Molecular epidemiology and drug resistance mechanism of Salmonella species especially in S. Typhi strains isolated in Bangladesh

Dr. Kaisar Ali Talukder
Senior Scientist
Icddr,b
This presentation will focus on:

- **Epidemiology** (Hospital and population based surveillance data)
- Serotyping and antibiogramme including MIC
- Outbreak investigation
- Food-borne pathogens investigation
- Molecular epidemiology:
  - molecular epidemiology of MDR S. Typhi
Samples used for this study:

Stools and blood samples collected from patients admitted at icddr,b hospital and different studies including population based

Stools and blood samples referred by physicians at clinical microbiology laboratory of icddr,b
Phenotypic and molecular methods used:

1. Serotyping
2. Antibiotic sensitivity testing using disk diffusion and MIC by E-test.
3. Plasmid profile analysis
4. PCR, conjugation
5. Sequencing analysis of quinolone resistance-determining region (QRDR) of *gyrA*, *gyrB* and *parC*.
6. Pulsed-field gel electrophoresis (PFGE)
7. Ribotyping,
8. Hybridization
Prevalence of *Salmonella* isolated between 1997 to 2012 (n=10369)
Comparison of culture and molecular method studied (n=1863) at Kumudini hospital Mirzapur from July 2011 to Nov 2012

- Culture: 3.5 (n=65)
- Molecular: 4.3 (n=80)
Antibiotic susceptibility pattern of *S. Typhi* from 1990 to 2004
Antibiotic resistance of S. Typhi isolated between 2009 to 2011
Objective:
This study was design to determine the frequency of MDR S. Typhi in Bangladesh and characterize using phenotypic and genotypic traits to understand the molecular epidemiology
Plasmid profiles of *S. Typhi* strains

MDR

M

All sensitive

140 MDa

140 MDa
Transfer of plasmid to *E. coli* K 12 by conjugation
Detection of TEM-1 gene in *Salmonella* Typhi

AMP resistant strains

All sensitive

971 bp
Southern hybridization analysis from both plasmid-less and plasmid-bearing ampicillin-resistant strain with TEM-1 gene probe.

MDR strains having 140 MDa plasmid

140 MDa plasmid

MDR strains without 140 MDa plasmid
Pulsed–Field Gel Electrophoresis to determine the clonal relationship among S. Typhi strains

Heterogeneous Clones

MDR

All sensitive

MDR

All sensitive
Ribotyping analysis of S. Typhi strains

Single clone → MDR → All sensitive → Heterogeneous Clones

R1  R1  R1  R1  R2  R3  R4  R5  R4  R2  R6  R7  R8
Findings:

- Of 11,364 strains of *Salmonella* isolated at icddr,b, S. Typhi was the most predominant except the years 1997-99 when it was replaced by *Salmonella* group B, followed by *Salmonella* Group C1, and others S. *enteritidis*.

- Resistance to all first line drugs (ampicillin, chloramphenicol, sulfamethoxazole-trimethoprim) of S. Typhi isolated in (1997-2011) increased from 31% in 1997 to 65% in 2008 but after that the isolation of MDR S. Typhi were decreased 25-30% in 2011.

- All MDR strains showed a MIC of >512 mg/L for ampicillin, trimethoprim and streptomycin, which was >256 mg/L, >128 mg/L and >256 mg/L for chloramphenicol, tetracycline and nalidixic acid (Nal), respectively.

- All MDR strains except for five harbored 140 MDa and/or 90 MDa plasmid.
Findings:

- 140 MDa plasmid was transferred independently into *E. coli* K-12 strains with the complete spectrum of resistance by conjugation.
- TEM-1 gene is present in both 140 MDa plasmid and chromosome of *S. Typhi*.
- All MDR *S. Typhi* were clonal whereas susceptible strains were heterogenous.
Drug resistance mechanism of fluoroquinolone resistant S. Typhi

Objective:

To characterize the molecular mechanism of fluoroquinolone resistance in S. Typhi strains recently isolated in Bangladesh
# MIC and Amino acid changes in *gyrA* and *parC* in S. Typhi

<table>
<thead>
<tr>
<th>Organism</th>
<th>Country</th>
<th>R factor</th>
<th>MIC (µg/ml)</th>
<th>Amino acid substitution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nal</td>
<td>Cip</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ser-83</td>
<td>Asp-87</td>
</tr>
<tr>
<td>S. Typhi</td>
<td>Bangladesh</td>
<td>All sensitive</td>
<td>2</td>
<td>0.008</td>
</tr>
<tr>
<td>S. Typhi</td>
<td>Bangladesh</td>
<td>MDR</td>
<td>&gt;256</td>
<td>0.5</td>
</tr>
<tr>
<td>S. Typhi</td>
<td>Bangladesh</td>
<td>MDR</td>
<td>&gt;256</td>
<td>0.5</td>
</tr>
<tr>
<td>S. Typhi</td>
<td>Bangladesh</td>
<td>MDR</td>
<td>&gt;256</td>
<td>6</td>
</tr>
<tr>
<td>S. Typhi</td>
<td>Bangladesh</td>
<td>Nal R</td>
<td>&gt;256</td>
<td>6</td>
</tr>
<tr>
<td>S. Typhi</td>
<td>Bangladesh</td>
<td>Nal R</td>
<td>&gt;256</td>
<td>16</td>
</tr>
<tr>
<td>S. Para Typhi A</td>
<td>Bangladesh</td>
<td>MDR</td>
<td>4</td>
<td>0.012</td>
</tr>
<tr>
<td>S. Para Typhi A</td>
<td>Bangladesh</td>
<td>MDR</td>
<td>&gt;256</td>
<td>0.5</td>
</tr>
</tbody>
</table>
Findings:

- All strains isolated before 1999 were susceptible to nalidixic acid (Nal), but resistance to Nal increased from 4% in 1999 to 98% in 2011.

- Of the Nal resistance strains 90-95% showed reduced susceptibility to Ciprofloxacin (MIC 0.25-0.5 mg/L) and only few strain (n=12) showed complete resistance to ciprofloxacin.

- Sequence analysis of QRDR of resistant strains revealed that all had mutations in \textit{gyrA} (Ser83 $\rightarrow$ Phe) and/or (Asp87 $\rightarrow$ Asn or Gly) and a single mutation in \textit{parC} (Ser80 $\rightarrow$ Ile).

- Of \textit{Salmonella} strains, 4% strains were found as ESBL producers.

- The prevalence of ESBL producers was very high in \textit{Salmonella} Group B (2%) and \textit{Salmonella} Group G (2%).

- Only one strain isolated in 2011 of the S. Typhi strain was positive for ESBLs.
Outbreak investigation

Objective:
To characterize S. Typhimurium isolated from two different outbreaks

1. icddr,b in 1998
2. Maltab in 2006

Used phenotypic and genotypic traits to understand the molecular epidemiology and clonal relationship between these two outbreaks.
PFGE analysis of *Salmonella* group B serover Typhimurium isolated in different epidemic in Bangladesh

Epidemic clone of 2006

Epidemic clone of 1998

Food Samples
Findings:

- Of 139 patients, S. Typhimurium strains were isolated from 93 stool samples and from the supplied food samples (proshad).

- All the strains were susceptible to all the antibiotics tested whereas the strains isolated from 1998 outbreak were MDR (Amp, Chl, Sxt, Nal, Cro)R.

- Plasmid analysis showed that all the recent outbreak (2006) strains did not contain any plasmid whereas MDR strains isolated in 1998 harbored 140 and or 90 MDa plasmid.

- PFGE analysis showed that the recent isolates (2006) from patients and food had an identical pattern.

- The PFGE pattern of S. Typhimurium strains isolated in 1998 belonged to a single type, which was completely different from the recent food borne outbreak strains.
Conclusion:

Different clones were responsible for these two outbreaks. Genetic analysis of food and patient isolated in 2006 the strains isolated from recent outbreak suggested close genetic relatedness, this data suggest that food is the likely source of the salmonella outbreak.
Investigation of food-borne pathogens

Objective:

The study was undertaken with a view to isolate *Salmonella* species from food samples and to characterize these strains using phenotypic and genotypic traits to understand the molecular epidemiology.
PFGE banding pattern of *Xba*I-digested chromosomal DNA of representative strains of *Salmonella* Paratyphi B

Juice isolates

Clinical isolates
PFGE banding pattern of XbaI-digested chromosomal DNA of representative strains of *Salmonella* Group C1
PFGE banding pattern of XbaI-digested chromosomal DNA of representative strains of *Salmonella* Group C2

- Juice isolates
- Clinical isolates
Findings:

- 36% strains were identified as S. paratyphi B, 9% as S. Group B, 27% as S. Group C1, 18% as S. Group C2 and 9% as S. Group H

- Strain isolated from food samples are mostly susceptible to all the antibiotics tested whereas clinical isolates were multidrug resistant

- Heterogeneous plasmid patterns were observed in both cases.

- PFGE pattern showed that S. paratyphi B and S. Group C1 isolated from juice sample and patients had an identical pattern.
Concluding remarks

Antibiotic resistance requires an urgent, sustained, multispectral, worldwide response including measures to:

- Restricted use of antibiotics in Livestock farming thus encouraging the use of legitimate alternatives e.g. Probiotics etc.
- Make precautionous selection of antibiotics as well as appropriate doses in clinical treatment purpose.
- Coordination among national and international policy makers, academia, consumers, advocacy groups and health care professionals, sharing information and effective strategies to reduce the requirement of antibiotics
- Implementation of intervention strategies to develop new and more effective antimicrobial drugs/antibiotics.
Acknowledgements

Dilip K. Dutta
Mohd. Shamim Iqbal
Ishrat J. Azmi
Abdus Salam Mondol
Mohammad Aslam
Mst. Mahmuda Akter
Trishita
Atanu
Halima
Parveen
M. Ansaruzzaman

Dilruba Ahmed
Aleya Naheed
S. Luby
Abdullah Brook
David A Sack
Alejandro Cravioto
A. S. G. Faruque
B. M A Hosshain
S. M. Faruque
Thanks