Recent emergence of cephalosporin-resistant *Salmonella Typhi* carrying IncFIB(K) plasmids encoding $bla_{CTX-M-15}$ gene in India

Tharani Priya T
Doctoral student | Christian Medical College, Vellore
Drug Resistant S. Typhi: The timeline

- **ChlR S. Typhi reported**
- **Emergence of MDR S. Typhi**
- **FQ Resistant S. Typhi outbreak**
- **2015: >90% FQR S. Typhi in South Asia**
- **2016: CTR S. Typhi outbreak in Pakistan**
- **2019: Emergence of AZM S. Typhi in South Asia**

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
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<tbody>
<tr>
<td>1945</td>
<td>Widespread ChlR S. Typhi</td>
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<tr>
<td>1950</td>
<td>Widespread MDR S. Typhi</td>
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<tr>
<td>1960</td>
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<td>1970</td>
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<td>1980</td>
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<td>1990</td>
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<td>2000</td>
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<tr>
<td>2010</td>
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<tr>
<td>2015</td>
<td>&gt;90% FQR S. Typhi in South Asia</td>
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<tr>
<td>2016</td>
<td>CTR S. Typhi outbreak in Pakistan</td>
</tr>
<tr>
<td>2019</td>
<td>Emergence of AZM S. Typhi in South Asia</td>
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Outbreak of Ceftriaxone Resistant S. Typhi in India?

- 08/2022: **Single isolate** of Ceftriaxone resistant S. Typhi from Pathocare lab
- 11/2022: 8 isolates from NSRL lab were confirmed as CefR @ CMC (June onwards)
- 05/2023: 11 isolates from Zydus hospital were confirmed as CefR @ CMC
- 06/2023: 10 isolates from the 2nd Batch (NSRL) confirmed as CefR
- 06/2023 onwards: ~100 isolates of Ceftriaxone resistant S. Typhi from different diagnostic centres across Vadodara
Timeline of the emergence of CEF-R S. Typhi from diagnostic centres in Gujarat, India

<table>
<thead>
<tr>
<th></th>
<th>2022</th>
<th>2023</th>
<th>No of cases</th>
</tr>
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<tbody>
<tr>
<td>Jan-Mar</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Apr-Jun</td>
<td>3</td>
<td>4</td>
<td></td>
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<tr>
<td>Jul-Sep</td>
<td>11</td>
<td>49</td>
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</tr>
<tr>
<td>Oct-Dec</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Jan-Mar</td>
<td>6</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Apr-Jun</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Jul-Sep</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Oct-Dec</td>
<td>0</td>
<td>86</td>
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Legend:
- Blue: Suburban Diagnostics India Pvt. Ltd, Mumbai
- Green: Pathocare Pathology Laboratory, Gujarat
- Orange: NSRL, Gujarat
- Yellow: Zydus hospitals, Gujarat
- Green: Toprani, Gujarat
- Black: Baroda unipath laboratory, Gujarat
Typhoid getting stubborn, recovery taking longer now

VADODARA: Typhoid, which is otherwise widely prevalent and treatable with known medicines is fast becoming become a nightmare for doctors and patients alike. Alarming, the bacterial infection is showing stubborn resistance to multiple drugs that have been used for decades to treat this disease that is caused by consuming contaminated water or food. As a result, the symptoms, which usually subside within seven to 10 days, are giving patients a harrowing time for as long as 20-25 days.
Methodology

Phenotypic characterization
- Antimicrobial susceptibility testing (DD, MIC)

Molecular characterization
- Whole Genome Sequencing
  - S. Typhi - 148 isolates
  - 16 contemporary isolates
- Genomic analysis
  - Comparative genomic analysis
  - Phylogenetic analysis
  - Plasmid characterization
Core Genome Phylogeny of global H58 S. Typhi isolates

Maximum likelihood tree of 553 S. Typhi (H58 and non-H58)

Vadodara Outbreak (n=142)

Population structure of H58 S. Typhi isolates (n=371)
Unrooted phylogenetic tree of CTR-R S. Typhi outbreak isolates
Genomic Characterization of CefR S. Typhi in India

Vadodara Outbreak

AMR genes in IncFIB(K) Plasmid

Plasmid: AMR genes
IncFIB(K): CTX-M, dfrA, sul, qnrS
QRDR
gyrA: S83F

H58 (4.3.1.2)

Reported from Delhi

AMR genes in IncY Plasmid

Plasmid: AMR genes
IncY: TEM1B, CTX-M, dfrA, sul, tet, qnrS
QRDR
gyrA: S83F
H58 (4.3.1)

Reported from Mumbai

AMR genes in IncX3 Plasmid

Plasmid: AMR genes
IncX3: SHV-12, qnrB
QRDR
gyrA: S83F, D87N, S80I

H58 (4.3.1.2)
Core Phylogenetic tree of global IncFIB(K) plasmids (n=276)

- Dataset includes plasmids from diverse bacterial hosts K. pneumoniae, S. Typhi, E. coli and other bacterial spp
- Cluster 1 carried IncFIB(K) plasmid from global collection similar to study isolates
- Core gene SNP of IncFIB(K) plasmid from other Enterobacteriales were similar to that of study isolates
Characterisation of IncFIB(K) plasmid

- IncFIB(K) plasmid confers resistance to 3GC by means of $\text{bla}_{\text{CTX-M-15}}$ gene
- Closer to IncFIB(K) plasmid of Tanzanian S. Typhi strain (LT904889) (isolated on 2008) and European E. coli (CP116920)
- IncFIB(K) plasmids carried by S. Typhi from Bangladesh showed less similarity
- Possible transmission from other Enterobacteriales
Key observation

✓ Outbreak of ceftriaxone-resistant S. Typhi detected from Vadodara & Ahmedabad, India

✓ These isolates carried three plasmids: IncFIB(K), IncX1 and IncFIB(pHCM2) where IncFIB(K) plasmid confers resistance to 3GC by means of $\text{bla}_{\text{CTX-M-15}}$ gene

✓ Other resistance determinants such as aph(3''), aph(6'), sul2, dfrA14 and tetA were identified

✓ Genotyping and phylogenetic analysis revealed that the outbreak isolates ($n=142$) belong to a distinct subclade (4.3.1.2.2) within genotype 4.3.1.2 (H58 lineage II)

✓ SNP-based phylogenetic analysis of the core genes in IncFIB(K) revealed the plasmid backbone is closely related to that of IncFIB(K) from other Enterobacteriales

✓ Possible transmission from other Enterobacteriales
Conclusion

• *S. Typhi* continues to improve its ability to remodel its genome
  - Acquisition of various mobile genetic elements and different genetic structures which are related to antibiotic resistance

• Exposure of third-generation cephalosporins during the treatment
  - Beginning of a new wave of ceftriaxone-resistant *S. Typhi* in India

• Measures to reduce the emergence of increasingly resistant strains of *S. Typhi*
  - Introduction of new typhoid conjugate vaccines as well as other control measures such as improved water, sanitation, and hygiene (WASH) systems
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Thank you...!