Molecular characterisation of a multiresistant strain of *Salmonella enterica* serotype Typhimurium DT204b responsible for an international outbreak of salmonellosis

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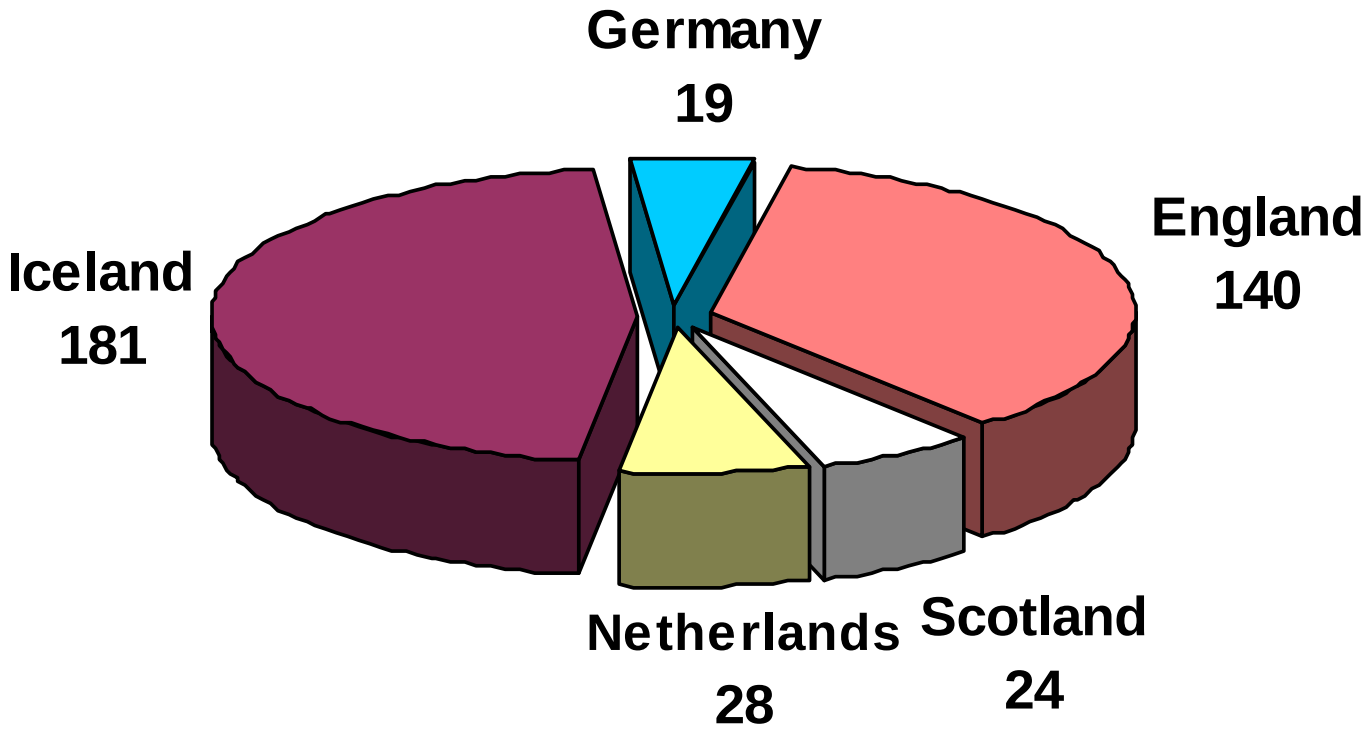
European Outbreak of S*almonella* Typhimurium DT204b

S. Typhimurium DT204b

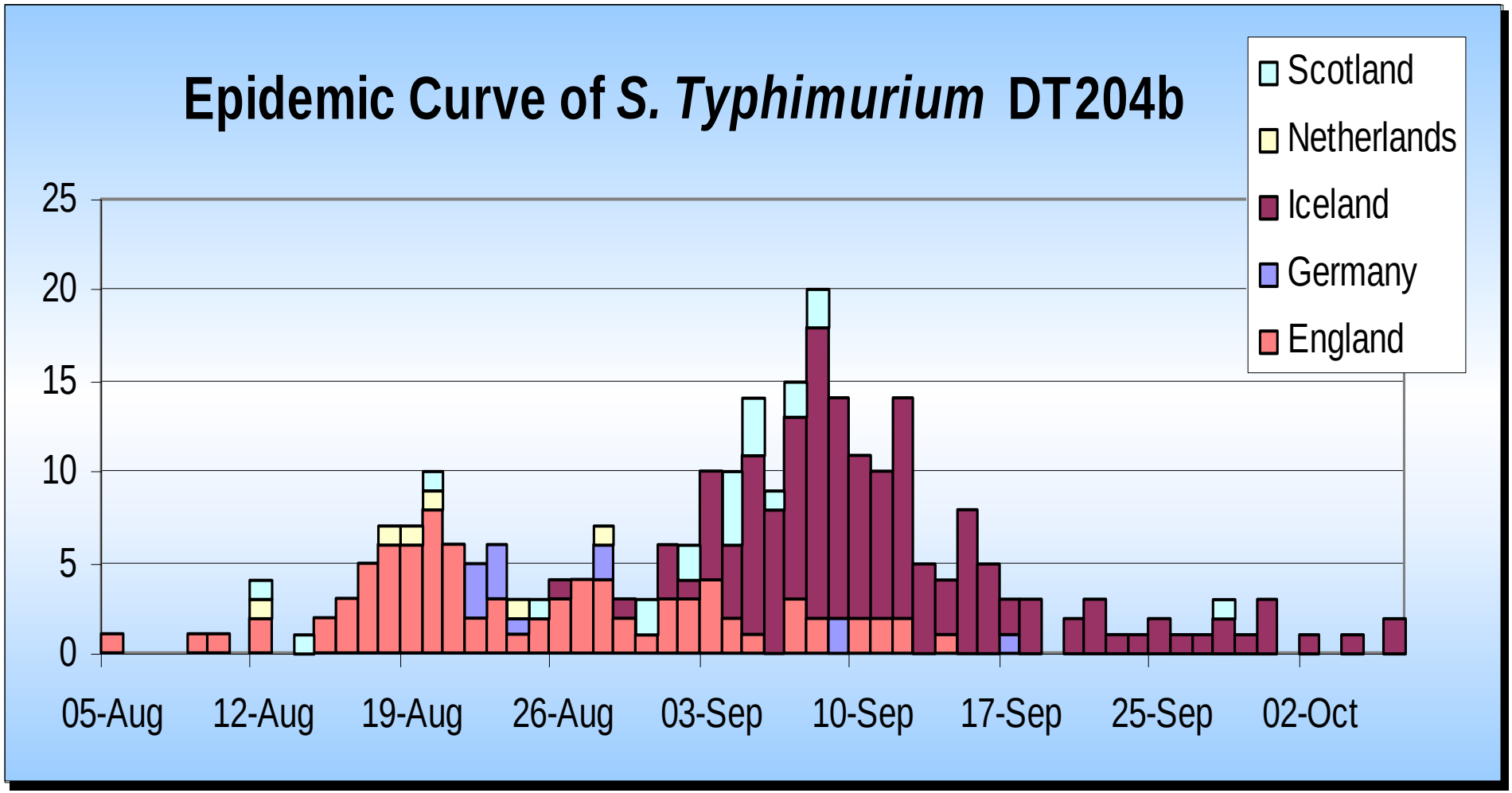
- **17 September 2000: Iceland report an outbreak of multi-resistant *S.* Typhimurium - ? DT104 ?**
- **18 September: 17 strains transferred to the LEP**
- **26 September: Confirmed as same strain as current E&W outbreak of *S.* Typhimurium 204b**
- **Resistance pattern: ACGKSSuSpTTmNxCp$_L$**
- **26 September: Enter-net message between Iceland and England**
- **28 September: European outbreak reported in *Eurosurveillance Weekly***

Descriptive Epidemiology

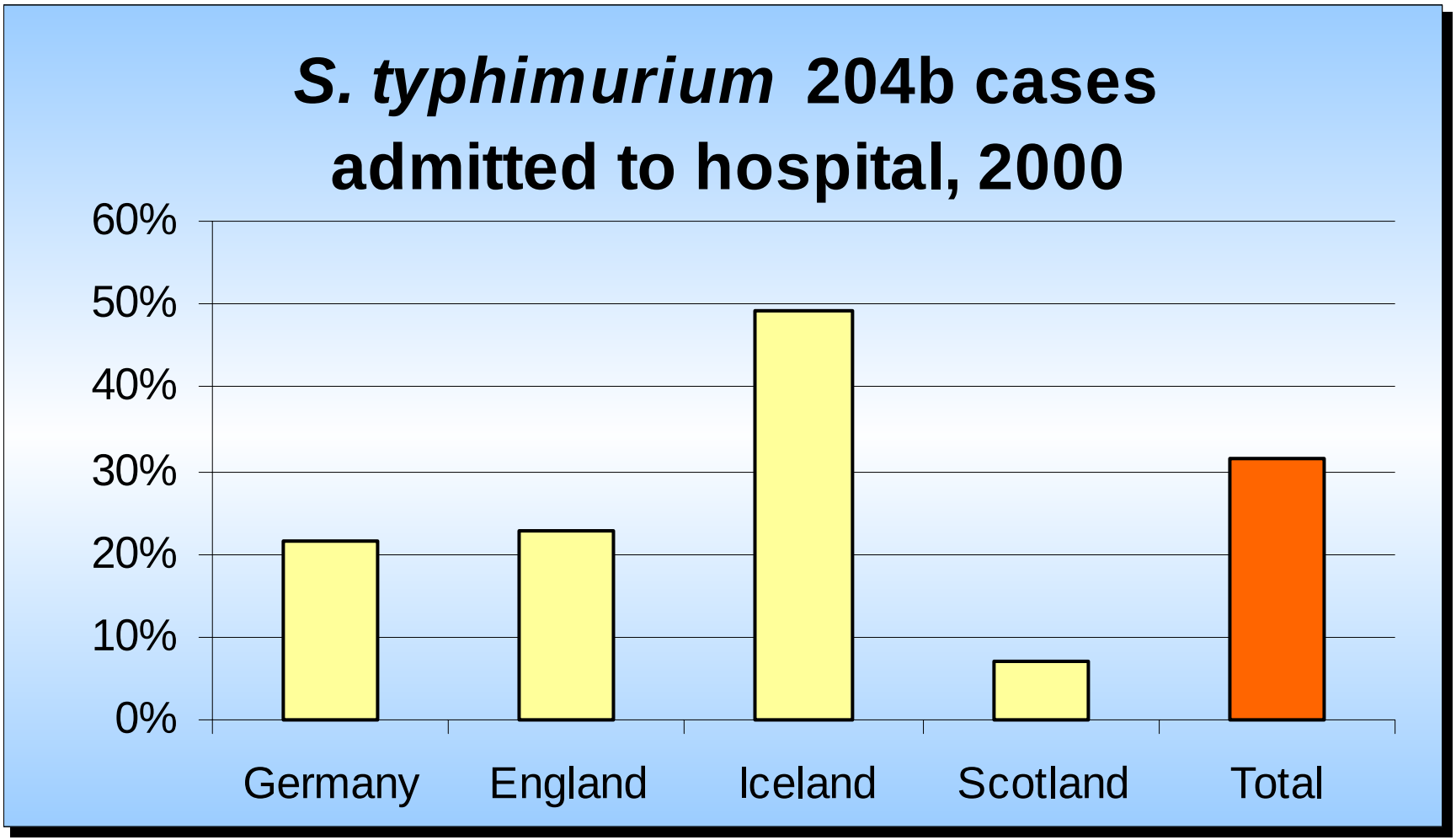
Case distribution by country: n=392



S. Typhimurium DT204b - Epidemic Curve



Frequency of hospitalisation



Epidemiological Investigations

- Lettuce implicated in Iceland
  - Case-control study (OR 40.8)
- Fast food in Germany
- Descriptive epidemiology in the UK implicated lettuce
- Probable cause - an internationally distributed foodstuff circulating in Europe

S. Typhimurium DT204b

SUMMER 2000

R-TYPE: ACGKSSuSpTTmNxCp$_L$

COUNTRIES

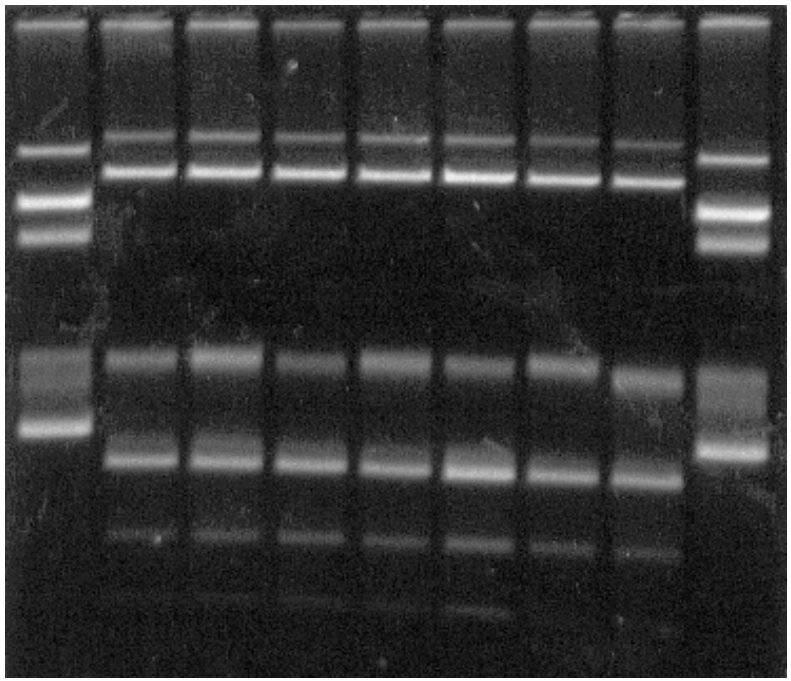
ENGLAND
SCOTLAND
ICELAND
GERMANY
NETHERLANDS

S. Typhimurium DT204b

LABORATORY INVESTIGATIONS

- PHAGE TYPING
- ANTIBIOGRAM ANALYSIS
- DRUG RESISTANCE TRANSFER
- Gyr A MUTATION ANALYSIS (GAMA)
- RESISTANCE GENE PROFILING
- PLASMID PROFILE ANALYSIS (PPT)
- PULSED FIELD GEL ELECTROPHORESIS (PFGE)
- FLUORESCENT AMPLIFIED FRAGMENT LENGTH POLYMORPHISM FINGERPRINTING (FAFLP)

MR *S.* TYPHIMURIUM DT 204b: PLASMID ANALYSIS



Lanes 1& 9: 39R861, control

Lane 2: UK

Lane 3: Scotland

Lane 4: Iceland

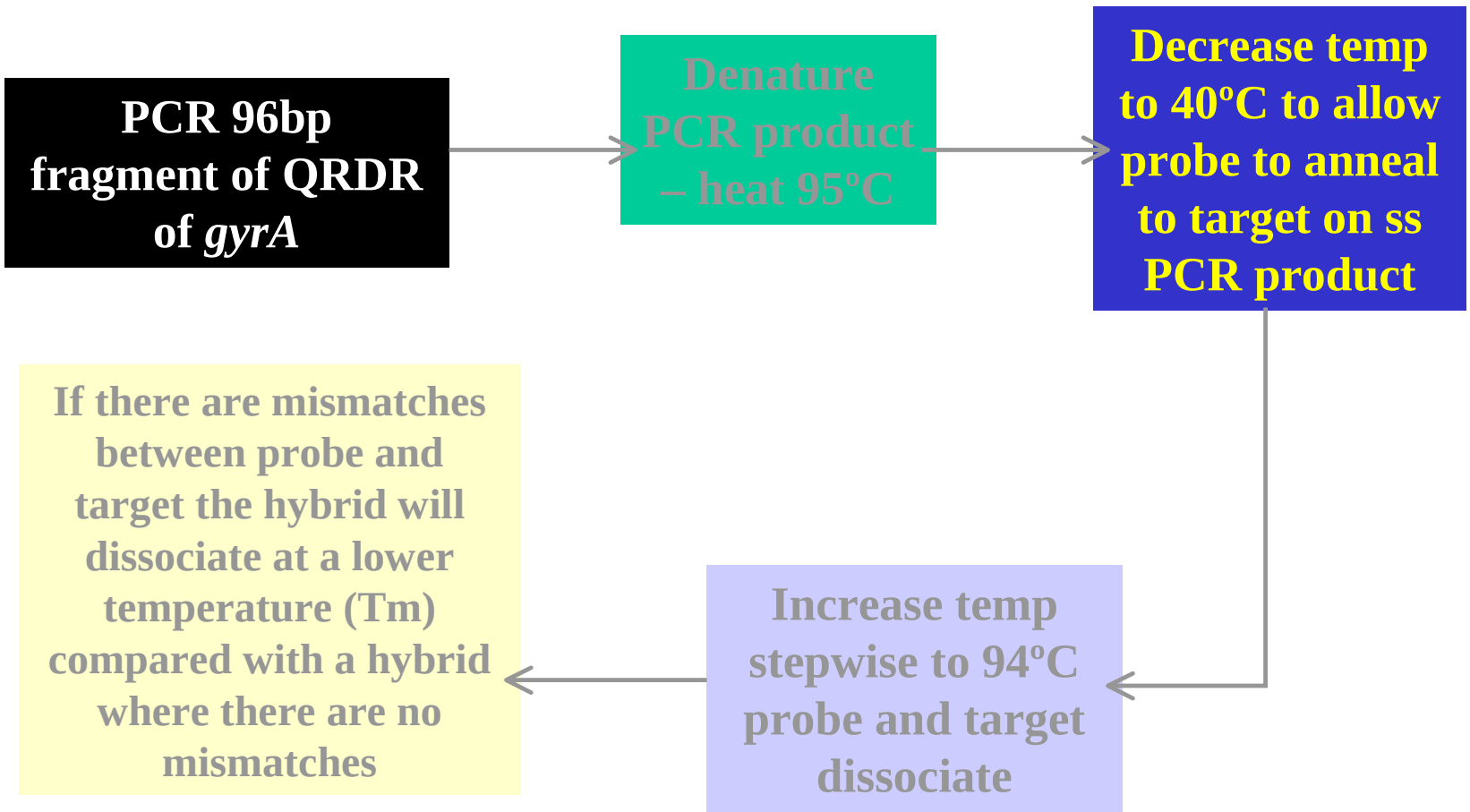
Lane 5: Netherlands

Lane 6: Visit to Greece

Lane 7: Visit to Germany

Lane 8: Visit to Netherlands

Summary of GAMA



Ciprofloxacin-resistant MR DT204b: European Outbreak, 2000

OUTBREAK: EUROPEAN: 2000

England, Scotland, Germany, Iceland, The Netherlands

STRAIN: *S.* Typhimurium DT 204b

R-type ACGKSSuSpTTmNxCp$_L$

***Gyr A* Mutation: Asp 87 → Asn**

PRIMER SEQUENCES FOR PCR AMPLIFICATION

Antiimicrobial	Gene/Primer	Sequence	Step 1	Denaturation	Step 2 Annealing	Extension	Step 3
Streptomycin/ Spectinomycin	*aadA2*	Forward: 5' TGTTGGTTACTGTGGCCGTA 3' Reverse: 5' GATCTCGCCTTTCACAAAGC 3'	94°C (3 m)	94°C (1m)	60°C (1m)	72°C (1min)	72°C (10 m)
Ampicillin	$bla_{\text{CARB-2}}$	Forward: 5' GCTTCGCAACTATGACTAC 3' Reverse: 5' GTTCACCATCCAAGACTC 3'	94°C (5 m)	94°C (25 s)	52°C (40 s)	72°C (50 s)	72°C (6 m)
Ampicillin	bla_{TEM}	Forward: 5' CATTTTCGTGTCGCCCTTAT 3' Reverse: 5' TCCATAGTTGCCTGACTCCC 3'	94°C (3 m)	94°C (1 m)	55°C (1 m)	72°C (1 m)	72°C (10 m)
Sulphonamides	*sul1*	Forward: 5' TCACCGAGGACTCCTTCTTC 3' Reverse: 5' AATATCGGGATAGAGCGCAG 3'	94°C (3 m)	94°C (1 m)	60°C (1 m)	72°C (1 m)	72°C (10 m)
Tetracyclines	*tet*A (classA)	Forward: 5' GCTACATCCTGCTTGCCTTC 3' Reverse: 5' CATAGATCGCCGTGAAGAGG 3'	94°C (3 m)	94°C (1 m)	60°C (1 m)	72°C (1 m)	72°C (10 m)
Tetracyclines	*tet*A (classG)	Forward: 5' CCGGTCTTATGGGTGCTCTA 3' Reverse: 5' CCAGAAGAACGAAGCCAGTC 3'	94°C (3 m)	94°C (1 m)	59°C (1 m)	72°C (1 m)	72°C (10 m)
Tetracyclines	*tet*A (class B)	Forward: 5' TTGGTTAGGGGCAAGTTTTG 3' Reverse: 5' GTAATGGGCCAATAACACCG 3'	94°C (3 m)	94°C (1 m)	60°C (1 m)	72°C (1 m)	72°C (10 m)
	Integron R1/L1	Forward: 5' AAGCAGACTTGACCTGA 3' Reverse: 5' GGCATCCAAGCAGCAAG 3'	94°C (5 m)	94°C (30 s)	63°C (1 m)	72°C (1 m)	

Resistance genes for *Salmonella* Typhimurium DT204b

Tetracyclines: *tet*A class A (-ve for *tet*B and *tet*A class G)

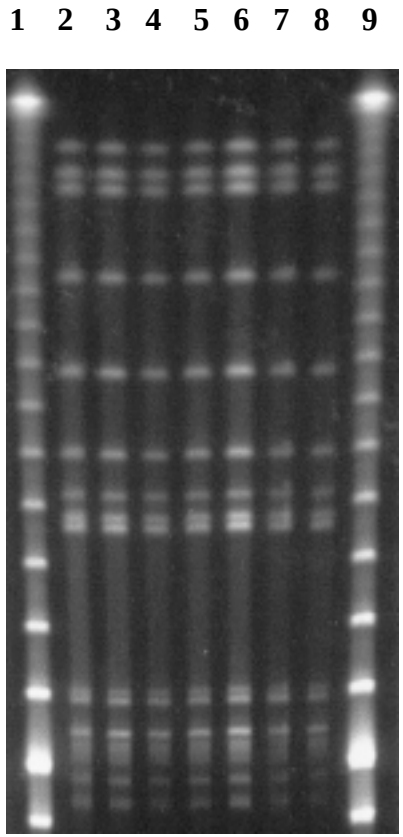
Streptomycin/spectinomycin: *aad*A2 (*ant*3"1a)

Sulphonamides: *sul*-1

Beta-lactams: *tem*-1 (-ve for *pse*-1)

Integrons: 1.6 kb (4 kb)

PFGE analysis of *Xba*I-digested genomic DNA from *S.* Typhimurium DT204b strains



Lanes 1& 9: 48.5 kb ladder;

Lane 2: UK

Lane 3: Scotland

Lane 4: Iceland

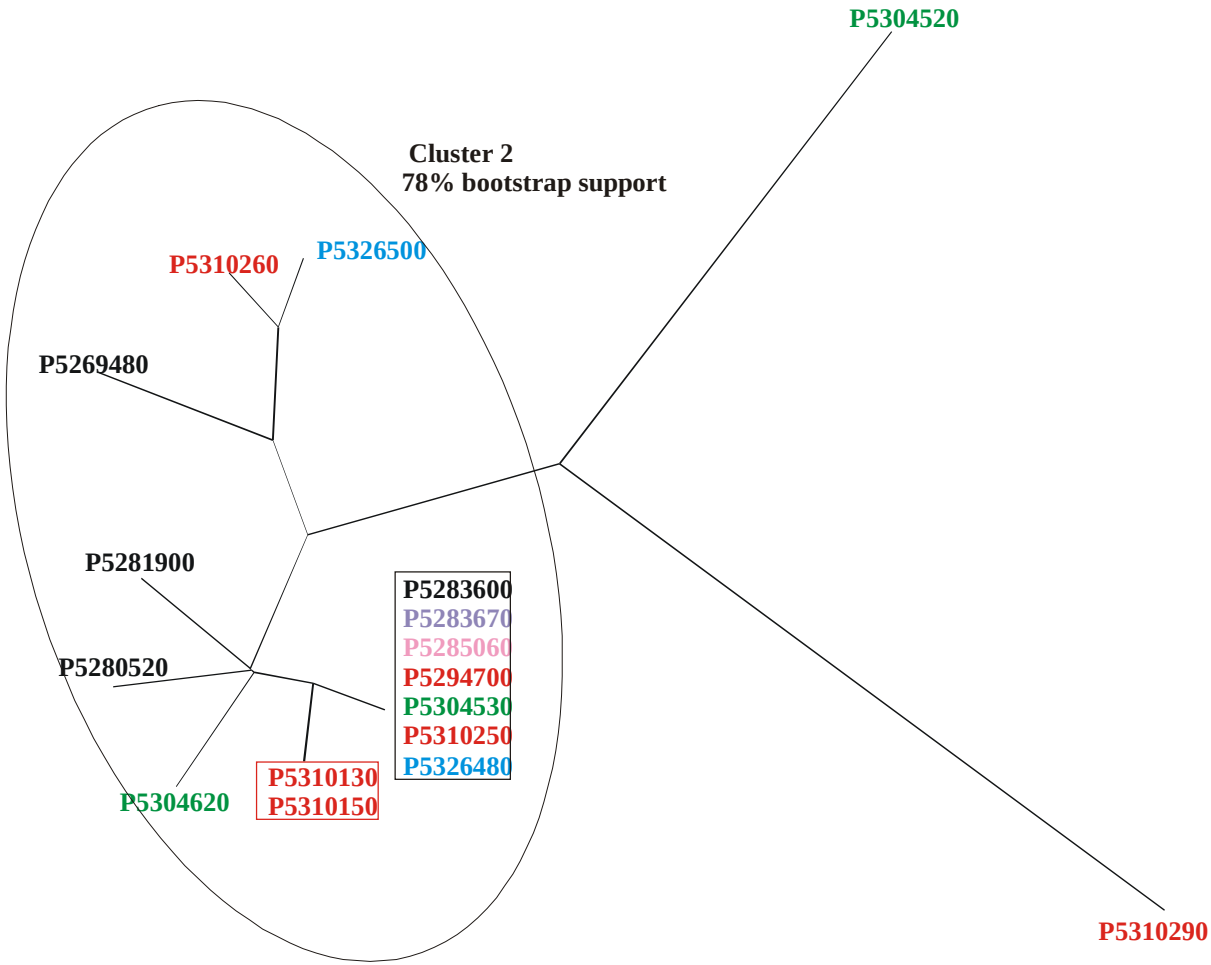
Lane 5: Netherlands

Lane 6: Visit to Greece

Lane 7: Visit to Germany

Lane 8: Visit to Netherlands

S. Typhimurium DT 204b - FAFLP



S. Typhimurium DT 204b outbreak

- PFGE and plasmid fingerprints were exchanged between England, Germany and Scotland in the tag image file format (TIFFs).
- Confirmation of molecular identity of widely-distributed outbreak strain
- Allowed a more rapid exchange of information regarding the outbreak.

Benefits

- Improved outbreak recognition
- More effective outbreak investigation
- Strengthening of national surveillance
- Reduction in risks from international food trade
- Interactive network of public health professionals

Summary

- Electronic communications have made international networks possible.
- Rapid transfer of data and information allows early recognition of international outbreaks.
- Trend information can quickly be analysed to provide an assessment of intervention measures.
- Exchange of TIFFs of PFGE and plasmid profiles can aid outbreak recognition without exchange of strains between laboratories

The International network for the surveillance of Enteric Infections - Salmonella and VTEC O157

Funded by the European Commission, DG Health and Consumer Protection

(previously by DG 12 under Framework 4)

References

- Fisher IST (on behalf of the Enter-net participants) The Enter-net international surveillance network – how it works. *Eurosurv* 1999; 4: 52-55.

- Lindsay EA, Lawson AJ, Walker RA, Ward LR, Smith HR, Scott FW, O'Brien SJ, Fisher IST, Crook PD, Wilson D, Brown DJ, Hardardottir H, Wannet WJB, Tschäpe H, Threlfall EJ, Molecular characterisation of a multiresistant strain of *Salmonella enterica* serotype Typhimurium DT204b responsible for an international outbreak of salmonellosis. *Emerg Infect Dis* 2002 (in press)