

# BMJ Open Disease severity of Shiga toxin-producing *E. coli* O157 and factors influencing the development of typical haemolytic uraemic syndrome: a retrospective cohort study, 2009–2012

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## ABSTRACT

**Objectives:** Assess the disease severity of Shiga toxin-producing *Escherichia coli* (STEC) O157 infection and factors influencing the development of typical haemolytic uraemic syndrome (tHUS).

**Design:** A retrospective cohort study using data collected through routine surveillance questionnaires between 2009 and 2012.

**Participants:** 3323 symptomatic cases of STEC O157.

**Main outcome measures:** Incidence of human STEC O157 and tHUS, proportion of cases reporting bloody diarrhoea, hospitalisation, tHUS and death. Odds of progression to tHUS and predicted percentage chance of developing tHUS based on case demographics, STEC O157 strain characteristics and clinical symptoms.

**Results:** From 2009 to 2012, 3323 cases of symptomatic STEC O157 with completed questionnaires were reported, of which 172 developed tHUS (5.18%). Being aged 1–4 years (OR 8.65, 95% CI 5.01 to 14.94,  $p=0.004$ ) or female (OR 1.61, 95% CI 1.12 to 2.30,  $p=0.009$ ), being infected with phage type (PT) 21/28 (OR 2.07, 95% CI 1.25 to 3.42,  $p=0.005$ ) or PT 2 (OR 2.18, 95% CI 1.06 to 4.50,  $p=0.034$ ), receiving  $\beta$ -lactam antibiotics (OR 4.08, 95% CI 1.43 to 11.68,  $p=0.009$ ) and presenting with vomiting (OR 3.16, 95% CI 2.16 to 4.62,  $p<0.001$ ) or bloody diarrhoea (OR 2.10, 95% CI 1.38 to 3.20,  $p=0.001$ ) were found to be significant risk factors for progression to tHUS. The predicted percentage chance of developing tHUS varied from under 1% to 50% if all risk factors were present.

**Conclusions:** The data from this study indicate the use of  $\beta$ -lactam antibiotics should be avoided in suspected cases of STEC infection in all age groups, particularly in those under the age of 5.

## INTRODUCTION

Shiga toxin-producing *Escherichia coli* (STEC) are a group of zoonotic bacteria characterised by possession of phage-encoded

## Strengths and limitations of this study

- Data used are a standardised, comprehensive data set and the largest used to evaluate Shiga toxin-producing *Escherichia coli* (STEC) O157 severity and risk factors for haemolytic uraemic syndrome (HUS) development.
- Clinical data were self-reported, so misclassification could occur.
- HUS is likely under-reported as questionnaires completed early in the course of infection.
- As with any observational study, causation cannot be determined and it is possible that cases of tHUS were treated with antibiotics due to the development of tHUS not for STEC infection.

Shiga toxins (Stx). In England, the most common serogroup associated with human disease is O157. Each year around 900 cases of human STEC infection are reported in England through the national surveillance system. These include apparently sporadic cases but also those associated with outbreaks. The main reservoirs for STEC are cattle and other ruminants. Transmission occurs through consumption of contaminated food or water, contact with infected animals or their environment or through person–person transmission. Clinical illness is characterised by diarrhoea,<sup>1</sup> ranging from mild and self-limiting to more severe bloody diarrhoea. Asymptomatic infection can also occur. Previous studies have reported between 6% and 14% of STEC cases go on to develop haemolytic uraemic syndrome (HUS),<sup>2–5</sup> usually 5–13 days after initial diarrhoeal symptoms.<sup>6</sup>

HUS is defined as microangiopathic haemolytic anaemia, thrombocytopenia and acute kidney injury. Typical HUS (tHUS) has

bacterial causes, most commonly STEC infection, although infection with *Shigella dysenteriae* serotype 1 or *Streptococcus pneumoniae* may also lead to tHUS. HUS may also have non-infectious causes, most frequently due to defects in the complement pathway. This is a rare form of HUS (termed atypical HUS), with poorer prognosis than tHUS. Strains of STEC O157 encode the protein intimin, which facilitates intimate attachment of the bacteria to the host gut mucosa.<sup>7</sup> During infection, STEC release Stx, the primary virulence factor responsible for the most serious clinical outcomes. The Stx are AB<sub>5</sub> toxins that target cells expressing the glycolipid globotriaosylceramide (Gb3), disrupting host protein synthesis and causing apoptotic cell death.<sup>7–9</sup> Renal epithelial cell membranes are enriched for Gb3 resulting in the kidneys bearing the brunt of Stx toxicity; damage to cardiovascular and neurological systems can also occur. Children under the age of 5 are at greatest risk of tHUS,<sup>4 10 11</sup> and a study of paediatric HUS cases in the UK and Ireland found that STEC infection was the cause of 80% tHUS cases.<sup>10</sup> While relatively rare, tHUS can cause long-term sequelae such as kidney dysfunction, hypertension and neurological abnormalities, and death.<sup>8 10–12</sup>

Strains of STEC O157 encoding the stx2-only toxin, specifically the stx subtype stx2a, are significantly associated with progression to tHUS.<sup>7 13–16</sup> Antibiotic usage is generally contraindicated for use in cases of STEC infection, due to the possibility that bacterial DNA damage may upregulate the production of Stx, particularly the stx2 subtype,<sup>14 17</sup> therefore increasing the risk of tHUS. Observational epidemiological studies,<sup>5 18</sup> and analysis of outbreak data have shown that the use of antibiotics increases the risk of tHUS.<sup>19 20</sup> However, the numbers of cases included in these studies are low. The aims of this study were to retrospectively examine disease severity of STEC O157 of a cohort of cases extracted from a large surveillance data set, and to determine and quantify factors which influence whether cases infected with STEC O157 go on to develop tHUS.

## METHODS

### Case ascertainment

Stool samples from patients presenting to healthcare with clinical symptoms indicative of STEC were sent to the local laboratory and cultured for the presence of STEC O157 (<http://www.hpa-standardmethods.org.uk/>).

Local laboratories reported presumptive isolates of STEC to local Public Health England (PHE) Centres in England, who arranged for an enhanced surveillance questionnaire (ESQ) to be administered to cases. The ESQ collected information on demographic details, clinical details, exposure history, epidemiological case classification (primary, co-primary, secondary or asymptomatic), household or other close contacts, and whether cases were in high-risk groups. Symptomatic contacts of cases

and contacts deemed to pose a risk of onward transmission were screened.

Bacterial cultures of *E. coli* O157 were then sent for confirmation at the PHE Gastrointestinal Bacterial Reference Unit (GBRU). Strains confirmed as *E. coli* O157 were phage typed, and presence of Stx genes (*stx1* and/or *stx2*) and the intimin (*eae*) gene were determined using real-time PCR.<sup>21</sup> In cases where no faecal specimens were available serum samples were sent to GBRU for detection of serological evidence of STEC O157 infection.<sup>22</sup>

HUS status was collected both on the ESQ and on laboratory submission forms accompanying isolates sent to GBRU. As ESQ data were obtained directly from cases or case relatives, no detailed clinical parameters were available and no clinical definition of HUS was applied.

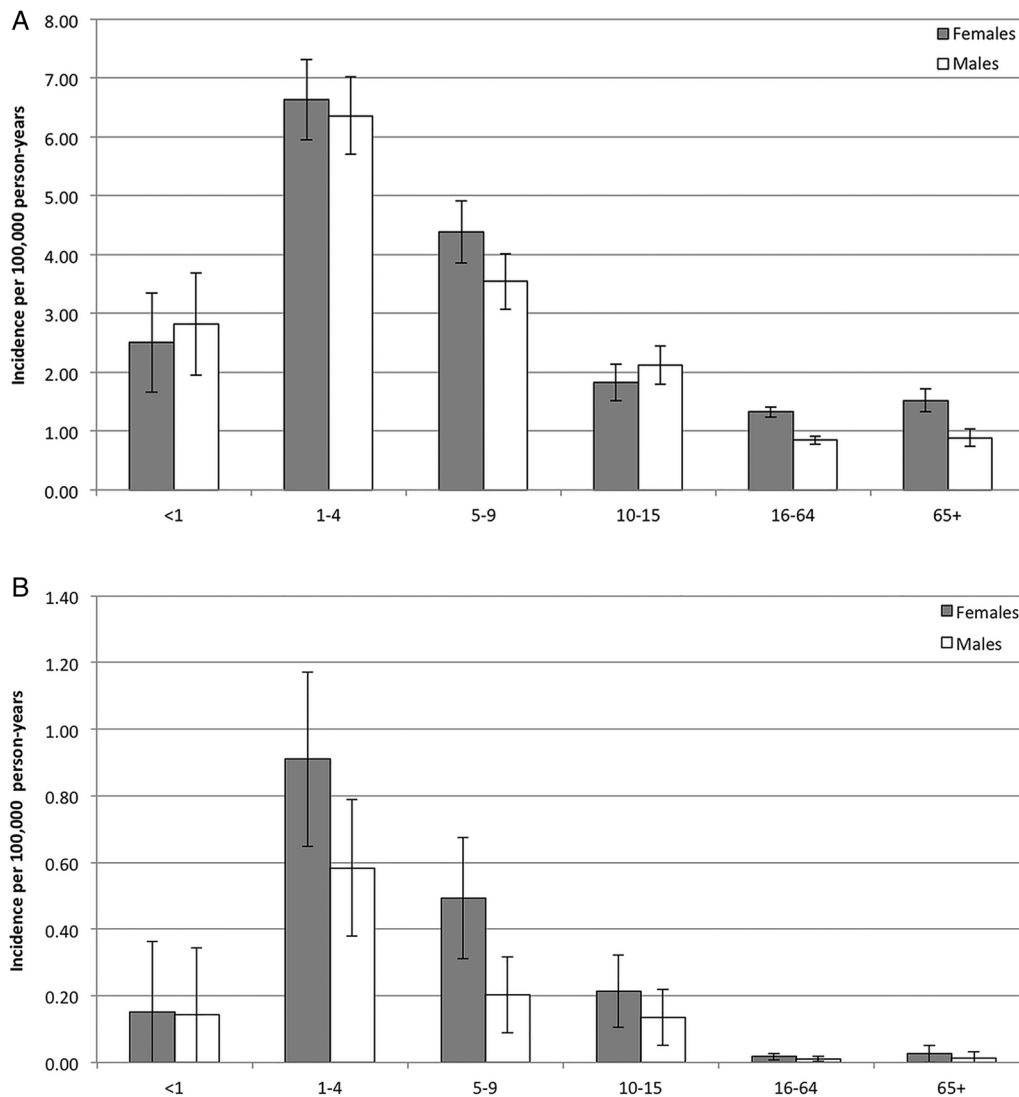
Data from ESQs were entered into the National Enhanced Surveillance System for STEC (NESSS). Microbiological results, including typing, were appended daily to NESSS and reconciled with ESQ data based on patient identifiable data.

### Data analyses

Data were extracted from NESSS for the period 2009–2012 inclusive and analysed using Microsoft Access and Excel, and STATA V.12.0 (STATA Corporation, Texas, USA). Variables for analysis included age group, gender, ethnicity, PHE region of residence, STEC O157 phage type (PT), STEC O157 Stx type, epidemiological case definition (ie, primary/secondary case), foreign travel status, season and year of infection, clinical illness and treatment (specific symptoms and use of antibiotics and antidiarrhoeal medication). Cases were categorised into the following age groups, based on a priori knowledge that children and the elderly are most at risk of infection; under 1, 1–4, 5–9, 10–15, 16–64, and 65 years and over. Ethnicity was collected in broad groups on the ESQ as follows: Caucasian, African-American/black British, Asian/Asian British, Chinese, mixed, other. Where clinical symptoms, treatment or travel status were blank on the ESQ, these were coded as negative responses, while missing ethnicity fields were coded as such. There were no missing values for any other variables.

Incidence rates of STEC O157 and tHUS were calculated for all cases and by age group and gender, using the Office for National Statistics mid-year population estimates for 2010,<sup>23</sup> as the denominator. Incidence rate ratios (IRRs) were calculated to compare incidence in different groups including age and gender, and a  $p < 0.05$  was considered statistically significant.

Logistic regression was used to calculate ORs for different outcomes and exposures: cases of STEC O157 who developed tHUS were compared with those who did not with respect to each variable. Variables with a  $p$  value of less than 0.2 were included in the multivariable model. Variables with no evidence of any association ( $p > 0.05$ ) in the multivariable regression models were



**Figure 1** (A) Incidence of symptomatic Shiga toxin-producing *Escherichia coli* (STEC) O157 infection by age and gender. (B) Incidence of symptomatic typical haemolytic uraemic syndrome attributable to STEC O157 infection by age and gender.

excluded sequentially until all exposures in the regression models provided evidence of an association. Variables in the final model were assessed for interactions—by adding interaction terms into the final logistic regression model. The final model was used to estimate the predicted percentage chance of developing tHUS under a range of variable levels using out-of-sample estimations with dummy data. Adults aged 16–64 were chosen as the reference group for age group as this group is known to be at the lowest risk for tHUS; other PTs were chosen to compare PT as the smallest group for analysis.

## RESULTS

From 2009 to 2012, 3672 (98.68%) STEC O157 cases were reported and ESQs were completed for 3564 of these (97.11%). Of those, 3323 (93.24%) cases were symptomatic, and 172 developed tHUS (5.18%). The

annual incidence of symptomatic STEC O157 infection was 1.59 cases per 100 000 person-years. Incidence was highest (6.49 cases/100 000 person-years) in those aged 1–4 years (figure 1A). The annual incidence of reported tHUS due to STEC O157 infection was 0.08 cases per 100 000 person-years, which also peaked in the 1–4-year age group (figure 1B), with an IRR of 50.30 ( $p<0.001$ ) compared with adults aged 16–64. The incidence of STEC O157 infection was higher in females than in males (1.77 vs 1.4 cases/100 000 person-years, IRR 1.25,  $p<0.001$ ), although this differed by age group (figure 1A). Development of tHUS was also higher in females than in males (0.1 vs 0.06 cases/100 000 person-years, IRR 1.6,  $p<0.001$ ), and this was true for every age group (figure 1B).

The majority of cases (3086, 92.87%) experienced diarrhoea, of which 65.68% ( $n=2027$ ) was bloody. Abdominal pain was frequently reported (81.73%,  $n=2716$ ), while fever, nausea and vomiting were reported

by less than half of cases. Bloody diarrhoea was most frequently reported in cases aged 65 years and over (252/366, 68.85%), followed by those aged 10–15 (192/284, 67.61%), while vomiting was most frequently reported in children aged 10–15 (136/284, 47.89%). In univariable analysis, children under 10 were less likely to report bloody diarrhoea, particularly those under 1 year of age, with 19.44% (14/72) of cases in this age group compared with 66.24% (973/1469) in adults aged 16–64 (OR 0.12, 95% CI 0.07 to 0.22,  $p < 0.001$ ). Bloody diarrhoea (OR 1.17, 95% CI 1.05 to 1.31,  $p = 0.006$ ) and vomiting (OR 1.24, 95% CI 1.09 to 1.40,  $p = 0.001$ ) were reported by a higher proportion of females than males, as were the non-specific symptoms of abdominal pain (OR 1.22, 95% CI 1.08 to 1.37,  $p = 0.001$ ), fever (OR 1.21, 95% CI 1.06 to 1.38,  $p = 0.004$ ) and nausea (OR 1.34, 95% CI 1.19 to 1.51,  $p < 0.001$ ). Bloody diarrhoea was more frequently reported in those prescribed antibiotics (OR 2.79, 95% CI 2.16 to 3.60,  $p < 0.001$ ) and antidiarrhoeal medication (OR 1.67, 95% CI 1.37 to 2.04,  $p < 0.001$ ).

Cases reporting treatment with antidiarrhoeal medication (OR 0.3, 95% CI 0.16 to 0.57,  $p < 0.001$ ) or travelling abroad in the 7 days prior to onset of symptoms (OR 0.34, 95% CI 0.20 to 0.58,  $p < 0.001$ ), or infected in 2011 (OR 0.50, 95% CI 0.34 to 0.75,  $p = 0.001$ ) were associated with a decreased odds of progression to tHUS. There were increased odds of cases developing tHUS in the spring season (March to May) which was weakly significant ( $p = 0.151$ ), although this peak was only observed in 2009. However, none of these factors were significantly associated with tHUS development in the final multivariable logistic regression model. No association between tHUS development and ethnicity or nausea was observed in single variable analysis, and therefore were not considered in multivariable analysis.

The most common PTs detected were PT 8 (1003 cases, 30.18%), PT 21/28 (969, 29.16%), PT 32 (251, 7.55%) and PT 2 (185, 5.57%). Isolates possessing *stx2* only accounted for 99.59% (965/969) of PT 21/28 isolates and 92.43% (171/185) of PT 2 isolates, whereas 93.72% (940/1003) of PT 8 isolates possessed *stx1+2*. Bloody diarrhoea was more frequently reported in cases infected with PT 2 than other PTs, and those infected with isolates carrying the *stx1+2* genes compared with those infected with strains carrying the *stx2* gene alone (OR 1.54, 95% CI 1.32 to 1.80,  $p < 0.001$ ).

A total of 394 cases reported antibiotic usage during their illness, of which 184 could not recall the type of antibiotic used. The most commonly used antibiotics were quinolones (81), metronidazole (47),  $\beta$ -lactams (42) and macrolides (41). In single variable analysis, only the use of  $\beta$ -lactams was significantly associated with HUS (OR 3.78, 95% CI 1.65 to 8.63,  $p = 0.002$ ).

In the final multivariable model (table 1), cases diagnosed serologically had the highest OR of 23.19 for developing tHUS. However, this is expected as serological testing is only performed when cases have tHUS or very severe symptoms which make it impossible to

**Table 1** Final multivariable logistic regression model for factors influencing progression to tHUS among cases of STEC O157

	OR	p Value	95% CIs
Age group			
<1	5.75	0.004	1.75 to 18.87
1–4	8.65	<0.001	5.01 to 14.94
5–9	4.83	<0.001	2.67 to 8.74
10–15	4.67	<0.001	2.40 to 9.10
16–64	Reference group		
65+	1.15	0.775	0.44 to 3.03
PT			
PT 8	0.42	0.028	0.20 to 0.91
PT 21/28	2.07	0.005	1.25 to 3.42
PT 32	0.89	0.805	0.37 to 2.17
PT 2	2.18	0.034	1.06 to 4.50
Other PT	Reference group		
Serology	23.19	<0.001	12.03 to 44.70
Antibiotics			
No antibiotics	Reference group		
Other antibiotics	1.20	0.538	0.67 to 2.16
$\beta$ -lactams	4.08	0.009	1.43 to 11.68
Region: North East	0.29	0.007	0.12 to 0.72
Female gender	1.61	0.009	1.12 to 2.30
Vomiting	3.16	<0.001	2.16 to 4.62
Bloody diarrhoea	2.10	0.001	1.38 to 3.20

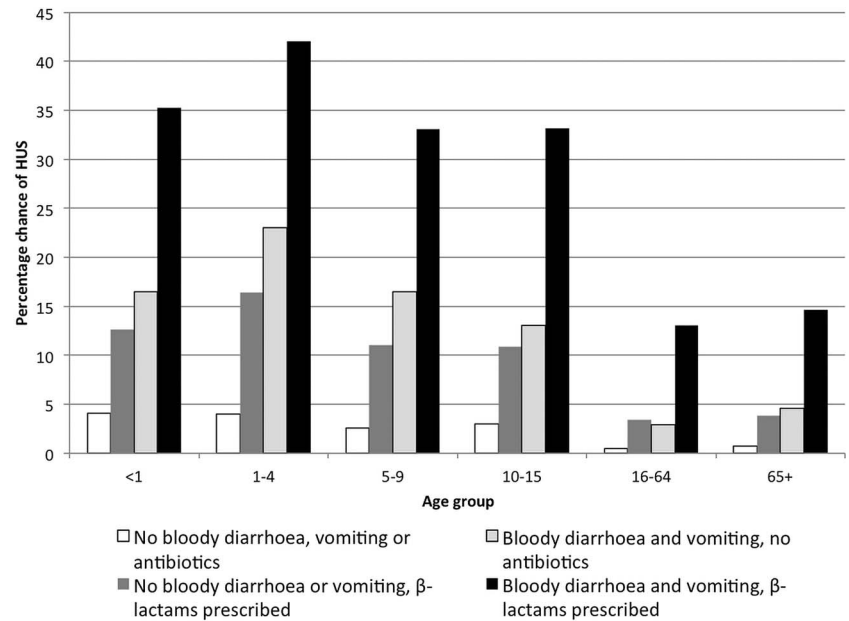
PT, phage type; STEC, Shiga toxin-producing *Escherichia coli*; tHUS, typical haemolytic uraemic syndrome.

obtain a faecal specimen. Following this, those aged 1–4 (OR 8.65) and those prescribed  $\beta$ -lactam antibiotics (OR 4.08) had the highest ORs for developing tHUS. Forty-two cases reported the use of  $\beta$ -lactam antibiotics, of which seven developed tHUS (OR 3.87, 95% CI 1.69 to 8.986,  $p = 0.001$ ). Of those prescribed  $\beta$ -lactam antibiotics, the majority ( $n = 33$ ) were penicillin derivatives, and these accounted for six of seven tHUS cases prescribed  $\beta$ -lactam antibiotics. No association was seen between tHUS and the prescription of non- $\beta$ -lactam antibiotics. Female cases were also at increased odds (OR 1.61) of developing tHUS.

Cases of STEC O157 PT 8 were less likely to develop tHUS than PT 21/28, PT 32 or PT 2. In the final model, cases infected with PT 21/28 (OR 2.07) or PT 2 (OR 2.18), cases who had reported vomiting (OR 3.16) and bloody diarrhoea (OR 2.10) had increased odds of developing HUS compared with the reference groups. Twelve cases were infected with strains carrying *stx1* only and none of these cases reported development of tHUS. When PT was replaced with *stx* type in the final multivariable model, cases infected with isolates carrying the *stx2* gene only were more likely to develop tHUS than those carrying *stx1+2* (OR 2.80, 95% CI 1.57 to 5.01,  $p < 0.0001$ ). When  $\beta$ -lactam antibiotics were replaced with penicillin derivatives, cases prescribed these had an OR of 3.85 (95% CI 1.19 to 12.49,  $p = 0.025$ ). Cases resident in the North East of England were at a decreased odds of tHUS (OR 0.29) compared with those in other regions.



**Figure 2** Predictive percentage chance of Shiga toxin-producing *Escherichia coli* O157 cases developing typical haemolytic uraemic syndrome (HUS) by age group, symptoms and antibiotic treatment.



Interactions were observed between PT 21/28 and vomiting (OR 4.69, 95% CI 1.41 to 15.65,  $p=0.012$ ) suggesting that the effect of vomiting on tHUS development may be underestimated in the model for cases infected with PT 21/28. An interaction was also observed between the 'other PT' category and bloody diarrhoea (OR 5.68, 95% CI 1.41 to 22.82,  $p=0.014$ ). As 'other PT' is a heterogeneous category this is difficult to interpret. No other interactions between the variables analysed were observed.

Based on the final logistic regression model, the percentage chance of developing tHUS ranged from 0.09% (95% CI 0.04% to 0.22%) in males infected with PT 8 aged 16–64 who have not experienced bloody diarrhoea or vomiting and have not been prescribed antibiotics to 50.31% (95% CI 24.61% to 77.57%) in a female child aged 1–4 who had bloody diarrhoea and vomiting, was prescribed  $\beta$ -lactam antibiotics and was infected with PT 21/28. For all age groups, the chance of developing tHUS is increased if an individual experiences bloody diarrhoea or vomiting, or is treated with  $\beta$ -lactam antibiotics (figure 2). If a child aged 1–4 has not experienced bloody diarrhoea and vomiting and has not been prescribed  $\beta$ -lactam antibiotics, the percentage chance of HUS development is estimated to be 4.01% (95% CI 2.22% to 7.13%), increasing to 23.05% (95% CI 15.69% to 33.08%) if they report bloody diarrhoea and vomiting. This increases further to 42.06% (95% CI 22.10% to 67.75%) if treated with  $\beta$ -lactam antibiotics.

## DISCUSSION

### Principle findings

The disease severity of STEC O157 infection and risk of tHUS development is dependent on a complex interplay of host, pathogen and environmental factors, including

age and gender of the host, strain characteristics, clinical presentation and antibiotic treatment. The predicted chance of developing tHUS varied from 0.1% to 50.3% depending on certain risk factors.

Our study found that around 5% of symptomatic STEC O157 cases developed tHUS, with 11% of cases aged 1–4 developing the syndrome. While children under the age of 5 are recognised as being at greater risk of developing tHUS,<sup>10 11</sup> our study indicates that it is specifically the 1–4 age group that is at highest risk, with lower incidence of tHUS and risk of developing tHUS in children under 1 year of age. Females had a higher odds than males of developing tHUS among all age groups.

The likelihood of developing tHUS in children aged 1–4, with bloody diarrhoea and vomiting caused by STEC O157 could be 23%, and when treated with  $\beta$ -lactam antibiotics increases to 42%. The use of  $\beta$ -lactam antibiotics, particularly penicillin derivatives, increased the risk of tHUS in all age groups, irrespective of presence of bloody diarrhoea and vomiting and strain characteristics.

### Strengths and weaknesses of the study

Our data provide standardised information on a range of microbiological, demographic, clinical and exposure details for cases of STEC O157 in England, and to our knowledge is the largest data set used to evaluate STEC O157 severity and risk factors for HUS development. Reporting of both microbiological and questionnaire data is complete for over 97% of STEC infections diagnosed in England, providing a large, comprehensive data set of STEC O157 cases for analysis.

As with all data reliant on laboratory surveillance, not all cases will seek healthcare or provide samples,

particularly those with mild illness. However, the proportion of cases with bloody diarrhoea is lower than has previously reported,<sup>1</sup> suggesting cases with milder symptoms are being captured by NESSS.

All clinical data were self-reported either by cases or family members, and therefore misclassification may have occurred. In particular, many cases did not know the names of the medications they had taken, and no clinical definition of HUS could be applied. It is likely that tHUS, hospitalisation and death are under-reported as questionnaires are completed to ensure that public health action is taken and represent the status of the patient at the time of completion. The majority of STEC cases in this data set had been ill for less than a week when the ESQ was completed, and were not followed up to determine the outcome of their infection.

As with any observational study, causation cannot be determined and as the date of antibiotic administration was not recorded, it is possible that cases were treated with antibiotics due to the development of tHUS or presentation of severe disease. However, antibiotic usage in cases that had already developed tHUS is unusual, and both bloody diarrhoea and vomiting have been included in the multivariable analysis as markers of severe disease. While our study found that those with bloody diarrhoea were more likely to have been treated with antibiotics, there was no significant association between the use of  $\beta$ -lactam antibiotics and bloody diarrhoea or vomiting.

The final multivariable model identified those cases diagnosed by serological testing as at an increased risk of tHUS. This is most likely due to reversed causality, as serological testing is more frequently undertaken in hospitalised patients, particularly those who may be unable to provide faecal samples due to tHUS. The model also identified those cases resident in the North East of England as at a lower risk of tHUS. The reasons for this are unclear; no interactions between region and other variables were observed in the model. It may be due to a reporting difference between the regions of England or that there are true differences in clinical prognosis, which may be resultant of differences in presentation and treatment of STEC O157 cases in primary care. Finally, an interaction between PT 21/28 and vomiting in the model may mean that the effect of vomiting on tHUS development is underestimated for cases infected with PT 21/28.

### Comparison with other studies

The proportion of cases of STEC O157 developing tHUS was 5.2%. This is similar to other surveillance studies, reporting 9.7% in Scotland and between 6.3% and 7.8% for the USA.<sup>2–4</sup> However, a prospective cohort study of STEC cases conducted in the USA found 14% of cases went on to develop tHUS.<sup>5</sup> Within NESSS, around a third of tHUS cases were microbiologically undiagnosed due to no available faecal specimens and this will have contributed to underascertainment of

STEC-HUS cases. In addition, collection of tHUS data on the laboratory referral form and ESQ may occur prior to a case developing tHUS leading to further underascertainment.

The multivariable analysis of factors influencing the development of tHUS is in agreement with other published studies, and the addition of predicted chances of tHUS development allows the quantification of risks across different risk groups. Our study identified female gender, young age, presence of bloody diarrhoea and vomiting and organism characteristics as risk factors for tHUS development, all of which have been reported as risk factors previously.<sup>2 4 5 11 18 24–26</sup> Our study suggests treatment with  $\beta$ -lactam antibiotics increases the risk of developing tHUS. Although not all studies have found an association between antibiotic use and tHUS,<sup>27</sup> when individual classes of antibiotics are studied, both our results and those of others suggest the use of  $\beta$ -lactams, particularly penicillin-based antibiotics, may increase the risk of tHUS development.<sup>5 17 18 28</sup>

The factors determining whether an individual case of STEC develops tHUS are poorly understood. It is likely that factors not considered in this study such as genetic predisposition,<sup>29</sup> pre-existing conditions and other clinical and treatment parameters such as white cell count are also risk factors for tHUS development.<sup>5</sup>

This study found that STEC O157 PT 21/28 and PT 2 were more likely to cause hospitalisation, tHUS, bloody diarrhoea and death than other PTs, and these PTs have been observed as more likely to cause tHUS in the UK and Ireland previously.<sup>10</sup> The pathogenicity of a PT is determined by the virulence factors, including Stx types, that they possess. It has been shown that expression of *stx2* increase the likelihood of tHUS,<sup>25 30</sup> and in our study, those isolates possessing *stx2* only appeared to be more pathogenic than those with *stx1+2*. Data on Stx subtypes has suggested that the *stx2a* subtype is associated with increased risk of tHUS.<sup>26 31</sup>

### Clinical impact

The data from this study and others suggest that the use of  $\beta$ -lactam antibiotics should be avoided in suspected cases of STEC infection in all age groups, and particularly in those under the age of 5. While the use of other classes of antibiotics was not significantly associated with development of HUS, this group of antibiotics were heterogeneous, and therefore this study does not exonerate their risk. Our data support the current guidelines in use in England,<sup>32</sup> which state that clinicians in primary care should seek specialist advice for cases of bloody diarrhoea in children under 5 and should avoid the use of antibiotics prior to referral. Once a suspected case of STEC has been referred for specialist care, while there may be clinical need to use antibiotics in cases of severe septic illness, this needs to be balanced with the increased risk of tHUS if the illness is due to STEC O157.<sup>33</sup>

The guidelines also stress that primary care practitioners should not treat with antimotility drugs prior to referral. While our study did not show any increased risk of tHUS development in cases given antidiarrhoeal medications, bloody diarrhoea was more often reported in those who had taken these, and previous studies have found an association with tHUS.<sup>34</sup> Therefore, treatment with these should also be avoided.

While bloody diarrhoea is an indicator of severe STEC infection, and is a predictor for tHUS, bloody diarrhoea was reported by under half of those under the age of 5, highlighting the need to consider STEC as a differential diagnosis in young children with non-bloody diarrhoea. The lower proportion of bloody diarrhoea in children may in part be a result of higher healthcare consultation rates in this age group and milder cases of diarrhoea being more readily investigated in young children.<sup>35</sup> This study and others also found that vomiting is a predictor for tHUS,<sup>5</sup> and therefore those presenting with suspected STEC O157 should be considered as at risk of tHUS development, particularly if other risk factors, such as young age, are present.

This study shows that certain STEC O157 PTs are associated with higher risk of severe disease. While laboratory results may not always be available, rapid diagnostics will aid clinical decisions on treatment. A recent case report from a case with long-term carriage of STEC showed the utility of determining strain pathogenicity through whole genome sequencing prior to antibiotic administration.<sup>36</sup>

### Further research

The results of this study have identified a number of areas for further research. This study has identified an increased risk of tHUS development following STEC O157 infection in females, and while this has been reported previously, the reasons for this are not understood. The reasons for a lower risk of tHUS development in infants than in young children are also unclear. Further work to evaluate the role of virulence factors, in particular Shiga toxin subtypes and the interplay with PT, is required. Finally, the investigators are reviewing the surveillance system and are evaluating the feasibility of short-term follow-up of cases to improve data capture of tHUS and other severe outcomes of disease, thereby increasing the power of the data collected.

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