Genotypic analysis of Shiga toxin-producing *Escherichia coli* clonal complex 17 in England and Wales, 2014-2022

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INTRODUCTION

Shiga toxin-producing *Escherichia coli* (STEC) is a zoonotic, gastrointestinal pathogen transmitted via the faecal-oral route

- Characterised by the presence of the Shiga toxin (*stx1* or *stx2)* gene
- Transmitted via food chain, contact with ruminant animals (e.g. cattle) or environmental sources contaminated with animal faeces[1]
- Symptoms range from mild to severe diarrhoea (often bloody), vomiting, abdominal pain, fever[1]
- Some STEC infections are associated with severe clinical outcomes e.g. haemolytic uraemic syndrome (HUS)[1]
  - ➢ STEC-HUS is the leading cause of renal failure in children <5 years of age and can be fatal

STEC serotype O157:H7 has been the most clinically significant serotype in the UK[2]

- In the past decade, there has been a decrease in incidence of STEC O157[3]
- PCR implementation at local hospital laboratories has led to an increase in detection of non-O157 STEC serogroups in recent years, such as O103[4,6]
- The US Food and Drug Administration has described the six key non-O157 STEC serogroups causing severe illness and death in humans globally – O26, O45, O103, O111, O121, O145[5]
- Increasing incidence of non-O157 STEC serogroups has highlighted the need for closer monitoring of these groups

Surveillance of STEC clonal complex (CC) 17 is necessary to:

- Understand the pathogenicity and public health burden of this clonal complex in the UK
  - ➢ STEC O103, one of the 'big six' serogroups associated with severe illness, belongs to CC17
- Facilitate earlier diagnosis, improve clinical management of patients, and the prevention and control of outbreaks

The aim of this study was to integrate genomic data with available epidemiological data to better understand the virulence and disease severity of CC17 in the UK.

METHODS

Isolates of *E. coli* belonging to CC17 (*n*=472 isolates from 421 patients) submitted to the Gastrointestinal Bacteria Reference Unit (GBRU), between January 2014 to December 2022, were included in this study.

- Whole genome sequencing was performed on all isolates in this study
- This was via paired-end sequencing using the Illumina Hiseq platform, as part of routine surveillance[6]

Bioinformatic pipelines were used for the analysis of raw sequencing data to infer:

- Identification (Kmer, ST, clonal complex)[6]
- Typing for strain discrimination (SNP address)[6]
- Characterisation[6]
  - ➢ virulence gene profiling
  - ➢ antimicrobial resistance gene determination

Genomic data was analysed retrospectively in conjunction with laboratory and epidemiological data

- Of the 472 total isolates, the dataset was de-duplicated to remove identical strains received from the same patient within a close timeframe
- Following de-duplication of data, 426 isolates subsequently underwent further analysis

RESULTS

From 2014 to 2022, 426 pathogenic *E. coli* cases belonging to CC17 were detected in England (*n*=393) and Wales (*n*=33)

- Majority of cases were female (*n*=246/426; 57.7%) and the age range was 0-100 years
  - ➢ Highest proportion of cases belonged to the 0-4 age group (*n*=82/422; 19.4%) (Figure 1)
- CC17 cases were detected in Wales and all regions of England
  - ➢ The South of England had the highest frequency (*n*=190/426; 44.6%), followed by London (*n*=65/426; 15.3%), and the North of England (*n*=63/426; 14.8%)
- Foreign travel was reported for 85/167 (50.9%) cases
  - ➢ 79/85 (92.9%) had travel information – travel to Mexico (*n*=23/85; 27.1%) and Egypt (*n*=12/85; 14.1%) had the highest incidence

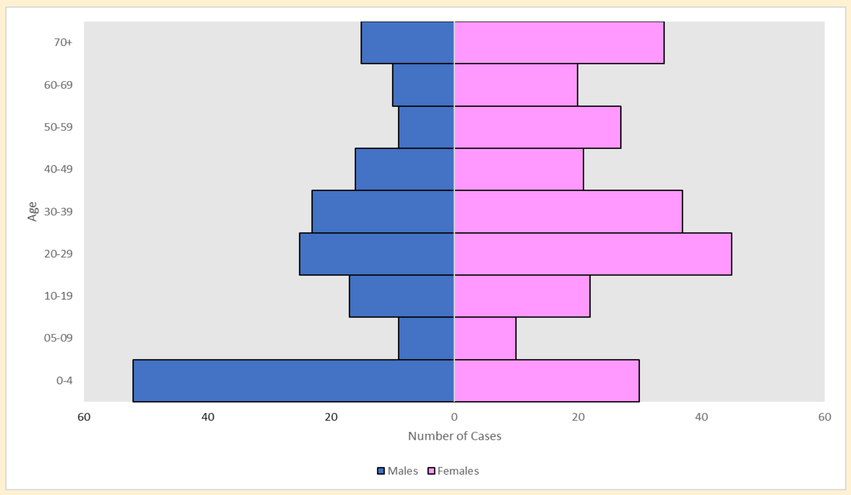

Figure 1. Age-sex distribution of STEC cases reported to UKHSA [isolates originating from England and Wales (*n*=422), where date of birth, sample receipt date and sex were available.

Overall, 359/426 (84.3%) isolates harboured 1 or more *stx* genes and were defined as STEC

- 67 isolates (15.7%) were enteropathogenic *E. coli* (EPEC) – positive for the intimin (*eae*) gene only and negative for *stx* genes
- Majority of STEC isolates (*n*=335/359; 93.3%) had the *stx1a/eae* virulence gene combination
- A minority of STEC isolates presented with other virulence profiles – *stx1a* (*n*=7; 1.9%); *stx1a/stx2a/eae* (*n*=3; 0.8%); *stx2a/eae* (*n*=10; 2.8%); *stx2d/eae* (*n*=2; 0.6%); *stx2f/eae* (*n*=1; 0.3%)

Reported cases of *E. coli* belonging to CC17 in England and Wales have increased overall each year from 2014 (*n*=16) to 2022 (*n*=165) (Figure 2)

- The highest number of CC17 cases were reported in 2022 (*n*=165/426):
  - ➢ England (*n*=151) and Wales (*n*=14)
- Cases decreased in 2020 (*n*=34) and 2021 (*n*=49) compared to total cases reported in 2019 (*n*=56)

Overall, there were 426 isolates of CC17 comprising five sequence types (STs):

- ST12 (*n*=1), ST17 (*n*=355), ST20 (*n*=9), ST376 (*n*=2) and ST386 (*n*=58)

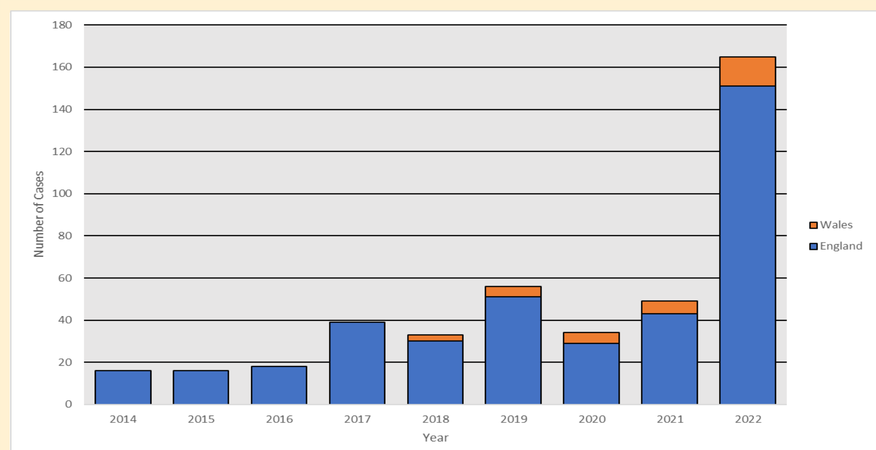

Figure 2. Annual number of cases belonging to CC17 reported to UKHSA, originating from England and Wales (*n*=426) from 2014 to 2022.

Seasonal variation in number of cases of STEC O103:H2 (*n*=258/426; 60.6%) and total CC17 cases (Figure 3)

- Two peaks in the number of STEC O103:H2 cases were observed throughout the year:
  - ➢ Summer peak from June to August
  - ➢ A second late Autumn peak in November

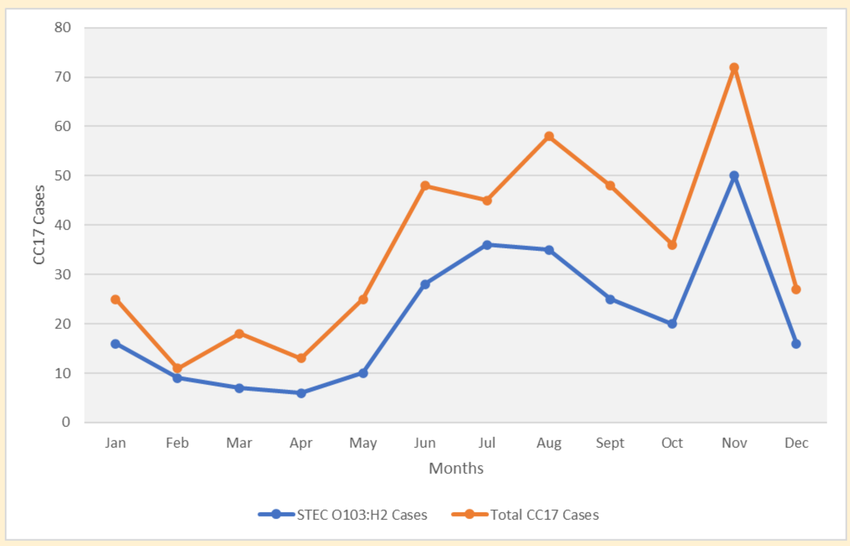

Figure 3. Number of STEC O103:H2 cases (*n*=258) and total CC17 cases (*n*=426) by months in England and Wales.

Genome-derived serotyping identified 5 established serotypes:

- O103:H2 was the predominant serotype (*n*=275/426; 64.6%)
- Other serotypes included:
  - ➢ O123:H2 (*n*=47/426; 11.0%)
  - ➢ O151:H2 (*n*=28/426; 6.6%),
  - ➢ O4:H2 (*n*=11/426; 2.6%)
  - ➢ O71:H2 (*n*=14/426; 3.3%)

Antimicrobial resistance (AMR) determinants:

- 83 of 426 CC17 isolates harboured AMR determinants
  - ➢ 5 harboured 1 AMR determinant
  - ➢ 11 harboured 2 AMR determinants
  - ➢ 67 harboured 3 or more AMR determinants
- AMR genes conferred resistance to:
  - ➢ β-lactams
  - ➢ Aminoglycosides
  - ➢ Macrolides
  - ➢ Sulfonamides
  - ➢ Chloramphenicol
  - ➢ Tetracyclines
- 343 isolates had no AMR determinants

DISCUSSION

Accurate assessment of true incidence and prevalence of STEC CC17 is challenging due to inconsistent surveillance

- Diagnosis of non-O157 STEC is inconsistent as algorithms vary between different regions
- Decrease of annual cases in 2020 and 2021 likely due to social distancing lockdown restrictions during the coronavirus disease 2019 (COVID-19) pandemic
- Cases were reported across all regions in England, with highest rates seen in South England and London
  - ➢ Higher proportion of local diagnostic laboratories in these regions have implemented PCR for detection of non-O157 STEC[1,7]
  - ➢ Hence geographical distribution of cases is dependent on which laboratories have implemented PCR, making interpretation based on geography difficult
- Strategies for the referral of PCR-positive faecal specimens to national reference laboratories for confirmation, culture and typing vary per region, impacting on surveillance[1]

Late autumn peak in seasonal variation of CC17 cases can be linked to outbreak of STEC O103:H2 in November 2022:

- 28/50 (56%) cases associated with outbreak in November 2022
- The temporal and geographical distribution of cases indicated the vehicle was foodborne and distributed by a national retailer
- The outbreak was investigated, and food exposure histories of the cases were reviewed, but no source was identified

CONCLUSIONS

- Nationwide implementation of PCR for the detection of all STEC serogroups is recommended, including improved consistency in strategies for the referral and follow-up confirmation/characterisation of PCR-positive faecal specimens
- More comprehensive and standardised policies regarding the sharing of sequencing data, including increased collaboration between public health agencies worldwide is recommended
- Enhanced surveillance is necessary to monitor changing trends in clinically significant STEC CC17 serogroups over time and facilitate the detection and investigation of future outbreaks
- Earlier detection will allow effective infection control and prevention approaches to reduce transmission of STEC and safeguard the public

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