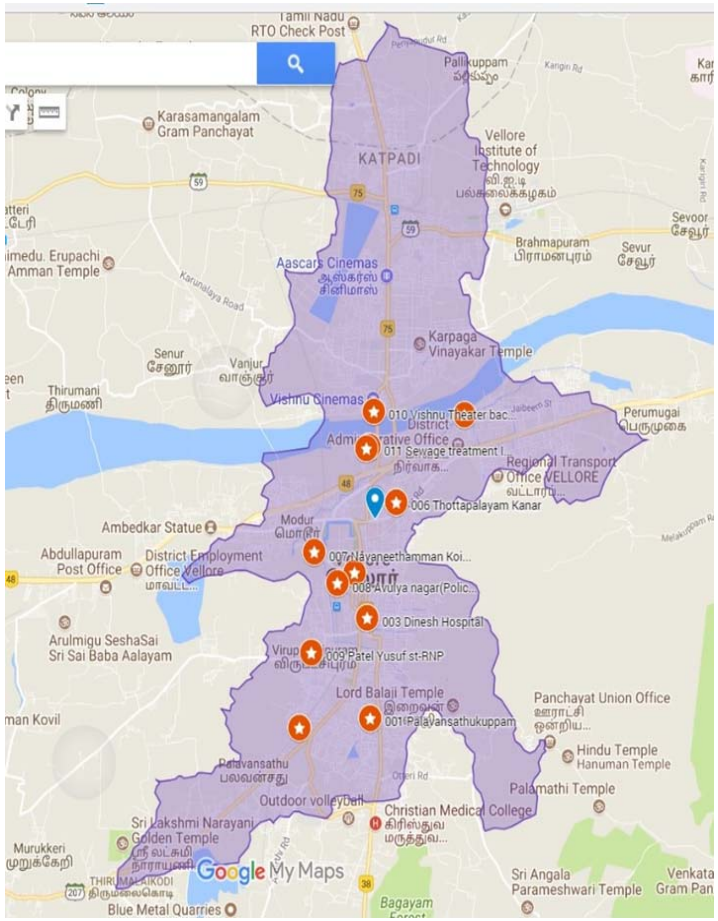
Objectives

- 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

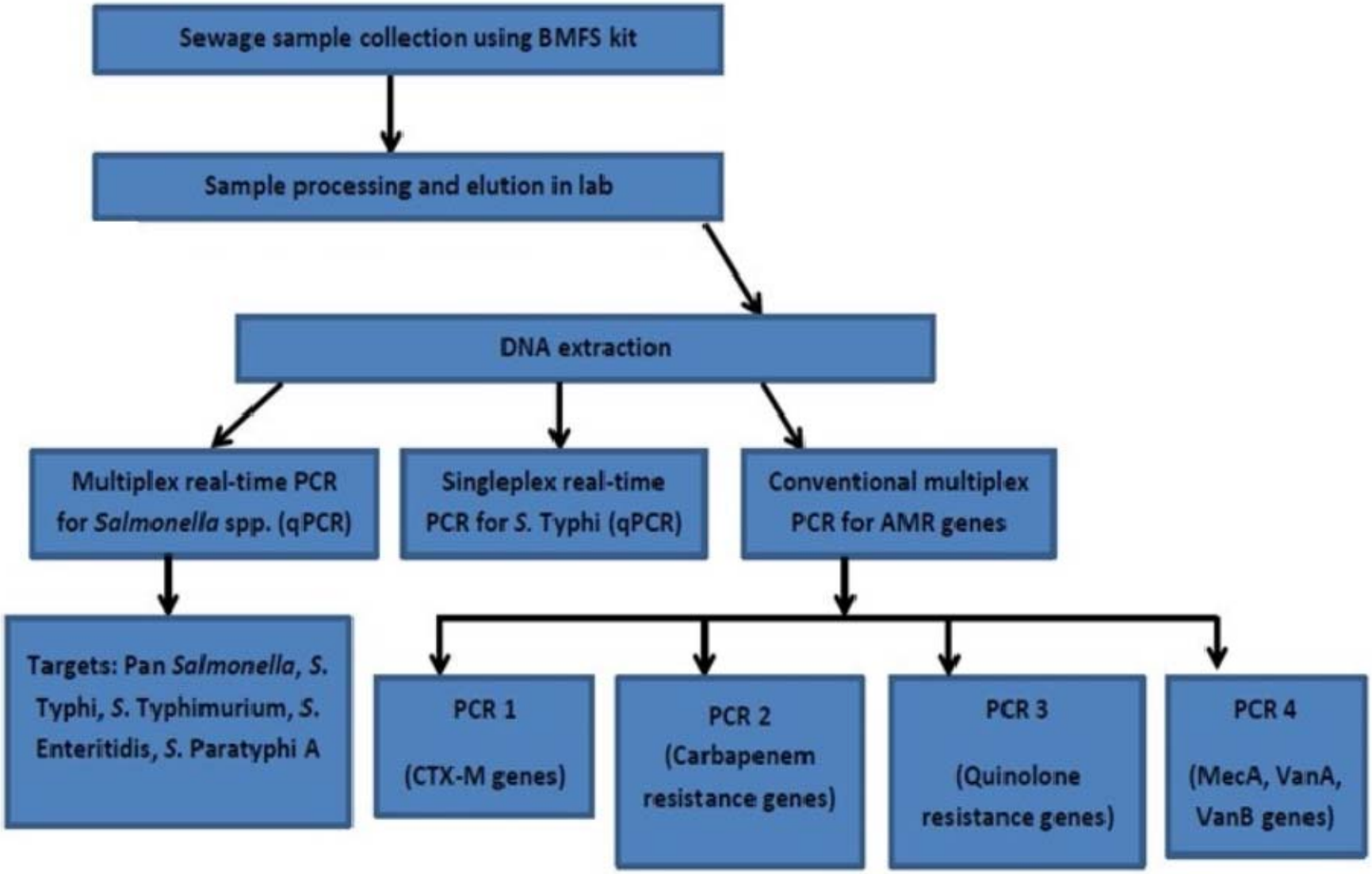
- Application of detection methods in Vellore town over a period of 1 year to explore spatial relationships within households, with potential hot-spots and within the community

ES- sampling locations in Vellore city



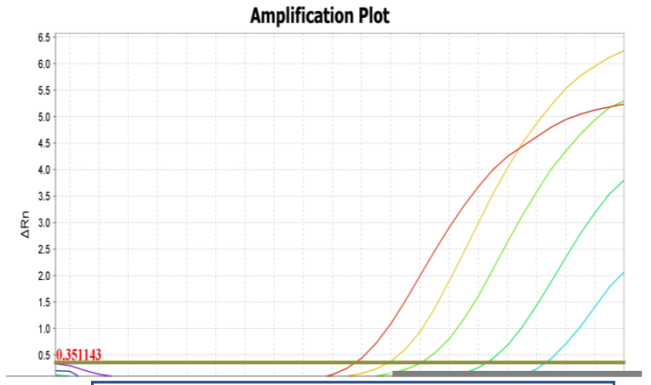
- **Generic locations** – convergence of sewage lines in the network in areas where typhoid disease burden estimation is carried out
- No big industries in the locality that could dump waste water which may contain chemicals
- Higher chances of obtaining fecal matter from children
- Relatively higher 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.

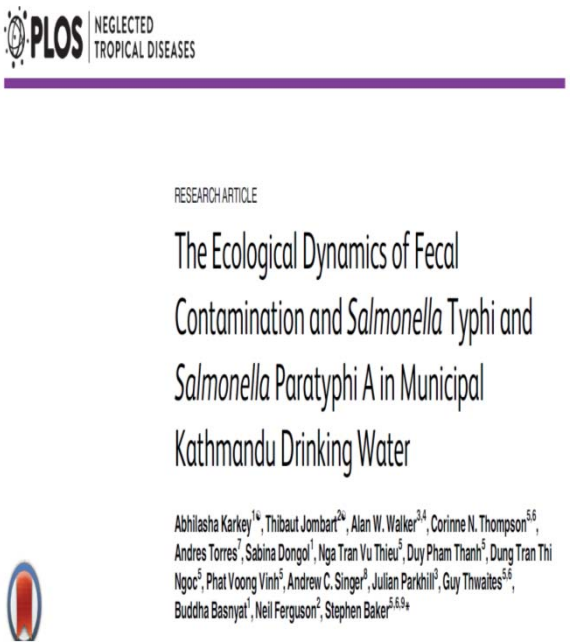
- Multiplex qPCR for detection of
  - Pan *Salmonella*
  - *S.* Typhi,
  - *S.* Enteridis,
  - *S.* Typhimurium,
  - *S.* Paratyphi A



Courtesy: Mami Taniuchi, UVA

- 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)


PLOS | NEGLECTED TROPICAL DISEASES

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Nga et al. BMC Infectious Diseases 2010, 10:125
http://www.biomedcentral.com/1471-2334/10/125

BMC Infectious Diseases

RESEARCH ARTICLE Open Access

The sensitivity of real-time PCR amplification targeting invasive *Salmonella* serovars in biological specimens

Tran Vu Thieu Nga[1,2], Abhilasha Karkey[3], Sabina Dongol[3], Hang Nguyen Thuy[1,2], Sarah Dunstan[1,4], Kathryn Holt[5], Le Thi Phuong Tu[1,2], James I Campbell[1,4], Tran Thuy Chau[1,2], Nguyen Van Vinh Chau[1], Amit Arjyal[3], Samir Koirala[3], Buddha Basnyat[3], Christiane Dolecek[1,4], Jeremy Farrar[1,4] and Stephen Baker*[1,4]

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 (28th January, 11th 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)

Sites	1-Feb	12-Feb	1-Mar	14-Mar	30-Mar	16-Apr	2-May	17-May	1-Jun	16-Jun	2-Jul	19-Jul	Sep-18	Oct-18	Nov-18	Dec-18	Jan-19	Feb-19
1																		
2																		
3																		
4																		
5																		
6																		
7																		
8																		
9																		
10																		
11	NC	NC	NC															
12	NC	NC	NC															

S.Typhi detection by multiplex qPCR

Sites	1-Feb	12-Feb	1-Mar	14-Mar	30-Mar	16-Apr	2-May	17-May	1-Jun	16-Jun	2-Jul	19-Jul	Sep-18	Oct-18	Nov-18	Dec-18	Jan-19	Feb-19
1																		
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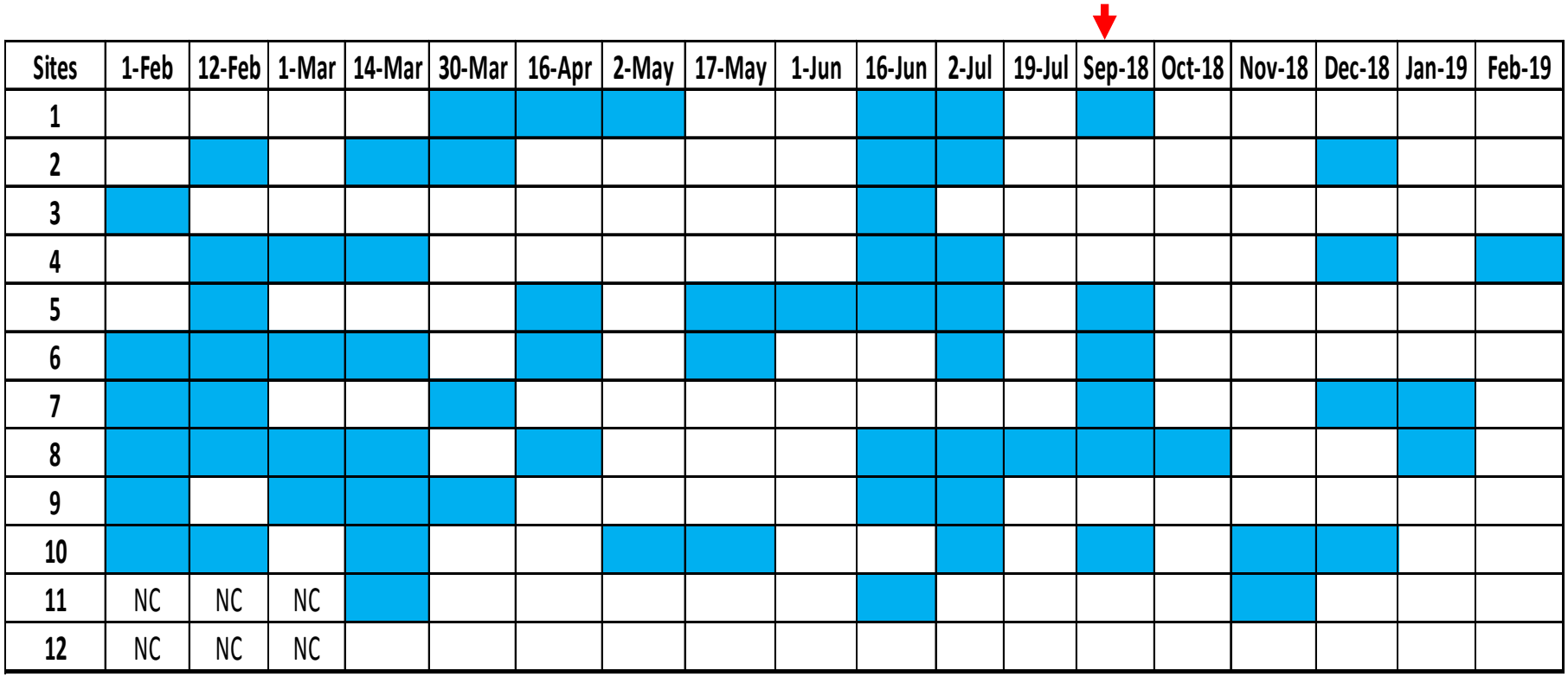
11	NC	NC	NC															
12	NC	NC	NC															

NC- not collected

Salmonella Typhimurium multiplex qPCR



Sites	1-Feb	12-Feb	1-Mar	14-Mar	30-Mar	16-Apr	2-May	17-May	1-Jun	16-Jun	2-Jul	19-Jul	Sep-18	Oct-18	Nov-18	Dec-18	Jan-19	Feb-19
1																		
2																		
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5																		
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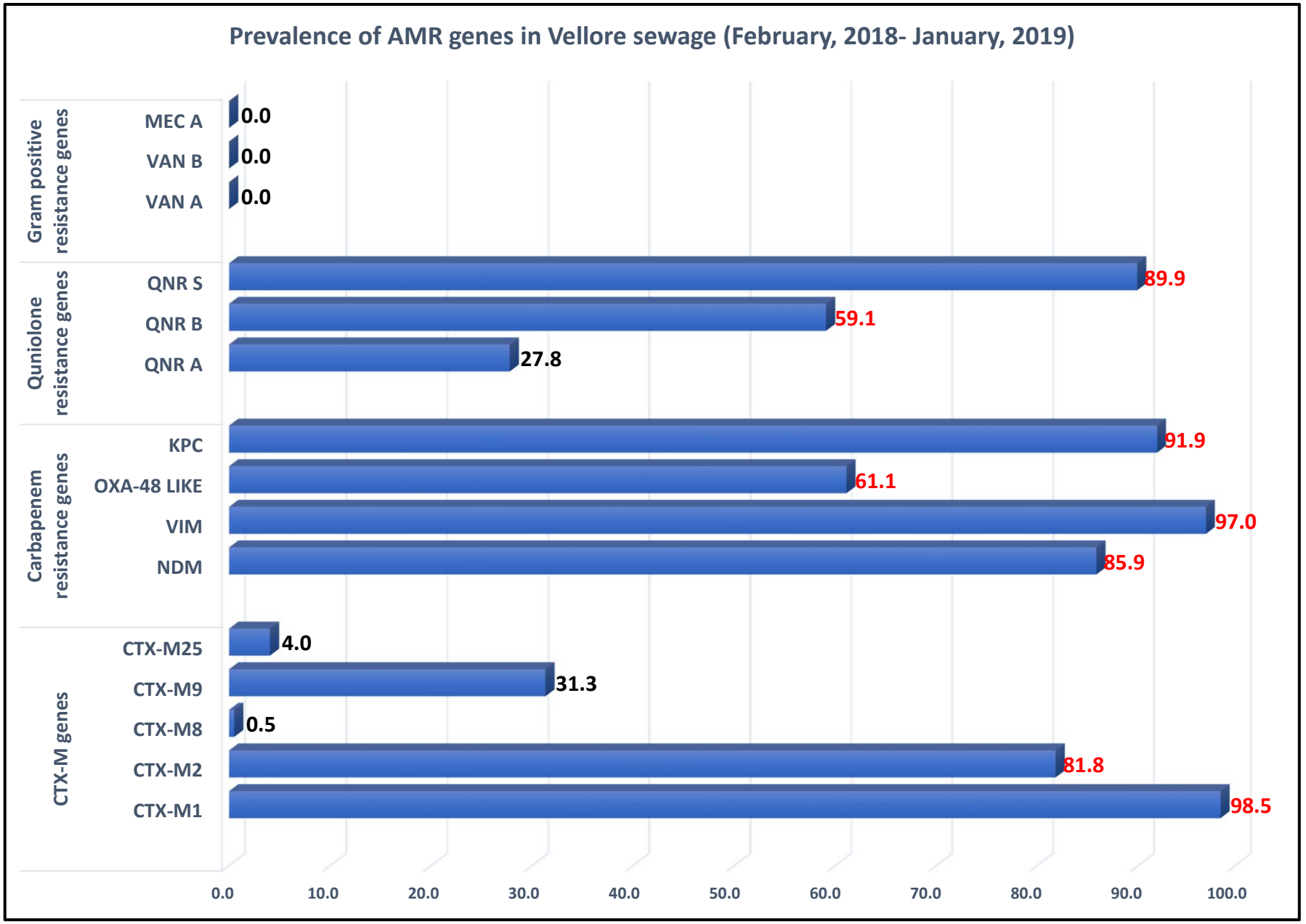
10																		
11	NC	NC	NC															
12	NC	NC	NC															

NC- not collected


Prevalence of AMR genes in Vellore sewage (February, 2018- January, 2019)
Gram positive resistance genes
MEC A
VAN B
VAN A
0.0
0.0
0.0
Quniolone resistance genes
QNR S
QNR B
QNR A
89.9
59.1
27.8
Carbapenem resistance genes
KPC
OXA-48 LIKE
VIM
NDM
91.9
61.1
97.0
85.9
CTX-M genes
CTX-M25
CTX-M9
CTX-M8
CTX-M2
CTX-M1
4.0
31.3
0.5
81.8
98.5
0.0
10.0
20.0
30.0
40.0
50.0
60.0
70.0
80.0
90.0
100.0

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)

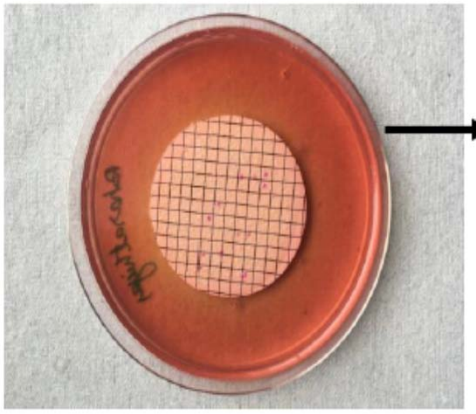
Community Drinking water

- **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 (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

- **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)
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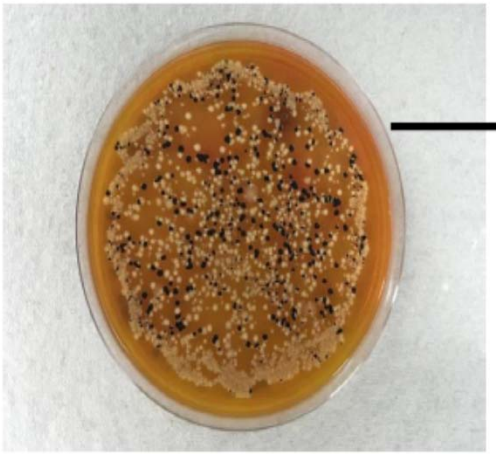
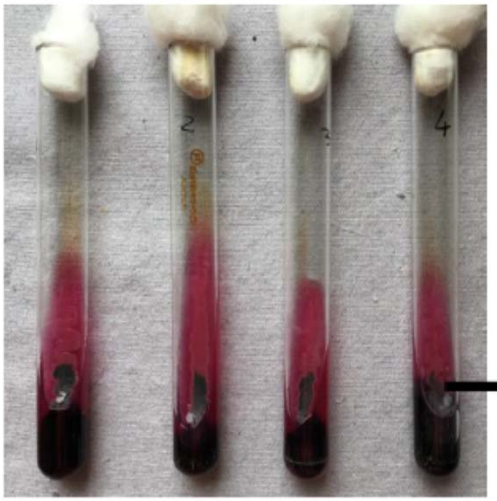


Culture on XLD agar



Abundant H_2S in TSI, not suggestive of *S*. Typhi

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*

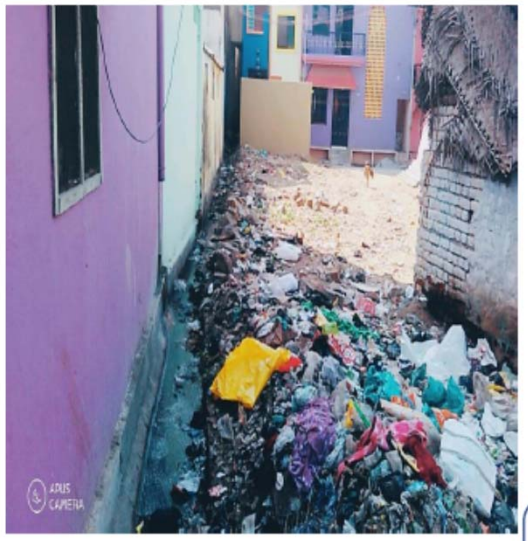
- 5 hospitals from Vellore included in the study

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

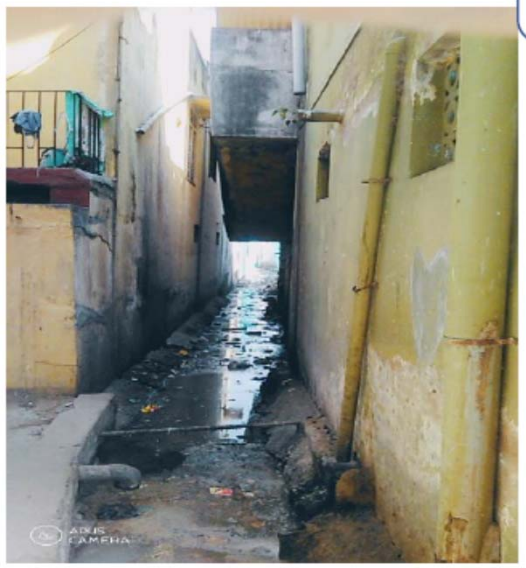
- Samples tested (*July, 2018- February, 2019)*: 96 samples
  - Pan *Salmonella*: 83.3% (80/96)
  - *S*. Typhi: 19.8% (19/96; singleplex and/or multiplex qPCR)
  - *S*. Typhimurium: 29.2% (28/96)
  - *S*. Enteritidis: 3.1% (3/96)
  - *S*. Paratyphi A: 0%

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 37^0C overnight
    - After incubation, broth used for culture and qPCR
  - Floor swab (EnviroMax swab)
    - In lab, 10ml selenite F broth inoculated & incubated at 37^0C 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

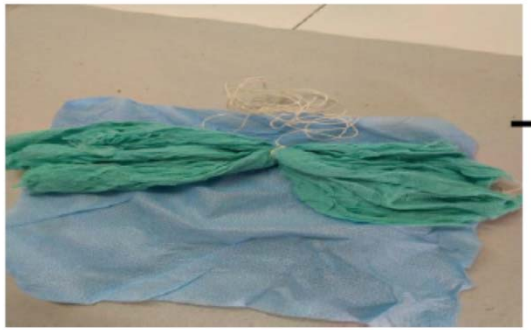


Household sewage



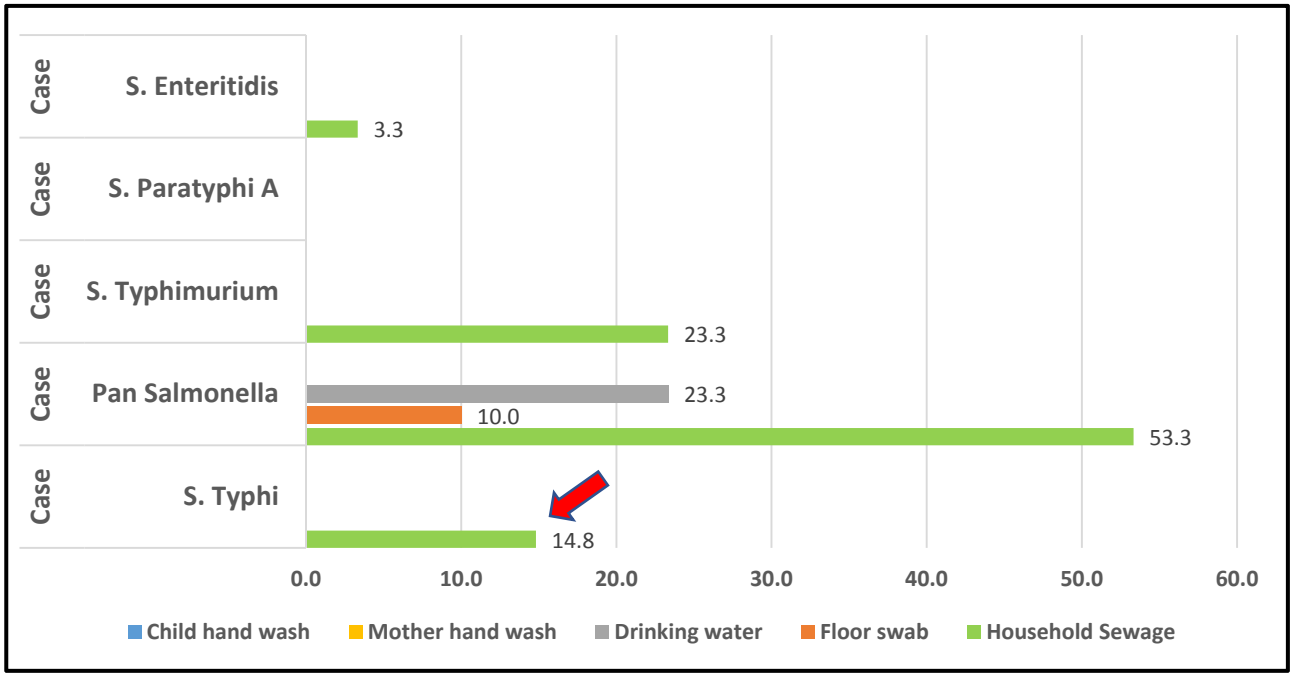
- Results available-
  - Drinking water- 30 pairs
  - Mother hand wash- 30 pairs
  - Child hand wash- 30 pairs
  - Floor swab- 30 pairs
  - Moore swab- 27 cases, 30 controls
  - BMFS (household sewage)- 14 cases, 15 controls (*collected started from end of July, 2018*)

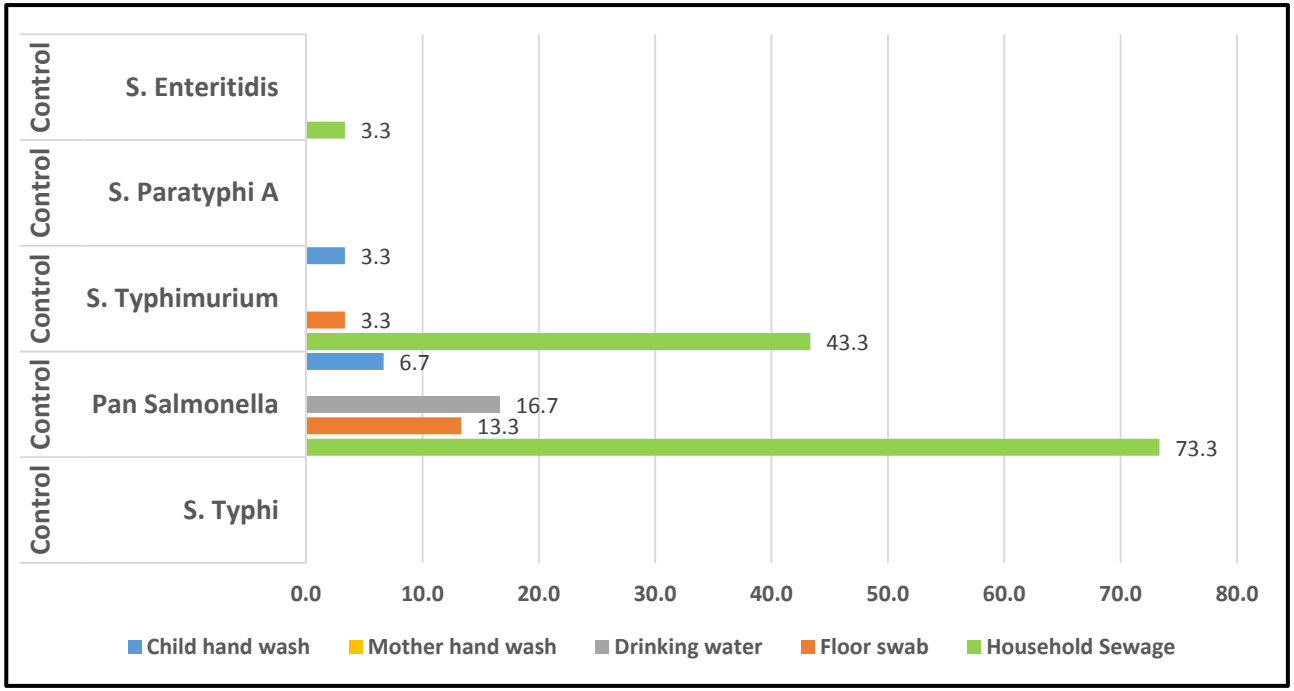
Household sewage collection using BMFS



Moore swab

Drinking water


Case
S. Enteritidis
3.3
Case
S. Paratyphi A
Case
S. Typhimurium
23.3
Case
Pan Salmonella
23.3
10.0
53.3
Case
S. Typhi
14.8
0.0
10.0
20.0
30.0
40.0
50.0
60.0
Child hand wash
Mother hand wash
Drinking water
Floor swab
Household Sewage


Control
S. Enteritidis
3.3
Control
S. Paratyphi A
Control
S. Typhimurium
3.3
3.3
43.3
Control
Pan Salmonella
6.7
16.7
13.3
73.3
Control
S. Typhi
0.0
10.0
20.0
30.0
40.0
50.0
60.0
70.0
80.0
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

Acknowledgement

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