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

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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)
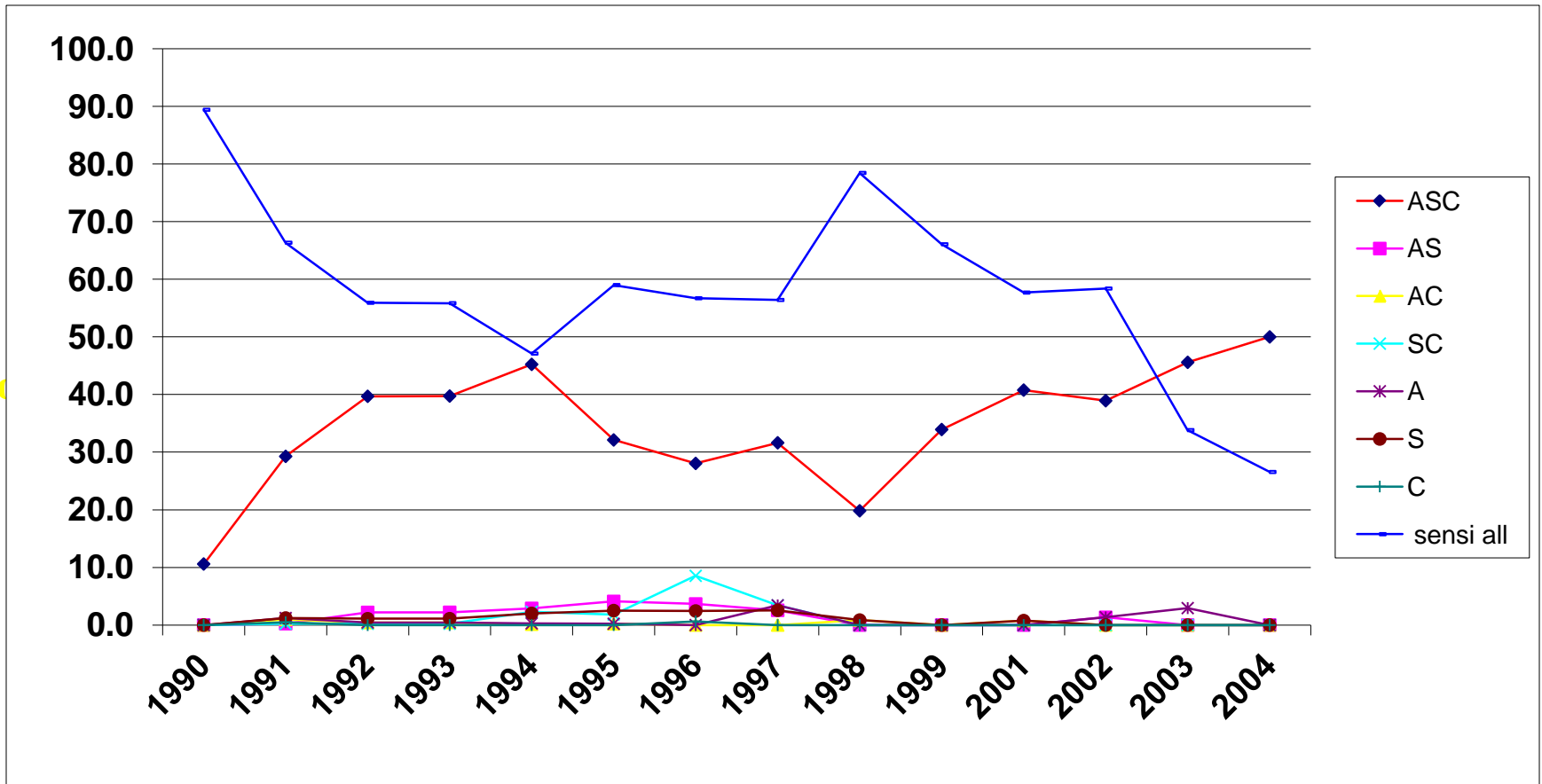7. Ribotyping,
8. Hybridization

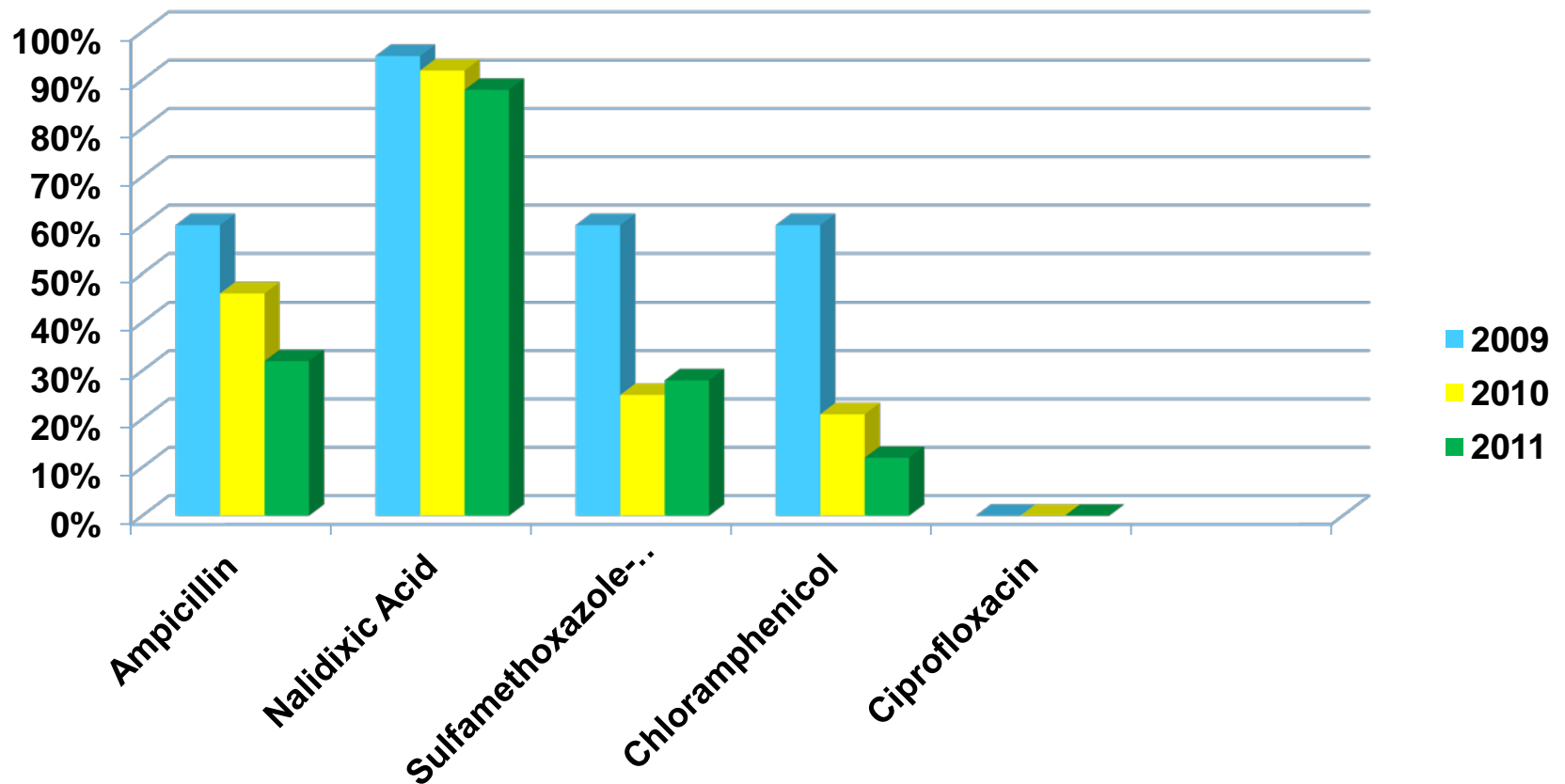# Prevalence of *Salmonella* isolated between 1997 to 2012 (n=10369)
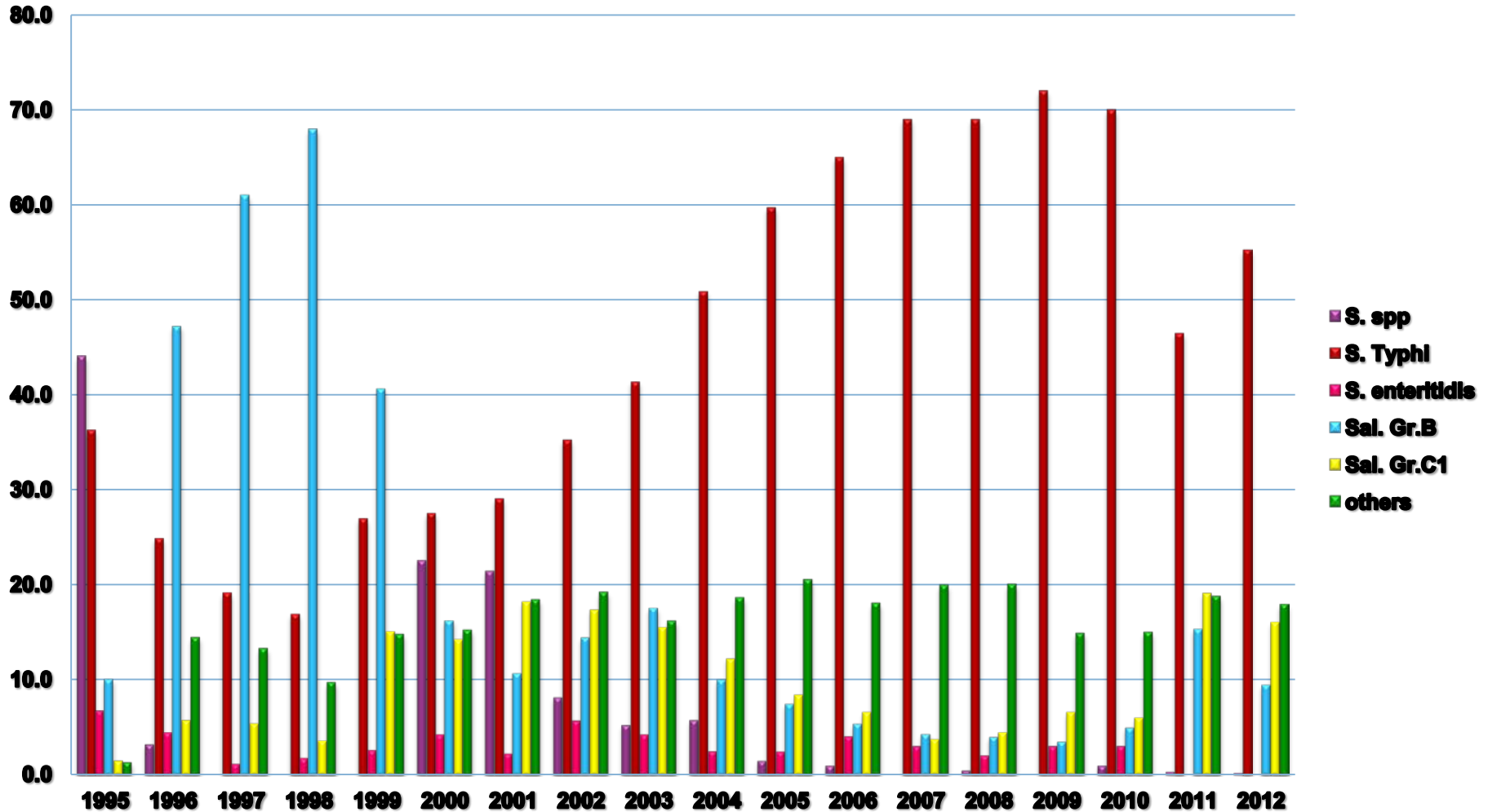
Comparison of culture and molecular method studied (n=1863) at Kumudini hospital Mirzapur from July 2011 to Nov 2012
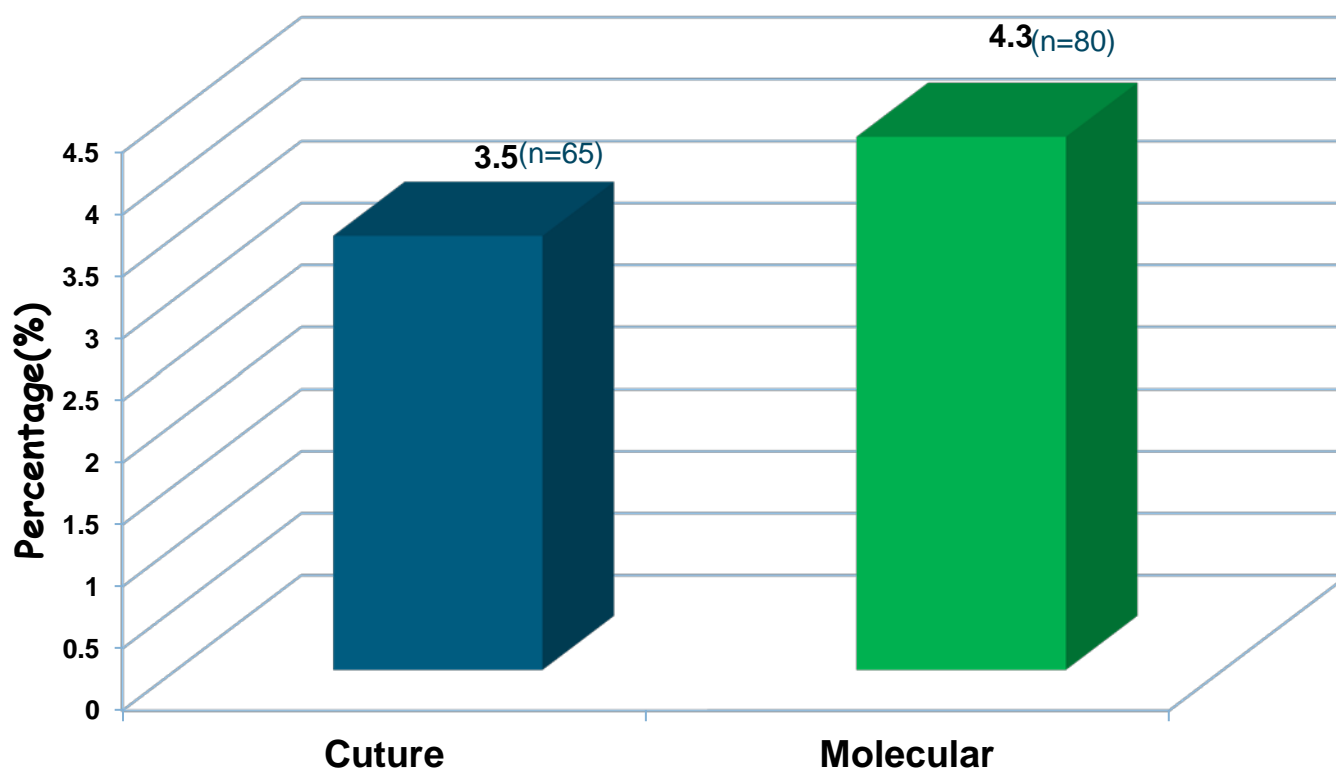
Antibiotic susceptibility pattern of *S. Typhi* from 1990 to 2004

Year

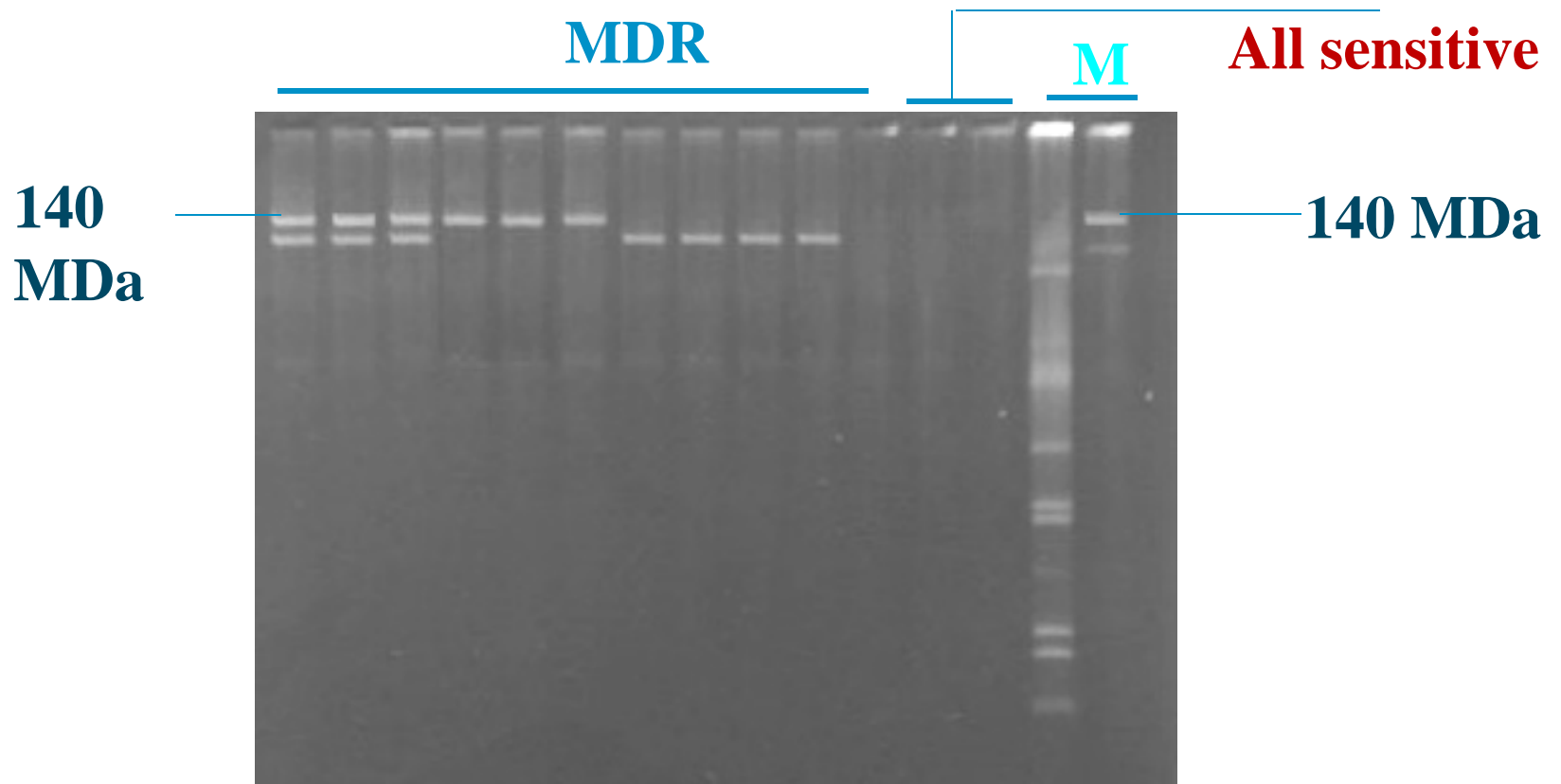# Antibiotic resistance of *S.* Typhi isolated between 2009 to 2011

Molecular Epidemiology of MDR S. Typhi
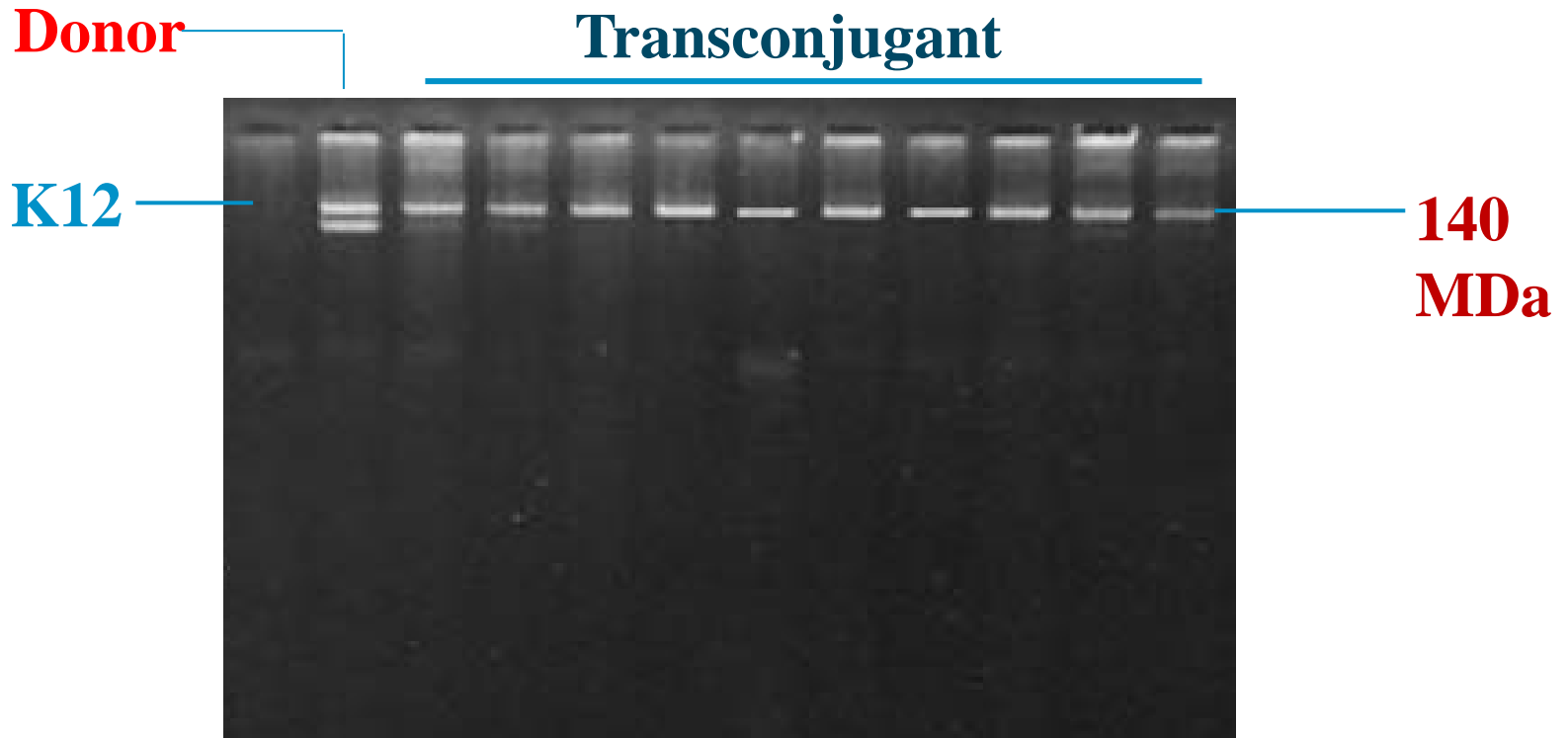
Objective:

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

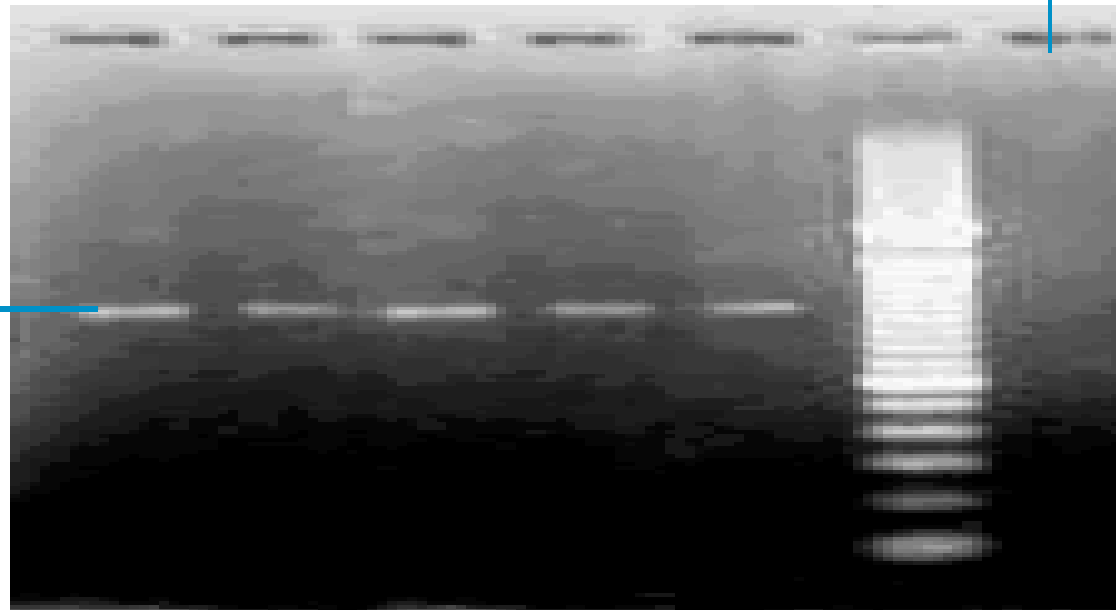# Transfer of plasmid to *E.coli* K 12 by conjugation

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
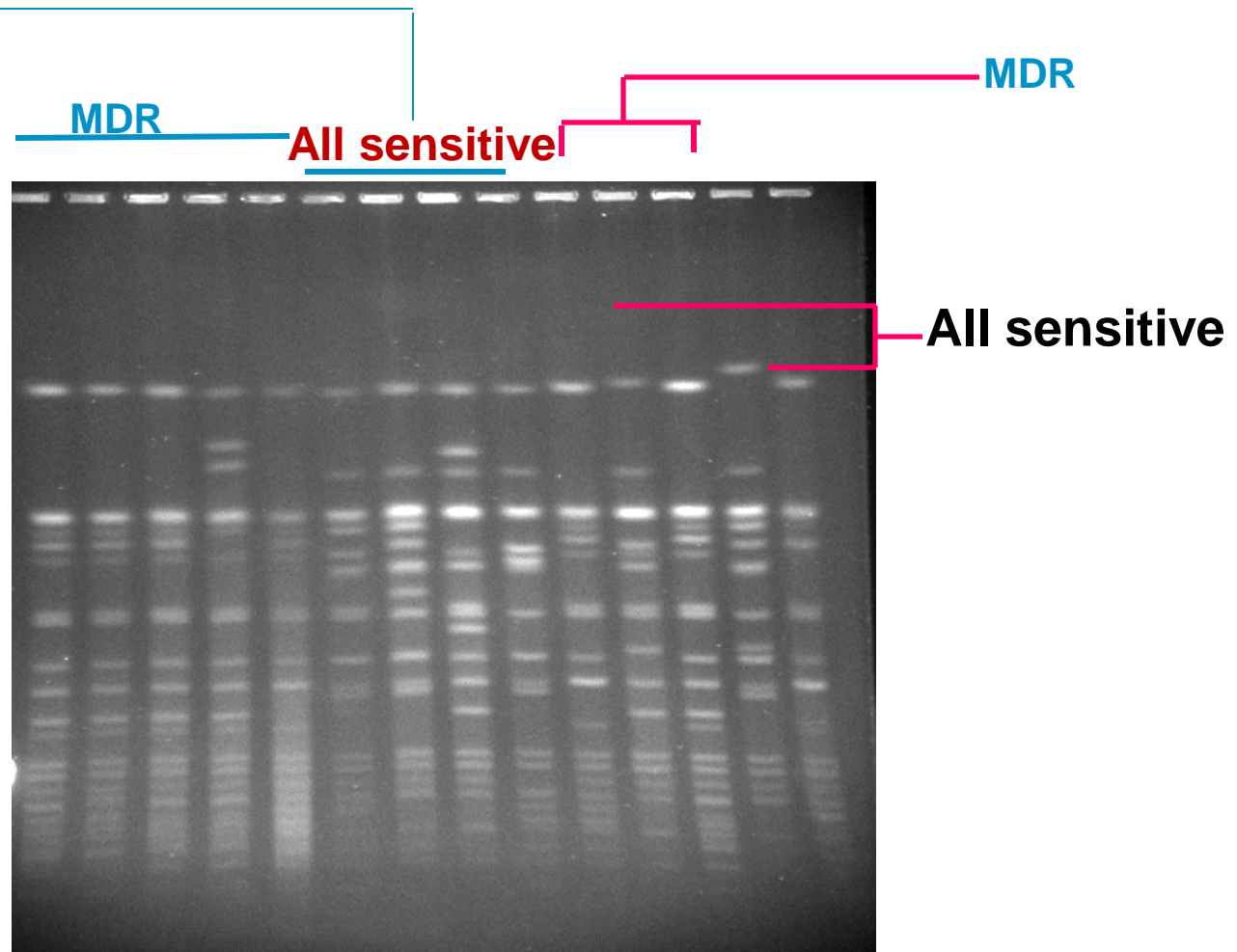
MDR strains having 140 MDa plasmid

140 MDa plasmid

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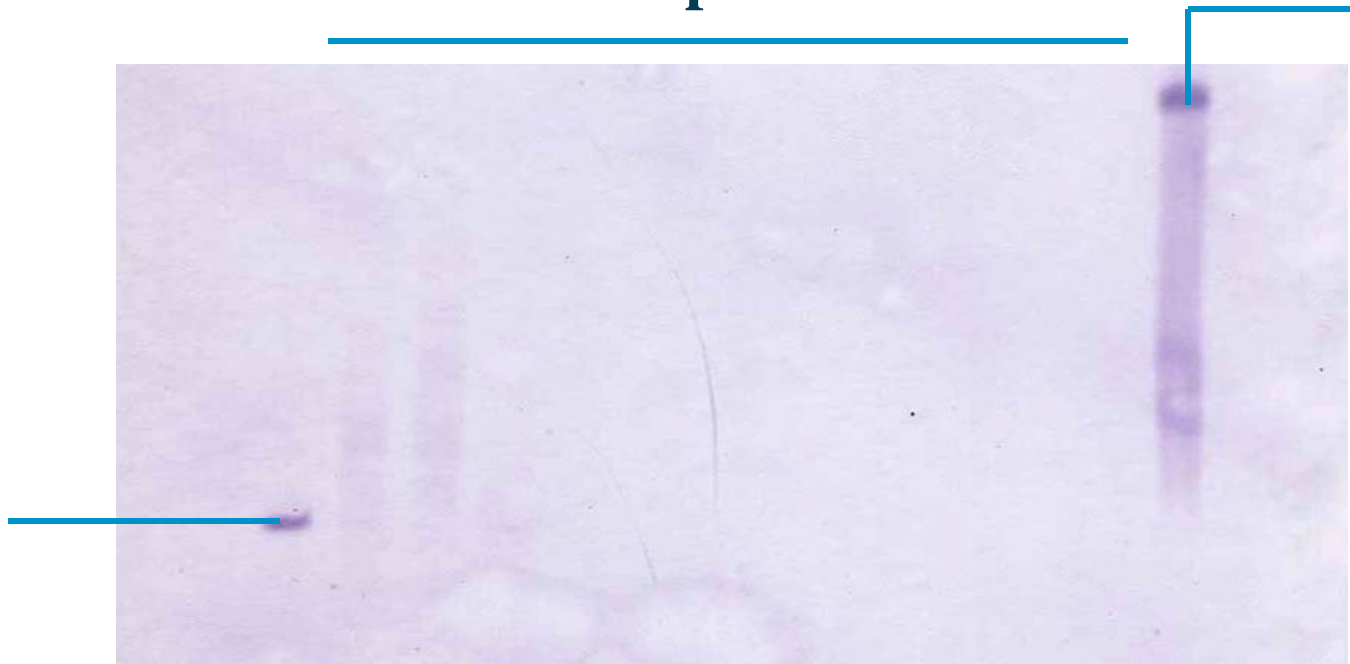
Pulsed–Field Gel Electrophoresis to determine the clonal relationship among S. Typhi strains

Heterogeneous Clones

MDR

All sensitive

MDR

All sensitive

Ribotyping analysis of S. Typhi strains

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

Organism	Country	R factor	MIC (μg/ml)		Amino acid substitiution		
			Nal	Cip	gyrA		parC
					Ser-83	Asp-87	Ser-80
S. Typhi	Bangladesh	All sensitive	2	0.008	-	-	-
S. Typhi	Bangladesh	MDR	>256	0.5	Phe	-	-
S. Typhi	Bangladesh	MDR	>256	0.5	Phe	-	-
S. Typhi	Bangladesh	MDR	>256	6	Phe	Asn	Ile
S. Typhi	Bangladesh	Nal [R]	>256	6	Phe	Gly	Ile
S. Typhi	Bangladesh	Nal [R]	>256	16	Phe	Asn	Ile
S. Para Typhi A	Bangladesh	MDR	4	0.012	-		-
S. Para Typhi A	Bangladesh	MDR	>256	0.5	Phe		-

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 *gyr*A (Ser83 → Phe) and/or (Asp87 → Asn or Gly) and a single mutation in *par*C (Ser80 → Ile)

➢ Of *Salmonella* strains, 4% strains were found as ESBL producers.

➢ The prevalence of ESBL producers was very high in *Salmonella* Group B (2%) and *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

I.   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
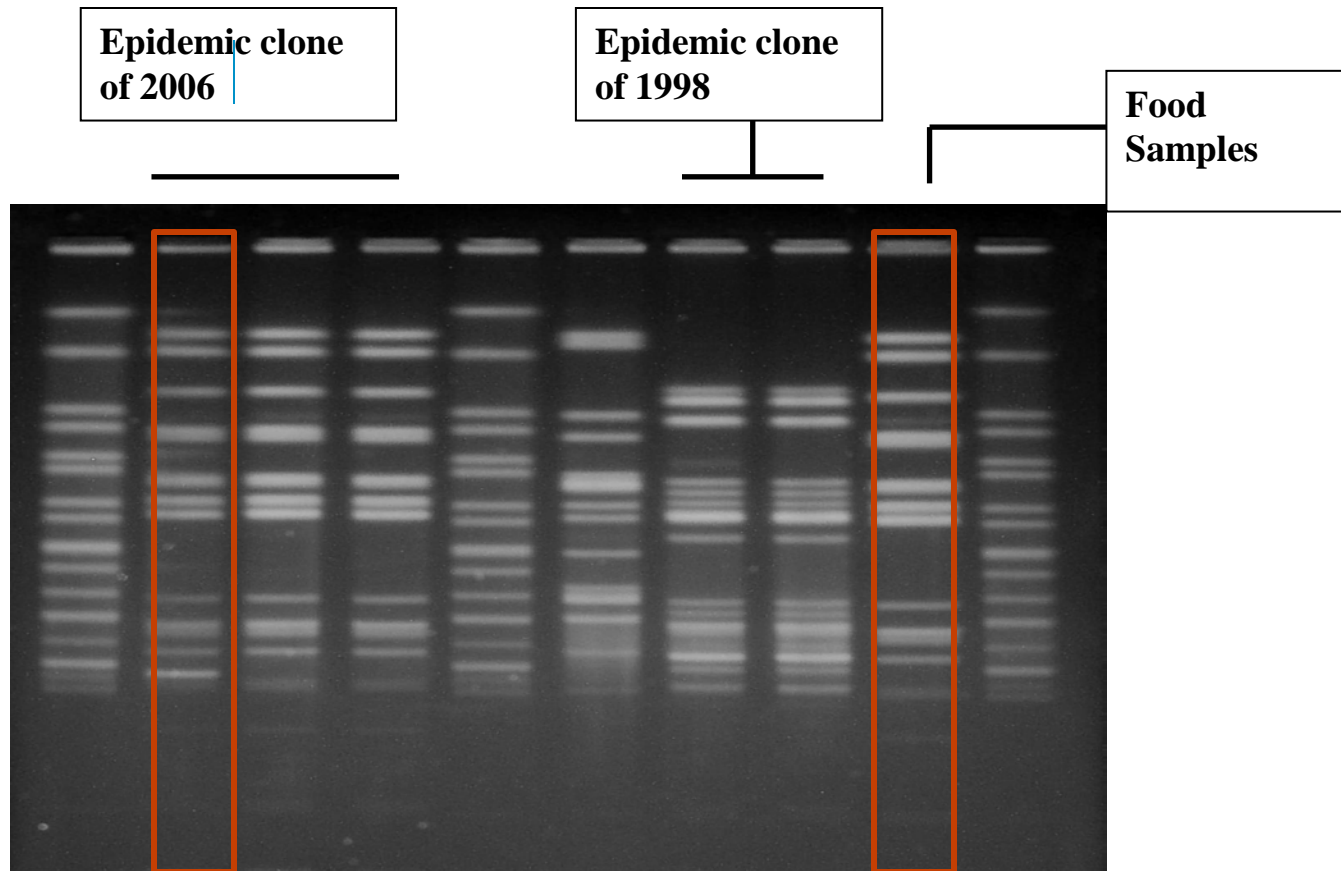
Findings:

➢ 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.
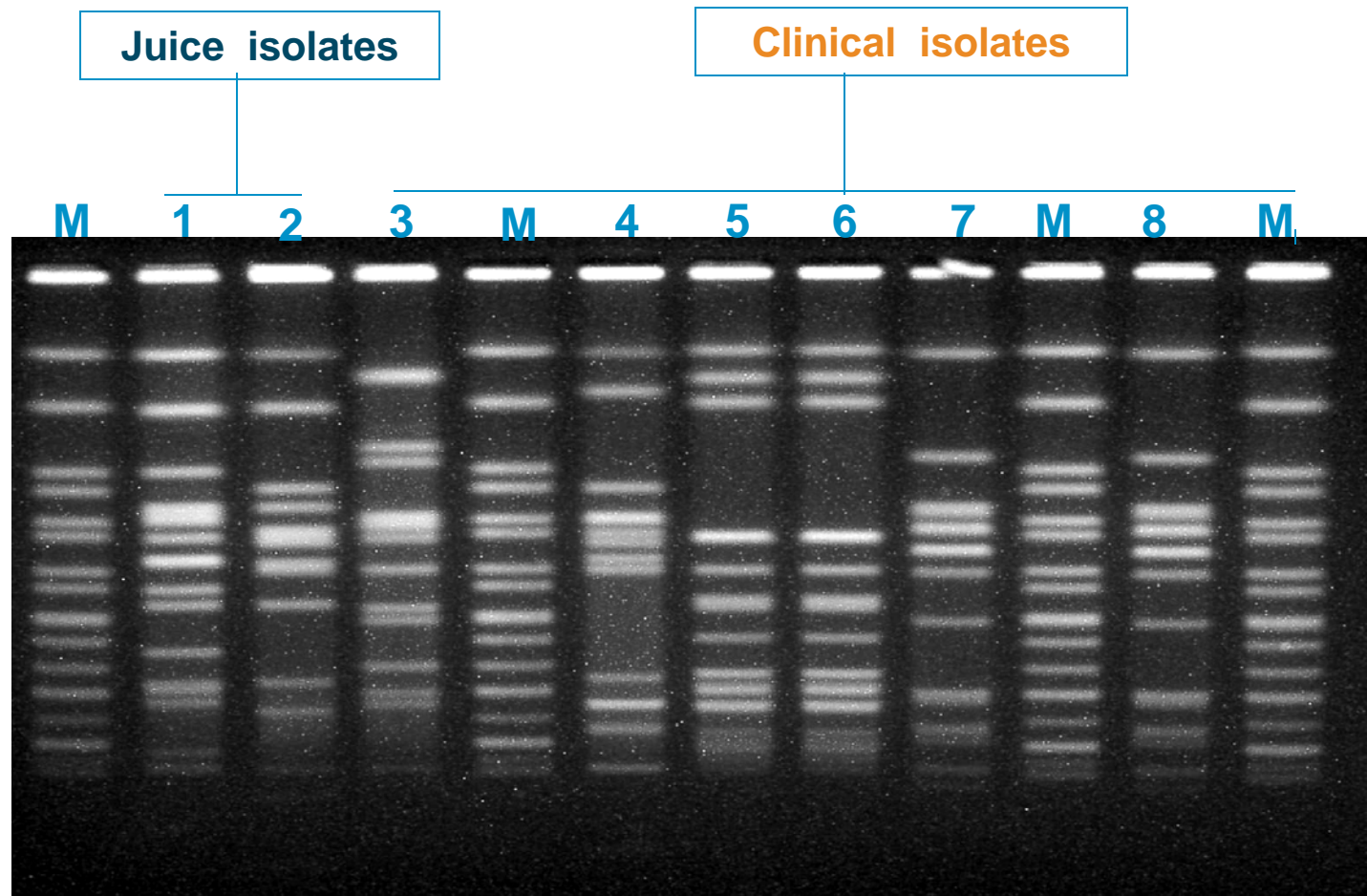
PFGE banding pattern of *Xba*I- digested chromosomal DNA of representative strains of *Salmonella* Paratyphi B
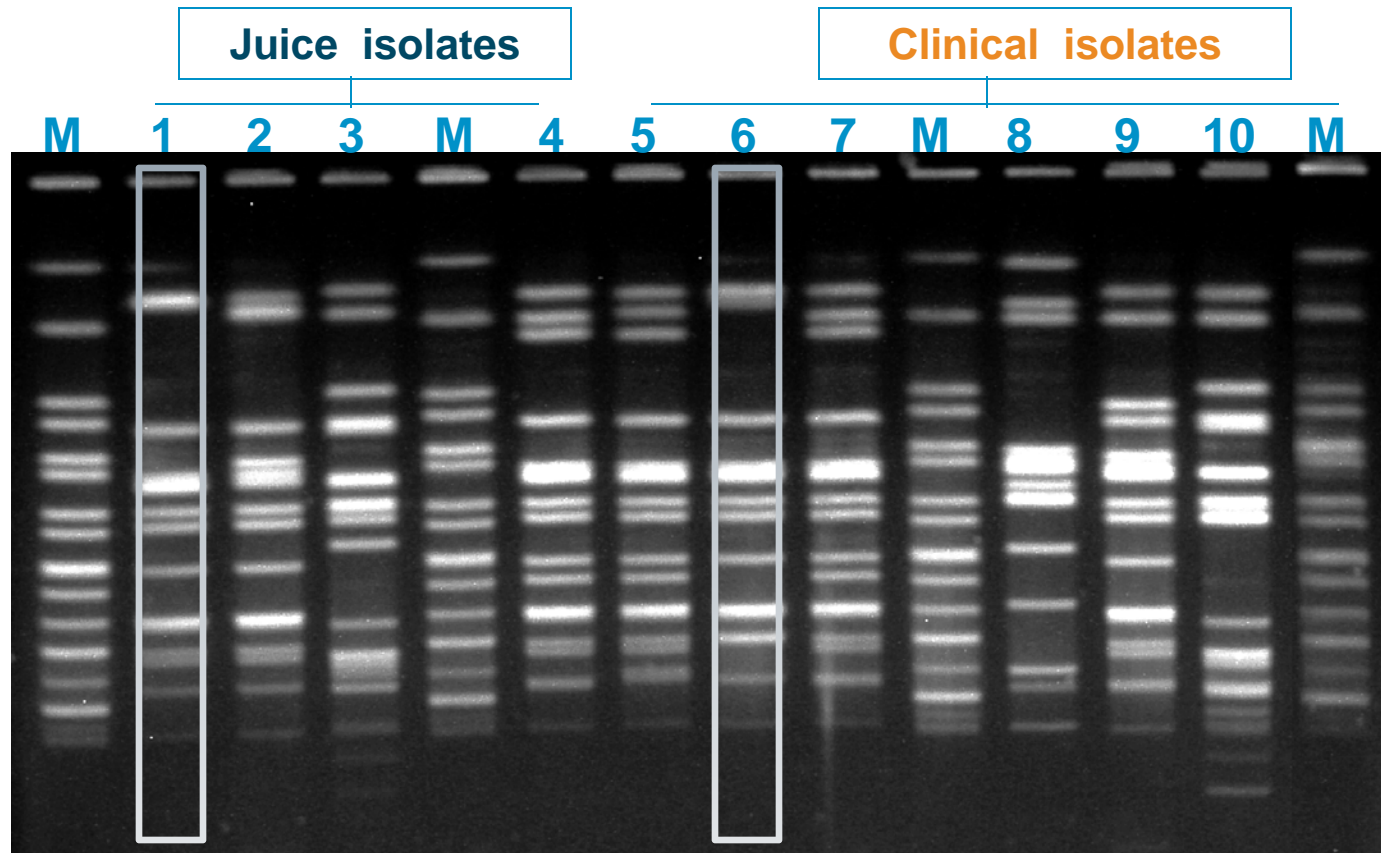
PFGE banding pattern of *XbaI*-digested chromosomal DNA of representative strains of *Salmonella* Group C1
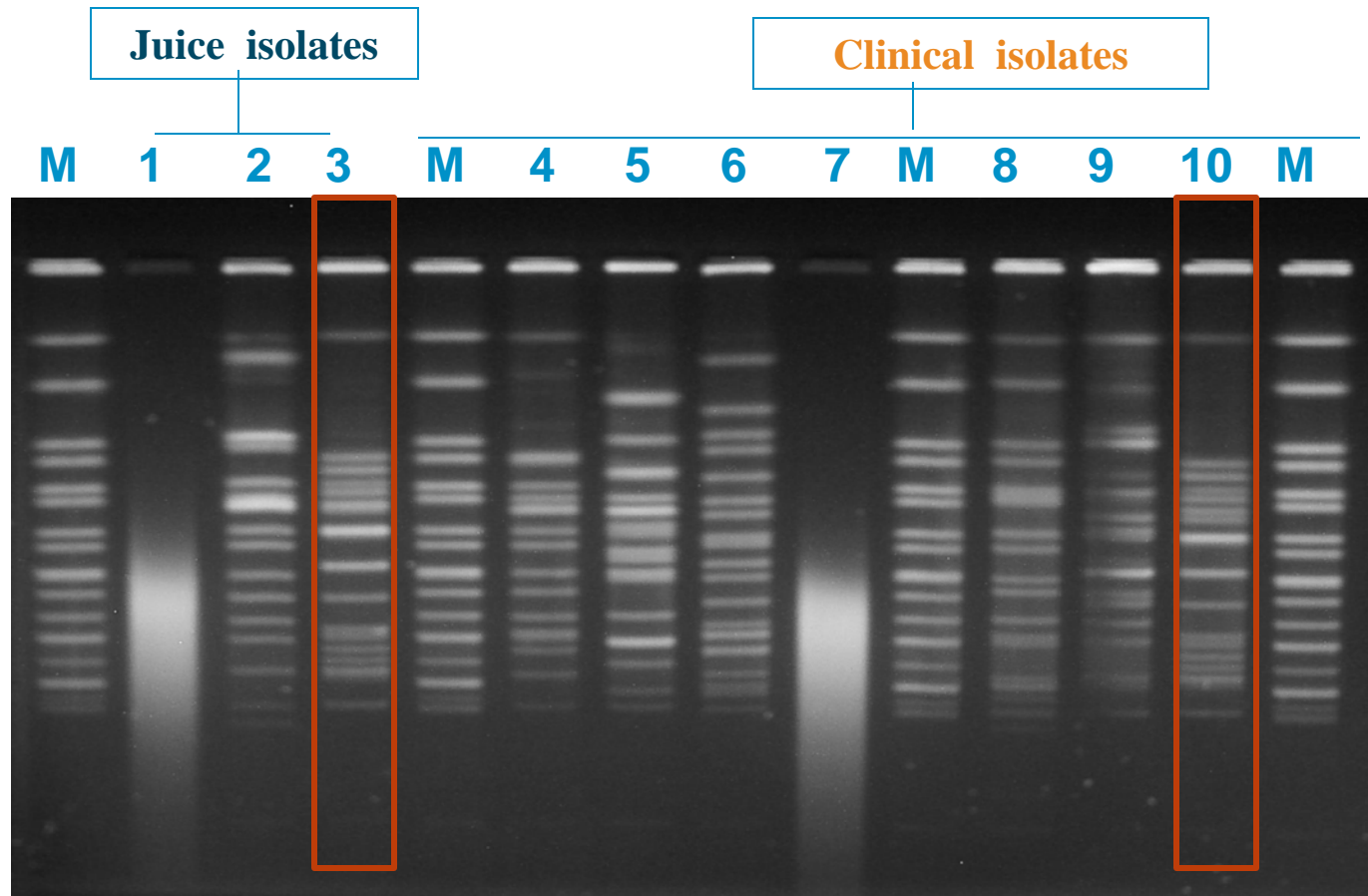
PFGE banding pattern of *XbaI*-digested chromosomal DNA of representative strains of *Salmonella* Group C2

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 precautious 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.

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