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 *Salmonella 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: ACGKSSuSpTTmNxCpL

- 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

- Iceland: 181 cases
- Germany: 19 cases
- Netherlands: 28 cases
- Scotland: 24 cases
- England: 140 cases
Epidemic Curve of *S. Typhimurium* DT204b

**Epidemic Curve of S. Typhimurium DT204b**

- Scotland
- Netherlands
- Iceland
- Germany
- England

**Timeline:**
- 05-Aug
- 12-Aug
- 19-Aug
- 26-Aug
- 03-Sep
- 10-Sep
- 17-Sep
- 25-Sep
- 02-Oct
Frequency of hospitalisation

**S. typhimurium** 204b cases admitted to hospital, 2000

- Germany
- England
- Iceland
- Scotland
- Total

- 0%
- 10%
- 20%
- 30%
- 40%
- 50%
- 60%
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: ACGKSSuSpTTmNxCpL

COUNTRIES

ENGLAND
SCOTLAND
ICELAND
GERMANY
NETHERLANDS
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

PCR 96bp fragment of QRDR of gyrA

If there are mismatches between probe and target the hybrid will dissociate at a lower temperature (Tm) compared with a hybrid where there are no mismatches

Denature PCR product – heat 95°C

Decrease temp to 40°C to allow probe to anneal to target on ss PCR product

Increase temp stepwise to 94°C probe and target dissociate
Ciprofloxacin-resistant MR DT204b: European Outbreak, 2000

OUTBREAK: EUROPEAN: 2000

England, Scotland, Germany, Iceland, The Netherlands

STRAIN: S. Typhimurium DT 204b

R-type ACGKSSuSpTTmNxCpL

Gyr A Mutation: Asp 87 → Asn
# PRIMER SEQUENCES FOR PCR AMPLIFICATION

<table>
<thead>
<tr>
<th>Antiimicrobial</th>
<th>Gene/Primer</th>
<th>Sequence</th>
<th>Step 1</th>
<th>Denaturation</th>
<th>Step 2</th>
<th>Annealing</th>
<th>Extension</th>
<th>Step 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptomycin/Spectinomycin</td>
<td>aadA2</td>
<td>Forward: 5’TGTGGTACTGTCGCTTA 3’ Reverse: 5’GATCTCGCCCTTCACAAAGC 3’</td>
<td>94°C (3 m)</td>
<td>94°C (1 m)</td>
<td>60°C (1 m)</td>
<td>72°C (1 min)</td>
<td>72°C (10 m)</td>
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<tr>
<td>Ampicillin</td>
<td>blaCARB-2</td>
<td>Forward: 5’GCTTGCACATGACTAC 3’ Reverse: 5’GTCACCATCAAGACTC 3’</td>
<td>94°C (5 m)</td>
<td>94°C (25 s)</td>
<td>52°C (40 s)</td>
<td>72°C (50 s)</td>
<td>72°C (6 m)</td>
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<tr>
<td>Ampicillin</td>
<td>blaTEM</td>
<td>Forward: 5’CATTTTCGTCTGCTTTAT 3’ Reverse: 5’TCCATAGTTGCGACTCCC 3’</td>
<td>94°C (3 m)</td>
<td>94°C (1 m)</td>
<td>55°C (1 m)</td>
<td>72°C (1 m)</td>
<td>72°C (10 m)</td>
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<tr>
<td>Sulphonamides</td>
<td>sul1</td>
<td>Forward: 5’TCACCGAGACTCTCTCTTC 3’ Reverse: 5’AATATCGGGATAGGCGCAG 3’</td>
<td>94°C (3 m)</td>
<td>94°C (1 m)</td>
<td>60°C (1 m)</td>
<td>72°C (1 m)</td>
<td>72°C (10 m)</td>
<td></td>
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<tr>
<td>Tetracyclines</td>
<td>tetA (class A)</td>
<td>Forward: 5’GCTACATCTGCTTGCTTCTTCTC 3’ Reverse: 5’AATATCGGGATAGGCGCAG 3’</td>
<td>94°C (3 m)</td>
<td>94°C (1 m)</td>
<td>60°C (1 m)</td>
<td>72°C (1 m)</td>
<td>72°C (10 m)</td>
<td></td>
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<tr>
<td>Tetracyclines</td>
<td>tetA (class G)</td>
<td>Forward: 5’CCGCTTATGCGTTCTA 3’ Reverse: 5’CCAGAAGAGCAAGACCAGTC 3’</td>
<td>94°C (3 m)</td>
<td>94°C (1 m)</td>
<td>59°C (1 m)</td>
<td>72°C (1 m)</td>
<td>72°C (10 m)</td>
<td></td>
</tr>
<tr>
<td>Tetracyclines</td>
<td>tetA (class B)</td>
<td>Forward: 5’TGGTGTTAGGGAAGGTGGTGTTT 3’ Reverse: 5’GTAATGGCGGCAATAACACCG 3’</td>
<td>94°C (3 m)</td>
<td>94°C (1 m)</td>
<td>60°C (1 m)</td>
<td>72°C (1 m)</td>
<td>72°C (10 m)</td>
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<td>Integron R1/L1</td>
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<td>Forward: 5’AAGCAGACTTGACCTGA 3’ Reverse: 5’GGCATCAGAAGCAGCAAG 3’</td>
<td>94°C (5 m)</td>
<td>94°C (30 s)</td>
<td>63°C (1 m)</td>
<td>72°C (1 m)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Notes:
- **aadA2** FORWARD: 5’TGTGGTACTGTCGCTTA 3’ REVERSE: 5’GATCTCGCCCTTCACAAAGC 3’
- **blaCARB-2** FORWARD: 5’GCTTGCACATGACTAC 3’ REVERSE: 5’GTCACCATCAAGACTC 3’
- **blaTEM** FORWARD: 5’CATTTTCGTCTGCTTTAT 3’ REVERSE: 5’TCCATAGTTGCGACTCCC 3’
- **sul1** FORWARD: 5’TCACCGAGACTCTCTCTTC 3’ REVERSE: 5’AATATCGGGATAGGCGCAG 3’
- **tetA (class A)** FORWARD: 5’GCTACATCTGCTTGCTTCTTCTC 3’ REVERSE: 5’AATATCGGGATAGGCGCAG 3’
- **tetA (class G)** FORWARD: 5’CCGCTTATGCGTTCTA 3’ REVERSE: 5’CCAGAAGAGCAAGACCAGTC 3’
- **tetA (class B)** FORWARD: 5’TGGTGTTAGGGAAGGTGGTGTTT 3’ REVERSE: 5’GTAATGGCGGCAATAACACCG 3’
- **Integron R1/L1** FORWARD: 5’AAGCAGACTTGACCTGA 3’ REVERSE: 5’GGCATCAGAAGCAGCAAG 3’
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 \textit{XbaI}-digested genomic DNA from \textit{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 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

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References

• Fisher IST (on behalf of the Enter-net participants)