The population structure of *E. coli* causing bacteraemia in the UK and Ireland between 2001 and 2010

Dr Michaela Day
Molecular Microbiologist
Antimicrobial Resistance and Healthcare Associated Infections (AMRHAI)
E. coli in bacteraemia

• Year on year increase in Gram-negatives bacteraemias
  • 38% increase in E. coli bacteraemia between 2004-2008
  • 5% increase between 2009-2010
  • 10% increase between 2010-2011

• 2011 new legislation made E. coli bacteraemia surveillance mandatory
Project aim: Elucidate the population structure of *E. coli* causing bacteraemias in the UK and Ireland

- 2168 *E. coli* strains from the British Society for Antimicrobial Chemotherapy (BSAC) bacteraemia resistance surveillance programme
- 2001-2010
- 18 centres across the UK and Ireland
Methods

• Molecular techniques
  – Phylogrouping
  – Multilocus Sequence Typing (MLST)
  – Pulsed Field Gel Electrophoresis (PFGE)
The population structure of E.coli causing bacteraemia in the UK and Ireland between 2001 and 2010

- Phylogrouping

- **A**
  - associated with commensal strains

- **B1**
  - associated with commensal strains

- **B2**
  - associated with virulent extra-intestinal infections

- **D**

  - Internal control
  - \( chuA \)
  - \( yjaA \)
  - TSPE4.C2
Improved Multiplex PCR Strategy for Rapid Assignment of the Four Major *Escherichia coli* Phylogenetic Groups

M. Doumith,* M. J. Day,* R. Hope,* J. Wain,*,<sup>c</sup> and N. Woodford,*

Microbiology Service-Collindale, Health Protection Agency, London, United Kingdom,* Health Protection Services-Collindale, Health Protection Agency, London, United Kingdom, and Norwich Medical School, University of East Anglia, Norwich, United Kingdom

Using data from whole-genome projects, an updated multiplex PCR strategy was developed to assign *Escherichia coli* isolates rapidly to major phylogenetic groups. This assay accommodates sequence variations detected within target sequences, thereby increasing sensitivity and reliability. It was validated using 185 isolates of known sequence types and showed improved congruence with multilocus sequence typing data.

Phylogenetic analyses have shown that *Escherichia coli* isolates fall into four main phylogenetic groups, groups A, B1, B2, and D (1). In 2000, Clermont et al. (1) described a triplex PCR strategy to assign *E. coli* isolates rapidly to one of these phylogroups. The strategy involved using three phylogenetic group markers, the *chtA* and *yjaA* genes encoding hypothetical proteins and the TSPE4C2 DNA sequences situated within a gene encoding a putative lipase esterase, and groups were assigned on the basis of different combinations of presence and/or absence of the three amplicons (1, 5). This strategy has within the annealing regions for the published PCR primers for these three marker fragments; for example, more than 60% of the analyzed sequences had one or two polymorphic nucleotides within the *yjaA* forward (59/97) and/or TSPE4C2 reverse (129/177) primers (Table 1). We sought therefore to update the primers used to accommodate these sequence variations and thereby to improve the coverage offered by this phylogrouping scheme.

New primers were designed to amplify conserved regions of the same three markers, while a new fourth primer pair specifically targeted the *E. coli* glutamate decarboxylase-alpha gene, *gadA*, as
E. coli phylogroups in bacteraemia

- A: 8.9%
- B1: 6.3%
- B2: 66.7%
- D: 18.1%

N=2168
E. coli phylogroups in bacteraemia
Achtman Multi Locus Sequence Typing (MLST) scheme

• Measures DNA sequence variations in 7 housekeeping genes
• Sequence of each gene compared to those stored in the database and assigned an allele number
• The combination of all 7 allele numbers gives you a sequence type
• Currently 3266 STs

<table>
<thead>
<tr>
<th>ST</th>
<th>adk</th>
<th>fumC</th>
<th>gyrB</th>
<th>icd</th>
<th>mdh</th>
<th>purA</th>
<th>recA</th>
<th>ST Complex</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST73</td>
<td>36</td>
<td>24</td>
<td>9</td>
<td>13</td>
<td>17</td>
<td>11</td>
<td>25</td>
<td>ST73 Cplx</td>
</tr>
<tr>
<td>ST73</td>
<td>36</td>
<td>24</td>
<td>9</td>
<td>13</td>
<td>17</td>
<td>11</td>
<td>25</td>
<td>ST73 Cplx</td>
</tr>
<tr>
<td>ST892</td>
<td>21</td>
<td>24</td>
<td>9</td>
<td>13</td>
<td>17</td>
<td>11</td>
<td>25</td>
<td>None</td>
</tr>
<tr>
<td>ST968</td>
<td>36</td>
<td>21</td>
<td>9</td>
<td>13</td>
<td>17</td>
<td>11</td>
<td>25</td>
<td>None</td>
</tr>
<tr>
<td>ST804</td>
<td>6</td>
<td>24</td>
<td>9</td>
<td>13</td>
<td>17</td>
<td>11</td>
<td>25</td>
<td>None</td>
</tr>
</tbody>
</table>

Escherichia coli MLST Database.
Minimum spanning (MS) trees

7, 12, 45, 6, 92, 1, 40

7, 12, 45, 9, 92, 1, 22

7, 12, 60, 23, 6, 9, 22

7, 12, 45, 6, 92, 1, 22

7, 12, 60, 23, 6, 9, 22

41, 2, 32, 19, 6, 9, 22
Minimum spanning tree

N=1923
Diversity of sequence types

- 1158 isolates:
- 283 different STs
- 14% of isolates belong to STs that appear only once over the 10 years
- 14% of isolates are new single locus and double locus variants of known STs
- 50% of isolates belong to the top 5 most prevalent STs
Minimum spanning tree

ST131

ST73

N=1923
ST73  \( (n=77) \)

- 30 distinct patterns (14 unique)
- Largest group is present in 2001 and 2010 in multiple centres across the UK and Ireland

- 20% of all isolates
- Phylogroup B2
- Associated with Urinary Tract Infections
ST131 (n=77)

- 11% of all isolates
- Phylogroup B2
- Associated with Urinary Tract Infections
- ST131 is a globally disseminated clone associated with ciprofloxacin resistance and CTX-M genes

- 57 distinct patterns (42 unique)
- The patterns cluster by year
The population structure of E.coli causing bacteraemia in the UK and Ireland between 2001 and 2010

**ST12, ST95, ST69**

- 3%, 10% and 6% of all isolates respectively
- Phylogroup B2 except for ST69, group D
- Associated with Urinary Tract Infections
- ST95 and ST12 have an association with birds
- ST69 and ST12 have a high prevalence of virulence associated genes
ST12, ST95, ST69

- **ST12**
  - 17 distinct patterns (13 unique)
  - 2 patterns occur in 2001 and 2010 in the same centres

- **ST95**
  - 18 distinct patterns (12 unique)
  - 6 patterns with multiple isolates that occur in 2001 and 2010 in multiple centres across the UK and Ireland

- **ST69**
  - 11 distinct patterns (8 unique)
  - 1 pattern occurs in 2001 and 2010 in different centres
Sequence types over time

% of total isolates

The population structure of E.coli causing bacteraemia in the UK and Ireland between 2001 and 2010

The population structure of E.coli causing bacteraemia in the UK and Ireland between 2001 and 2010
Conclusions

• Phylogroup B2 is the most prevalent in bacteraemia

• The top 5 sequence types account for 50% of *E. coli* all bacteraemias in the UK in Ireland between 2001-2010
  – ST73
  – ST131
  – ST95
  – ST69
  – ST12

• These sequence types are also prevalent in UTIs

• There is a lot of diversity within sequence types by PFGE, although with the exception of ST131, the same patterns reoccur over the 10-year period
Acknowledgments

• Funded by Public Health England, National Institute for Health Research
• Michel Doumith, Russell Hope, John Wain, Jane Turton, Anthony Underwood, Rosy Reynolds, David Livermore, and Neil Woodford
• BSAC providing isolates