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The epidemiology of Shiga toxin-producing *Escherichia coli* O26:H11 (clonal complex 29) in England, 2014–2021

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SUMMARY

Objectives: We aimed to describe the genomic epidemiology of the foodborne gastrointestinal pathogen, Shiga toxin-producing *Escherichia coli* (STEC) serotype O26:H11 belonging to clonal complex 29 (CC29) in England.

Methods: Between 01 January 2014 and 31 December 2021, 834 human isolates belonging to CC29 were sequenced at the UK Health Security Agency, and the genomic data was integrated with epidemiological data.

Results: Diagnoses of STEC O26:H11 in England have increased each year from 19 in 2014 to 144 in 2021. Most isolates had the Shiga toxin subtype profiles *stx1a* (47%), *stx1a,stx2a* (n = 24%) or *stx2a* (n = 28%). Most cases were female (57%), and the highest proportion of cases belonged to the 0–5 age group (38%). Clinical symptoms included diarrhoea (93%), blood-stained stool (48%), and abdominal pain (74%). Haemolytic Uraemic Syndrome (HUS) was diagnosed in 40/459 (9%) cases and three children died. All isolates causing STEC-HUS had *stx2a* either alone (n = 33) or in combination with *stx1a* (n = 7).

Conclusions: STEC O26:H11 are a clinically significant, emerging threat to public health in England. Determining the true incidence and prevalence is challenging due to inconsistent national surveillance strategies. Improved diagnostics and surveillance algorithms are required to monitor the true burden, detect outbreaks and to implement effective interventions.

Introduction

Gastrointestinal infections cause an estimated 17 million cases in the UK each year.[1] Outbreaks of zoonotic, foodborne bacterial gastroenteritis, such as campylobacteriosis, salmonellosis, yersiniosis and Shiga toxin-producing *Escherichia coli* (STEC) occur every year, with the highest number of incidents detected in late summer and early autumn.[2,3] Although the vast majority of cases report mild, self-limiting diarrhoea, symptoms can be persistent and severe, and can include abdominal pain, fever, vomiting and blood-stained stools. Patients infected with STEC, particularly the elderly and young children are at risk of developing haemolytic uraemic syndrome (HUS), a systemic condition involving renal failure and sometimes cardiac and/or neurological complications that can be fatal.[4–7] Although the annual number of STEC cases are lower than other more common GI pathogens, such as *Campylobacter* and *Salmonella* species, STEC remains a public health priority because of the severe clinical outcomes associated with infection and the risk of progression to HUS.[3,8]

For nearly 40 years STEC serotype O157:H7 has been the dominant STEC serotype in the UK and public health surveillance systems focused on detecting and monitoring this serotype.[9] Elsewhere, other STEC, most notably STEC O26:H11, were recognised as clinically significant, pathogenic serotypes.[10,11] In 2013, Bielaszewska et al.[12] described the emergence of a virulent clone of STEC O26:H11 in Europe associated with the acquisition of a bacteriophage encoding the Shiga toxin (Stx) gene variant, *stx2a*. More recently, STEC O26:H11 harbouring the *stx2d* variant have been described.[13] The

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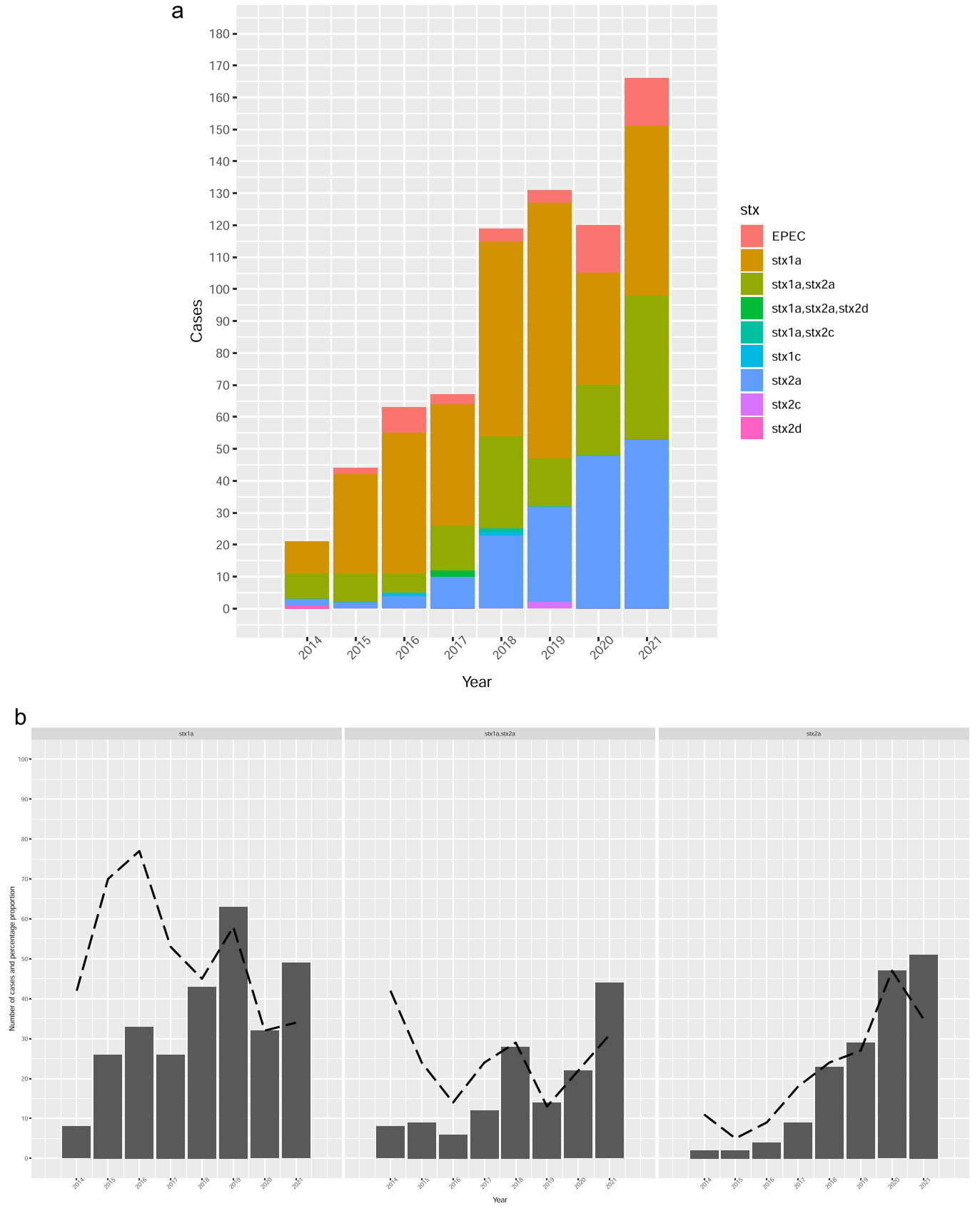

Fig. 1. a. Cases per year of *E. coli* belonging to CC29 (n = 731) corresponding to the left y-axis. The *stx* subtype breakdown across the years is also represented as the different stacks in each bar. b. Distribution of the three most reported stx profiles (*stx1a*, *stx1a,stx2a* and *stx2a*) in STEC O26:H11 of CC29 (n = 590). The bars indicate number of cases for each *stx* subtype profile each year. The line indicates what percentage proportion that *stx* subtype count was, out of the total number of STEC O26:H11 counts for each year.

Table 1
Diversity of serotypes of EPEC and STEC within the sequence types of CC29 (n = 731). Other STs (n = 6): ST295, ST1732, ST5429, ST6266, ST6277, ST11341.

Serotype	ST21	ST29	ST16	Other ST's	Total
O26:H11	562	54	0	5	621
O111:H8	0	0	41	0	41
O177:H11	0	19	0	0	19
O unidentifiable:H11	6	1	0	0	7
O69:H11	7	0	0	0	7
O123:H11	1	5	0	0	6
O118-O151:H16	5	0	0	0	5
O71:H11	3	1	0	0	4
O151:H16	3	0	0	0	3
O123-O186:H11	0	2	0	0	2
O82:H11	0	2	0	0	2
O92:H11	0	1	0	0	1
O111:H40	0	0	1	0	1
O34:H9[a]	0	4	0	0	4
O70:H11[a]	0	4	0	0	4
O unidentifiable:H8[a]	0	1	0	0	1
O49:H8[a]	0	1	0	0	1
O84:H27[a]	0	0	0	1	1
O96:H11[a]	0	1	0	0	1
Total	587	96	42	6	731

[a] No isolates in this serotype had *stx* (EPEC only serotype).

ability to produce Stx is the defining pathogenic feature of STEC and there are at least 10 different Stx variants or subtypes.[14,15] The Stx subtypes *stx2a* and *stx2d* are significantly associated with STEC that have the potential to cause STEC-HUS.[16] Like STEC O157:H7, STEC O26:H11 carry a pathogencity island called the locus of enterocyte effacement (LEE),[17] encoding proteins that facilitate intimate attachment of STEC to the host gut mucosa.[18]

Animal studies have shown that the zoonotic reservoir for STEC O26:H11 is ruminants, although other animals can act as transient vectors.[19,20] Transmission to humans can occur via direct or indirect contact with animal faeces or the consumption of contaminated food.[21–23] Foodborne outbreaks of STEC O26 have been described in the UK and elsewhere.[24–26] Like STEC O157:H7, the infectious dose is low and person-to-person transmission in households and childcare setting have been described.[27,28]

Since 2012, the number of diagnostic microbiology laboratories in the UK using molecular diagnostic assays for GI pathogens targeting *stx*, has increased from none to at least 30 laboratories.[29] This has improved the detection of all STEC serotypes, and consequently, we have observed an increase in the number of diagnoses of non-O157 STEC serotypes, with STEC O26:H11 being the most frequently detected.[29] STEC O26:H11 belongs to clonal complex 29, one of four STEC clonal complexes (CC) along with CC11, CC32 and CC165, most commonly associated with causing STEC-HUS in the UK.[8,30,31] Genomic analyses of the population structure of STEC O26:H11 has been described globally and in England, previously,[32,33] with data identifying lineages and sub-lineages associated with travel, and domestic acquisition, and *stx* profiles.[32,33]

The aim of this study was to integrate genomic data with epidemiological information from cases of STEC CC29 resident in England to gain insight regarding source and transmission routes, virulence profiles, disease severity, and populations most as risk of infection with this clinically significant STEC clonal complex.

Methods

Microbiology

In England, all stool samples from hospitalised patients and community-acquired gastrointestinal (GI) infections are tested for STEC O157:H7 using cefixime-tellurite sorbitol MacConkey agar (CTSMAC) agar, and non-sorbitol fermenting colonies agglutinating with *E. coli* O157 antisera are referred to the Gastrointestinal Bacteria Reference Unit (GBRU), UK Health Security Agency (UKHSA) for confirmation and typing. Where local laboratories have implemented a commercial GI PCR targeting *stx*, all faecal specimens are tested for all STEC serotypes. *Stx*-positive samples are cultured on CTSMAC and/or Chromoagar for STEC, and colonies exhibiting characteristics indicative of STEC may be referred to GBRU. Alternatively, the *stx*-positive faecal specimen may be referred to GBRU for PCR and culture. Microbiological results are stored in the Gastro Data Warehouse (GDW).

STEC surveillance

The STEC operational guidance recommends that the STEC Enhanced Surveillance Questionnaires (ESQ) is administered to all cases, however, cases of STEC O157, and patients infected with STEC harbouring *stx2* and *eae* or *aggR*, and/or children under the age of six are prioritised. The guidance was updated in July 2021 to include STEC O26 in the prioritisation. The collected data includes travel and food histories, environmental and animal exposures and clinical symptoms. These epidemiological data is paired with genomic and microbiological data for each case and stored in the National Enhanced STEC Surveillance System (NESSS).

STEC epidemiological data were analysed to understand clinical presentation, food history, and animal exposures. Travel-associated infections were investigated for all STEC belonging to CC29, and travel data was extracted from NESSS, and where cases were lost to follow up, travel data (where available) was extracted from GDW. For age sex analysis, the patients infected with STEC serotypes O26:H11, STEC O111:H8 and STEC O117:H11 were included.

DNA extraction and genomic processing

Genomic DNA was extracted and sequenced on Illumina HiSeq 2500 platform[32] and held in the UKHSA in-house data warehouse. Post whole genome sequencing (WGS), isolates are processed through an in-house pipeline that determines serotype and *stx* subtype using GeneFinder (https://github.com/phe-bioinformatics/gene_finder).[34] Multilocus sequence typing (MLST) was performed using Metric Orientated Sequence Typer (https://github.com/phe-bioinformatics/MOST), as described in Tewolde et al.[35] Enterobase[36] was used to confirm the ST of isolates that were flagged as a SLVs of an ST in the UKHSA database (Supplementary Table 1). Antimicrobial resistance was determined using GeneFinder (https://github.com/phe-bioinformatics/gene_finder) and the UKHSA in-house database.

AMR profiles are displayed using Upset R (http://gehlenborglab.org/research/projects/upsetr/).

As described by Rodwell et al.,[31] in previous studies; SnapperDB v0.2.6[37] is the UKHSA in-house database that holds variant data, obtained from genomic DNA sequencing, relative to an appropriate reference for *Escherichia coli* CCs. SnapperDB v0.2.6[37] was employed to generate a whole genome alignment of isolates representing the t:250 level of CC29. Gubbins v2.0.0[38] was used on the whole genome alignment to identify recombinant regions, which were then masked during the building of a second alignment of isolates within this study, where variant positions that belonged to a minimum of 80% of strains in the alignment. This alignment was examined by IQTree v2.0.4[39] which produced a maximum-likelihood phylogeny that was visualised in ITOL v5.7.[40]

SNP cluster and phylogenetic clade designation and identification

SNP (single nucleotide polymorphism) addresses (described by Dallman et al.[29]) are generated as part of the processing pipeline in UKHSA for all isolates submitted to GBRU and are used to identify closely related strains and subsequently, clusters. As described previously,[32] the SNP address is based on a pairwise clustering approach

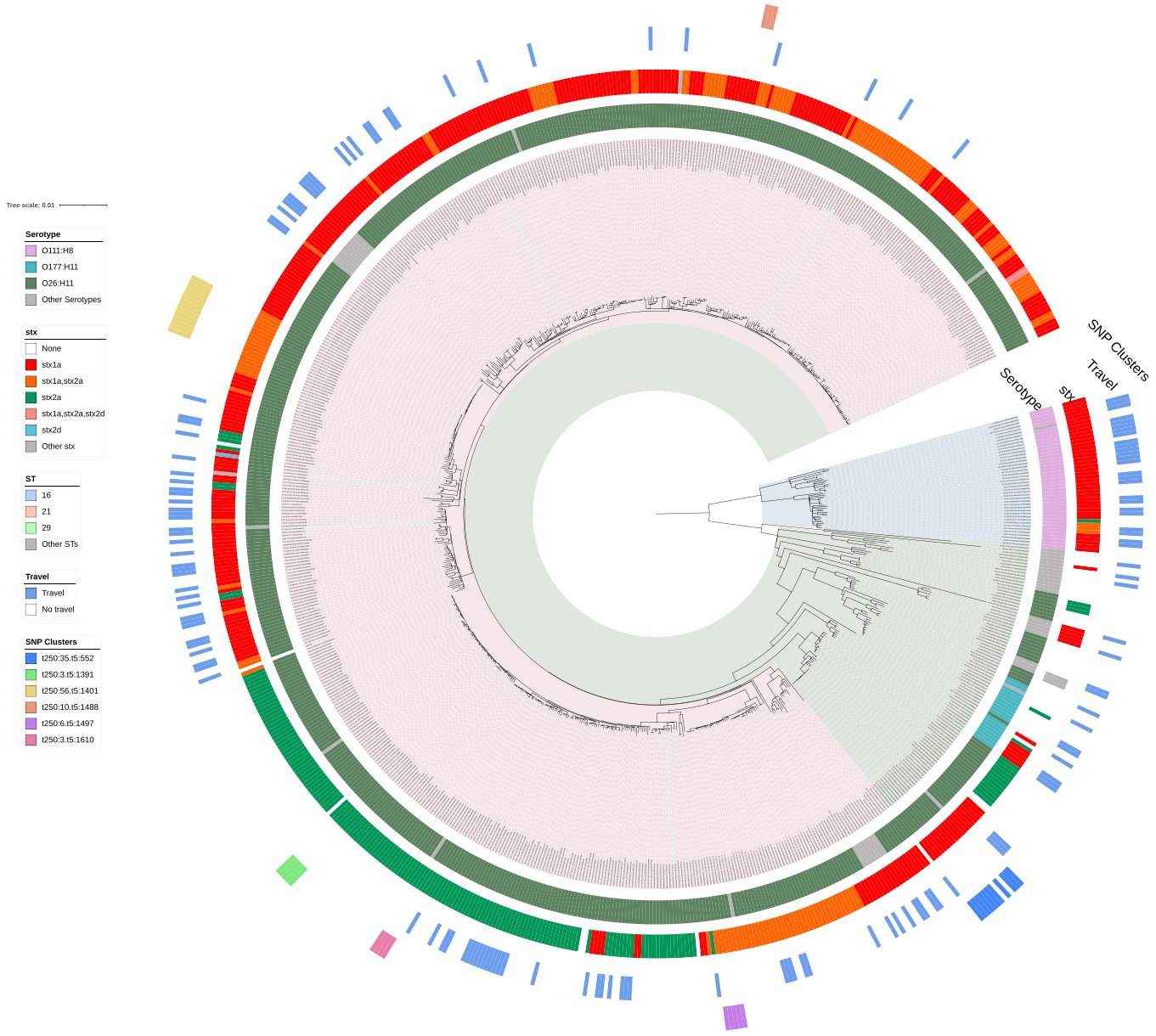

Fig. 2. Population structure of CC29 in England, where ST, serotype, *stx* profile, travel and the 6 SNP clusters are indicated, from the inward colour to outward bars.

to assign distance threshold levels that descend: Δ250, Δ100, Δ50, Δ25, Δ10, Δ5, Δ0. Each isolate within one level is no more than that many SNPs apart from the next isolate within the same level. For this study, clusters were defined as 3 or more isolates that differ in the 0 SNP or 5 SNP level.

Previous studies have shown that ST16, ST21 and ST29 form distinct lineages within the CC29 population structure.[32,33] ST21 subdivides into two clades as defined by Ogura,[33] ST21C1 and ST21C2, and we previously showed that in England, ST21C1 can be differentiated into four clades, defined at the t250 SNP cluster level as t:10, t:3, t:6, t:56.[32]

Results

Overview of CC29 in England

There were 834 human isolates belonging to CC29 reported in England from January 2014 to December 2021, from 724 patients. Seventy-six patients had multiple isolations of the same strain (defined as the same *stx* profile and SNP type) and 4 patients had multiple isolates of different strains of CC29, making a total of 731 unique strains. Of these, 680/731 (93%) had *stx* and *eae*, 50/731 (7%) had *eae* but not *stx*, and were designated enteropathogenic *E. coli* (EPEC), 1/731 (0.1%) had *stx* but not *eae* and 1/731 (0.1%) isolates that had neither *eae* or *stx* (Supplementary Table 1). Diagnoses of infection caused by CC29 increased every year with the exception of 2020 (Fig. 1a). The seasonality of STEC O26:H11 follows similar patterns to STEC O157:H7, where cases rise in the summer months, peak in August and decrease to baseline levels during the winter months (Supplementary Fig. 1).

The most common serotype was O26:H11 (621/731, 85%), followed by O111:H8 (41/731, 6%), and O177:H11 (19/731, 3%). The remaining isolates belonged to 16 different serotypes (50/731, 7%) (Table 1, Supplementary Table 1). Most isolates belonged to ST21 (587/731, 80%), ST29 (96/731, 13%) and ST16 (42/731, 6%) (Fig. 2). There were six additional STs (ST295, ST1732, ST5429, ST6266, ST6277, ST11341), each with only 1 isolate (Table 1, Supplementary Table 1 and Fig. 2). Shiga toxin subtyping of isolates of CC29 revealed

Table 2
Diversity of serotypes of EPEC and STEC and the associated stx subtype profiles of CC29 (n = 731). *No isolates in this serotype had *stx* (EPEC only serotype).

Serotype	stx-negative	*stx1a*	*stx1a,stx2a*	*stx1a,stx2a,stx2d*	*stx1a,stx2c*	*stx1c*	*stx2a*	*stx2c*	*stx2d*	Total
O26:H11	25	280	143	2	1	0	167	2	1	621
O111:H8	0	37	3	0	0	0	1	0	0	41
O177:H11	11	6	0	0	0	0	2	0	0	19
O unidentifiable:H11	0	3	2	0	0	0	2	0	0	7
O69:H11	0	7	0	0	0	0	0	0	0	7
O123:H11	1	4	0	0	0	1	0	0	0	6
OgC4-O118-O151:H16	0	5	0	0	0	0	0	0	0	5
O34:H9*	4	0	0	0	0	0	0	0	0	4
O70:H11*	4	0	0	0	0	0	0	0	0	4
O71:H11	1	3	0	0	0	0	0	0	0	4
O151:H16	0	3	0	0	0	0	0	0	0	3
O123-O186:H11	0	2	0	0	0	0	0	0	0	2
O82:H11	1	1	0	0	0	0	0	0	0	2
O unidentifiable:H8*	1	0	0	0	0	0	0	0	0	1
O111:H40	0	1	0	0	0	0	0	0	0	1
O49:H8*	1	0	0	0	0	0	0	0	0	1
O84:H27*	1	0	0	0	0	0	0	0	0	1
O92:H11	0	0	0	0	0	1	0	0	0	1
O96:H11*	1	0	0	0	0	0	0	0	0	1
Total	51	352	148	2	1	2	172	2	1	731

that the most common *stx* subtype profile was *stx1a* (352/680, 52%), followed by *stx2a* (172/680, 25%), and *stx1a,stx2a* (148/680, 22%) (Fig. 1, Table 2, Supplementary Table 1 and Fig. 2). The *stx* profiles of the remaining eight isolates included *stx1a,stx2a,stx2d* (2/680, 0.3%), *stx1c* (2/680, 0.3%), *stx2c* (2/680, 0.3%), *stx1a,stx2c* (1/680, 0.1%) and *stx2d* (1/680, 0.1%) (Fig. 2).

The most common subtype detected for STEC O26:H11, STEC O111:H8 and STEC O177:H11 was *stx1a*, accounting for 45% (280/621), 90% (37/41) and 32% (6/19) of the respective isolates. The detection of STEC O26:H11 isolates that had *stx2a* either alone or in combination with *stx1a,* followed an increasing trend during the course of this study (Fig. 1b) whereas STEC O26:H11 isolates harbouring *stx1a* decreased as a percentage proportion of total STEC cases each year (Fig. 1b).

Epidemiology of STEC belonging to CC29

Cases of STEC belonging to CC29 were located in regions across England, with the South East of England reporting the most cases (265/680, 39%), followed by London (131/680, 19%). The remaining cases resided in regions across England in the North West (53/680, 8%), West Midlands (46/680, 7%), South West (44/680, 7%), East of England (42/680, 6%), North East (41/680, 6%), East Midlands (36/680, 5%) and Yorkshire and Humber (22/680, 3%) (Fig. 3).

The age-sex distribution of the most common STEC serotype, O26:H11 (where age and sex were available, n = 591), revealed that 57% of cases were female (339/591) with a median age of 21 years (IQR: 3–46, and the median age for males was 13 (IQR: 1–31). Additionally, the highest proportion of cases belonged to the 0–5 age group for both males (107/252, 42%) and females (117/339, 35%) (Fig. 4). For STEC O111:H8 and STEC O177:H11, there was a higher proportion of male cases (21/41, 52% and 6/8, 75% respectively) (Supplementary Fig. 2).

We analysed the distribution of age-sex data in relation to the three common *stx* subtypes exhibited by STEC O26:H11 (Fig. 5). The proportion of females was highest in cases infected with a STEC O26:H11 strain that had *stx1a* (n = 169/278, 61%), or both *stx1a,stx2a* (n = 82/140, 59%). The male:female ratio for cases infected with a strain harbouring *stx2a* only was 1:1 (females: 84/167 (50%), males: 83/167 (50%). The median ages of both males and females were higher in those cases associated with *stx1a* (21 years both) and for females with stx1a,stx2a (22 years) than those with an *stx2a* strain (4 and 9 years, respectively) and males with *stx1a,stx2a* (13 years) (Fig. 5).

Clinical outcome based on serotype and stx profile

The proportion of STEC O26:H11 cases reporting nausea, vomiting and fever were consistent irrespective of the *stx* profile, whereas cases infected with STEC O26:H11 *stx1a*, either alone or in combination with *stx2a*, were more likely to report having blood-stained diarrhoea (Table 2). A higher proportion of HUS was reported by cases infected with STEC O26:H11 *stx2a*, either alone (32/162, 20%) or in combination with *stx1a* (7/127, 6%) when compared with those infected with STEC O26:H11 *stx1a* only (0/157, 0%). All but one of the cases that had HUS were infected with STEC O26:H11 (39/40), the exception being infected with STEC O177:H11 *stx2a* (1/40). Of the patients that developed STEC-HUS, 21/40 (53%) were male. The median age profile for these HUS cases was similar for both genders (male = 4 (range 0–20); female = 3 (range 1–29) and most of the cases were aged 5 or under (male 15/21, 75%; female 14/19, 74%). During the study period, the number of cases of HUS increased from 2 in 2014 to 11 in 2021 (2014 = 2/18, 11%; 2015 = 0/33; 2016 = 0/41; 2017 = 2/36, 5%; 2018 = 6/71, 8%; 2019 = 8/69, 11%; 2020 = 11/76, 16%; 2021 = 11/133, 8%).

Travel

Within CC29, there were 133/680 (20%) STEC cases reporting recent travel outside the UK in the 7 days prior to onset of symptoms (O26:H11, 91/680, 13%; O111:H8, 25/680, 4%; O177:H11, 4/680, 0.6%; O69:H11, 3/680, 0.4%; O71:H11, 2/680, 0.3%; O123-O186:H11, 2/680, 0.3%; O151:H16, 2/680, 0.3%; O118-O151:H16, 2/680, 0.3%; O123:H11, 1/680, 0.1%; O unidentifiable:H11, 1/680, 0.1%). The most common international travel destinations included Egypt (n = 16), Turkey (n = 15), Morocco (n = 11), Mexico (n = 9), Romania (n = 6), Ireland (n = 6), India (n = 5), France (n = 5), Portugal (n = 4). Travel data was available for 9 EPEC cases, where destinations were France, Pakistan, Egypt (2 cases each), Mexico, Dominica and Peru (1 case each).

The proportion of travel-related cases varied by serotype and by clade (Fig. 2). Of the three most common serotypes, the highest proportion of travellers had O111:H8 (25/41, 61%) compared to 15% and 21% for O26:11 (91/621) and O177:H11 (4/19), respectively. For STEC O26:H11, the clade designated t250:1 by Dallman et al.[32] had the highest proportion of cases reporting international travel (21/44, 48%) to Europe (13/21, 62%), Latin America (5/21, 24%), and Africa (2/21, 10%) and Oceania (1/21, 5%) (Fig. 2). Although the majority of cases in the clade designated t250:3 were domestically acquired, there were sub-clades of cases linked to travel, with common

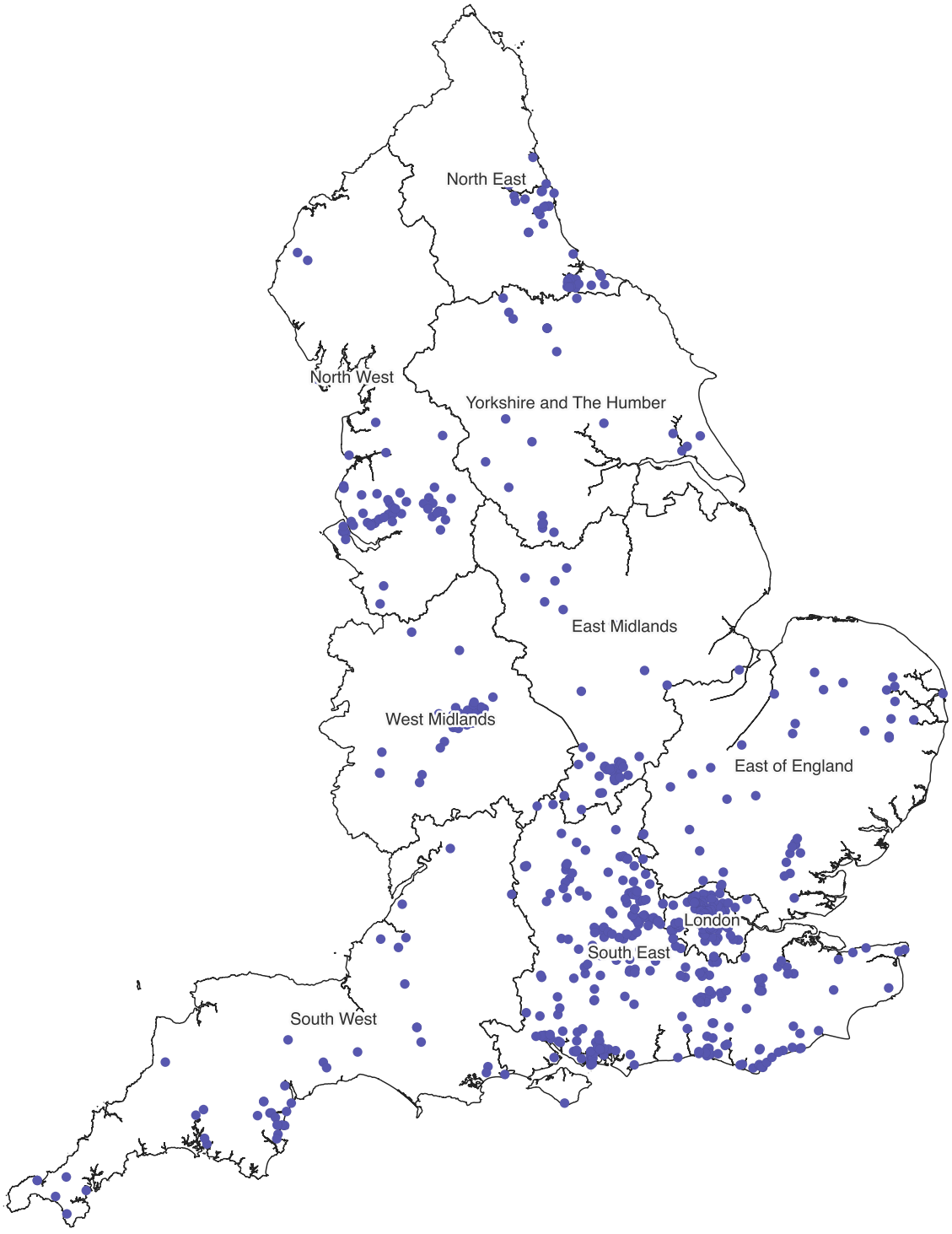

Fig. 3. Distribution of STEC cases belonging to CC29 in England, where postcode was available (n = 668).

destinations being Egypt (10/23, 43%), Turkey (5/23, 22%) and Romania (2/23, 9%) Fig. 2).

Outbreaks of STEC O26:H11 in England

Community clusters and outbreaks of O26:H11 in England prior to 2020 have been described previously[32] and included six travel-related clusters and one outbreak linked to the salad component of prepacked sandwiches.[24] Between 01 January 2020 and 31 December 2021, 6 additional 5 SNP single linkage clusters comprising three or more cases were detected during routine surveillance activities (Table 3). Two clusters were geographically linked (max distances: 5 km and 17 km) and 4 were geographically dispersed across different regions in England (max distances: 160 km, 190 km, 481 km and 591 km). The two largest clusters comprised cases identified over a wider time frame, 16 cases identified over 14 months for t5:1401 and 11 cases over 44 months for t5:552 (Supplementary Fig. 3). Most cases linked to these two clusters were adult females (t5:1401 adults 16/16, 100%, females 10/16, 63%; t5:552 adults 11/11, 100%, females 7/11, 64%), and were geographically and temporally dispersed with cases recurring on a yearly basis. For both clusters, there was epidemiological evidence

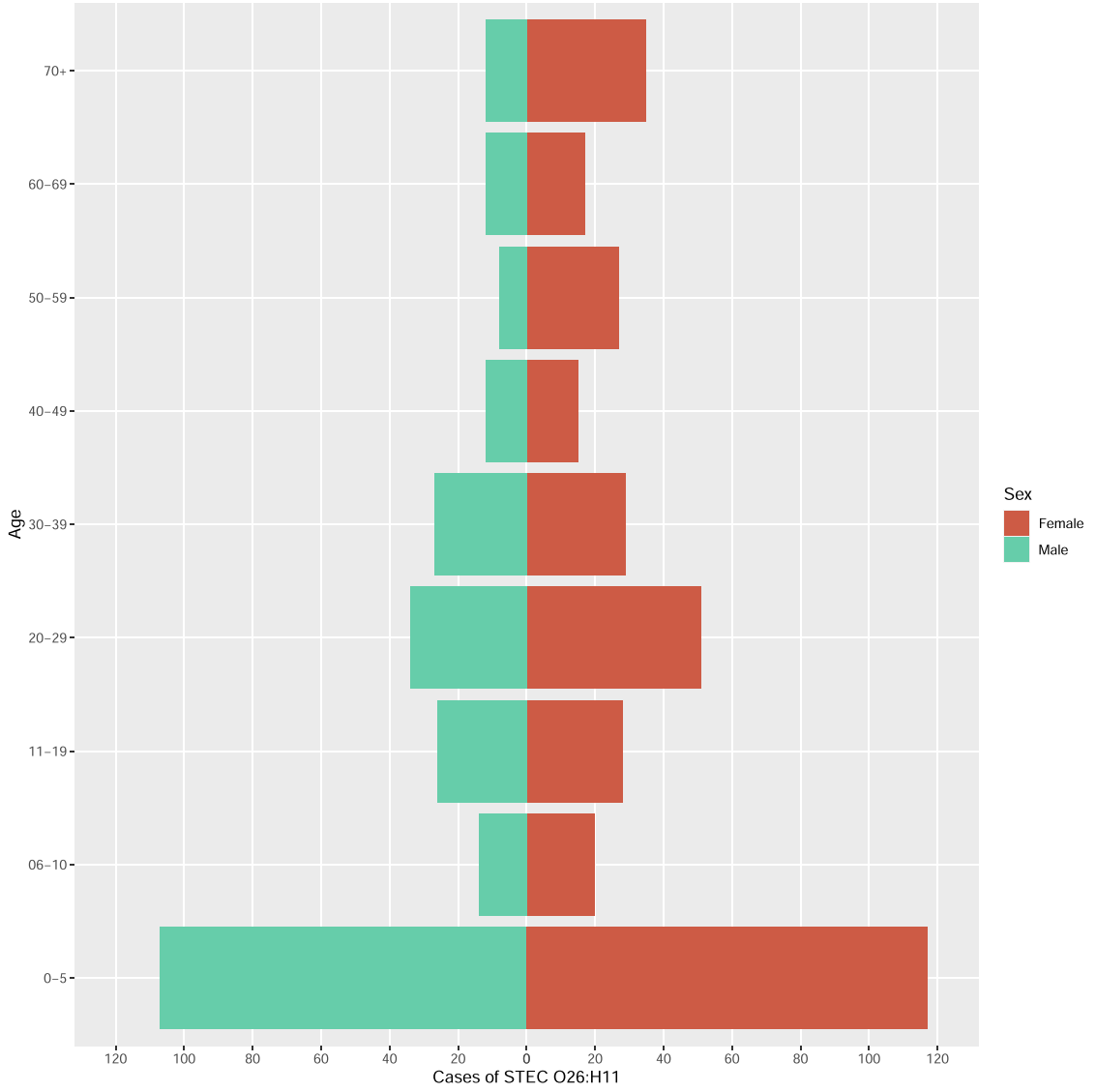

Fig. 4. Age-sex distribution of STEC O26:H11 cases in England, where age and sex were both available (n = 591). Red indicates female data and green indicates male data.

implicating salad items as a potential vehicle of infection, but this association was not confirmed (UKHSA in-house data). Of the remaining four clusters, one (designated t5:1488) was associated with a petting farm and the potential source of infection was not identified for the other three.

Antimicrobial resistance

Of the STEC isolates we profiled *in silico*, 458/679 (67.5%) did not harbour any resistance genes in the reference database, and full susceptibility to all 8 classes of antibiotics included in this analysis was inferred. There were 162/679 (24%) isolates that were multidrug (MDR) resistant, defined as harbouring three or more AMR determinants. AMR determinants known to confer resistance to the beta-lactams were $bla_{\text{TEM-1}}$ (n = 75/679, 11%) and $bla_{\text{CTX-M-15}}$ (n = 6/679, 0.8%) (Supplementary Table 2). The most common profiles conferring aminoglycoside and tetracycline resistance, respectively, were *strA,strB* (n = 184/679, 27%) *tetA/tetA-1* (n = 150/679, 22%). There were 183/679 (27%) isolates predicted to resistant to sulphonamide based on the presence of *sul1* and/or *sul2*, and variants of *dfrA* conferring trimethoprim resistance were detected in 48/679 (7%) isolates (Supplementary Table 2). Mutations in the quinolone resistance determining region of *gyrA* known to confer reduced susceptibility to fluoroquinolones were present in 21 isolates either on its own (n = 18) or in combination with a mutation in *parC* (n = 3). There were 15 isolates with resistance-associated genes including *mphA* (3/679, 0.4%) and *mphB* (12/679, 2%) that confer resistance to macrolides. The most common resistance profile was resistance determinants associated with aminoglycoside, sulphonamide and tetracyclines resistance was in 66/679 (10%) isolates (Fig. 6). The next common profile was genes associated with aminoglycoside, sulphonamide, tetracycline and beta-lactam (n = 23) Fig. 6).

Discussion

In England, the number of cases of CC29 have increased over the last 10 years, with the number of notifications in 2021 being eight times that recorded in 2013. This increase is likely due for the most part to the increasing number of local hospital laboratories in England that have adopted a PCR approach to GI diagnostics, facilitating their capacity to detect non-O157 STEC serotypes, including STEC O26:H11.[29,41]

There is evidence, however, that PCR implementation may only attribute to part of the increase in the burden of gastrointestinal infections caused by of STEC in England. Previous studies showed that historically UK cattle were colonised with STEC O26:H11

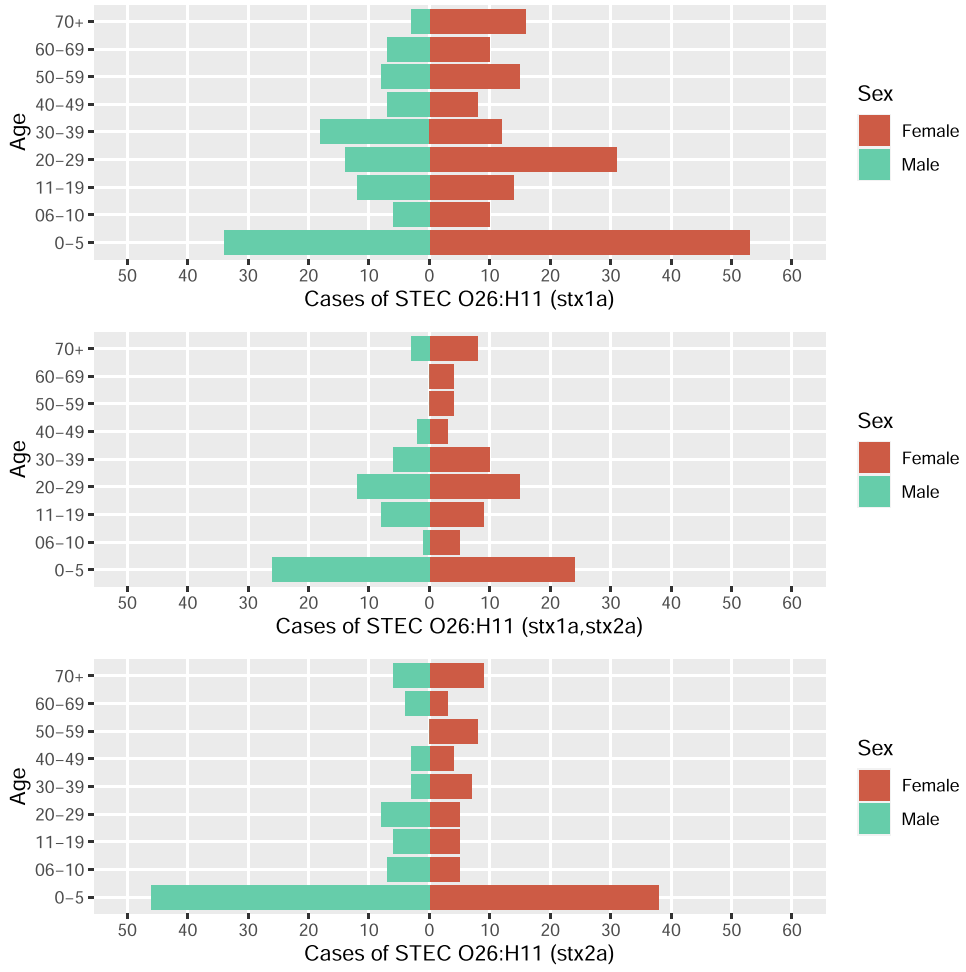

Fig. 5. Age-sex distribution of cases of STEC O26:H11 three most common *stx* profiles. From top to bottom, right, the data reflects *stx1a* (n = 278), *stx1a,2a* (n = 140) and *stx2a* (n = 167). Red indicates female data and green indicates male data.

harbouring *stx1a*.[19,42] These strains have been causing gastrointestinal diseases in humans for over 40 years; in fact, early work in this laboratory of *stx* phage was performed on such strains.[43,44] However, the phylogenetic analysis presented here and in our previous studies suggest that certain clades within these domestic sublineages have acquired *stx2a* encoding bacteriophage, associated with causing more severe clinical outcomes including STEC-HUS.[32] In certain clades, this corresponded with the loss of *stx1a*, while others carried both *stx* subtypes. It is also possible that previously described *stx2a* encoding clones of STEC O26:H11 were imported from elsewhere and become endemic in the UK cattle population.[12,45,46] Data analysed in this study provide evidence that over

Table 3
Clinical symptoms reported by patients where available, displayed as the three common serotypes (O26:H11 (highlighted in bold), O111:H8 and O177:H11) and *stx* subtypes *stx1a*, *stx1a,stx2a* and *stx2a* within CC29.

	stx2a						*stx1a,stx2a*				*stx1a*						Total	
	STEC O26:H11 (n = 162)		STEC O111:H8 (n = 1)		STEC O177:H11 (n = 2)		**STEC O26:H11 (n = 127)**		STEC O111:H8 (n = 3)		**STEC O26:H11 (n = 157)**		STEC O111:H8 (n = 21)		STEC O177:H11 (n = 4)		(n = 477)	
Symptom	**Yes**	**%**	Yes	%	Yes	%	**Yes**	**%**	Yes	%	**Yes**	**%**	Yes	%	Yes	%	Yes	%
Asymptomatic	**12**	**7**	0	0	0	0	**5**	**4**	0	0	**1**	**1**	0	0	0	0	18	4
Symptomatic	**150**	**93**	1	100	2	100	**122**	**96**	3	100	**156**	**99**	21	100	4	100	459	96
Diarrhoea	**127**	**78**	1	100	2	100	**119**	**94**	2	66	**154**	**98**	20	95	4	100	429	93
Abdominal pain	**96**	**59**	1	100	1	50	**99**	**78**	3	100	**121**	**77**	16	80	4	100	341	74
Blood stool	**55**	**34**	1	100	1	50	**71**	**56**	1	33	**84**	**54**	7	35	1	25	221	48
Nausea	**58**	**36**	0	0	1	50	**43**	**34**	1	33	**68**	**43**	4	20	2	50	177	39
Vomiting	**59**	**36**	0	0	1	50	**41**	**32**	0	0	**50**	**32**	3	15	1	25	155	34
Fever	**40**	**25**	1	100	1		**37**	**29**	1	33	**48**	**31**	4	20	4	100	136	30
HUS	**32**	**20**	0	0	1	50	**7**	**6**	0	0	**0**	**0**	0	0	0	0	40	9
Admitted to hospital	**61**	**38**	0	0	1	50	**37**	**29**	1	33	**41**	**26**	1	5	1	25	143	31
Outcome died	**3**	**2**	0	0	0	0-	0	0	0	0	**0**	**0**	0	0	0	0	3	1

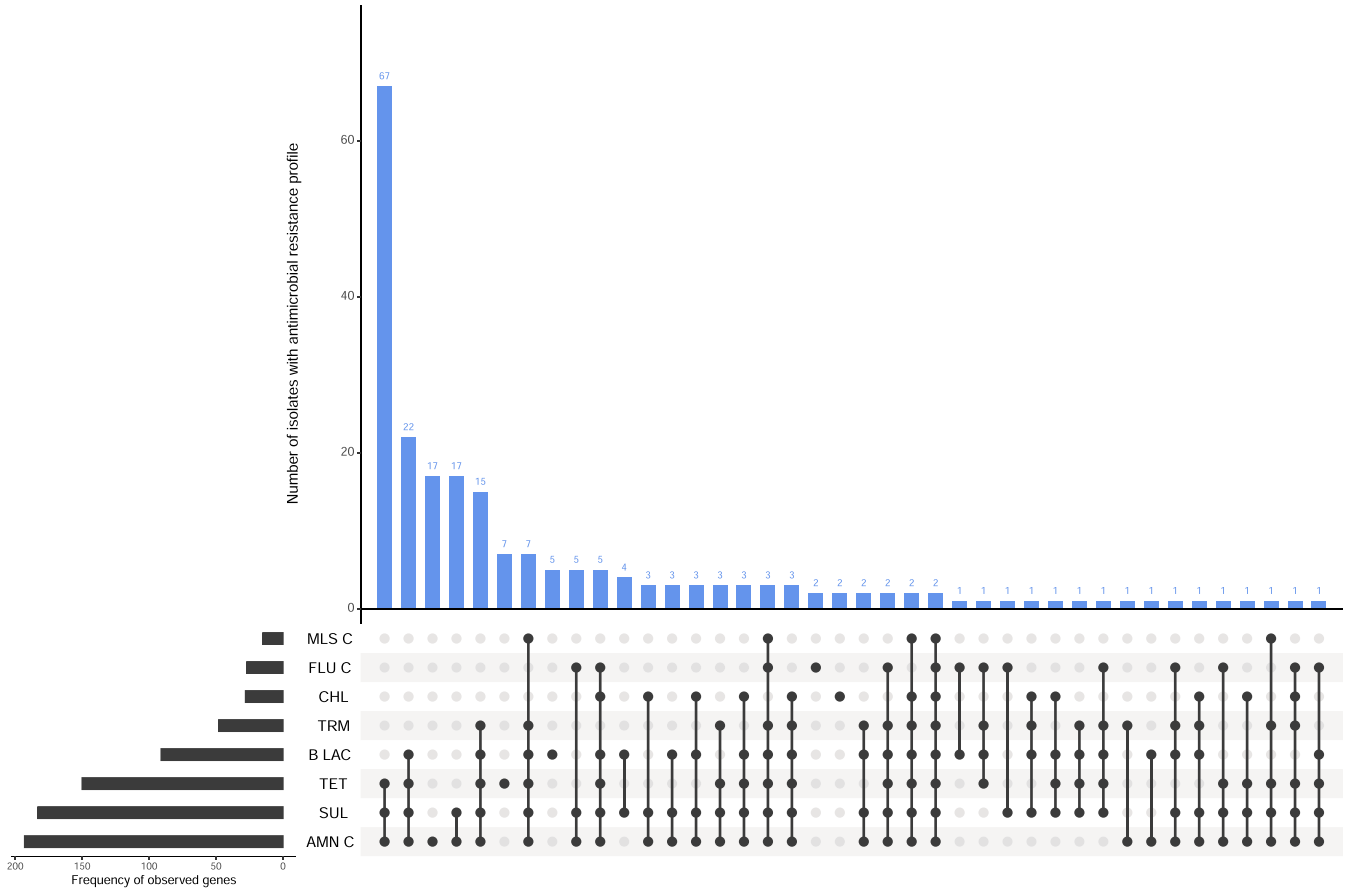

Fig. 6. Antimicrobial resistance profiles of CC29 679 STEC isolates. In silico detection of an AMR-associated gene was performed using gene-finder and the UKHSA AMR gene database, displayed using Upset in R. Gene data is found in Supplementary Table 2. Abbreviations are: B-LAC (beta-lactam), AMN-C (aminoglycoside), FLU-C (fluoroquinolone), MLS-C (macrolide), TRM (trimethoprim), TET (tetracycline), SUL (sulphonamide), CHL (chloramphenicol).

the last decade, the proportion of isolates of STEC O26:H11 harbouring *stx2a* alone or in combination with *stx1a*, and the number of cases of STEC-HUS caused by STEC O26:H11, has increased.

Overall, as previously described for STEC O157:H7 and STEC O145:H28, a higher proportion of cases of STEC O26:H11 were female, and the incidence was highest among children between 0 and 5 years old.[24,31] The highest rates were South East and London, where a higher proportion of local diagnostic laboratories have implemented PCR and this pattern has been seen with other clonal complexes of non-O157 STEC in England.[30,31] There is evidence from previous studies that cattle are an important zoonotic reservoir of STEC O26:H11, in the UK and elsewhere,[19–21] and analysis of outbreak data indicates that routes of transmission mirror those of STEC O157. Previous outbreaks in the UK and elsewhere have been associated with contaminated beef/dairy products and salad/raw vegetable produce,[22,23] in addition to contact with animals and person to person spread within households and nursery school settings.[27,28] Of the recent outbreak of STEC O26:H11 in England, contaminated salad items were implicated as the vehicle of infection in one previously published outbreak[24] and two of the clusters of cases described in this study.

Diarrhoea, vomiting and hospitalisation rates were comparable to those of STEC O157:H7 infection.[8] Although the proportion of cases reporting blood-stained stools, abdominal pain and fever was lower than for cases of STEC O157:H7, like STEC O145:H28 the risk of developing HUS was higher.[8,31] All STEC isolated from the cases of STEC-HUS had *stx2a*, either alone or in combination with *stx1a*. In a previous study, we found that STEC O157:H7 harbouring *stx2a* were most commonly associated with severe clinical outcomes such as HUS and hospital admission, which is consistent with our findings on STEC O26:H11 harbouring stx2a in the current study.[16] Clinical symptoms of gastroenteritis presenting in children are more often referred to healthcare, and this may reflect the ascertainment of STEC positive and HUS diagnoses of those in the under 5 age group.

Prior to 2021, the UKHSA STEC Operational Guidance focused on public health follow up with respect to administering an enhanced surveillance questionnaire (ESQ) and requiring microbiological clearance of patients in risk groups of those cases with STEC harbouring *stx2*. In light of the data presented here, which showed STEC O26:H11 that had *stx1a* leads to severe clinical outcomes (over half reported having bloody diarrhoea with a quarter being hospitalised) the guidance has been amended include public health follow up on all cases of STEC O26:H11, regardless of the *stx* profile.[47]

In a previous study, we concluded that three of the clades (designed t250:10, t250:3, t250:6 in Dallman et al., 2021,[32]) had most likely colonised the UK cattle population and were endemic in the UK. We also hypothesised that t:1 and t:8, were travel-associated clades. In this study, superimposing case travel histories on to the phylogeny, indicated that isolates belonged to serotype O111:H8 (ST16), and O26:H11 (ST21) t250:1, were more likely to be travel-associated or linked to an imported food vehicle, and those belonging to ST21 t250:10 (O26:H11) and ST21 t250:6 (O26:H11) were more likely to be domestically acquired. During outbreaks of foodborne disease, information on the likely origin of the outbreak strain can direct the investigation as to whether the vehicle is an imported food item or domestically produced.[48]

The majority of isolates belonging to CC29 were predicted to be fully susceptible to the eight classes of antibiotic included in the

testing algorithm, based on the genome derived AMR profiles. The most commonly detected genes conferred resistance to the aminoglycosides, beta-lactams, sulphonamides and tetracyclines, and these classes of antimicrobials account for the majority of antimicrobials sold for veterinary use. Historically, antimicrobial use in animal husbandry prior to the introduction of industry regulations and guidance in the 1990s, was a potential driver for the acquisition of the resistance determinants observed in STEC O26:H11 today.[31,49,50]

With the exception of 2020 when numbers were most likely impacted by social distancing restrictions implement due to the COVID pandemic, there has been a year-on-year increase of notification of cases of STEC O26:H11 in England. The burden of human disease caused by this zoonotic, foodborne pathogen is a public health concern for the farming community, food business operators and health practitioners. The increasing proportion of strains harbouring *stx2a*, associated with the potential to cause STEC HUS is cause for further concern. It is essential that both the diagnostic capabilities at local levels and sensitivity of national surveillance of non-O157 STEC continue to improve[41] to assess the true burden of these serotypes on the health care system.[7]

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Declaration of Competing Interest

None.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jinf.2023.04.006.

References

1. Tam CC, Rodrigues LC, Viviani L, Dodds JP, Evans MR, Hunter PR, et al. *Longitudinal study of infectious intestinal disease in the UK (IID2 study): incidence in the community and presenting to general practice. Gut* 2012;**61**(1):69–77.
2. Public Health England. *Gastrointestinal infections: guidance, data and analysis [Internet];* 2021. Available from: ⟨https://www.gov.uk/government/collections/gastrointestinal-infections-guidance-data-and-analysis⟩.
3. European Food Safety Authority, European Centre for Disease Prevention and Control. *The European Union one health 2020 zoonoses report. EFSA J* 2021;**19**(12):e06971.
4. Gasser C, Gautier E, Steck A, Siebenmann R, Oechslin R. *Hemolytic-uremic syndrome: bilateral necrosis of the renal cortex in acute acquired hemolytic anemia. Schweiz Med Wochenschr* 1955;**85**(38–39):905–9.
5. Obrig TG. *Escherichia coli Shiga toxin mechanisms of action in renal disease. Toxins* 2010;**2**(12):2769–94.
6. Lingwood CA. *Verotoxin/globotriaosyl ceramide recognition: angiopathy, angiogenesis and antineoplasia. Biosci Rep* 1999;**19**(5):345–54.
7. Jenkins C, Byrne L, Vishram B, Sawyer C, Balasegaram S, Ahyow L, et al. *Shiga toxin-producing Escherichia coli haemolytic uraemic syndrome (STEC-HUS): diagnosis, surveillance and public-health management in England. J Med Microbiol* 2020;**69**(7):1034–6.
8. Butt S, Jenkins C, Godbole G, Byrne L. *The epidemiology of Shiga toxin-producing Escherichia coli serogroup O157 in England, 2009-2019. Epidemiol Infect* 2022;**150**:e52. https://doi.org/10.1017/S0950268822000206
9. Adams NL, Byrne L, Smith GA, Elson R, Harris JP, Salmon R, et al. *Shiga toxin-producing Escherichia coli O157, England and Wales, 1983-2012. Emerg Infect Dis* 2016;**22**(4):590–7.
10. Michelacci V, Montalbano Di Filippo M, Gigliucci F, Arancia S, Chiani P, Minelli F, et al. *Population analysis of O26 Shiga toxin-producing Escherichia coli causing hemolytic uremic syndrome in Italy, 1989-2020, through whole genome sequencing. Front Cell Infect Microbiol* 2022;**12**:842508.
11. European Centre for Disease Prevention and Control. *Shiga toxin-producing Escherichia coli (STEC) infection annual epidemiological report for 2019* [Internet]; 2021. Available from: ⟨https://www.ecdc.europa.eu/sites/default/files/documents/AER-STEC-2019.pdf⟩.
12. Bielaszewska M, Mellmann A, Bletz S, Zhang W, Köck R, Kossow A, et al. *Enterohemorrhagic Escherichia coli O26:H11/H-: a new virulent clone emerges in Europe. Clin Infect Dis* 2013;**56**(10):1373–81.
13. Delannoy S, Mariani-Kurkdjian P, Bonacorsi S, Liguori S, Fach P. *Characteristics of emerging human-pathogenic Escherichia coli O26:H11 strains isolated in France between 2010 and 2013 and carrying the stx2d gene only. J Clin Microbiol* 2015;**53**(2):486–92.
14. Croxen MA, Law RJ, Scholz R, Keeney KM, Wlodarska M, Finlay BB. *Recent advances in understanding enteric pathogenic Escherichia coli. Clin Microbiol Rev* 2013;**26**(4):822–80.
15. Scheutz F, Teel LD, Beutin L, Piérard D, Buvens G, Karch H, et al. *Multicenter evaluation of a sequence-based protocol for subtyping Shiga toxins and standardizing Stx nomenclature. J Clin Microbiol* 2012;**50**(9):2951.
16. Byrne L, Adams N, Jenkins C. *Association between Shiga toxin– producing Escherichia coli O157:H7 stx gene subtype and disease severity, England, 2009–2019. Emerg Infect Dis* 2020;**26**(10):7.
17. Elliott SJ, Sperandio V, Wainwright L, Hutcheson SW, Mcdaniel TK, Kaper JB. *The locus of enterocyte effacement (LEE)-encoded regulator controls expression of both LEE- and non-LEE-encoded virulence factors in enteropathogenic and enterohemorrhagic Escherichia coli. Infect Immun* 2000;**68**:12.
18. Stevens MP, Frankel GM. *The locus of enterocyte effacement and associated virulence factors of enterohemorrhagic Escherichia coli. Microbiol Spectr* 2014;**2**(4). [cited 2020 Nov 10]. ⟨http://www.asmscience.org/content/journal/microbiolspec/10.1128/microbiolspec.EHEC-0007-2013⟩.
19. Pearce MC, Jenkins C, Vali L, Smith AW, Knight HI, Cheasty T, et al. *Temporal shedding patterns and virulence factors of Escherichia coli serogroups O26, O103, O111, O145, and O157 in a cohort of beef calves and their dams. Appl Environ Microbiol* 2004;**70**(3):1708–16.
20. Leomil L, Pestana de Castro AF, Krause G, Schmidt H, Beutin L. *Characterization of two major groups of diarrheagenic Escherichia coli O26 strains which are globally spread in human patients and domestic animals of different species. FEMS Microbiol Lett* 2005;**249**(2):335–42.
21. Krüger A, Lucchesi PMA, Sanso AM, Etcheverría AI, Bustamante AV, Burgán J, et al. *Genetic characterization of Shiga toxin-producing Escherichia coli O26:H11 strains isolated from animal, food, and clinical samples. Front Cell Infect Microbiol* 2015;**5**:74.
22. Shakerian A, Rahimi E, Emad P. *Vegetables and restaurant salads as a reservoir for Shiga toxigenic Escherichia coli: distribution of virulence factors, O-serogroups, and antibiotic resistance properties. J Food Prot* 2016;**79**(7):1154–60.
23. Ross CM, Rapp D, Cave VM, Brightwell G. *Prevalence of Shiga toxin-producing Escherichia coli in pasture-based dairy herds. Lett Appl Microbiol* 2019;**68**(2):112–9.
24. Butt S, Allison L, Vishram B, Greig DR, Aird H, McDonald E, et al. *Epidemiological investigations identified an outbreak of Shiga toxin-producing Escherichia coli serotype O26:H11 associated with pre-packed sandwiches. Epidemiol Infect* 2021;**149**:e178.
25. Vasser M, Barkley J, Miller A, Gee E, Purcell K, Schroeder MN, et al. *Notes from the field: multistate outbreak of Escherichia coli O26 infections linked to raw flour – United States, 2019. MMWR Morb Mortal Wkly Rep* 2021;**70**(16):600–1.
26. Minary K, Tanne C, Kwon T, Faudeux C, Clave S, Langevin L, et al. *Outbreak of hemolytic uremic syndrome with unusually severe clinical presentation caused by Shiga toxin-producing Escherichia coli O26:H11 in France. Arch Pediatr* 2022;**29**(6):448–52.
27. Moran-Gilad J, Rokney A, Danino D, Ferdous M, Alsana F, Baum M, et al. *Real-time genomic investigation underlying the public health response to a Shiga toxin-producing Escherichia coli O26:H11 outbreak in a nursery. Epidemiol Infect* 2017;**145**(14):2998–3006.
28. Scavia G, Gianviti A, Labriola V, Chiani P, Maugliani A, Michelacci V, et al. *A case of haemolytic uraemic syndrome (HUS) revealed an outbreak of Shiga toxin-2-producing Escherichia coli O26:H11 infection in a nursery, with long-lasting shedders and person-to-person transmission, Italy 2015. J Med Microbiol* 2018;**67**(6):775–82.
29. Vishram B, Jenkins C, Greig DR, Godbole G, Carroll K, Balasegaram S, et al. *The emerging importance of Shiga toxin-producing Escherichia coli other than serogroup O157 in England. J Med Microbiol Microbiol Soc* 2021;**70**:001375. Available from ⟨https://www.microbiologyresearch.org/content/journal/jmm/10.1099/jmm.0.001375⟩.
30. Rodwell EV, Vishram B, Smith R, Browning L, Smith-Palmer A, Allison L, et al. *Epidemiology and genomic analysis of Shiga toxin-producing Escherichia coli clonal complex 165 in the UK. J Med Microbiol* 2021;**70**(12).
31. Rodwell EV, Chan YW, Sawyer C, Carroll A, McNamara E, Allison L, et al. *Shiga toxin-producing Escherichia coli clonal complex 32, including serotype O145:H28, in the UK and Ireland. J Med Microbiol* 2022;**71**(8).
32. Dallman TJ, Greig DR, Gharbia SE, Jenkins C. *Phylogenetic context of Shiga toxin-producing Escherichia coli serotype O26:H11 in England. Microb Genom* 2021;**7**(6). [cited 2021 May 4]. ⟨https://www.microbiologyresearch.org/content/journal/mgen/10.1099/mgen.0.000551⟩.
33. Ogura Y, Gotoh Y, Itoh T, Sato MP, Seto K, Yoshino S, et al. *Population structure of Escherichia coli O26:H11 with recent and repeated stx2 acquisition in multiple lineages. Microb Genom* 2017;**3**(11).
34. Joensen KG, Engsbro ALØ, Lukjancenko O, Kaas RS, Lund O, Westh H, et al. *Evaluating next-generation sequencing for direct clinical diagnostics in diarrhoeal disease. Eur J Clin Microbiol Infect Dis* 2017;**36**(7):1325–38.
35. Tewolde R, Dallman T, Schaefer U, Sheppard CL, Ashton P, Pichon B, et al. *MOST: a modified MLST typing tool based on short read sequencing. PeerJ* 2016;**4**:e2308.

36. Zhou Z, Alikhan NF, Mohamed K, Fan Y, Achtman M. *The EnteroBase user's guide, with case studies on Salmonella transmissions, Yersinia pestis phylogeny, and Escherichia core genomic diversity. Genome Res* 2020;**30**(1):138–52.
37. Dallman T, Ashton P, Schafer U, Jironkin A, Painset A, Shaaban S, et al. *SnapperDB: a database solution for routine sequencing analysis of bacterial isolates.* In: Berger B, editor. . *Bioinformatics.* 34. 2018. p. 3028–9.
38. Croucher NJ, Page AJ, Connor TR, Delaney AJ, Keane JA, Bentley SD, et al. *Rapid phylogenetic analysis of large samples of recombinant bacterial whole genome sequences using Gubbins. Nucleic Acids Res* 2015;**43**(3).
39. Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, et al. *IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. Mol Biol Evol* 2020;**37**(5):1530–4.
40. Letunic I, Bork P. *Interactive Tree Of Life (iTOL) v4: recent updates and new developments. Nucleic Acids Res* 2019;**47**(W1):W256–9.
41. Jenkins C, Perry NT, Godbole G, Gharbia S. *Evaluation of chromogenic selective agar (CHROMagar STEC) for the direct detection of Shiga toxin-producing Escherichia coli from faecal specimens. J Med Microbiol* 2020;**69**(3):487–91.
42. Jenkins C, Pearce MC, Smith AW, Knight HI, Shaw DJ, Cheasty T, et al. *Detection of Escherichia coli serogroups O26, O103, O111 and O145 from bovine faeces using immunomagnetic separation and PCR/DNA probe techniques. Lett Appl Microbiol* 2003;**37**(3):207–12.
43. Scotland SM, Smith HR, Willshaw GA, Rowe B. *Vero cytotoxin production in strain of Escherichia coli is determined by genes carried on bacteriophage. Lancet* 1983;**2**(8343):216.
44. Scotland SM, Willshaw GA, Smith HR, Rowe B. *Properties of strains of Escherichia coli O26:H11 in relation to their enteropathogenic or enterohemorrhagic classification. J Infect Dis* 1990;**162**(5):1069–74.
45. Jenkins C, Evans J, Chart H, Willshaw GA, Frankel G. *Escherichia coli serogroup O26–a new look at an old adversary. J Appl Microbiol* 2008;**104**(1):14–25.
46. Hoyle DV, Keith M, Williamson H, Macleod K, Mathie H, Handel I, et al. *Prevalence and epidemiology of non-O157 Escherichia coli serogroups O26, O103, O111, and O145 and Shiga toxin gene carriage in Scottish Cattle, 2014-2015. Appl Environ Microbiol* 2021;**87**(10).
47. Public Health England. *Shiga toxin-producing Escherichia coli: public health management* [Internet]. Available from: ⟨https://www.gov.uk/government/publications/shiga-toxin-producing-escherichia-coli-public-health-management⟩.
48. Dallman TJ, Jalava K, Verlander NQ, Gally D, Jenkins C, Godbole G, et al. *Identification of domestic reservoirs and common exposures in an emerging lineage of Shiga toxin-producing Escherichia coli O157:H7 in England: a genomic epidemiological analysis. Lancet Microbe* 2022;**3**(8):e606–15.
49. Day M, Doumith M, Jenkins C, Dallman TJ, Hopkins KL, Elson R, et al. *Antimicrobial resistance in Shiga toxin-producing Escherichia coli serogroups O157 and O26 isolated from human cases of diarrhoeal disease in England, 2015. J Antimicrob Chemother* 2017;**72**(1):145–52.
50. Gentle A, Day MR, Hopkins KL, Godbole G, Jenkins C. *Antimicrobial resistance in Shiga toxin-producing Escherichia coli other than serotype O157: H7 in England, 2014–2016. J Med Microbiol* 2020;**69**(3):379–86.