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

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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

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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

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Methods

- Molecular techniques
  - Phylogrouping
  - Multilocus Sequence Typing (MLST)
  - Pulsed Field Gel Electrophoresis (PFGE)

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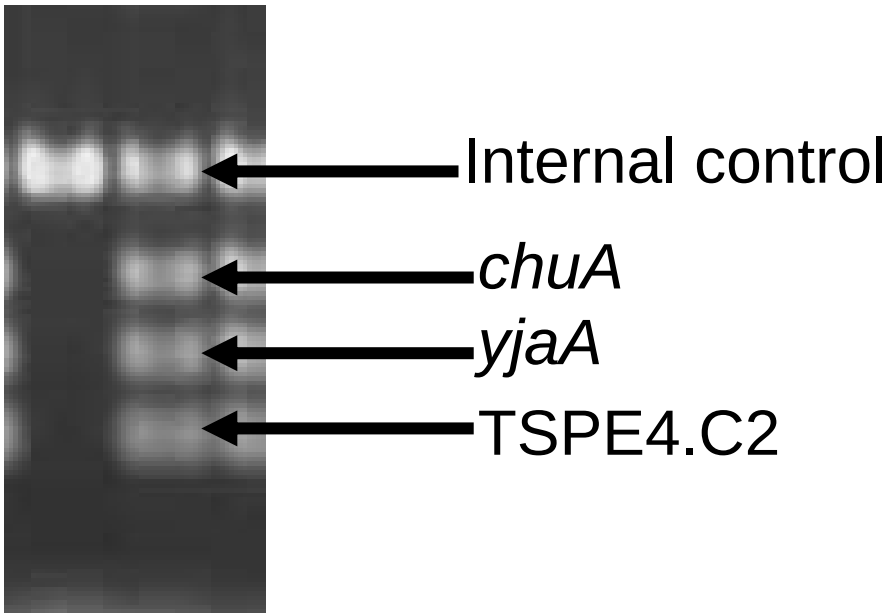
-Phylogrouping

- A
- B1

associated with commensal strains

- B2
- D

associated with virulent extra-intestinal infections



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Improved Multiplex PCR Strategy for Rapid Assignment of the Four Major *Escherichia coli* Phylogenetic Groups

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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 *chuA* and *yjaA* genes encoding hypothetical proteins and the TSPE4.C2 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 TSPE4.C2 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

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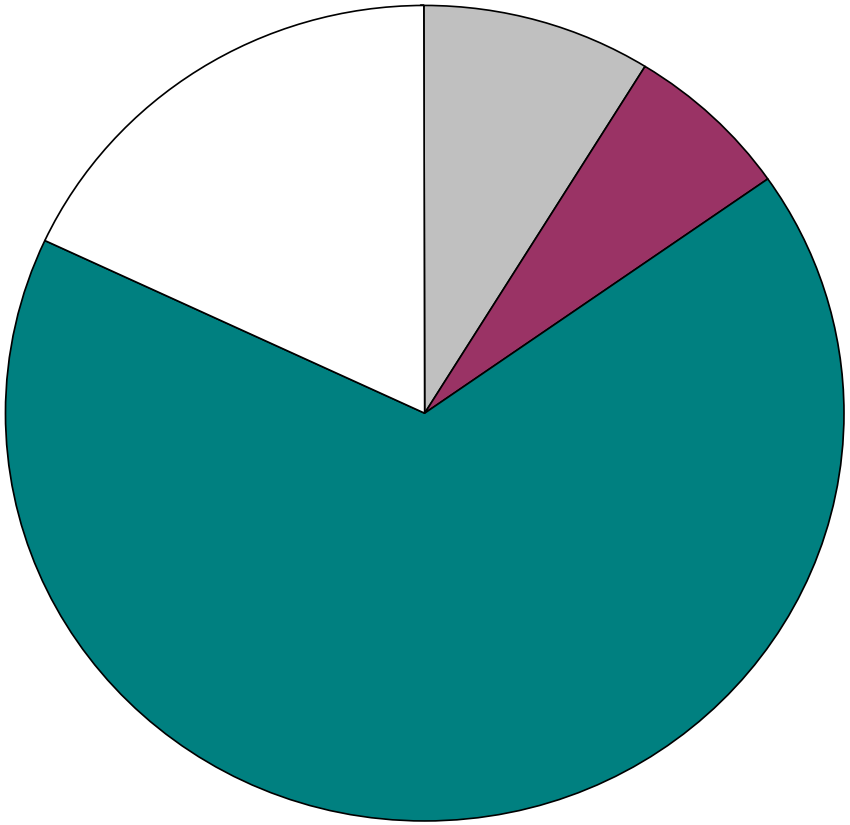
E. coli phylogroups in bacteraemia



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

N=2168

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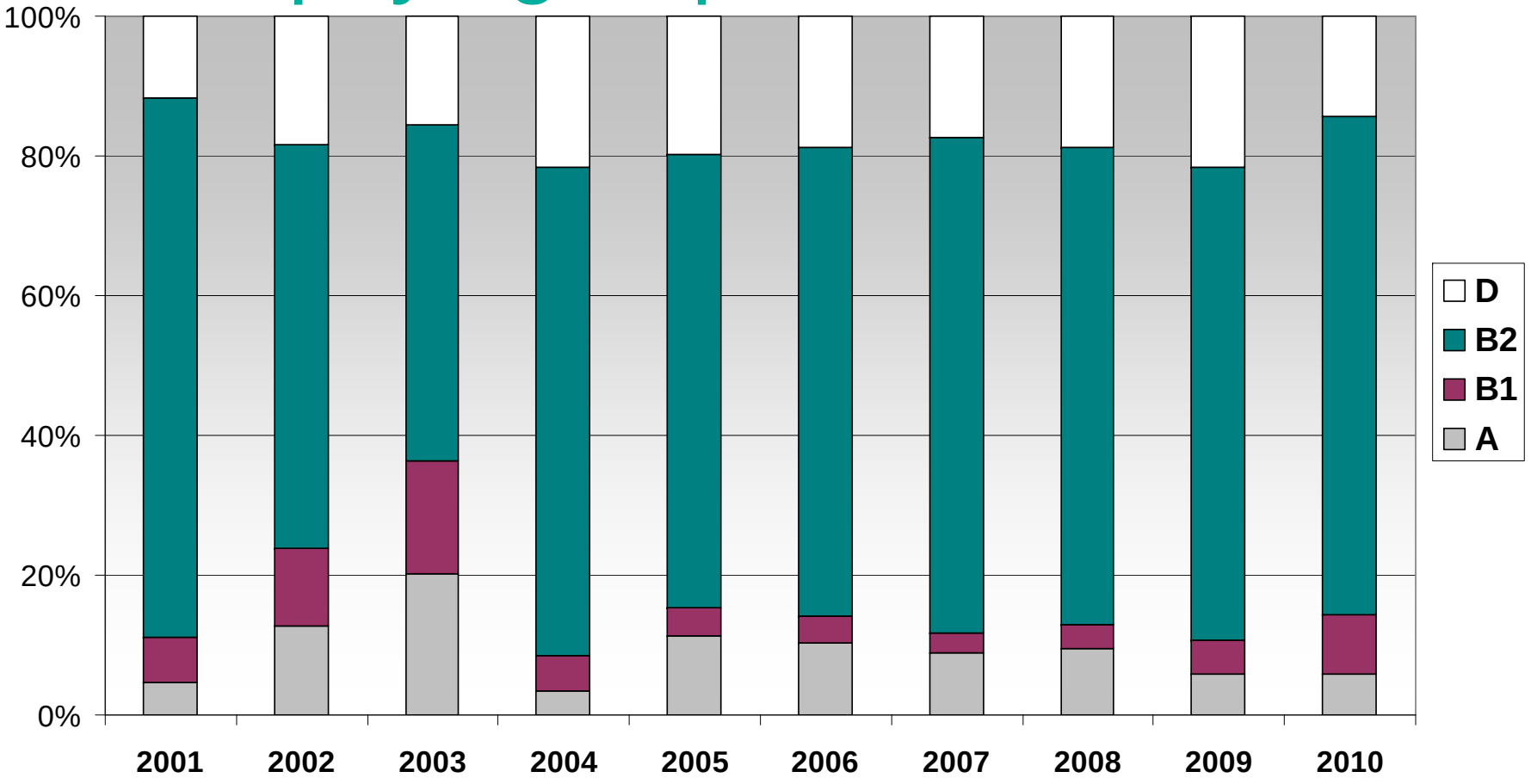
E. coli phylogroups in bacteraemia



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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

adk	adenylate kinase
fumC	fumarate hydratase
gyrB	DNA gyrase
icd	isocitrate/isopropylmalate dehydrogenase
mdh	malate dehydrogenase
purA	adenylosuccinate dehydrogenase
recA	ATP/GTP binding motif

Escherichia coli **MLST Database**.

ST	*adk*	*fumC*	*gyrB*	*icd*	*mdh*	*purA*	*recA*	ST Complex
ST73	36	24	9	13	17	11	25	ST73 Cplx
ST73	36	24	9	13	17	11	25	ST73 Cplx
ST992	21	24	9	13	17	11	25	None
ST989	36	21	9	13	17	11	25	None
ST968	36	24	9	13	17	128	25	None
ST804	6	24	9	13	17	11	25	None

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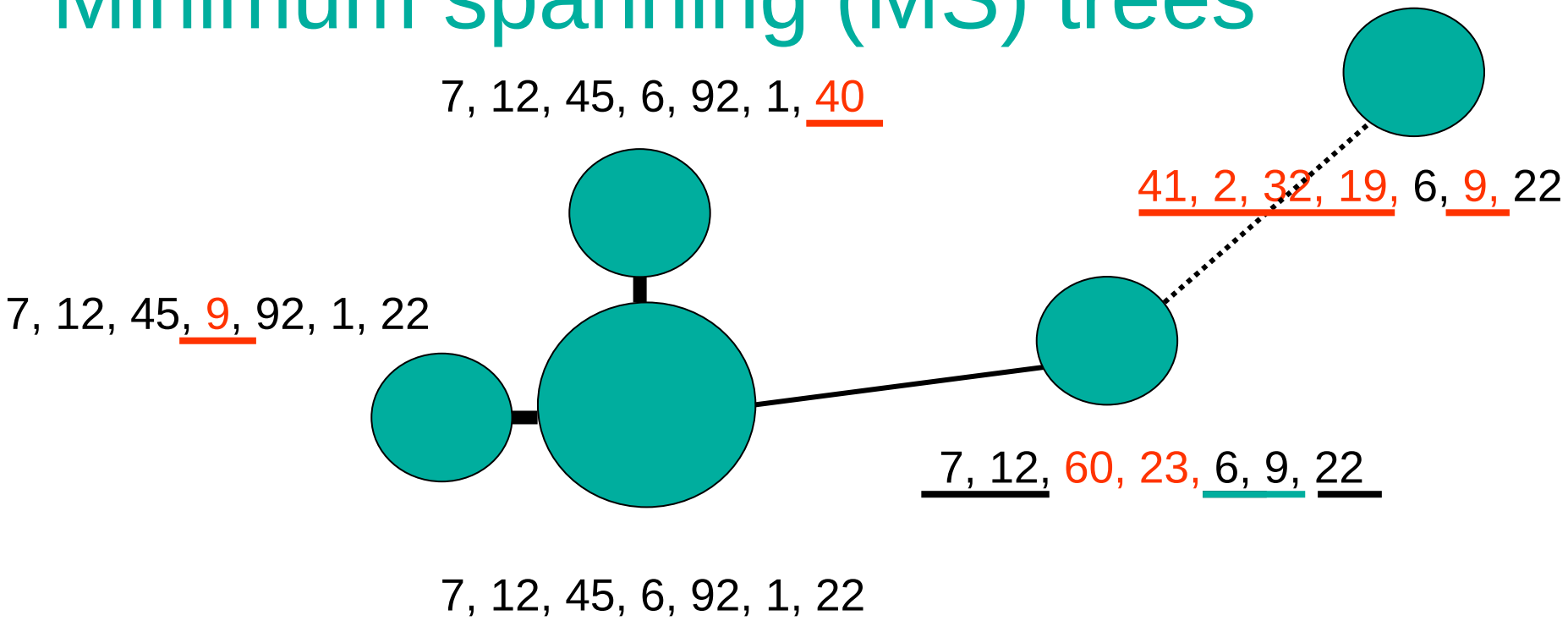
Minimum spanning (MS) trees



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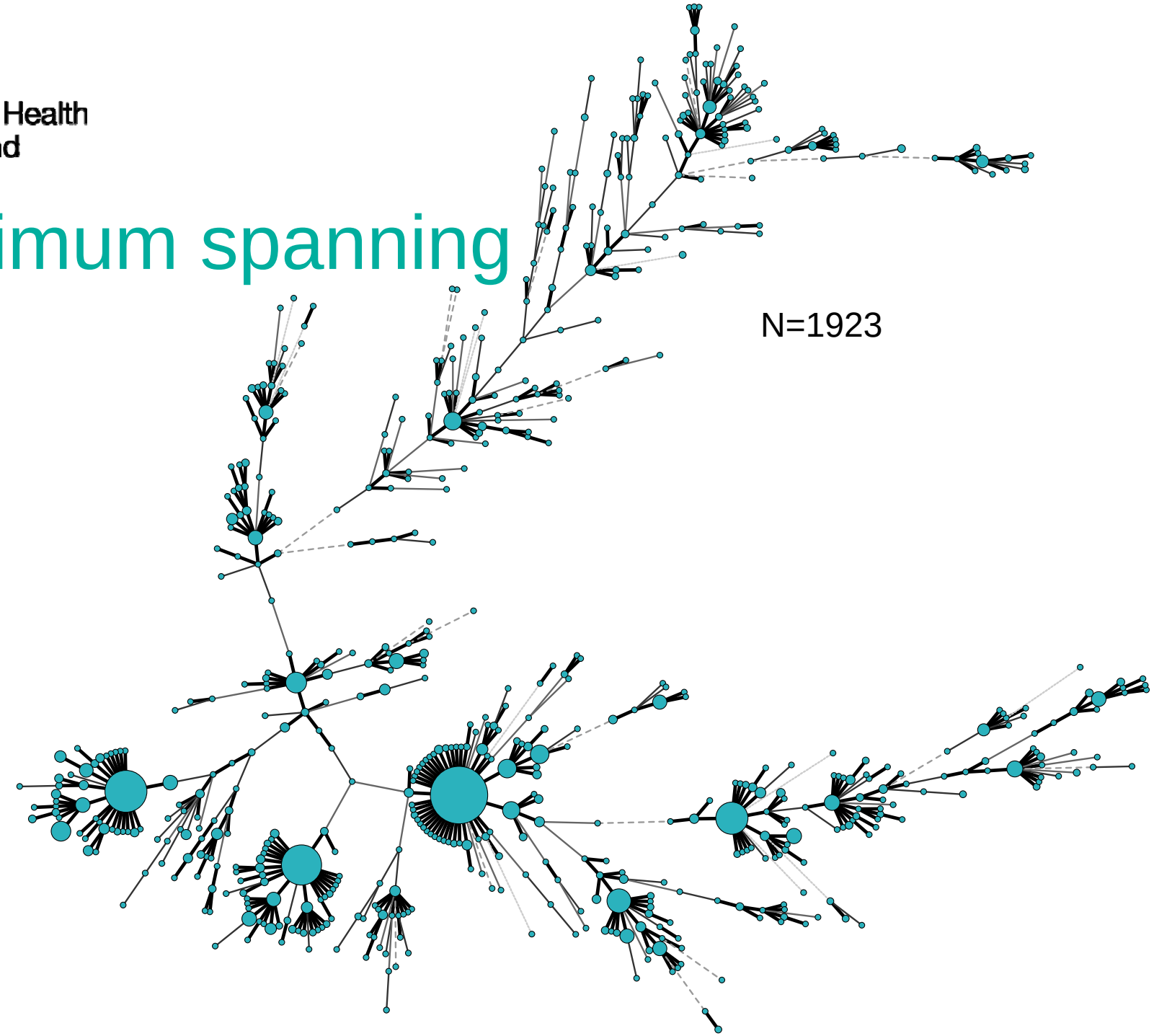
Minimum spanning tree

N=1923



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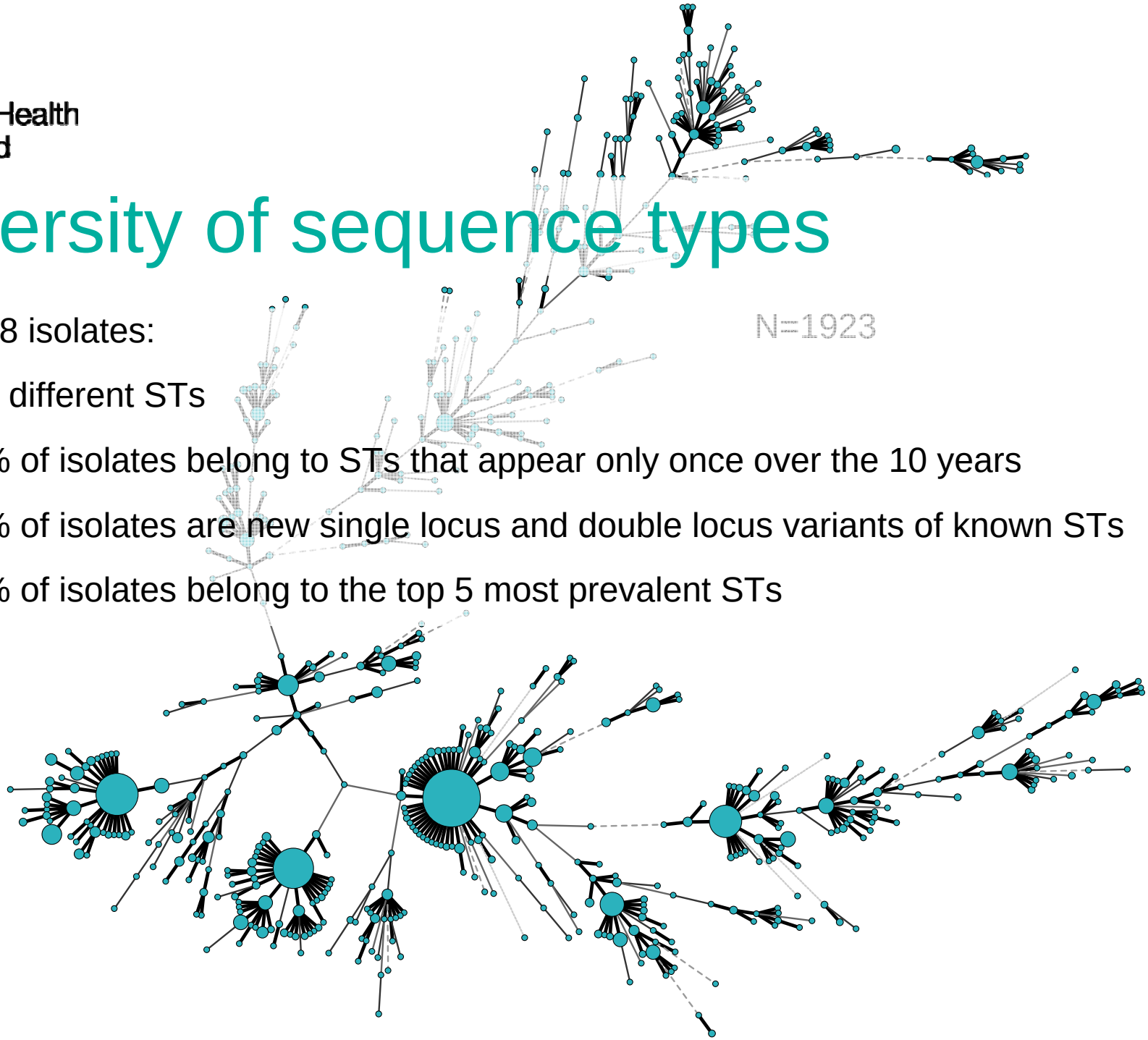
Diversity of sequence types

- 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



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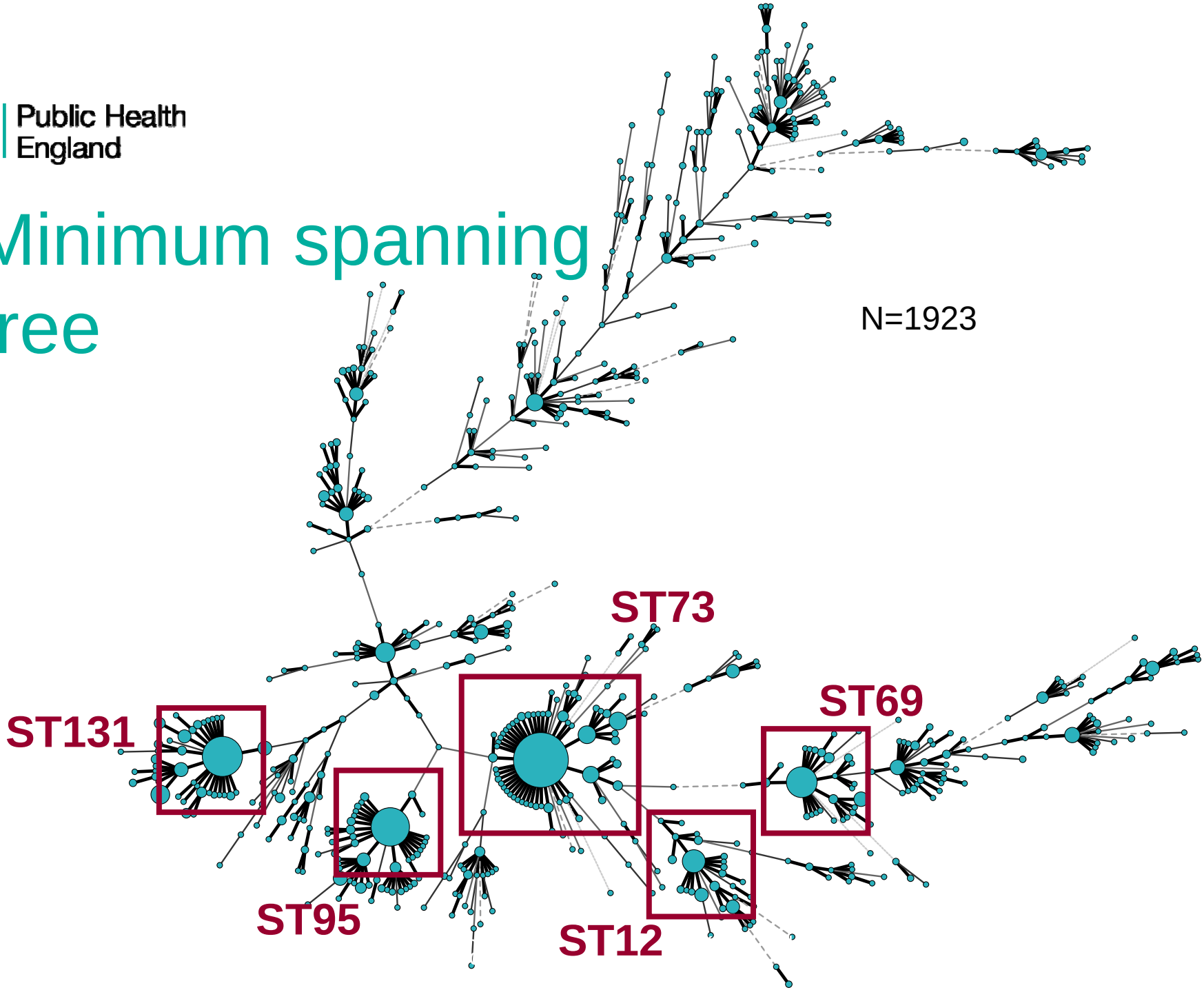
Minimum spanning tree

N=1923



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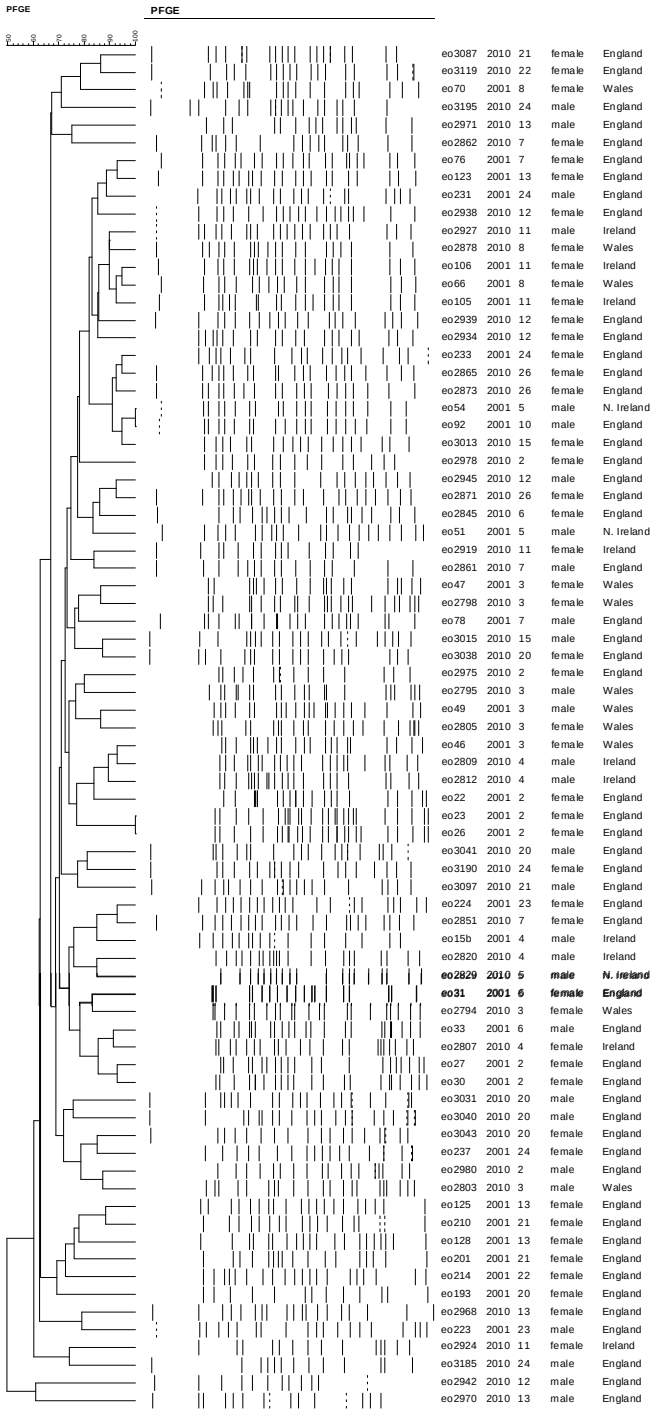
ST73 (n=77)

- 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



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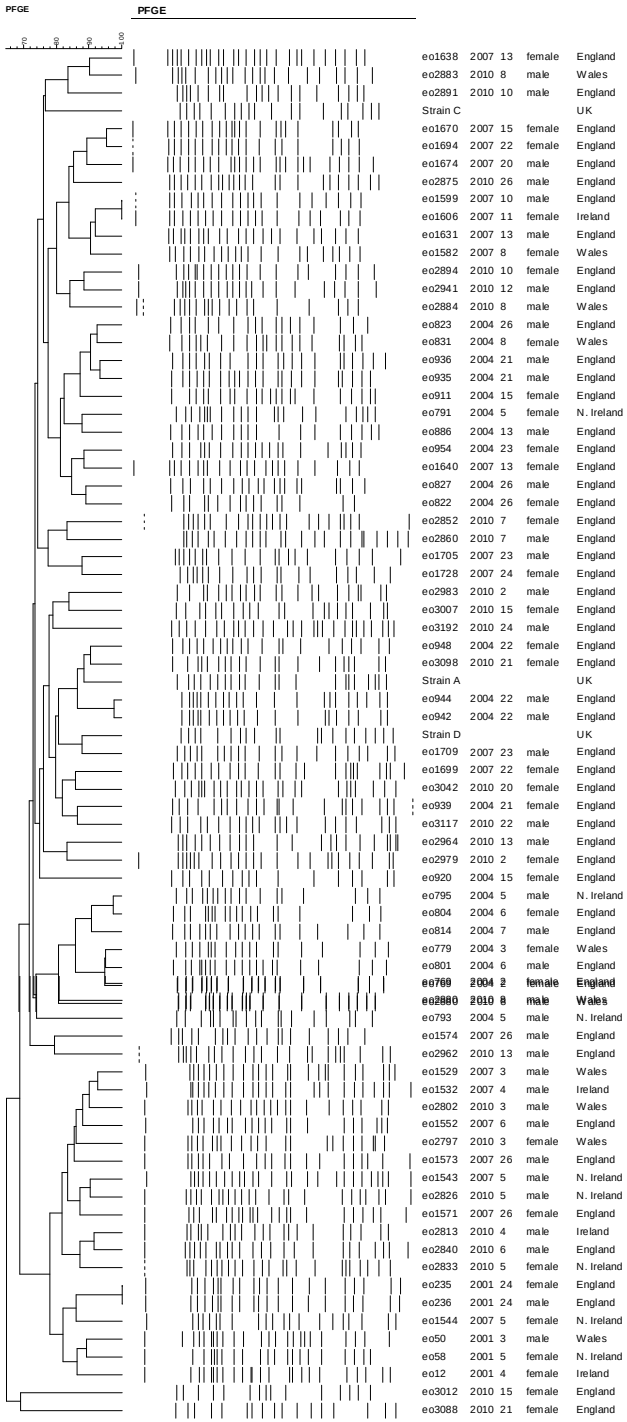
ST131 (n=77)

- 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



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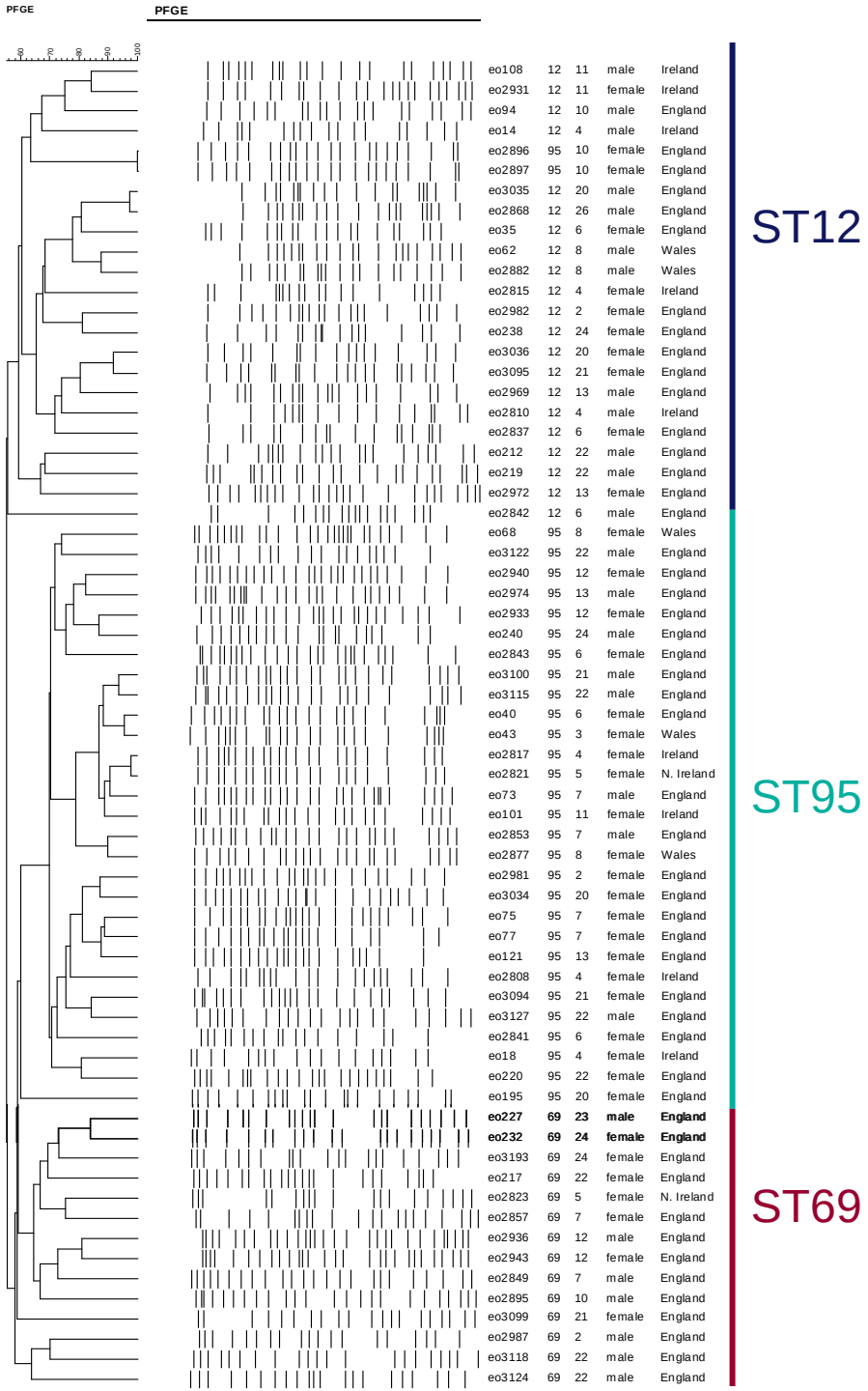
ST12, ST95, ST69

- 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



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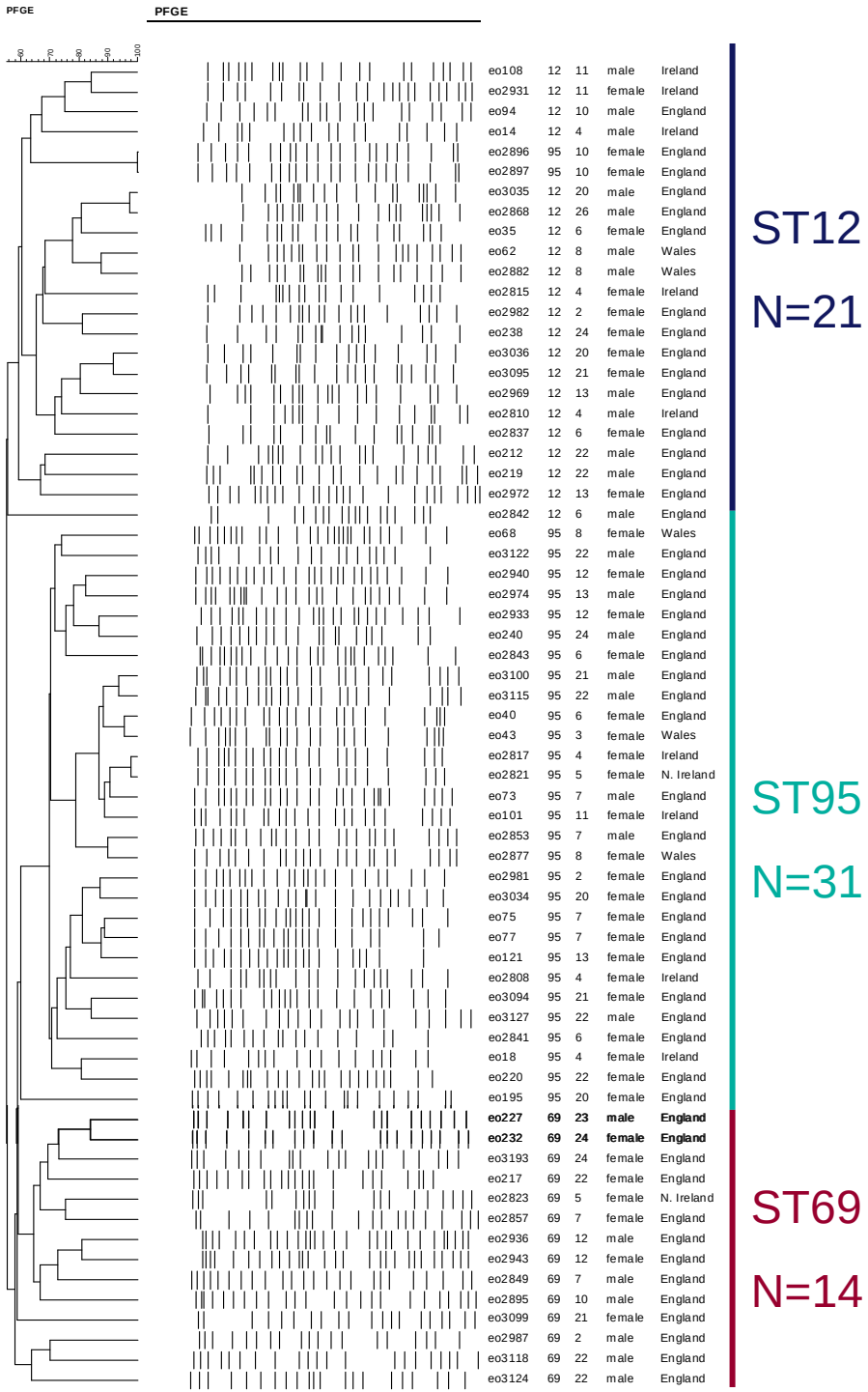
ST12, ST95, ST69

- 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



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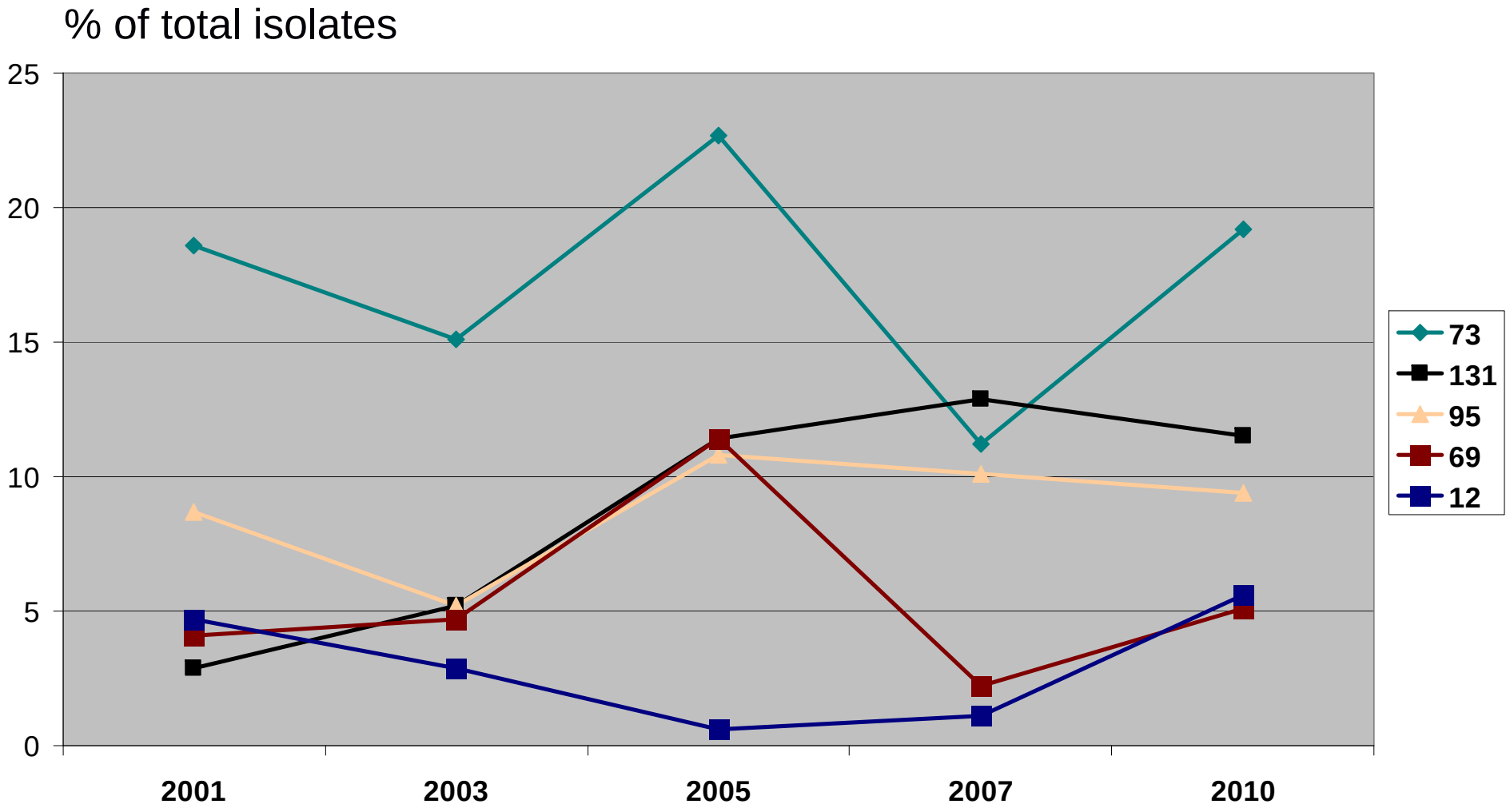
Sequence types over time



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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

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