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: ACNKSSuSpTTmNxCp
- 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
- Germany: 19
- Netherlands: 28
- Scotland: 24
- England: 140
S. Typhimurium DT204b - Epidemic Curve

Epidemic Curve of S. Typhimurium DT204b

Scotland
Netherlands
Iceland
Germany
England
Frequency of hospitalisation

S. typhimurium 204b cases admitted to hospital, 2000

<table>
<thead>
<tr>
<th>Country</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germany</td>
<td>20%</td>
</tr>
<tr>
<td>England</td>
<td>20%</td>
</tr>
<tr>
<td>Iceland</td>
<td>50%</td>
</tr>
<tr>
<td>Scotland</td>
<td>10%</td>
</tr>
<tr>
<td>Total</td>
<td>30%</td>
</tr>
</tbody>
</table>
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
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><em>aadA2</em></td>
<td>Forward: 5’TGTGGTTACTGTGGCCGTA 3’ Reverse: 5’GATCTCGCCTTTCAACAAGC 3’</td>
<td>94°C (3 m)</td>
<td>94°C (1 m)</td>
<td>60°C (1 m)</td>
<td>72°C (1min)</td>
<td>72°C (10 m)</td>
<td></td>
</tr>
<tr>
<td>Ampicillin</td>
<td><em>bla</em>&lt;sub&gt;CARB-2&lt;/sub&gt;</td>
<td>Forward: 5’GCTTCGAAACTATGACTAC 3’ Reverse: 5’GTTCCACATCAAAGACTC 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>
<td></td>
</tr>
<tr>
<td>Ampicillin</td>
<td><em>bla</em>&lt;sub&gt;TEM&lt;/sub&gt;</td>
<td>Forward: 5’CATTTCGTGTCGCCCTAT 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>
<td></td>
</tr>
<tr>
<td>Sulphonamides</td>
<td><em>sul1</em></td>
<td>Forward: 5’TCACCAGAGACTCTTTTCTC 3’ Reverse: 5’AATATCGGGATAGGCAGCAG 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>
</tr>
<tr>
<td>Tetracyclines</td>
<td><em>tetA</em> (class A)</td>
<td>Forward: 5’GCTACACCTGTGCTTTCTCTC 3’ Reverse: 5’CATAGATCGCGTGAAGCGC 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>
</tr>
<tr>
<td>Tetracyclines</td>
<td><em>tetA</em> (class G)</td>
<td>Forward: 5’CCGCTCTTTATGGGCTCTTA 3’ Reverse: 5’CCAGAAGAAGCGAGCCAGTC 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><em>tetA</em> (class B)</td>
<td>Forward: 5’TTGGTAGGGCGAAGTTGTTG 3’ Reverse: 5’GTAATGGGCAAATAACACGC 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>
</tr>
<tr>
<td>Integron R1/L1</td>
<td></td>
<td>Forward: 5’AAGCAGACTTGACCTGA 3’ Reverse: 5’GGCATCCAAGCAGCAAG 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>
Resistance genes for *Salmonella Typhimurium* DT204b

Tetracyclines: *tetA* class A (-ve for *tetB* and *tetA* class G)

Streptomycin/spectinomycin: *aadA2* (*ant3”1a*)

Sulphonamides: *sul-1*

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

Integrons: 1.6 kb (4 kb)
PFGE analysis of *XbaI*-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 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
