Monitoring acquisition and loss of virulence and antimicrobial resistance genes in pathogenic *Escherichia coli*

Ella V. Rodwell[1,2,3], David R. Greig[1,2,4], Noel McCarthy[1,5], Timothy J. Dallman[1,2], Claire Jenkins[1,2]

@rodwell_ella

[1]HPRU in Gastrointestinal Infections at University of Liverpool, [2]National Infection Service, Public Health England, [3]Warwick Medical School, University of Warwick, [4]Division of Infection and Immunity, The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, [5]Public Health and Primary Care, Trinity College Dublin

Introduction

Shiga toxin-producing *E. coli* (STEC) infections can lead to the development of a fatal condition called Haemolytic Uraemic Syndrome (HUS)

STEC-HUS is the leading cause of renal failure in the under 5 age group

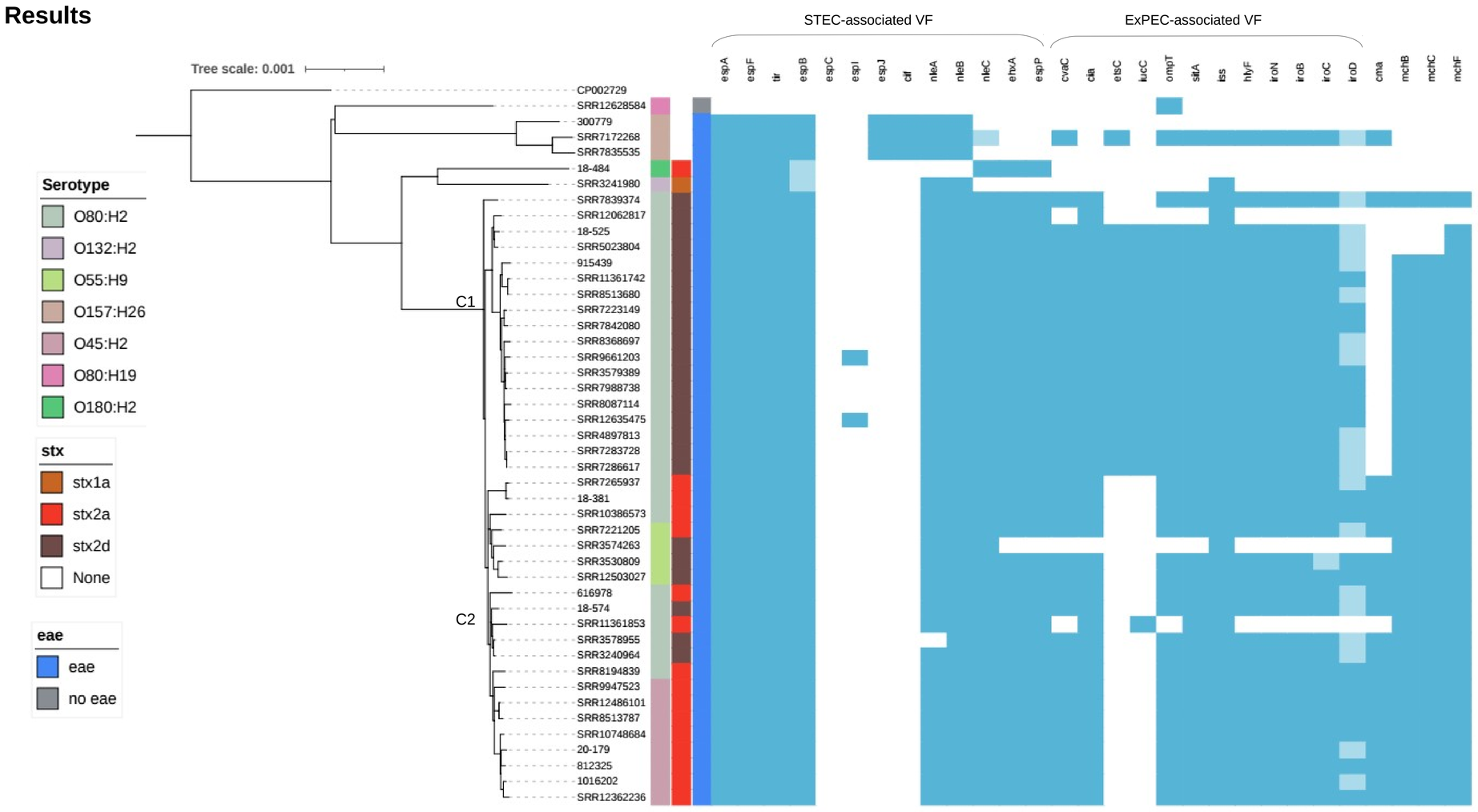
A highly pathogenic STEC has emerged in the UK and other European countries, belonging to Clonal Complex (CC)165

CC165 harbours STEC-associated virulence factors (VF), as well as VF associated with extra-intestinal *E. coli,* and an array of antimicrobial resistance (AMR) genes

Methods

- DNA Extraction: QiaSymphony — Revolugen Fire Monkey
- DNA Quantification: Qubit
- DNA Preparation: Nextera XT — Library Prep Rapid Barcoding SQK-RBK004
- DNA Sequencing: Illumina HiSeq 2500; ONT MinION
- Read Trimming and Post Sequencing Processing: Trimmomatic v0.27 — Guppy V3.2.10, Deepbinner v0.20, Nanoplot v1.24.0, Porechop v0.2.4, Filtlong v0.2.0
- Serotype, Sequence Type, *stx* Profiling: Ashton *et al.*, 2015, Chattaway *et al.*, 2016, Tewolde *et al.*, 2016 — Assembly and Correction: Flye v2.8, Minimap2 v2.17, Nanopolish v0.11.3, Pilon v1.22, BWA v0.7.17, Racon v1.3.3, Samtools v1.7
- GeneFinder: Ashton *et al.*, 2015, Chattaway *et al.*, 2016, Tewolde *et al.*, 2016; Gene Detection; Annotation: Prokka v.1.14.6
- SNP Typing and Phylogeny Production: SnapperDB v0.2.6, Gubbins 2.0.0, IQTree2 2.0.4, ITOL v6 — Phage Extraction and Phylogeny Production: Propi v0.0.1. Prokka v.1.13, Mash v2.2, FigTree v1.4.4

Results



There were 48 isolates of CC165 in the public health archives. Forty-three were STEC, four isolates had *eae* (intimin) but no *stx* (Enteropathogenic *E. coli*) and one isolate had no *stx* or *eae*

The majority of STEC isolates belonged to two sub-clusters, C1 and C2. C1 isolates harbour *stx2d* subtype whereas C2 are both *stx2a* and *stx2d* (Figure 1)

Isolates that had *eae* also had the Locus of Enterocyte Effacement (LEE) and harboured a variety of LEE and non-LEE effectors associated with the attachment to the gut mucosa

Extra-intestinal *E. coli* (ExPEC) associated genes were located on a plasmid

Loss and acquisition of VFs was observed between C1 and C2

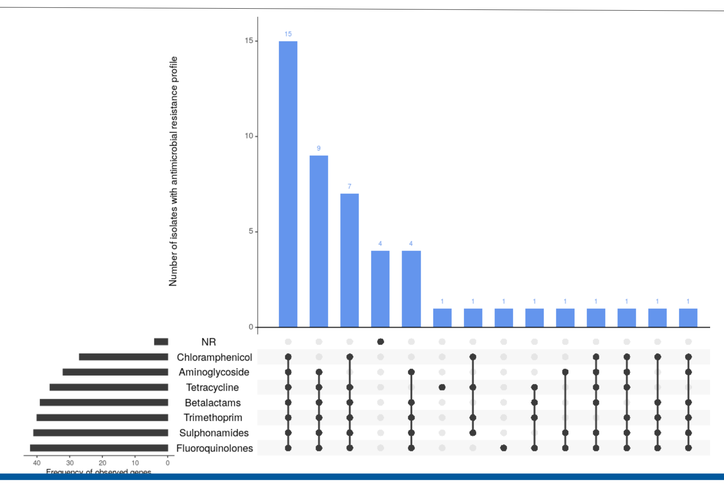
Serotype	N	%
O132:H2	1	2.3
O180:H2	1	2.3
O45:H2	9	21
O55:H9	4	9.3
O80:H2	28	65

STEC isolates (n=43) exhibited serotype variation, including 5 different STEC serotypes.

Analysis of epidemiological data revealed 25% of STEC infections* resulted in HUS development

HUS cases were infected with either STEC serotype O45:H2 (n=2), O55:H9 (n=2) or O80:H2 (n=4)

*where extended questionnaire data for cases in England was available



AMR genes were detected in high levels, with the most common profile being resistant to seven antimicrobials, exhibited by 15 isolates

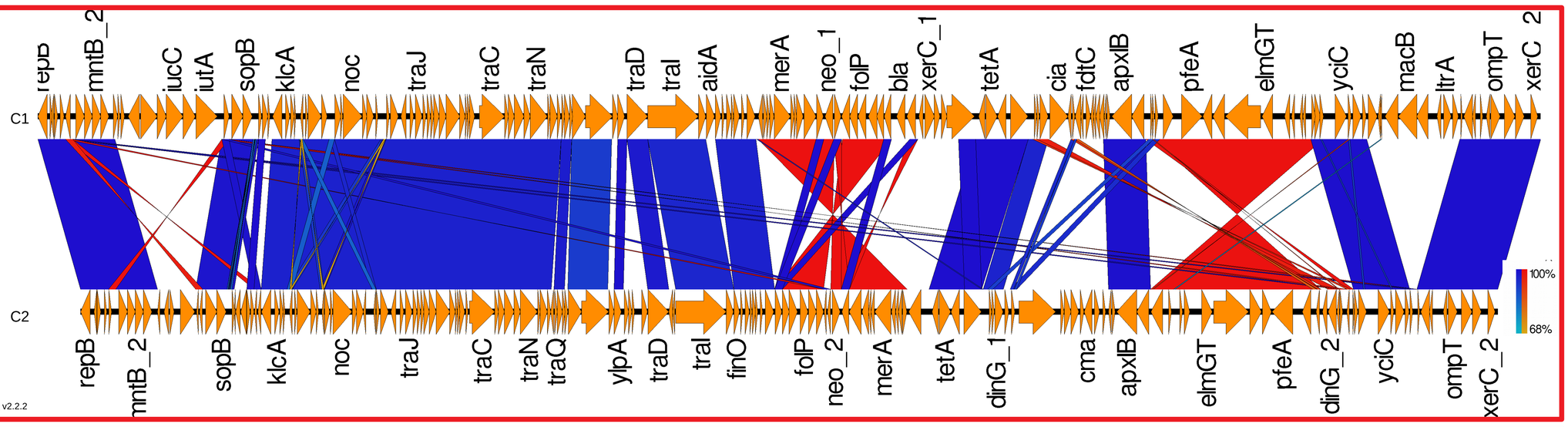
We observed cluster based acquisition of trimethoprim gene *dfrA36,* predominantly in C2. *dfrA46* was first described in the literature in 2019 but isolates of CC165 harbouring this gene in the PHE archive date back to 2013.

Long read sequencing of six isolates identified plasmids that harboured virulence factors that are associated with extra-intestinal *E. coli*

Plasmids encoding ExPEC VFs ranged from 150kb to 160kb in size

Long read analysis confirmed gene acquisition and loss of VFs genes between STEC sub-clusters

Two inversions were observed on the plasmid encoding the ExPEC VFs, between C1 and C2.



Plasmids orientated to repB using circulator fixstart v1.5.5 and visualised using EasyFig v2.2.2

Discussion

- The emergence of STEC-HUS CC with ExPEC virulence genes and multi-drug resistance is a public health concern
- Short read sequencing can detect the presence and absence of virulence and AMR genes within a population. Long read sequencing supplements this data and identifies the co-location of genes
- This study highlights the dynamic nature of the STEC genome and provides insight into to the emergence of STEC that cause HUS

References

Ashton, P.M., Perry, N., Ellis, R., Petrovska, L., Wain, J., Grant, K.A., Jenkins, C., Dallman, T.J., 2015. Insight into Shiga toxin genes encoded by *Escherichia coli* O157 from whole genome sequencing. PeerJ 3, e739. https://doi.org/10.7717/peerj.739. Chattaway, M.A., Dallman, T.J., Gentle, A., Wright, M.J., Long, S.E., Ashton, P.M., Perry, N.T., Jenkins, C., 2016. Whole Genome Sequencing for Public Health Surveillance of Shiga Toxin-Producing Escherichia coli Other than Serogroup O157. Front. Microbiol. 7. https://doi.org/10.3389/fmicb.2016.00258. Tewolde, R., Dallman, T., Schaefer, U., Sheppard, C.L., Ashton, P., Pichon, B., Ellington, M., Swift, C., Green, J., Underwood, A., 2016. MOST: a modified MLST typing tool based on short read sequencing. PeerJ 4, e2308. https://doi.org/10.7717/peerj.2308

The research was funded by the National Institute for Health Research Protection Research Unit (NIHR HPRU) in Gastrointestinal Infections at University of Liverpool in partnership with Public Health England (PHE), in collaboration with University of Warwick. The views expressed are those of the author(s) and not necessarily the NIHR, the Department of Health and Social Care or Public Health England.