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

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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 *gyrA/ B* and *parC/ E* genes.

[1] Britto CD et al 2018. [2] Klemm EJ et al 2018.

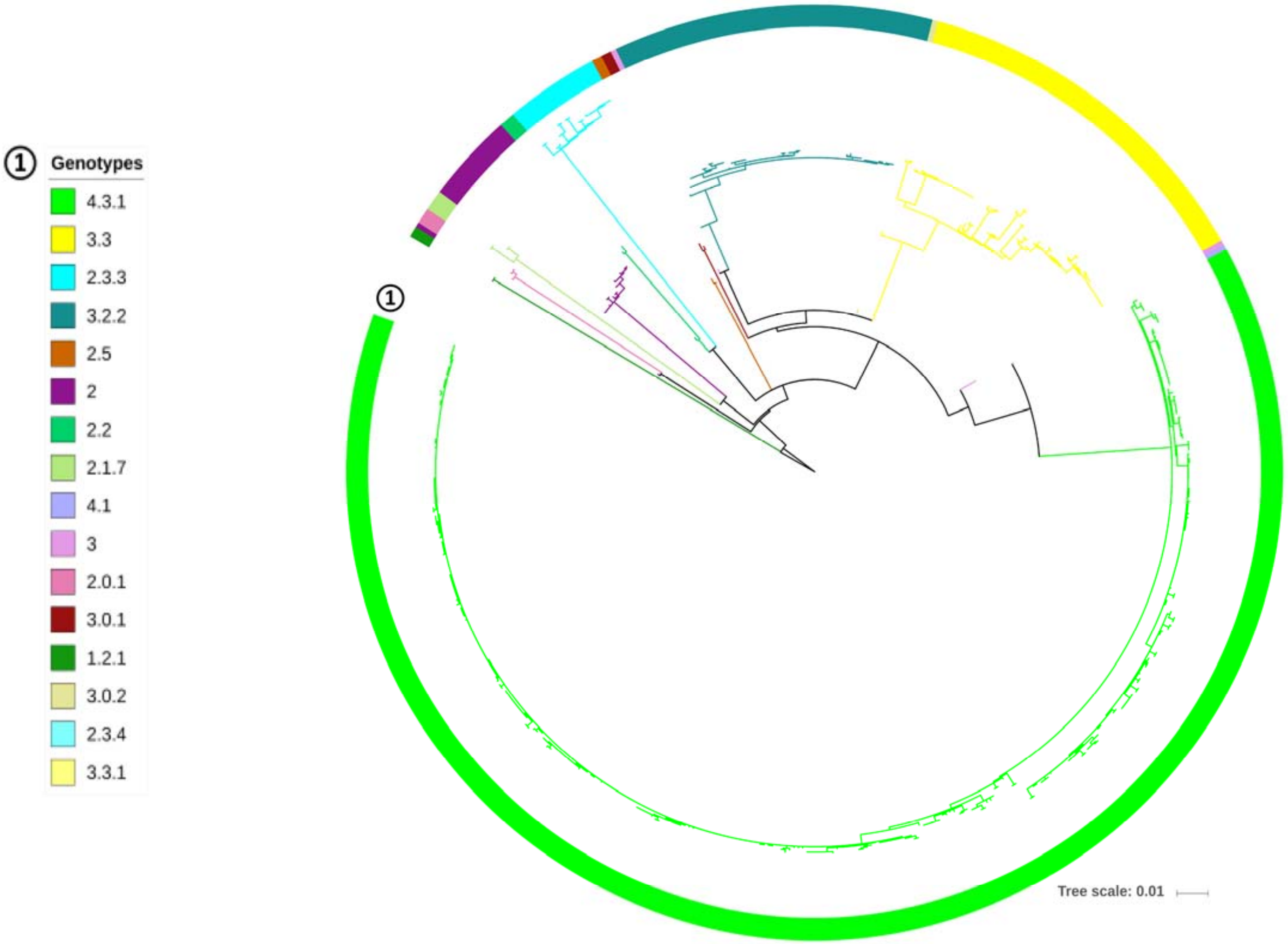
# Results: Prevalent genotypes

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

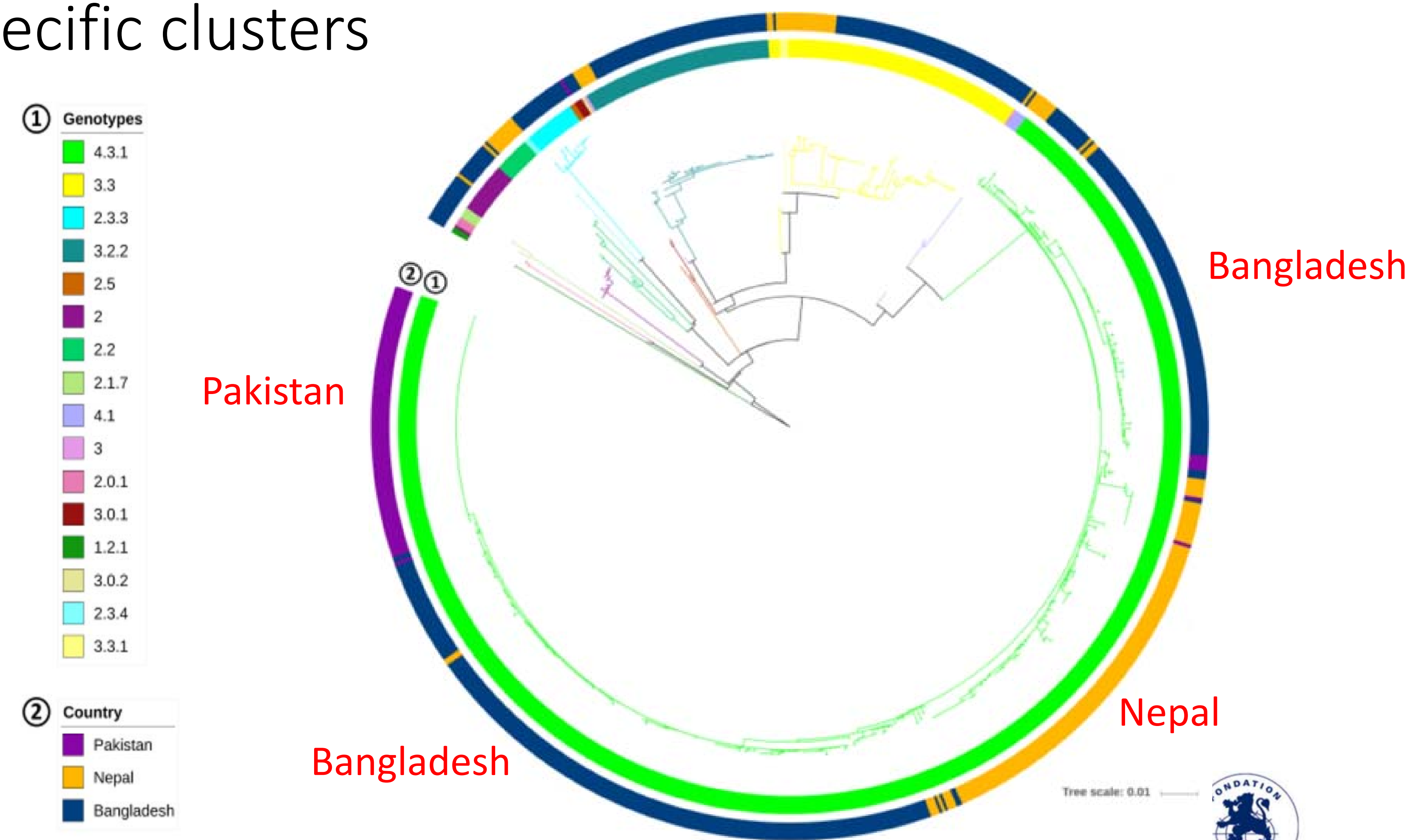
| Genotype | Number | % | Haplotype |
|---|---|---|---|
| **4.3.1** | 350 | 65.30 | H58 |
| **3.3** | 69 | 12.87 | H1 |
| **3.2.2** | 61 | 11.38 | H1 |
| **2.0** | 18 | 3.36 | NT |
| **2.3.3** | 18 | 3.36 | NT |
| **Total** | 536 | 100 | |

**Table.** Five most frequent genotypes in Bangladesh

# Phylogeny shows genotype-specific subclades



# Comparison with Nepal and Pakistan show country-specific clusters



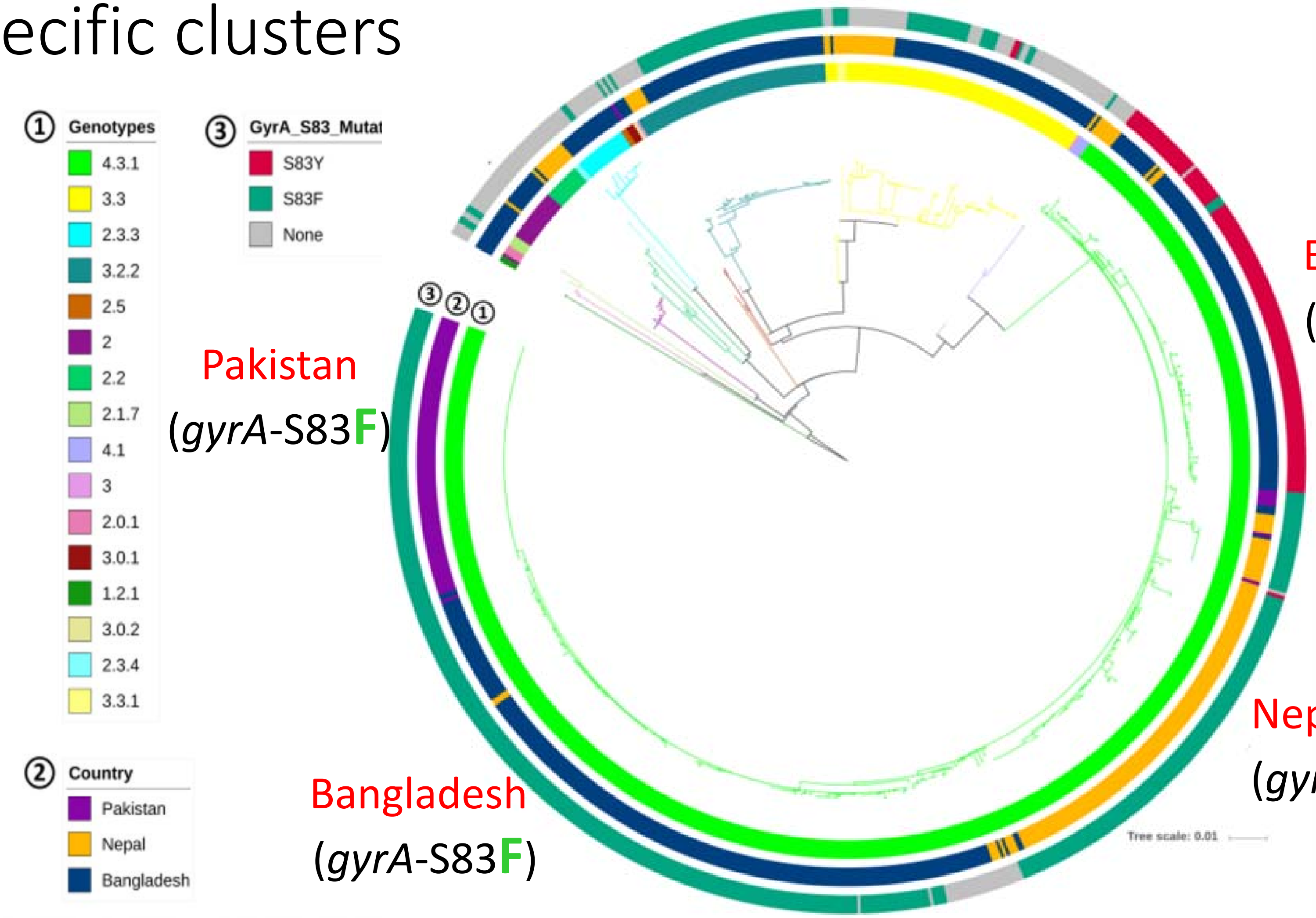
Bangladesh

Pakistan

Bangladesh

Nepal

# Comparison with Nepal and Pakistan show country-specific clusters



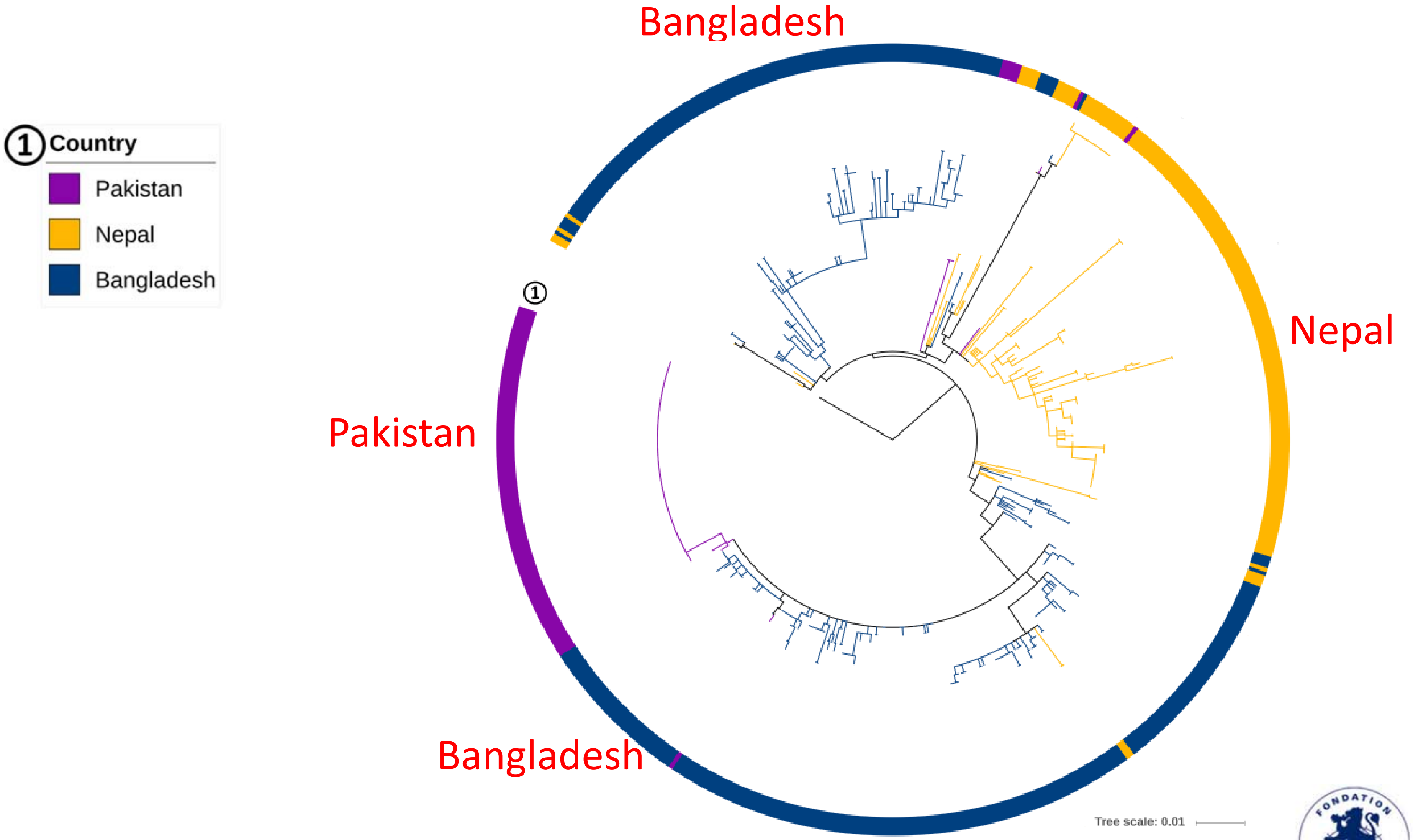
Bangladesh
(*gyrA*-S83**Y**)

Pakistan
(*gyrA*-S83**F**)

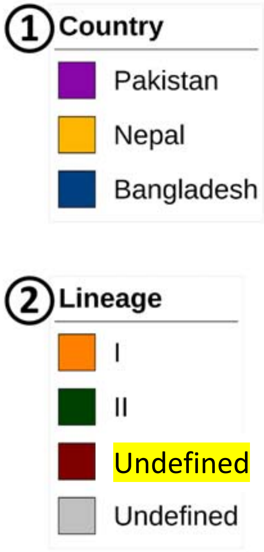
Nepal
(*gyrA*-S83**F**)

Bangladesh
(*gyrA*-S83**F**)

# Phylogeny of genotype 4.3.1 shows new lineage



# Phylogeny of genotype 4.3.1 shows new lineage

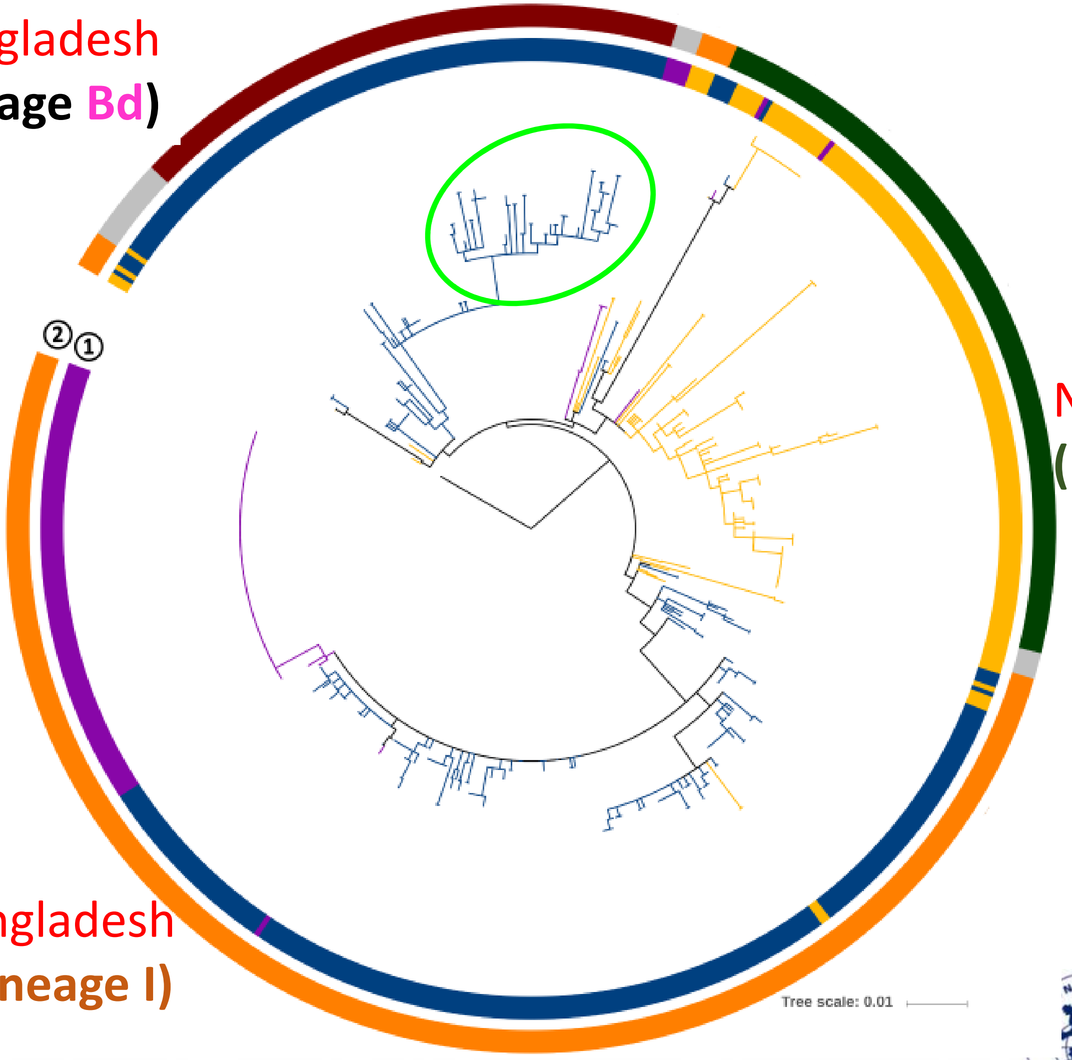


Bangladesh
**(Lineage Bd)**

Pakistan
**(Lineage I)**

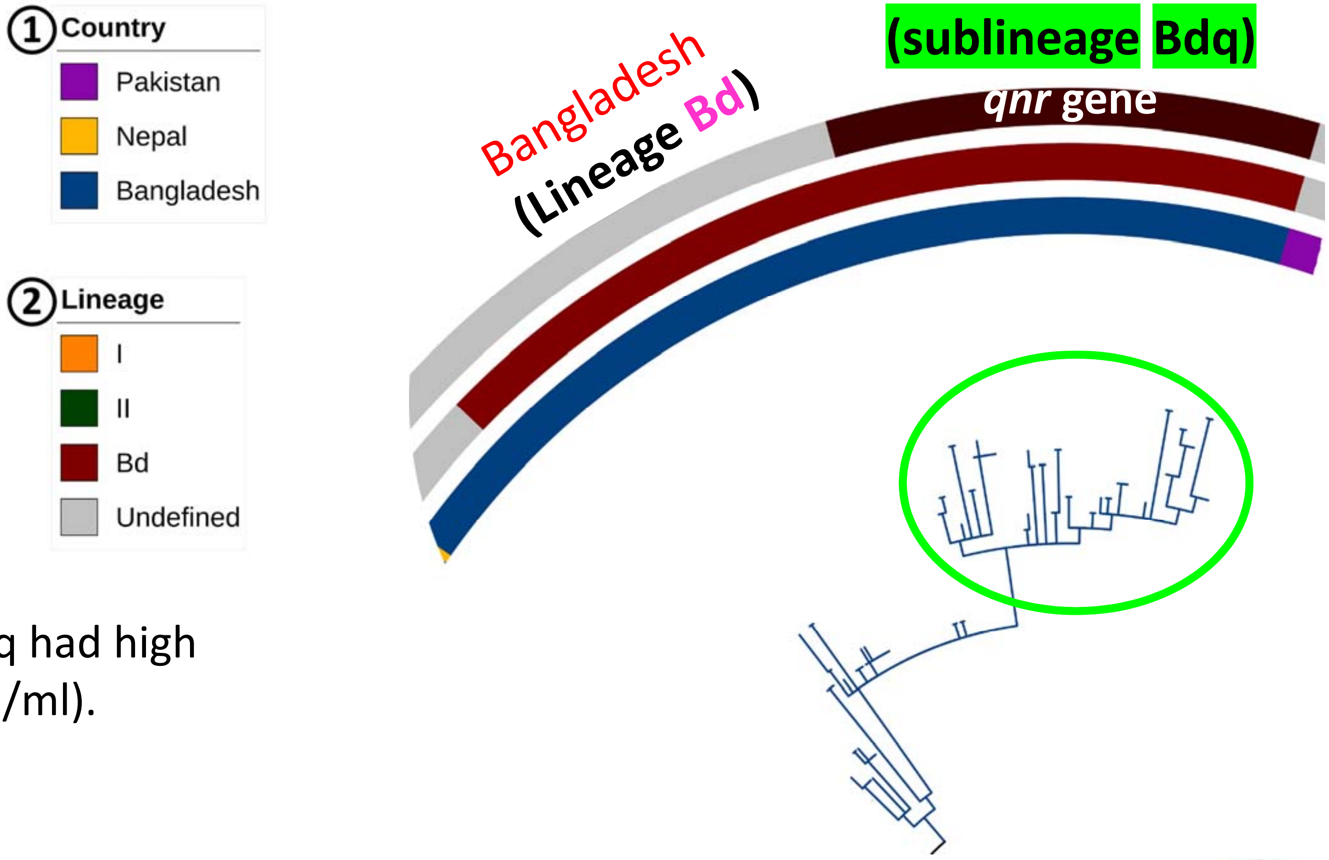
Nepal
**(Lineage II)**

Bangladesh
**(Lineage I)**



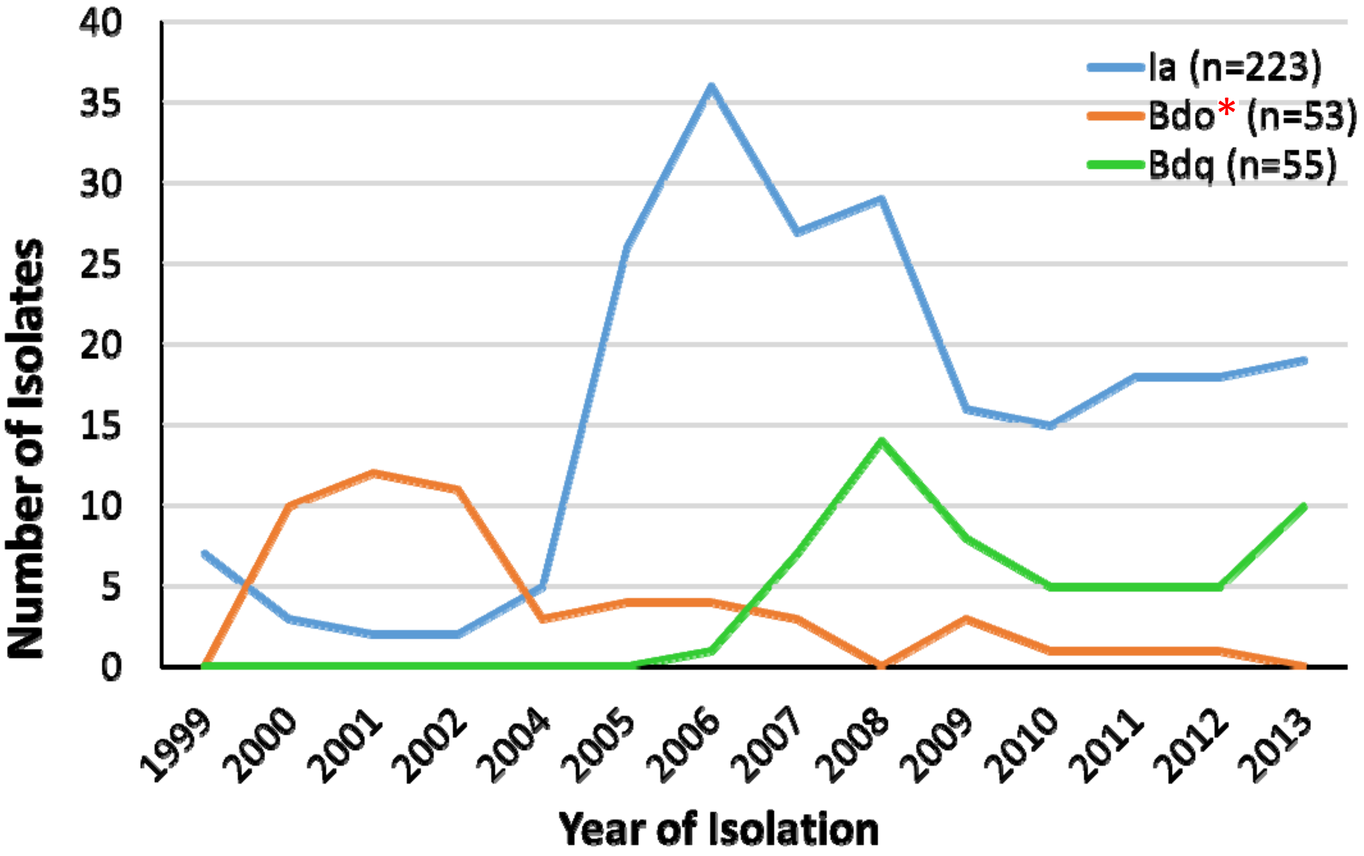
- Lineage Bd exclusively has the ***gyrA*-S83Y** mutation.

# 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.

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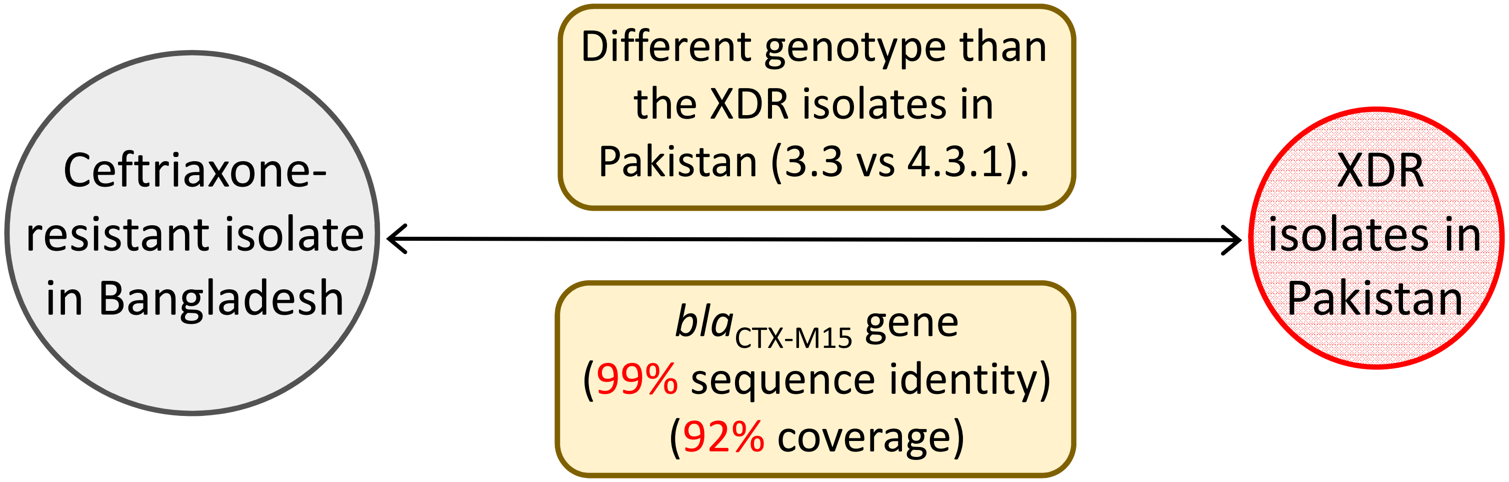
# Efficiency of WGS data to predict the AMR phenotypes

The 2nd objective

| Antimicrobial agent | Sensitivity (%) | Specificity (%) |
|---|---|---|
| Ampicillin | 99.6 | 97.4 |
| Chloramphenicol | 99.2 | 97.6 |
| Cotrimoxazole | 99.1 | 91.4 |
| Ciprofloxacin | 98.1 | 52.2 |
| Ceftriaxone | 100 | 100 |

# Ceftriaxone-Resistance in Bangladesh doesn't match with XDR in Pakistan



Ceftriaxone-resistant isolate in Bangladesh

Different genotype than the XDR isolates in Pakistan (3.3 vs 4.3.1).

*bla*$_{\text{CTX-M15}}$ gene (99% sequence identity) (92% coverage)

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.

Erasmus MC
University Medical Center Rotterdam

Child Health Research Foundation
CHRF *Prevent Infections, Save Lives*

Compare

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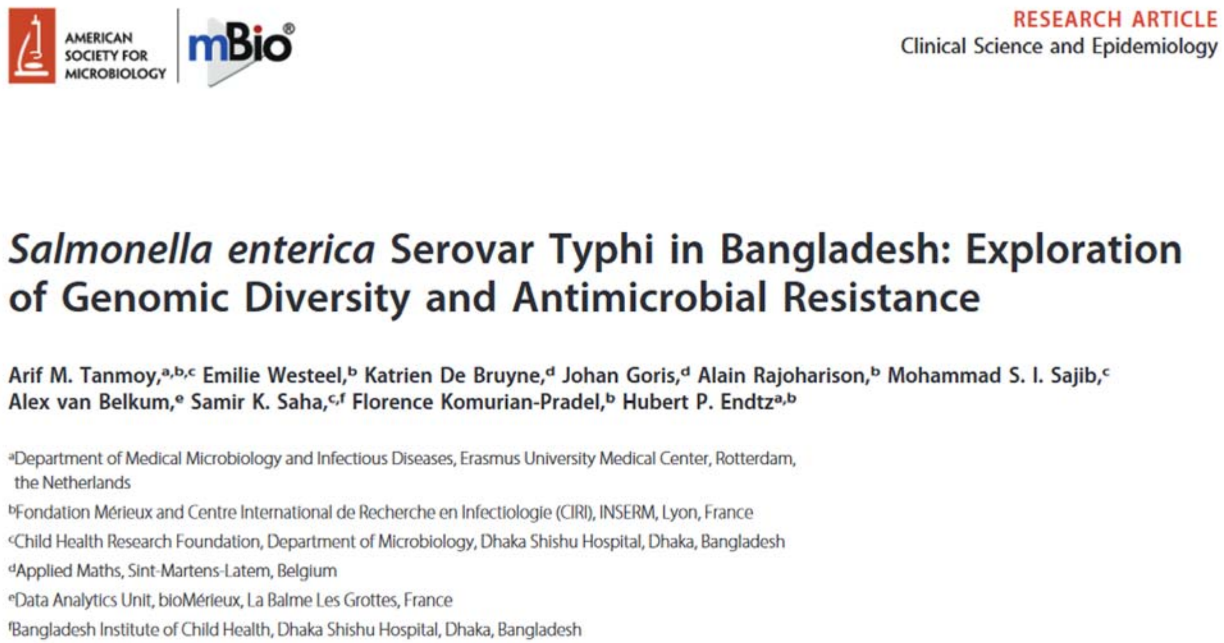
# Thank You



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## *Salmonella enterica* Serovar Typhi in Bangladesh: Exploration of Genomic Diversity and Antimicrobial Resistance

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