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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

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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
Serodiagnostics
Toxin detection
All diarrhoeagenic *E. coli* pathotypes

Current Identification Methods for STEC

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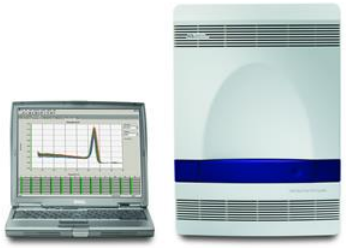
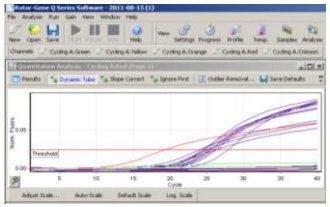
Gene Detection

Day 1



Real time TaqMan® PCR assays
- target four different genes

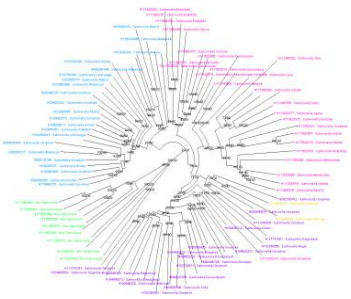
PCR targeting:
stx1, stx2
eae (intimin), O157



Day 4-8

Identification

Day 3



OmniLog® ID System (Biolog)
- phenotypic microarray

Differentiation from *Shigella* spp. and other Enterobacteriaceae

Serotyping

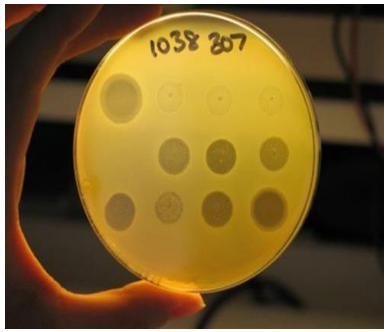
Agglutination with specific antisera against LPS & flagella (O & H antigens)

- **Slide agglutination**
- **Microtitre plates**
- H typing can take up to 14 days

Sub-typing Methods for O157 STEC

Phage Typing

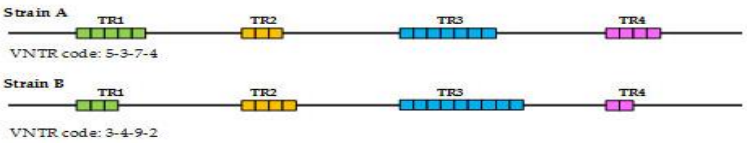
Day 3

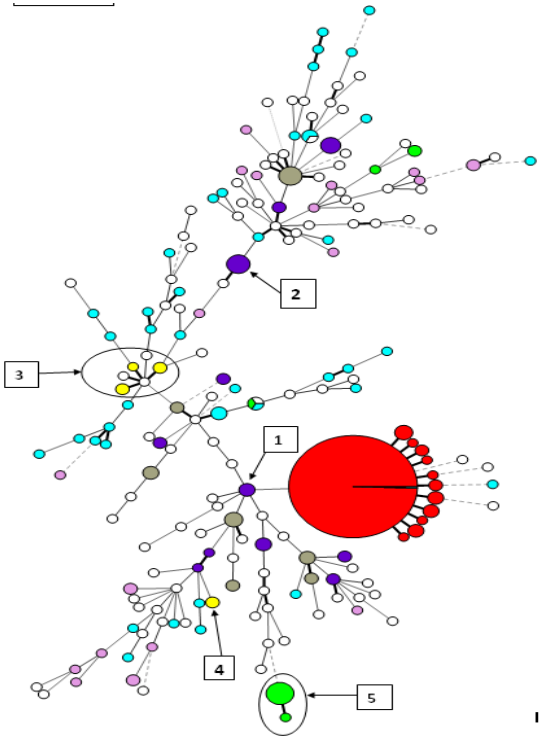


e.g. PT8 and PT21/28 most common in the UK

Multi-locus Variable Number Tandem Repeat Analysis (MLVA)

Day 3-5



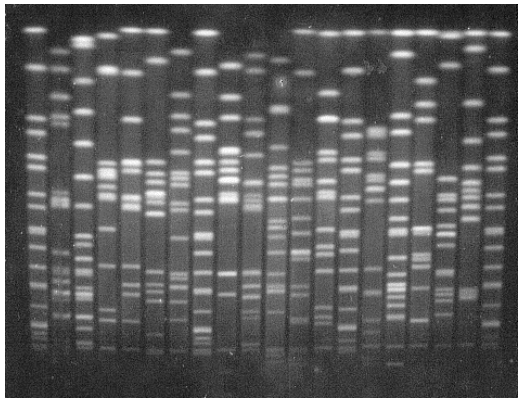


Both techniques useful in outbreak detection but not available for non-O157 STEC

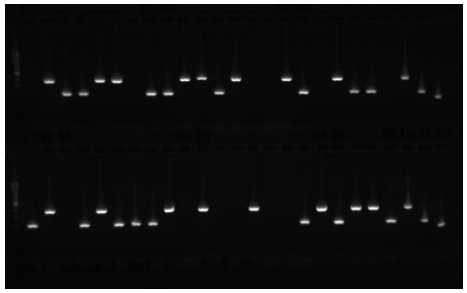
Sub-typing Methods for STEC

Pulsed-field gel electrophoresis (PFGE)

Day 4-7



Laborious, technically demanding , validation needed for each serogroup



***stx* subtyping**

Day 2

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

Whole Genome Sequencing

Day 5-7

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

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Sequencing

Bioinformatics

DNA extraction
Day 1

↓

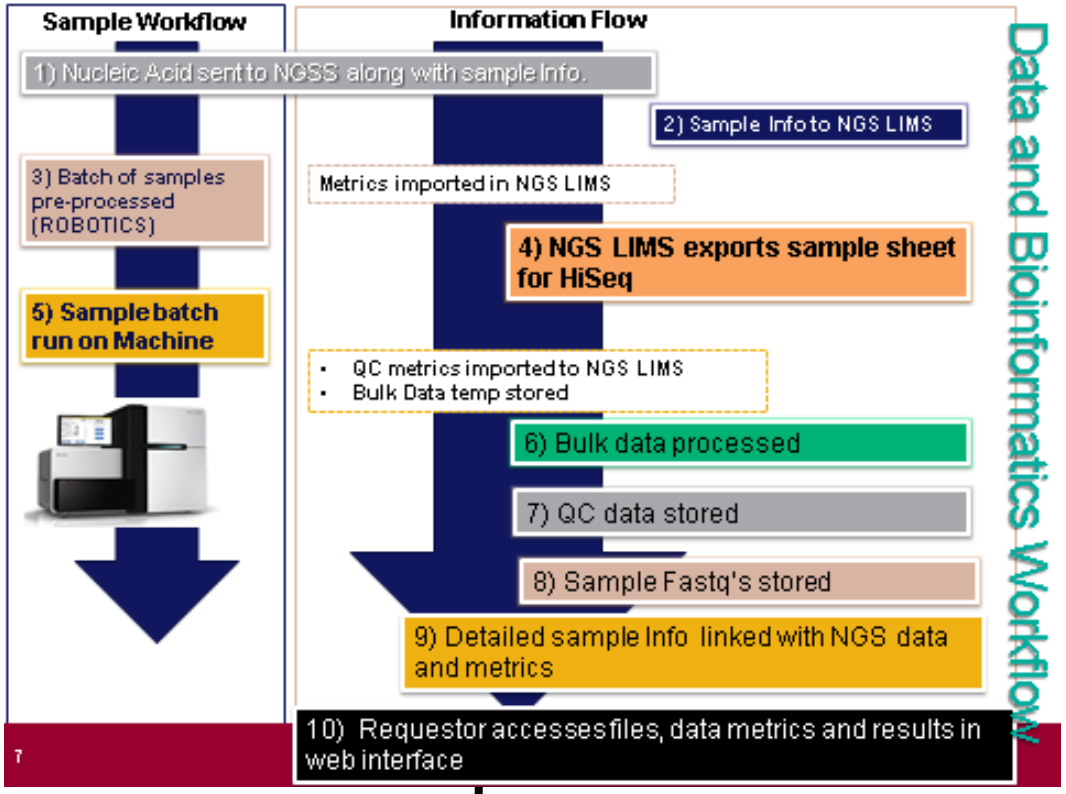
Library Prep
Day 2-3

↓

Sequencing
Day 4-5

↓

Bioinformatics Validation Reporting
Day 6-7



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Validation

↓

Reporting to customer (**communication**)

Jon Green –Head Bioinformatics

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Impact on Public Health
Improved Data – Example 2014

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

Test	Traditional	WGS	Mismatches
O antigen/*rfb*	102 identified 26 untypable 13 Rough	138 identified 1 untypable 1 O153/O178 1 O123/O186	10 (7%)
H antigen / *fliC*	69 identified 42 untypable 30 not motile	139 identified 2 untypable	3 (2%)
stx subtyping	141 identified 8 double positives (2 stx) 2 triple positives (2 *stx*)	141 identified 1 double positive due to recombination (2b/d)	14 (9%)
Outbreak analysis	Serotyping *stx* subtype	rfb/fliC *stx* subtype Core SNP phylogeny SNP address	-

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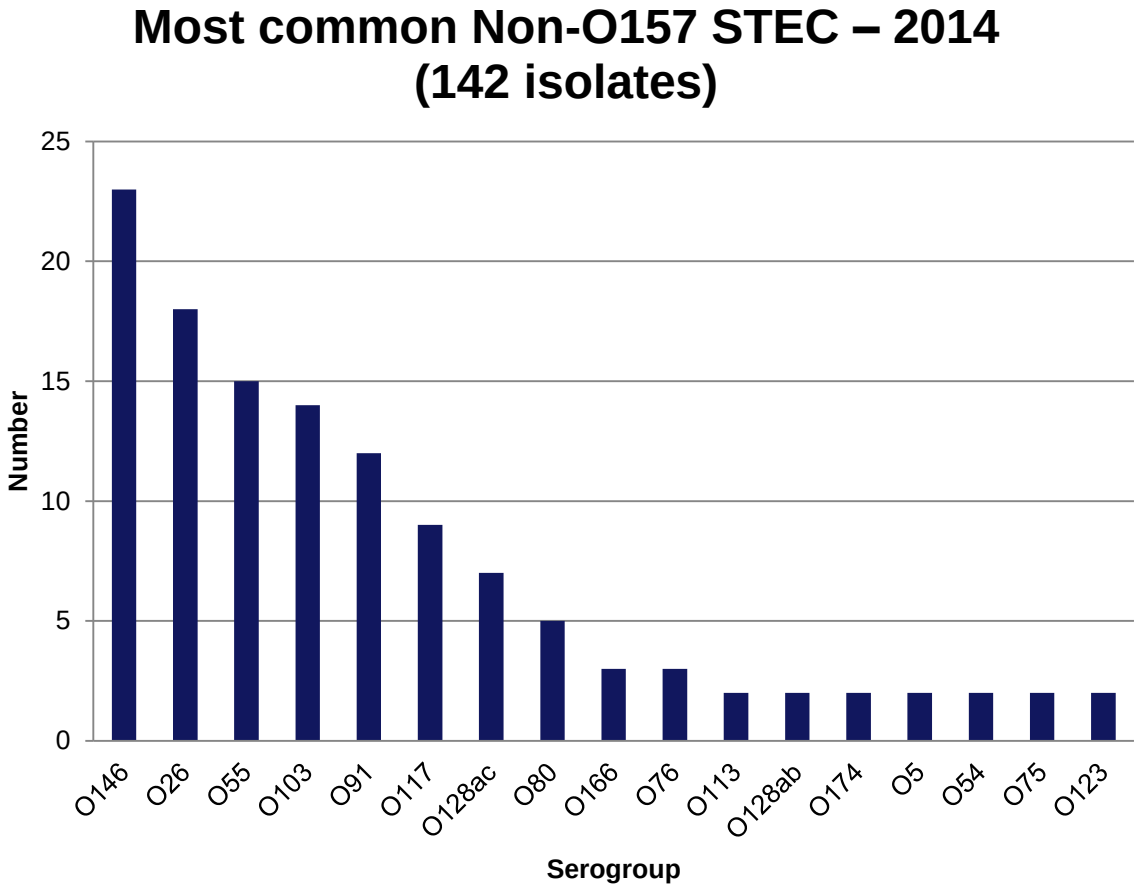
Impact on Public Health

Understanding trends in Epidemiology



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

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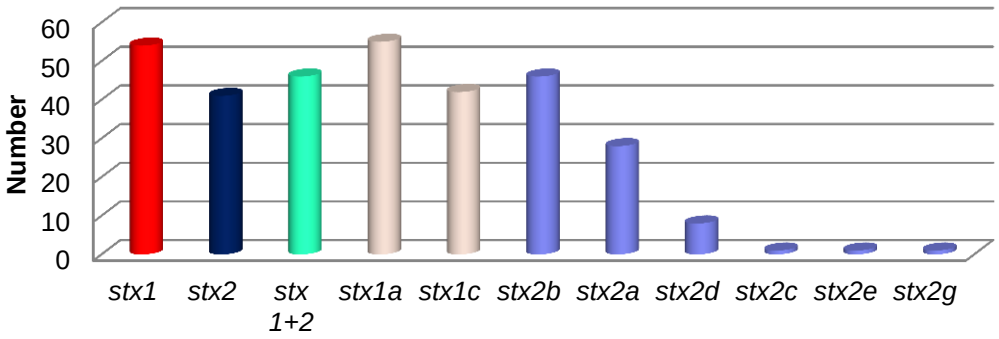
Impact on Public Health

Understanding trends in Epidemiology

stx sub-type in non-O157 - 2014



- *stx*1b 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*

Serogroup	ST	ST1	ST2	EAE	*stx1*	*stx2*
O103:H2	386	+	-	-	*stx1a*	-
O145:H28	SLV-137	-	+	+	-	*stx2a*
O26:H11	21	-	+	+	-	*stx2a*
O26:H11	21	+	+	+	*stx1a*	*stx2a*
O54:H45	491	-	+	-	-	*stx2b*
O55:H7	335	-	+	+	-	*stx2a*
O55:H7	335	+	+	-	*stx1c*	*stx2d*
O55:H7	335	-	+	+	-	*stx2a*
O55:H7	335	-	+	+	-	*stx2a*
O75:H5	550	-	+	+	-	-

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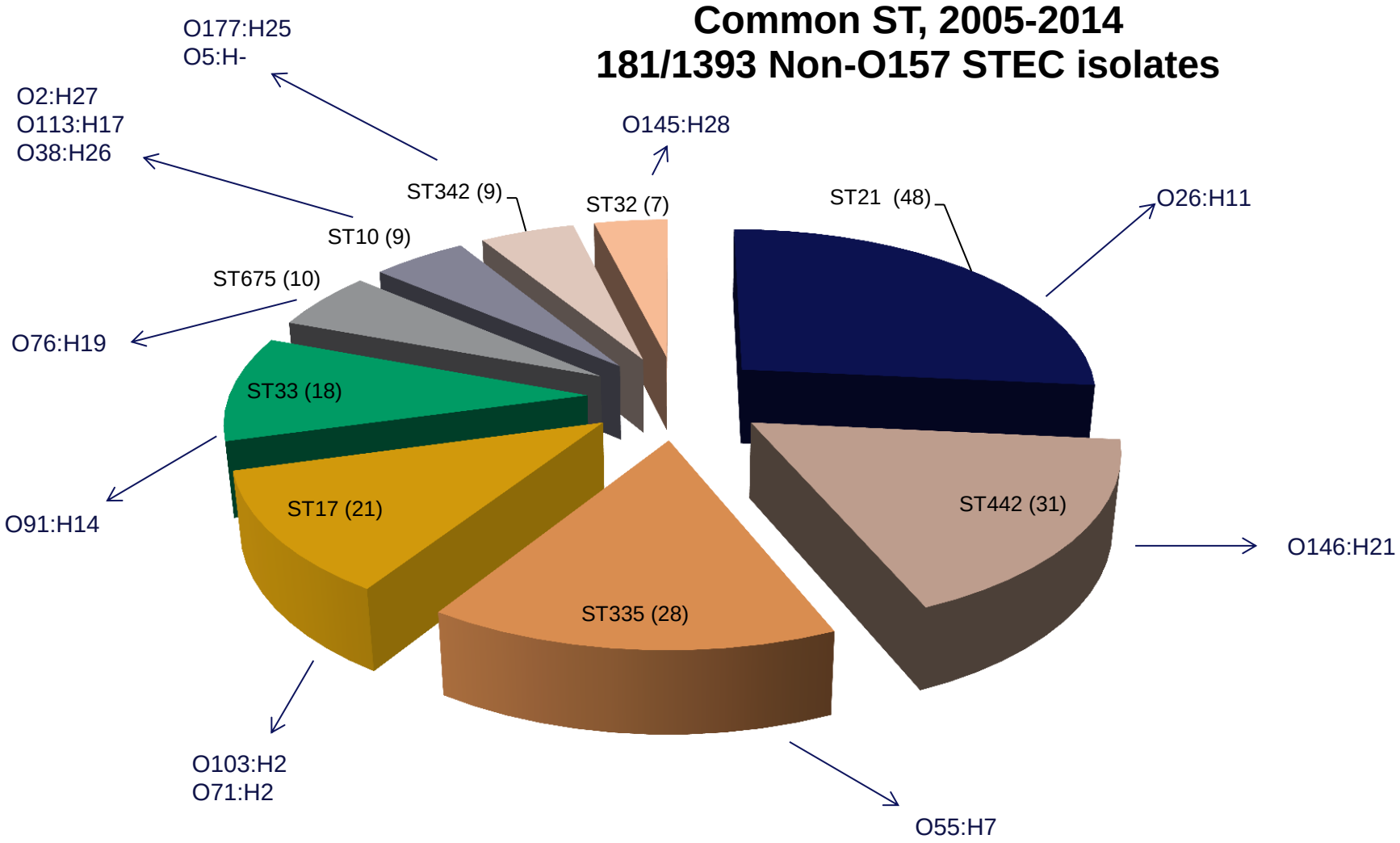
PHE in the 21st Century

Changing the way we view data



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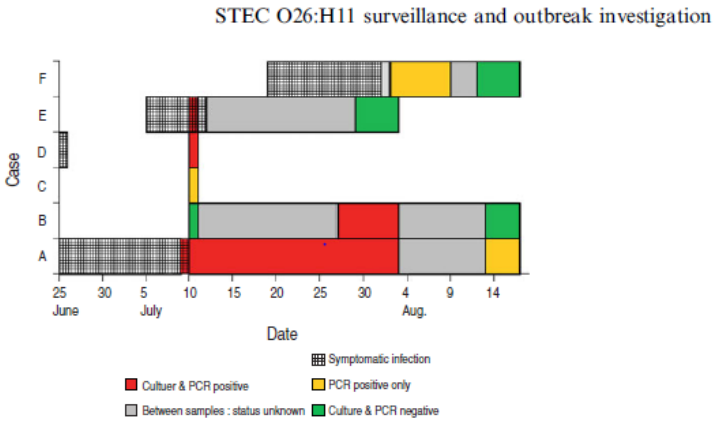
Impact on Public Health

Higher resolution in outbreak analysis



Dallman et al. 2014. Epidemiol infect.

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

Reference no.	*Stx* profile	MLST	Date culture isolated	Sex/ age	Travel	Additional information*
181/09	1&2	21	Feb. 2009	F/2	No travel	Outbreak 2009
461/09	1&2	21	Feb. 2009	M/14	No travel	Outbreak 2009
460/09	1&2	21	Feb. 2009	F/0	No travel	Outbreak 2009
259/10	1&2	21	Sept. 2010	M/3	No travel	HUS
605/10	1&2	21	Sept. 2010	F/3	France	HUS
467/10	2	21	Nov. 2010	M/13	Turkey	HUS
519/11	1&2	21	July 2011	M/4	No travel	
624/12	2	21	Apr. 2012	F/13	Egypt	HUS
165/12	1&2	21	May 2012	F/42	No travel	Fatal case
483/12	2	21	July 2012	M/35	Ireland and Switzerland	Outbreak 2012
482/12	2	21	July 2012	M/4	No travel	Outbreak 2012
626/12	2	21	July 2012	M/4	No travel	Outbreak 2012
627/12	2	21	July 2012	M/2	No travel	Outbreak 2012
2270–502/12	2	21	July 2012	F/1	Ireland	
2290–502/12	—	29	July 2012	M/40	No data	
670/13	—	29	Aug. 2013	F/0	Egypt	HUS
680/13	—	29	Sept. 2013	F/3	Italy	Hospitalized with severe bloody diarrhoea
075/13	2	21	Sept. 2013	M/0	Albania	
637/13	2	21	Sept. 2013	F/7	No data	

MLST, Multilocus sequence typing; HUS, haemolytic uraemic syndrome.
* Additional information includes whether or not cases were associated with an outbreak and clinical symptoms.

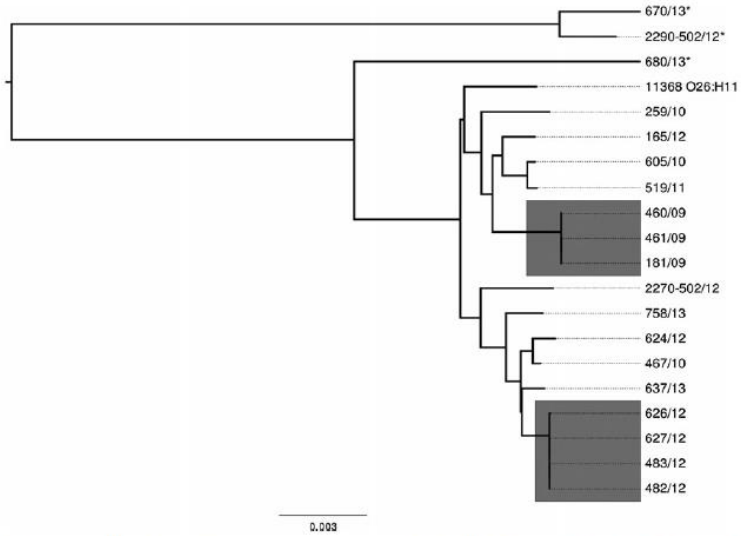


Fig. 2. Phylogenetic relationship of 19 strains of *E. coli* O26 isolated at GBRU between 2009 and 2013. Outbreak strains of STEC O26 from 2009 and 2012 are highlighted in grey. The scale represents number of nucleotide substitutions per site. * Strains negative for the *stx* genes.

- 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.

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October 2015

Switching to WGS for routine testing of E. coli

Web-based Gasto database available to Public Health Team

Molis ID	NGS LIMS ID	NGS RUN ID	Sequencing Date	Workflow	Yield	Postive Control	RAG	ID	Mixed	ST	MLST PROFILE	O	H	S. flexneri Serotype	EAE	BFPA	IPAH	AGGR	AAIC	LTCA	STA1	STB	STX	SNAPPERDB Flag	SNP Address
H153360699	147826	AHKYLVADXX	2015-08-26					Escherichia coli EC1848 89.58358	nothing	335	29,12,8,12,15,2,2	O55	H7	-	+	-	-	-	-	-	-	-	stx2a		71.730.1046.1948.2205.2254.2457
H153360699	147826	AHK3FNADXX	2015-08-24					Escherichia coli EC1848 89.207489	nothing	335	29,12,8,12,15,2,2	O55	H7	-	+	-	-	-	-	-	-	-	stx2a		71.730.1046.1948.2205.2254.2457
H153020735	136729	Ahjwtjadxx	2015-07-28					Escherichia coli EC1848 89.076042	nothing	335	29,12,8,12,15,2,2	O55	H7	-	+	-	-	-	-	-	-	-	stx2a		71.730.1046.1948.2205.2254.2457
H152720005	129383	AHJV27ADXX	2015-07-13					Escherichia coli EC1848 89.641296	nothing	335	29,12,8,12,15,2,2	O55	H7	-	+	-	-	-	-	-	-	-	stx2a		71.730.1046.1948.2205.2254.2457
H152720004	129382	AHJV27ADXX	2015-07-13					Escherichia coli EC1848 89.459167	nothing	335	29,12,8,12,15,2,2	O55	H7	-	+	-	-	-	-	-	-	-	stx2a		71.730.1046.1948.2205.2254.2457

ID by Kmer

MLST
Inouye 2012

Serotype
Joensen 2015

E.coli
virulence genes
Doumith

stx subtype
Ashton

SnapperDb
Dallman

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Conclusion

- 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.

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Acknowledgements

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