Non-O157 Shiga toxin-producing *Escherichia coli*—A poorly appreciated enteric pathogen: Systematic review

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**A R T I C L E   I N F O**

Article history:
Received 16 July 2018
Received in revised form 4 September 2018
Accepted 5 September 2018
Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Keywords:
Shigatoxin producing E. coli
hemolytic uremic syndrome
dysentery

**A B S T R A C T**

Non-O157 strains of Shiga toxin-producing *Escherichia coli* (STEC) are more common causes of acute diarrhea than the better-known O157 strains and have the potential for large outbreaks. This systematic review of the literature identified 129 serogroups as well as 262 different O and H antigen combinations of STEC in cases of epidemic and sporadic disease worldwide. Excluding the results from a single large outbreak of STEC O104:H4 in Germany and France in 2011, the reported frequency of dysenteric illness in patients was 26% (119 of 464) for epidemic disease and 25% (646 of 2588) for sporadic cases. Hemolytic uremic syndrome was identified in 14% of epidemic disease cases and 9% of sporadic illness cases. With the increasing use of PCR-based diagnostics, STEC strain identification may not be possible. Rapid diagnostics are needed for STEC infections to aid the clinician while allowing epidemiologists the opportunity to identify outbreaks and to trace the source of infection.

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**Introduction**

Shiga toxin-producing *Escherichia coli* (STEC), also known as verocytotoxigenic *E. coli* (VTEC) and enterohemorrhagic *E. coli* (EHEC), is a unique pathotype of *E. coli* that produces a Shiga toxin,
which is related molecularly to the toxin expressed by *Shigella dysenteriae* type 1 (the Shiga bacillus). The most common illness produced by STEC strains is hemorrhagic colitis.

STEC first emerged as a cause of foodborne illness in 1982 when a multi-state outbreak of illness occurred as a result of serotype O157:H7 contaminating hamburgers. Laboratory methods for detection are readily available, and since becoming reportable in 1995, O157 strains have become established as causes of outbreaks and sporadic symptomatic and asymptomatic infections worldwide, in many cases associated with the passage of bloody stools (dysentery). First documented in the USA in 1994, non-O157 STEC strains have grown in importance as a cause of illness, peaking with a unique and well-publicized outbreak of a strain of STEC O104:H4 in northern Germany in May and June 2011.

Estimates from the US Centers for Disease Control and Prevention (CDC) indicate that although largely undetected, non-O157 STEC strains as a group outnumber O157 strains as causes of human illness (Hadler et al., 2011; Hale et al., 2012).

This review of the literature was performed to determine the importance of non-O157 STEC as a cause of diarrhea in humans, and to compare the rates of dysentery and hemolytic uremic syndrome (HUS) in infections caused by non-O157 strains of STEC with those seen in infections caused by O157 strains. In reporting the percentages of these, this is the number with either dysentery or HUS divided by the number analyzed for that specific condition \( \times 100 \).

This review is timely since no review of the global importance of non-O157 STEC illness has been published since 2006 (Johnson et al., 2006), specific rates of dysenteric disease or the frequency of developing HUS in infections caused by specific serogroups and serotypes of non-O157 STEC (Bettelheim, 2007) have not been published, and non-O157 strains of STEC continue to be ignored as etiological agents in non-outbreak diarrhea (Clogher et al., 2012). Furthermore, with the increasing use of nucleic acid identification of STEC infections, we are concerned that epidemiology of these infections needed for the institution of prompt control measures will not be possible.

**Methods**

A search of the PubMed database for relevant articles on “non-O157 Shiga toxin-producing *Escherichia coli*” was performed on June 20, 2017, and the titles of articles identified were reviewed. The search included O157; shiga-toxigenic escherichia coli; escherichia coli; toxigenic escherichia coli. This search retrieved articles on STEC, VTEC, and EHEC cases. In order to obtain all significant articles not covered in the initial search, an additional search was performed for each of the top six serogroups by including each serogroup with the term “Shiga Toxin Producing *Escherichia coli* Outbreak”.

The abstracts of all titles of interest and the full texts were reviewed if the publications dealt with STEC epidemiology, etiology, the clinical features of diarrhea, or the development of dysenteric diarrhea and hemolytic uremic syndrome (HUS). Special attention was made to eliminate studies reporting the same patients in multiple studies.

The literature was reviewed to provide data on non-O157 strains of STEC as causes of well-defined outbreaks involving two or more cases, or as causes of sporadic disease in populations not involved with a known outbreak. For analysis, the studies were divided into two groups: well-defined outbreaks versus isolated cases of apparently non-epidemic disease. Whenever possible, complete typing of strains was included if this was reported in the papers in terms of the flagellar (H) and somatic (O) antigens, in order to determine any differences between strains of STEC. Disease caused by non-O157 serotypes became nationally notifiable in the USA in 2000, and it was observed that more outbreaks around the world were reported in the literature after that year.

Additionally, the annual reports compiled by the CDC – National Surveillance of Bacterial Foodborne Illness (Enteric Disease) and National Shiga Toxin-Producing *Escherichia coli* (STEC) 2002–2014—were reviewed.

**Results**

The following sections deal with outbreaks and sporadic cases of non-O157 STEC on a global basis, looking at clinical and epidemiological features.

**Epidemic disease reportedly due to non-O157 strains of STEC**

The reported outbreaks are summarized chronologically in Table 1. In this table, the outbreaks begin in 1995 when these strains were first identified and end with the most recent 2017 outbreaks. A total of 674 outbreaks worldwide caused by non-O157

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**Table 1**

A description of the global reports of outbreaks of two cases or more of non-O157 strains of Shiga toxin-producing *Escherichia coli* along with the reported frequency of dysentery and hemolytic uremic syndrome where these data were available, and the implicated vehicle of transmission, 1995–2017.

<table>
<thead>
<tr>
<th>Years</th>
<th>Number of confirmed cases</th>
<th>Median number of people per outbreak (range)</th>
<th>Serogroups/types</th>
<th>Number reporting dysentery (%)</th>
<th>Number reporting HUS (%)</th>
<th>Implicated vehicle of transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000–2004</td>
<td>26</td>
<td>11/[2–13]</td>
<td>O26:H11, O148:H8</td>
<td>0/26 (0%)</td>
<td>26/183 (7.7%)</td>
<td>Mutton, beef ice cream, farm animals, eating outside of home, restaurant, beef sausage, mutton</td>
</tr>
<tr>
<td>3816</td>
<td>3816</td>
<td></td>
<td>O104:H4*</td>
<td>141/161 (88%)</td>
<td>845/3816 (22%)</td>
<td>Multiple restaurant chains</td>
</tr>
<tr>
<td>2015–2017</td>
<td>Total minus STEC O104:H4*</td>
<td>60/674</td>
<td>0/60 (0%)</td>
<td>119/464 (26%)</td>
<td>78/544 (14%)</td>
<td></td>
</tr>
</tbody>
</table>

HUS, hemolytic uremic syndrome; STEC, Shiga toxin-producing *Escherichia coli*.

*Outbreak in Germany and France in 2011.*
strains of STEC have been documented since 1995, excluding the large 2011 O104:H4 outbreak in Germany and France. A majority of patients affected were children and young adults, and complete serotype identification was sought for all outbreak strains. Serogroup O26:H11 was the most prevalent during outbreaks occurring between 1995 and 2014. In STEC outbreak cases for which the presence or absence of dysentery was reported, on average 26% of cases were found to be affected by dysentery. The time period 2005 to 2009 had the greatest rate of dysentery (68%), due to outbreaks involving serogroups O26:H11, O45, O103:H25, O104:H4, O111:H8, O121, and O145:NM. HUS occurred on average in 14% of STEC outbreaks. The highest rate of HUS, in cases for which this condition was reported (37%), was seen during 2005 to 2009, involving contaminated farm animals, eating outside the home, ice cream, beef sausage, and mutton.

**Sporadic disease reportedly caused by non-O157 strains of STEC**

Table 2 summarizes published non-outbreak data of non-O157 STEC infections by region where reported, serotype, and rates of dysentery and HUS in those with and without dysentery and HUS. Table 2 reports a total of 2748 people with infections caused by non-O157 strains of STEC in an endemic setting; in cases where at least five people were infected with the same serogroup/serotype, the serogroup/serotype is reported separately, along with the number of individuals affected. Six serogroups/serotypes were found to have caused illness in > 100 patients: O26 (n = 622), O111 (n = 336), O103 (n = 335), O45 (n = 211), O145 (n = 184), and O121 (n = 117) (data not summarized in the table). There were 1512 (55%) reported cases in the USA and Canada. Rates of dysentery caused by non-O157:H7 serogroups in sporadically identified cases varied by region from 7.9% in South and Central America to 87% in Australia. HUS was reported to have occurred in 8.7% of infected cases for all regions combined.

**Surveillance of non-O157 STEC strains in the USA 2002–2014**

The serogroups of non-O157 STEC strains reported to the CDC from both outbreaks and sporadic cases over a period of 13 years are provided in (Figure 1). The top three serogroups in descending order of importance were O26, O103, and O111. In patients with enteric infection, 129 different O serogroups and a total of 262 serotypes of non-O157 STEC were identified. According to data from the CDC, the most common non-O157 serogroups from 2004 to 2014 were O26 (31%), O103 (27%), O111 (21%), O121 (7%), O45 (5%), and O145 (5%) (Figure 1). These strains have potential for outbreaks that can be quite large.

**Figure 1.** Annual Reporting to the U.S. Centers for Disease Control and Prevention of non-O157 STEC Strains by Major Serogroups.

**Number of non-O157 STEC serogroups**

This review identified 129 different O serogroups of STEC associated with clinical cases of diarrhea. The number of non-O157 STEC serotypes identified considering the different flagellar (H) groups within the various serogroups was 262. Four or more H groups were seen for the following O groups: O6, O8, O20, O53, O60, O75, O84, O91, O103, O104, O111, O113, O118, and O174.

**World regions where STEC infections occur**

Most outbreaks and sporadic disease cases were reported from industrialized countries including the USA, Canada, Western Europe, Australia, Brazil, Japan, and Argentina, where diagnostic reagents are available and public health notification is required.

**Acute dysentery caused by STEC strains: non-O157 versus O157**

In the O104 STEC outbreak in Germany in 2011, 141 of 161 (88%) patients monitored for a history of passage of bloody stools reported dysentery. Clinical features of the outbreak in Germany were more severe than those seen with other STEC, which was possibly related to the unique inclusion of virulence gene in the O104:H4 STEC strain derived from the pathogen enteroaggregative E. coli (Rangel et al., 2005). In the various STEC outbreaks, excluding the O104:H4 cases, 119 of 464 (26%) patients with epidemic disease and 646 of 2588 (25%) patients with sporadic disease reported having dysentery. Strains of O157 STEC are associated with

**Table 1**

Non-outbreak reports of non-O157 STEC strains by global region, serogroup, number reported, and numbers with dysentery and hemolytic uremic syndrome of those observed for these complications, 1995–2017.

<table>
<thead>
<tr>
<th>Regions</th>
<th>Serogroups/types (number reported)</th>
<th>Number reported</th>
<th>Number of reported cases of dysentery (%)</th>
<th>Number of reported cases of HUS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA and Canada</td>
<td>O26 (292), O45 (211), O111 (201), O103 (207), O121 (110), O145 (75), other serogroups/types (416)</td>
<td>1512</td>
<td>432/1454 (30%)</td>
<td>45/1460 (3.1%)</td>
</tr>
<tr>
<td>Europe</td>
<td>O26 (216), O63 (11), O103 (71), O111 (54), O145 (51), O146 (20), other serogroups/types (103)</td>
<td>526</td>
<td>95/512 (19%)</td>
<td>60/512 (12%)</td>
</tr>
<tr>
<td>South and Central America</td>
<td>O26 (107), O103 (43), O111 (52), O145 (55), O146 (10), O174 (3), other serogroups/types (297)</td>
<td>567</td>
<td>38/481 (7.9%)</td>
<td>119/567 (21%)</td>
</tr>
<tr>
<td>Australia</td>
<td>O26 (7), O103 (1), O111 (14), O113 (1), O172 (1), other serogroups/types (45)</td>
<td>69</td>
<td>58/67 (87%)</td>
<td>7/69 (10%)</td>
</tr>
<tr>
<td>Japan</td>
<td>O65 (5), O103 (13), O111 (15), O121 (7), O145 (3), O165 (5), other serogroups/types (26)</td>
<td>74</td>
<td>23/74 (31%)</td>
<td>4/74 (5.4%)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>2748</td>
<td>646/2588 (25.0%)</td>
<td>234/2682 (8.7%)</td>
</tr>
</tbody>
</table>

HUS, hemolytic uremic syndrome; STEC, Shiga toxin-producing *Escherichia coli*. 
dysentery in approximately 85% of infected adults and children in industrialized regions.

The lower frequency of dysentery for non-O157 strains compared with O157 strains in cases of illness has been noted previously (Hedican et al., 2009; Kappeli et al., 2011).

Rates of HUS complicating enteric infection due to non-O157 versus O157 STEC strains

The rate of development of HUS in the outbreak of 0104:H4 in Germany was uniquely high at 22%. The rate of development of HUS for other non-O157 strains was 14% (78 of 544) in epidemic disease and 9% (235 of 2682) in endemic disease.

Rates of HUS development in enteric infection due to O157 strains ranged from 2% to 15% in publications appraised for this review. These rates are similar to those seen in this review for both outbreak disease and endemic disease.

Sources of non-O157 STEC outbreaks

Sources of infection in the reported outbreaks (see Table 1) included the consumption of meat products, unpasteurized drinks or unidentified foods consumed at a restaurant, and exposure to contaminated water from ice or a recreational lake. These sources resemble those associated with O157:H7 human outbreak illness. Swimming in contaminated water and the consumption of dairy products, spinach and sprouts, unpasteurized apple cider, undercooked beef, and even raw cookie dough have all been documented as causing O157:H7 outbreaks (Rangel et al., 2005). In the setting of the outbreak in Germany caused by E. coli O104:H4, sprout seeds from Egypt appeared to serve as the vehicle of disease transmission (Karch et al., 2012).

Diagnostic challenges in STEC infection

O157:H7 strains of E. coli isolated from human fecal samples can be assumed to be Shiga toxin producers. These strains are customarily identified in the USA by culturing stools on sorbitol MacConkey agar (SMA) with subsequent study of sorbitol-negative E. coli for somatic O antigen 157 in the laboratory. If positive, the E. coli colonies are sent to a reference laboratory for flagellar (H) 7 serotyping. The non-O157 strains generally ferment sorbitol like commensal E. coli, requiring that susptexts be examined for the presence of Shiga toxin in broth culture of stool by commercial enzyme immunoassay (EIA), or PCR without the need for culturing stool on SMA (Frias et al., 1996). With a positive fecal test for Shiga toxin, E. coli colonies are sent to a local health department for complete serotyping using group and type-specific antisera and agglutination methods.

Improved diagnostic accuracy is needed for STEC infections. One approach recommended by the US CDC has been published (Gould et al., 2009). The lack of reagents and a focus on O157 STEC has led to under-diagnosis of non-O157 strains (Stigi et al., 2012). The detection of both forms of STEC (O157 and non-O157) in patients with acute diarrhea is important when considering management of the illness. The current mainstay of treatment for patients with STEC-associated diarrhea (O157 and non-O157) and its complications includes aggressive hydration and supportive care (Serna and Boeckeker, 2008). Antimicrobial therapy may actually increase the rate of HUS in patients with STEC infection (Wong et al., 2000), although this remains an area for further research due to inconsistent data (Safdar et al., 2002).

Improved diagnostic reagents are needed to accurately screen and identify patients with infection due to the broad range of STEC strains.

Discussion and conclusions

Non-O157 strains of STEC are more common causes of acute diarrhea than the better known O157 strains. The incidence of non-O157 STEC infections in the USA has risen from 0.19 per 100 000 in 2007 to 0.79 per 100 000 in 2014 and thus demands greater attention (Figure 2).

The detection of STEC strains remains a challenge. Biomarkers of STEC may provide epidemiological value. The STEC virulence factor plasmid-encoded enterohemolysin (ehxA), which is often associated with severe clinical disease in humans, has been used as a possible epidemiological marker for pathogenic STEC. Among 208 cow and calf non-O157 STEC isolates, 79% had stx1, 79% had stx2, and 50% had both. Five percent of the isolates were positive for eaeA and 82% were positive for ehlyA (Ekiri et al., 2014).

Detecting STEC strains in the feces of cattle, the source of many of these infections, is challenging; however, researchers have begun identifying non-O157 strains using qPCR (Shridhar et al., 2016). In a study comparing culture versus PCR-based methods in detecting non-O157 strains, researchers found that each method detected one or more serogroups that the other method found negative, concluding that both methods are required for accurate detection (Noll et al., 2015). An optical immunoassay has recently been developed to identify O157 and the top six non-O157 serogroups in contaminated food products with the aim of curbing an outbreak faster than with traditional detection methods (Mondani et al., 2016). qPCR provides the greatest sensitivity and specificity among STEC detection methods, followed by EIA (Qin et al., 2015).

The reservoirs and vehicles of transmission of non-O157 E. coli strains resemble those seen for O157 strains. Cattle, goats, and sheep, which harbor the pathogen in their hindguts and shed them in feces (Shridhar et al., 2016), have been shown to be important reservoirs for non-O157 as well as O157 STEC. Researchers examining naturally infected beef cows and steer calves found that non-O157 STEC shedding is highest in younger animals at the stage of post-weaning and before entry into the feedlot (Ekiri et al., 2014). A study of adult houseflies near feedlots and dairy farms in the US showed that 34% of flies carried one or more of six non-O157 serogroups, indicating a potential role of this insect as a vector and reservoir for disease (Puri-Giri et al., 2017). Although ruminants are known to be the major reservoirs of STEC, non-ruminant food-producing animals like swine can harbor STEC (Iwu et al., 2016). The consumption of beef has been found to be the main risk factor for developing STEC illness, but consumption of sheep, pigs, dairy, and produce have also been linked to STEC infection. Person-to-person spread is most common in childcare settings.

**Figure 2.** Incidence of non-O157:H7 Shiga Toxin Producing Escherichia coli Cases per 100,000 Persons in United States, 2006–2014. Summarized from the annual reports by the CDC, Atlanta, National Surveillance of Bacterial Foodborne Illness (Enteric Disease), National Shiga Toxin-Producing Escherichia coli (STEC) Surveillance (6–16).
Strains of non-O157 STEC produce lower rates of dysentery than are seen with infections due to O157 strains. In a retrospective study examining STEC cases reported in Michigan from 2001 to 2012, the odds of hospitalization were twice as high in O157 STEC cases relative to non-O157 STEC cases (Tseng et al., 2016). However, a study from England found that non-O157 STEC strains were associated with higher hospitalization and HUS rates than O157 STEC strains (Byrne et al., 2015). This latter study demonstrated that the incidence of STEC was four times higher in rural areas of England where exposure to livestock was twice as common as in urban areas.

HUS is the most serious complication of STEC infection. A single-center study examining HUS in the pediatric population determined that 90% of HUS cases were attributable to E. coli O157 and 10% to non-O157 STEC (Schindler et al., 2014). However, non-O157 strains still caused significant morbidity. Non-O157 STEC accounted for 81% of all STEC gastroenteritis and 32% of STEC-associated HUS cases in Germany from 2008 to 2012 (Kuehne et al., 2016). Among single-epitope non-O157 STEC outbreaks in the USA in 2010, a greater percentage of individuals infected by Shiga toxin 2-positive strains had HUS compared to those infected with Shiga toxin 1-positive strains (Luna-Gierke et al., 2014), as has been shown for infection caused by Shiga toxin-producing E. coli O157: H7 (Ostroff et al., 1989).

With the increasing diagnosis of infectious diarrhea using multiplex PCR methods (Freeman et al., 2017), clinicians will be helped to make an efficient clinical diagnosis, but will lose the opportunity to identify non-O157 strains of STEC, hindering outbreak investigations that depend upon identification of organisms followed by molecular fingerprinting of the strains. In order for public health agencies to document the sources of epidemics of STEC infection, as they are required to do, it is necessary to isolate STEC strains for molecular typing. The current multiplex PCR methods are extremely sensitive but unfortunately identify low concentrations of genetic material that cannot always be confirmed by careful culture of stools in a research laboratory (Chao et al., 2017). It is important that we continue to develop improved assays to properly identify this group of pathogens in laboratories evaluating diarrhea cases, in order to quickly provide information to care providers, but we need to support public health agencies that need molecular data to stop epidemics rapidly if they are to achieve their public health mission.

Key points

- Non-O157 strains of STEC are important causes of acute diarrhea, dysentery, and HUS. In epidemic diarrhea, when illness is severe and associated with dysentery or HUS, stools should be processed for O157 and non-O157 strains.

Funding source

Funds needed for this review were provided by the University of Texas School of Public Health and McGovern Medical School and by the Kelsey Research Foundation.

Ethical approval

This review of the literature was not approved by an ethics committee.

Conflict of interest

No authors have any conflicts of interests to disclose.

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