Salmonella Typhi in Bangladesh: exploration of genomic diversity and antimicrobial resistance

Arif Mohammad Tanmoy

PhD Candidate
Department of Medical Microbiology and Infectious Diseases,
Erasmus MC, Rotterdam, The Netherlands.

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Background

- Typhoid fever is caused by *Salmonella enterica* serovar Typhi.
- Endemic in tropical countries (Africa and South-Southeast Asia).

- The increasing antimicrobial resistance (AMR) is limiting the treatment options.
- The current usual treatment choices are Ceftriaxone and Azithromycin.
- The first large-scale outbreak of extensively drug resistant (XDR) *S. Typhi* is ongoing in Pakistan (ceftriaxone resistant).

- Several large *S. Typhi* genomic studies are there.
- However, only few of them had country-specific focus, which is required for public interventions.
Objectives

Study a well-characterized set of *S.* Typhi isolates from an endemic country (Bangladesh).

Explore the genotypic diversity and phylogenetic relationships among the isolates and compare with neighboring countries.

Study the efficiency of WGS data to predict the AMR phenotypes of multiple antibiotics.

CHRF has been running Hospital surveillance in Bangladesh since 1999

~4000 *S.* Typhi strains from suspected enteric fever patients.

With antimicrobial susceptibility data
Method (overview)

Generated a WGS dataset of 536 S. Typhi strains, mostly isolated from blood of pediatric cases (1999 – 2013).

Studied the genotypic diversity and phylogenetic relationships using wgSNP analysis.

Compared with neighboring countries –
- A hospital-based surveillance in Nepal (198 isolates, 2008-2016)\(^1\)
- XDR S. Typhi outbreak in Pakistan\(^2\)

Compared WGS data with AMR phenotypes
- Acquired resistance genes.
- Mutations in the \(\text{gyrA/B}\) and \(\text{parC/E}\) genes.

Results: Prevalent genotypes

- Dominant genotype: 4.3.1 (Haplotype 58, H58)
- 3.3 and 3.2.2 are second and third most prevalent.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Number</th>
<th>%</th>
<th>Haplotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.3.1</td>
<td>350</td>
<td>65.30</td>
<td>H58</td>
</tr>
<tr>
<td>3.3</td>
<td>69</td>
<td>12.87</td>
<td>H1</td>
</tr>
<tr>
<td>3.2.2</td>
<td>61</td>
<td>11.38</td>
<td>H1</td>
</tr>
<tr>
<td>2.0</td>
<td>18</td>
<td>3.36</td>
<td>NT</td>
</tr>
<tr>
<td>2.3.3</td>
<td>18</td>
<td>3.36</td>
<td>NT</td>
</tr>
<tr>
<td>Total</td>
<td>536</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

Table. Five most frequent genotypes in Bangladesh
Phylogeny shows genotype-specific subclades
Comparison with Nepal and Pakistan show country-specific clusters
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Phylogeny of genotype 4.3.1 shows new lineage
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- **Bangladesh (Lineage Bd)**
- **Pakistan (Lineage I)**
- **Nepal (Lineage II)**

- Lineage Bd exclusively has the *gyrA-S83Y* mutation.
Phylogeny of genotype 4.3.1 shows new lineages

- Sublineage Bdq had high cip-MIC (≥4 µg/ml).
New sublineage Bdq could be recently emerged

* Isolates belong to lineage Bd, but not sublineage Bdq.
Efficiency of WGS data to predict the AMR phenotypes

The 2\textsuperscript{nd} objective

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>99.6</td>
<td>97.4</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>99.2</td>
<td>97.6</td>
</tr>
<tr>
<td>Cotrimoxazole</td>
<td>99.1</td>
<td>91.4</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>98.1</td>
<td>52.2</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>
Ceftriaxone-Resistance in Bangladesh doesn’t match with XDR in Pakistan

- Different genotype than the XDR isolates in Pakistan (3.3 vs 4.3.1).
- $bla_{CTX-M15}$ gene (99% sequence identity) (92% coverage)

Ceftriaxone-resistant isolate in Bangladesh

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XDR isolates in Pakistan
Conclusions

We report –

- Lineage Bd, a previously unknown local variant of genotype 4.3.1.
- Recently emerged sub-lineage Bdq, with high ciprofloxacin resistance.

- High sensitivity and specificity for prediction of ampicillin, chloramphenicol, cotrimoxazole and ceftriaxone resistance phenotypes.
  - Needs further improvement for ciprofloxacin.
- Ceftriaxone resistance in Bangladesh may have different origin than the XDR isolates in Pakistan.

- The emergence of these highly resistant S. Typhi (e.g. XDR, lineage Bdq) reconfirm the necessity for preventive measures in endemic countries.
  - Public intervention should be initiated.
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aDepartment of Medical Microbiology and Infectious Diseases, Erasmus University Medical Center, Rotterdam, the Netherlands
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Thank You