Surveillance and outbreak investigation of Shiga toxin-producing *Escherichia coli* using whole genome sequencing - time for a change!

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Overview

- Role of Gastrointestinal Bacteria Reference Unit
- Current methodology of non-O157 STEC detection and surveillance
- New high-throughput WGS work stream
- Impact on public health
- Overview of comparison data 2014
- Understanding trends in epidemiology
- Improved surveillance and outbreak detection
- Changing the way we view data
- Conclusions
Gastrointestinal Bacteria Reference Unit

- National reference service for gastrointestinal bacterial pathogens
- Specialist diagnostic tests for rare infections/intoxications
- National surveillance, outbreak detection and investigation
- Research and development
- Expert advice, training and education
- Responsible for setting up EU-wide standards for routine microbiological procedures and testing methods at a national level

Identification, typing
- STEC, *Salmonella*
- *Listeria monocytogenes*
- *Campylobacter*, *Shigella, Yersinia, Vibrio, Clostridia, Bacillus, Helicobacter*,

Specialist diagnostic services
- Botulism
- Serodiagnosics
- Toxin detection
- All diarrhoeagenic *E. coli* pathotypes
Current Identification Methods for STEC

**Gene Detection**
- Real time TaqMan® PCR assays - target four different genes
  - PCR targeting: stx1, stx2, eae (intimin), O157

**Identification**
- Day 3
  - OmniLog® ID System (Biolog) - phenotypic microarray
    - Differentiation from *Shigella* spp. and other Enterobacteriaceae

**Serotyping**
- Day 4-8
  - Agglutination with specific antisera against LPS & flagella (O & H antigens)
    - Slide agglutination
    - Microtitre plates
    - H typing can take up to 14 days

**Day 1**
- PCR targeting: stx1, stx2, eae (intimin), O157
Sub-typing Methods for O157 STEC

Phage Typing
Day 3

- e.g. PT8 and PT21/28 most common in the UK

Multi-locus Variable Number Tandem Repeat Analysis (MLVA)
Day 3-5

Both techniques useful in outbreak detection but not available for non-O157 STEC
Sub-typing Methods for STEC

**Pulsed-field gel electrophoresis (PFGE)**

Day 4-7

*Laborious, technically demanding, validation needed for each serogroup*

**Whole Genome Sequencing**

Day 5-7

*Introduced in 2012 for outbreak analysis, now used routinely on all STEC*

**Block based multiple PCRs** – time consuming and not practical for routine use

**stx subtyping**

Day 2
DNA extraction
Day 1

Library Prep
Day 2-3

Sequencing
Day 4-5

Bioinformatics
Validation
Reporting
Day 6-7

Jon Green – Head Bioinformatics

Validation

Reporting to customer (communication)
Impact on Public Health

Improved Data – Example 2014

Traditional versus WGS data for 2014 – 141 non-O157 strains tested

<table>
<thead>
<tr>
<th>Test</th>
<th>Traditional</th>
<th>WGS</th>
<th>Mismatches</th>
</tr>
</thead>
<tbody>
<tr>
<td>O antigen/rfb</td>
<td>102 identified 26 untypable 13 Rough</td>
<td>138 identified 1 untypable 1 O153/O178 1 O123/O186</td>
<td>10 (7%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H antigen / fliC</td>
<td>69 identified 42 untypable 30 not motile</td>
<td>139 identified 2 untypable</td>
<td>3 (2%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>stx subtyping</td>
<td>141 identified 8 double positives (2 stx) 2 triple positives (2 stx)</td>
<td>141 identified 1 double positive due to recombination (2b/d)</td>
<td>14 (9%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outbreak analysis</td>
<td>Serotyping stx subtype</td>
<td>rfb/fliC stx subtype Core SNP phylogeny SNP address</td>
<td>-</td>
</tr>
</tbody>
</table>
Impact on Public Health
Understanding trends in Epidemiology

Most common Non-O157 STEC – 2014
(142 isolates)

Implementation of PCR at the hospitals has improved isolation of Non-O157 STEC

WGS is helping to understanding the data.

Common groups isolated in 2014 are O146, O26, O55, O103, O91, O117, O128ac & O80

O104 does not appear to be circulating in the community
Impact on Public Health

Understanding trends in Epidemiology

• *stx1b* and *stx2f* not associated with clinical non-O157 isolates
• 38% associated with *stx1* only
• *stx1a* and *stx2b* are most common sub-types
• All *stx1a* (only) are *eae* negative
• Majority (16/17) of O146 (ST442/738) are *eae* negative and not associated with severe disease

Table of strains associated with HUS

7% (10/141) associated with HUS

80% (8/10) are *eae* positive

70% (7/10) are *stx2* only of which 70% (5/7) are *stx2a*
Common ST, 2005-2014
181/1393 Non-O157 STEC isolates

O177:H25
O5:H-  
O2:H27
O113:H17
O38:H26

O76:H19

O91:H14

O103:H2
O71:H2

O145:H28

ST10 (9)
ST32 (7)

ST342 (9)

ST33 (18)

ST675 (10)

ST17 (21)

ST335 (28)

ST21 (48)

ST442 (31)

O26:H11

O146:H21

O55:H7

O145:H28

O177:H25
O5:H-  
O2:H27
O113:H17
O38:H26

O76:H19

O91:H14

O103:H2
O71:H2

O145:H28

ST10 (9)
ST32 (7)

ST342 (9)

ST33 (18)

ST675 (10)

ST17 (21)

ST335 (28)

ST21 (48)

ST442 (31)

O26:H11

O146:H21

O55:H7
Impact on Public Health

Higher resolution in outbreak analysis


Table 1. Molecular and epidemiological data associated with strains of E. coli O26:H11 isolated at GBRU between 2009 and 2013

<table>
<thead>
<tr>
<th>Reference no.</th>
<th>Strain profile</th>
<th>MLST</th>
<th>Date culture isolated</th>
<th>Sex/age</th>
<th>Travel</th>
<th>Additional information*</th>
</tr>
</thead>
<tbody>
<tr>
<td>181/09</td>
<td>1A2</td>
<td>21</td>
<td>Feb. 2009</td>
<td>F/2</td>
<td>No travel</td>
<td>Outbreak 2009</td>
</tr>
<tr>
<td>461/09</td>
<td>1A2</td>
<td>21</td>
<td>Feb. 2009</td>
<td>M/14</td>
<td>No travel</td>
<td>Outbreak 2009</td>
</tr>
<tr>
<td>460/09</td>
<td>1A2</td>
<td>21</td>
<td>Feb. 2009</td>
<td>F/10</td>
<td>No travel</td>
<td>Outbreak 2009</td>
</tr>
<tr>
<td>259/10</td>
<td>1A2</td>
<td>21</td>
<td>Sept. 2010</td>
<td>M/3</td>
<td>No travel</td>
<td>HUS</td>
</tr>
<tr>
<td>567/10</td>
<td>1A2</td>
<td>21</td>
<td>Sept. 2010</td>
<td>F/3</td>
<td>France</td>
<td>HUS</td>
</tr>
<tr>
<td>467/10</td>
<td>2</td>
<td>21</td>
<td>Nov. 2010</td>
<td>M/13</td>
<td>Turkey</td>
<td>HUS</td>
</tr>
<tr>
<td>519/11</td>
<td>1A2</td>
<td>21</td>
<td>July 2011</td>
<td>M/4</td>
<td>No travel</td>
<td></td>
</tr>
<tr>
<td>634/12</td>
<td>2</td>
<td>21</td>
<td>Apr. 2012</td>
<td>F/13</td>
<td>Egypt</td>
<td></td>
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<tr>
<td>165/12</td>
<td>1A2</td>
<td>21</td>
<td>May 2012</td>
<td>F/42</td>
<td>No travel</td>
<td>Fatal case</td>
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<tr>
<td>403/12</td>
<td>2</td>
<td>21</td>
<td>July 2012</td>
<td>M/55</td>
<td>Ireland and Switzerland</td>
<td>Outbreak 2012</td>
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<tr>
<td>412/12</td>
<td>2</td>
<td>21</td>
<td>July 2012</td>
<td>M/4</td>
<td>No travel</td>
<td>Outbreak 2012</td>
</tr>
<tr>
<td>634/12</td>
<td>2</td>
<td>21</td>
<td>July 2012</td>
<td>M/6</td>
<td>No travel</td>
<td>Outbreak 2012</td>
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<tr>
<td>627/12</td>
<td>2</td>
<td>21</td>
<td>July 2012</td>
<td>M/2</td>
<td>No travel</td>
<td>Outbreak 2012</td>
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<tr>
<td>2270-502/12</td>
<td>2</td>
<td>21</td>
<td>July 2012</td>
<td>F/1</td>
<td>Ireland</td>
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<tr>
<td>2290-502/12</td>
<td>2</td>
<td>29</td>
<td>July 2012</td>
<td>M/40</td>
<td>No data</td>
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<tr>
<td>670/13</td>
<td>—</td>
<td>29</td>
<td>Aug. 2013</td>
<td>F/50</td>
<td>Egypt</td>
<td>HUS</td>
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<tr>
<td>680/13</td>
<td>—</td>
<td>29</td>
<td>Sept. 2013</td>
<td>F/3</td>
<td>Italy</td>
<td>Hospitalized with severe bloody diuresis</td>
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<tr>
<td>075/13</td>
<td>2</td>
<td>21</td>
<td>Sept. 2013</td>
<td>M/0</td>
<td>Albania</td>
<td></td>
</tr>
<tr>
<td>637/13</td>
<td>2</td>
<td>21</td>
<td>Sept. 2013</td>
<td>F/7</td>
<td>No data</td>
<td></td>
</tr>
</tbody>
</table>

MLST, Multilocus sequence typing; HUS, haemolytic uraemic syndrome.

* Additional information includes whether or not cases were associated with an outbreak and clinical symptoms.

- Only main local outbreaks are picked up epidemiologically where there is either an increase of HUS or where PCR is available at the hospital
- SNP address will pick up national outbreaks that may not have obvious epidemiological link.
- This still relies on initial PCR detection.
Web-based Gasto database available to Public Health Team

Switching to WGS for routine testing of E. coli

ID by Kmer
MLST Inouye 2012
Serotype Joensen 2015
E.coli virulence genes Doumith
stx subtype Ashton
SnapperDb Dallman

<table>
<thead>
<tr>
<th>Molec ID</th>
<th>NGS LIMS ID</th>
<th>NGS RUN ID</th>
<th>Sequencing Date</th>
<th>Workflow</th>
<th>Whole</th>
<th>Positive Control</th>
<th>NAG</th>
<th>ID</th>
<th>Mixed ST</th>
<th>MLST PROFILE</th>
<th>O H</th>
<th>Virulence genes</th>
<th>stx subtype</th>
<th>SNP Flag</th>
<th>SNP Address</th>
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<tbody>
<tr>
<td>H153360699 147826 AHKLYVADXX 2015-08-26</td>
<td>Escherichia coli EC1848 89.58358</td>
<td>nothing</td>
<td>335</td>
<td>29,12,8,12,15,2,2 065 H7</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>stb2a</td>
<td>71.730.1046.1948.2205.2254.2457</td>
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<tr>
<td>H153360699 147826 AHKGFNADXX 2015-08-24</td>
<td>Escherichia coli EC1848 89.207480</td>
<td>nothing</td>
<td>335</td>
<td>29,12,8,12,15,2,2 065 H7</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>stb2a</td>
<td>71.730.1046.1948.2205.2254.2457</td>
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<tr>
<td>H153020735 130729 Abjoji1xx 2015-07-28</td>
<td>Escherichia coli EC1846 89.079042</td>
<td>nothing</td>
<td>335</td>
<td>29,12,8,12,15,2,2 065 H7</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>stb2a</td>
<td>71.730.1046.1948.2205.2254.2457</td>
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<tr>
<td>H152720005 129383 AHNZ27ADXX 2015-07-13</td>
<td>Escherichia coli EC1848 89.641296</td>
<td>nothing</td>
<td>335</td>
<td>29,12,8,12,15,2,2 065 H7</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>stb2a</td>
<td>71.730.1046.1948.2205.2254.2457</td>
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<tr>
<td>H152720004 129382 AHNZ27ADXX 2015-07-13</td>
<td>Escherichia coli EC1848 89.459157</td>
<td>nothing</td>
<td>335</td>
<td>29,12,8,12,15,2,2 065 H7</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>stb2a</td>
<td>71.730.1046.1948.2205.2254.2457</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
• WGS Reference typing of all *E.coli* will be implemented October 2015
• All typing information will be available to public health team via a web based Gastrointestinal database.
• Cost should be reduced to the NHS customer from £120
• Turnaround time reduced from 14 days to 7 days
• PHE will proactively detect local and national non-O157 outbreaks in real time.
• Additional data gathered will be assessed to understand genetic associations with severe disease.
• Improvement of isolation is still needed, only a fraction of hospitals (<10%) use PCR to detect STEC and isolated is carried out at GBRU.
• Ideally the detection and isolation of non-O157 STEC will be implemented at the regional laboratories for an accurate number of non-O57 STEC infections.

**Conclusion**
Acknowledgements

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Genomic Service Unit

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