

Non-O157 Shiga Toxin–Producing *Escherichia coli* Infections in the United States, 1983–2002

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Background. Shiga toxin–producing *Escherichia coli* (STEC) O157:H7 is a well-recognized cause of bloody diarrhea and hemolytic-uremic syndrome (HUS). Non-O157 STEC contribute to this burden of illness but have been underrecognized as a result of diagnostic limitations and inadequate surveillance.

Methods. Between 1983 and 2002, 43 state public health laboratories submitted 940 human non-O157 STEC isolates from persons with sporadic illnesses to the Centers for Diseases Control and Prevention reference laboratory for confirmation and serotyping.

Results. The most common serogroups were O26 (22%), O111 (16%), O103 (12%), O121 (8%), O45 (7%), and O145 (5%). Non-O157 STEC infections were most frequent during the summer and among young persons (median age, 12 years; interquartile range, 3–37 years). Virulence gene profiles were as follows: 61% *stx*₁ but not *stx*₂; 22% *stx*₂ but not *stx*₁; 17% both *stx*₁ and *stx*₂; 84% intimin (*eae*); and 86% enterohemolysin (*E-hly*). *stx*₂ was strongly associated with an increased risk of HUS, and *eae* was strongly associated with an increased risk of bloody diarrhea. STEC O111 accounted for most cases of HUS and was also the cause of 3 of 7 non-O157 STEC outbreaks reported in the United States.

Conclusions. Non-O157 STEC can cause severe illness that is comparable to the illness caused by STEC O157. Strains that produce Shiga toxin 2 are much more likely to cause HUS than are those that produce Shiga toxin 1 alone. Improving surveillance will more fully elucidate the incidence and pathological spectrum of these emerging agents. These efforts require increased clinical suspicion, improved clinical laboratory isolation, and continued serotyping of isolates in public health laboratories.

More than 100 serotypes of Shiga toxin–producing *Escherichia coli* (STEC) have been associated with human disease [1–3], causing illnesses that range from

mild diarrhea to bloody diarrhea, hemorrhagic colitis, and hemolytic-uremic syndrome (HUS) [4, 5]. *E. coli* O157:H7 is the STEC most frequently isolated in North America and is the serotype most often associated with bloody diarrhea and HUS. Non-O157 STEC have also caused sporadic illness and outbreaks of bloody diarrhea and HUS, both in the United States [6–10] and overseas [11–21]. It has been estimated that STEC O157:H7 causes 73,000 illnesses annually in the United States and that non-O157 STEC serotypes cause at least 37,000 illnesses [22]. Surveys from North America demonstrate that STEC causes diarrhea at frequencies similar to those of other important enteric bacterial pathogens (e.g., *Salmonella* and *Shigella* species), depending on the population studied and its geographic location, with STEC O157 isolated in one-half of the illnesses and a variety of non-O157 STEC serotypes isolated in the other half [23–34]. In continental Europe, infections with non-O157 STEC serotypes are more common than infections with O157:H7 STEC

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[35, 36]. Not all non-O157 STEC cause human illness, and some non-O157 STEC isolates recovered from diarrheal stool are likely not pathogens. The full spectrum of pathogenic non-O157 serogroups and the illnesses they cause remain poorly defined.

When infection with a non-O157 STEC is suspected, clinical laboratories may refer isolates to public health laboratories for identification and further characterization. The Foodborne and Diarrheal Diseases Laboratory of the Centers for Disease Control and Prevention (CDC) serves as the national reference laboratory for the confirmation of suspect non-O157 STEC isolates submitted by clinical laboratories to state and local public health departments and is one of the few laboratories in North America with the capacity to serotype confirmed isolates. Until 2000, non-O157 STEC infections were not a nationally notifiable disease. Since 2000, the Council of State and Territorial Epidemiologists has requested that public health departments report STEC infections to the National Notifiable Diseases Surveillance System. In the present article, we summarize the data from a large convenience sample of sporadic human non-O157 STEC isolates from the United States that have been confirmed by the CDC, describing the frequencies of serotypes, their seasonality, age distribution, the presence of various virulence factors, and associations between virulence factors and clinical syndromes.

SUBJECTS, MATERIALS, AND METHODS

Data collection. We reviewed the records for non-O157 STEC isolates forwarded by state public health laboratories to the CDC's reference laboratory between 1983 and 2002 for confirmation and serotyping. The documentation required when submitting specimens to the CDC included the name of the submitting state, the date of illness and/or specimen collection, the source (e.g., stool or blood), and the age and sex of the person from whom the specimen was collected. A presumptive diagnosis (e.g., diarrhea or HUS) and illness symptoms were requested but not required. Some of the isolates included in this national data set (<10% total) may have been included in previous analyses from contributing jurisdictions [31, 32, 34, 37]. The present investigation followed the guidelines of the US Department of Health and Human Services with regard to the protection of human subjects.

Laboratory procedures. Submitted isolates were streaked onto tryptose blood plates with washed sheep blood (Smith River Biologicals) and were incubated at 35°C for 18–24 h [38]. The plates were examined at 4 and 18 h for production of enterohemolysin. Individual colonies, both hemolytic and non-hemolytic, were then plated on trypticase soy agar with 5% sheep blood, incubated for 18–24 h, and tested by polymerase chain reaction for gene sequences encoding the following virulence factors: Shiga toxins 1 and 2 (*stx₁* and *stx₂*) [39–41],

intimin (*eae*) [42], and enterohemolysin (*E-hly*) [43]. Isolates that were positive for either or both Shiga toxins were serologically characterized for O and H antigens [44].

Statistical methods. We created a data set with a unique record for each isolate submitted, eliminating duplicate isolates that had been submitted from the same person for the same illness episode. We limited our descriptive analysis to sporadic disease by excluding any non-O157 STEC isolates that had been sent to the CDC from the few known US outbreaks. We analyzed dichotomous variables by the χ^2 test and Fisher's exact test and continuous variables by the Wilcoxon rank sum test (SAS; version 8.0e; SAS Institute). Multivariate analyses were performed by logistic regression with a backward-elimination process. We considered associations to be significant if the 95% confidence interval (CI) excluded 1.0 and $P \leq .05$ (2-tailed).

RESULTS

Between 1983 and 2002, the CDC confirmed 940 non-O157 STEC isolates that had been submitted by 42 state public health laboratories and the District of Columbia; 866 (92%) of the isolates were received after 1996 (figure 1). For 801 (85%) isolates, the clinical specimen source was provided: 794 were from stool, 4 were from urine, and 3 were from blood. The O-groups for 123 (13%) isolates could not be determined (i.e., they were nontypeable or formed rough colonies). The remainder included 209 (22%) O26:H11 or NM; 152 (16%) O111:H8 or NM; 117 (12%) O103:H2, H11, H25, or NM; 80 (8%) O121:H19 or H7; 63 (7%) O45:H2 or NM; 43 (5%) O145:NM; and 147 other isolates from 55 O-groups (each accounting for $\leq 1\%$ of all isolates) (table 1). The 6 most common serogroups accounted for 664 (71%) of all isolates. No cases were identified in which >1 STEC strain was isolated during a single illness episode. Most isolates were collected during the North American summer, between June and September. Incidence peaked in August, when 18% of all isolates were collected (figure 2). Other than this seasonality, there was no other evident clustering of isolates from any serotype in time or space in a fashion that would have suggested an unrecognized outbreak. We found no statistically significant differences in the proportional distributions of the 6 most common serotype north and south of latitudes 36°N and 42°N (data not shown).

Of 676 isolates from persons whose sex was included on the specimen submission form, 372 (55%) were from women. Of 501 (53%) isolates collected from persons for whom age was provided, the median age was 12 years (interquartile range [IQR], 3–37 years); 285 (57%) isolates were from persons ≥ 10 years old. There were 357 persons of known age from whom isolates belonging to the 6 most common serogroups were isolated. The median age of these persons was significantly lower than that of the 61 persons with isolates belonging to other serogroups (10 vs. 21 years; $P = .04$), after exclusion of 83

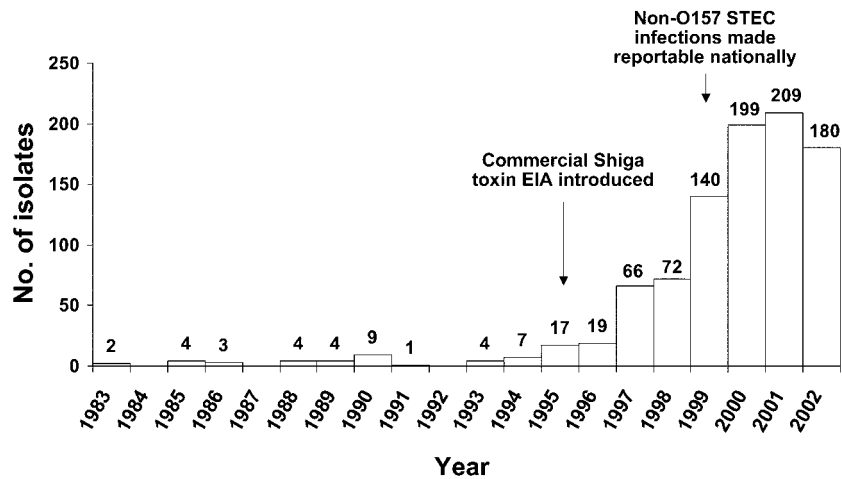


Figure 1. Human non-O157 Shiga toxin-producing *Escherichia coli* (STEC) isolates submitted to the Centers for Disease Control and Prevention for confirmation, by year, 1983–2002 ($n = 940$).

persons of known age from whom non-O157 STEC isolates of undetermined serogroup were isolated.

Of 292 (31%) isolates submitted with definitive clinical data, 21 (7%) were from persons with HUS and 75 (26%) were from persons with bloody diarrhea but not HUS (table 2). Data on age were available for 13 (62%) of the 21 persons from whom isolates associated with HUS were collected; among these 13, the median age was 6 years (IQR, 2–10 years). Data on age were available for 49 (65%) of the 75 persons from whom isolates associated with bloody diarrhea but not HUS were collected; among these 49, the median age was 17 years (IQR, 5–51 years).

STEC O111 was the only serogroup statistically associated with HUS; it was isolated from 10 (48%) of the 21 persons with HUS, compared with 42 (16%) of the 271 persons without HUS (relative risk [RR], 4.20 [95% CI, 1.18–9.36]; $P = .001$). STEC O121 was the only serogroup statistically associated with bloody diarrhea; it was isolated from 13 (17%) of the 75 persons with bloody diarrhea but not HUS, compared with 9 (4%) of the 217 persons without bloody diarrhea (RR, 2.57 [95% CI, 1.71–3.88]; $P = .001$).

Multivariate analyses examining the relationships between virulence gene profiles (singly and in all permutations, up to and including the combination of all 4 genes evaluated) and HUS demonstrated a statistically significant increased risk of HUS associated with *stx*₂: 20 (93%) of the 21 persons with HUS provided isolates with detectable *stx*₂, compared with 88 (33%) of the 271 persons without HUS (estimated odds ratio [OR], 32.0 [95% CI, 4.26–240]; $P = .008$). Similar analyses demonstrated a statistically significant increased risk of bloody diarrhea without HUS associated with *eae*: 70 (93%) of the 75 persons with bloody diarrhea but without HUS provided isolates with detectable *eae*, compared with 184 (86%) of the 214

persons without bloody diarrhea (OR 5.26 [95% CI, 1.60–17.3]; $P = .006$). All associations remained robust and of comparable magnitude when the univariate and multivariate analyses included all isolates, regardless of clinical data.

DISCUSSION

This comprehensive evaluation of non-O157 STEC isolates from persons in the United States found that 6 (O26, O111, O103, O121, O45, and O145) of the 61 serogroups identified accounted for 71% of the isolates recovered from 1983 to 2002. Three of these serogroups (O26, O111, and O103) accounted for 50% of the isolates. The non-O157 STEC demonstrated a summer seasonality, similar to that for the STEC O157:H7 [45, 46], and were isolated more frequently from children.

The virulence gene *stx*₂ was significantly associated with an increased risk of HUS in persons infected with non-O157 STEC in the United States, which is consistent with similar findings reported from the United Kingdom [47]. Surveys have shown that the vast majority of North American STEC O157:H7 isolates associated with HUS possess *stx*₂ alone or in combination with *stx*₁ and that only a small fraction possess *stx*₁ but not *stx*₂ [4, 46, 48]; we found a similar distribution of Shiga toxin genes among the non-O157 STEC isolates associated with HUS. Studies conducted elsewhere in the world examining the association between HUS and virulence factors in STEC collections, some of which included O157:H7, have similarly shown that *stx*₂ is the factor most strongly associated with the development of HUS [20, 47, 49–54]. Although the mechanism by which Shiga toxin 2 causes HUS is not fully understood, the active site of this molecule is considerably more accessible than the active site of Shiga toxin 1, which may explain the stronger association between this virulence factor and disease [55].

Table 1. Human non-O157 Shiga toxin–producing *Escherichia coli* isolates submitted to the Centers for Disease Control and Prevention (CDC) for confirmation and prevalence of associated virulence genes, by serotype, 1983–2002.

| Serogroup | Age of donor, median (IQR), years ^a | Shiga toxin | | | <i>eae</i> | E- <i>hly</i> |
|-----------------------------|--|-------------------------------|-------------------------------|---|---------------|---------------|
| | | <i>stx</i> ₁ alone | <i>stx</i> ₂ alone | <i>stx</i> ₁ and <i>stx</i> ₂ | | |
| O26 (n = 209) | 6 (2–18) | 182/208 (88) | 4/208 (2) | 22/208 (11) | 198/202 (98) | 195/202 (96) |
| O111 (n = 152) | 6 (2–21) | 65/152 (43) | 0/152 (0) | 87/152 (57) | 146/149 (98) | 139/149 (93) |
| O103 (n = 117) | 17 (3–31) | 117/117 (100) | 0/117 (0) | 0/117 (0) | 115/115 (100) | 114/115 (99) |
| O121 (n = 80) | 10 (4–34) | 1/80 (1) | 72/80 (90) | 7/80 (9) | 73/75 (97) | 69/75 (92) |
| O45 (n = 63) | 25 (13–48) | 62/63 (98) | 0/63 (0) | 1/63 (2) | 62/63 (98) | 60/63 (95) |
| O145 (n = 43) | 18 (6–42) | 18/43 (42) | 19/43 (44) | 6/43 (14) | 42/42 (100) | 42/42 (100) |
| O165 (n = 14) | 20 (8–66) | 0/14 (0) | 7/14 (50) | 7/14 (50) | 13/14 (93) | 13/14 (93) |
| O118 (n = 9) | 36 (2–52) | 9/9 (100) | 0/9 (0) | 0/9 (0) | 8/8 (100) | 8/8 (100) |
| O91 (n = 8) | 42 (30–46) | 4/8 (50) | 1/8 (12) | 3/8 (38) | 0/8 (0) | 4/8 (50) |
| O113 (n = 8) | 19 (10–41) | 0/8 (0) | 6/8 (75) | 2/8 (25) | 0/8 (0) | 5/8 (62) |
| O153 (n = 7) | 10 (9–11) | 7/7 (100) | 0/7 (0) | 0/7 (0) | 7/7 (100) | 7/7 (100) |
| O146 (n = 6) | 69 (64–74) | 5/6 (83) | 1/6 (17) | 0/7 (0) | 0/6 (0) | 2/6 (33) |
| O174 (n = 6) | 51 (21–75) | 0/6 (0) | 6/6 (100) | 0/6 (0) | 0/6 (0) | 0/6 (0) |
| Other (n = 95) ^b | 27 (7–62) | 29/95 (30) | 46/95 (48) | 20/95 (20) | 29/94 (31) | 39/93 (42) |
| Undetermined (n = 123) | 27 (7–62) | 71/123 (58) | 38/123 (31) | 14/123 (11) | 82/121 (68) | 96/121 (79) |
| Total (n = 940) | 15 (5–41) | 570/939 (61) | 200/939 (21) | 169/939 (18) | 775/918 (84) | 793/917 (86) |

NOTE. Data are proportion (%) of isolates, unless otherwise noted. IQR, interquartile range.

^a For isolates for which the age of the person providing the specimen was included with submission to the CDC.

^b For O22, O28, and O88, 5 isolates each (<1%); for O119, O128, and O172, 4 isolates each (<1%); for O6, O8, O60, O63, O104, O117, and O126, 3 isolates each (<1%); for O2, O14, O49, O50, O55, O73, O75, O96, O109, O110, O137, and O163, 2 isolates each (<1%); and for O1, O4, O5, O21, O38, O46, O48, O51, O77, O79, O83, O84, O86, O116, O124, O125, O140, O142, O143, O159, O168, O171, and O53/O117, 1 isolate each (<1%).

We also identified an independent association between *eae* and bloody diarrhea; *eae* encodes the protein intimin, which facilitates attaching and effacing lesions on the gut epithelium and may explain, in part, the association between this virulence factor and bloody diarrhea. Bloody diarrhea has also been associated with *stx*₂, with or without other factors [52, 56]. The association with *stx*₂ remains controversial [34, 57], with some authors proposing that the bloody diarrhea observed during STEC infections may more likely be related to virulence factors other than Shiga toxins [34, 58].

Our data demonstrate that STEC O111 is the second most common bacterial cause of HUS in the United States, after STEC O157:H7. STEC O111 was identified as the etiological agent for 3 of 7 reported outbreaks of non-O157 STEC serotypes; 2 of the STEC O111 outbreaks included cases of HUS. Eleven (52%) of the 21 non-O157 STEC isolates associated with HUS were STEC O111; none were isolates from the 3 recognized outbreaks. An association between STEC O111 and HUS has also been observed in other countries [59].

The association we observed between HUS and *stx*₂ was not, however, a marker for infection with STEC O111, even though 9 (90%) of the 10 STEC O111 isolates associated with HUS were *stx*₂ positive. Our multivariate analysis included as variables only virulence genes, which (like other investigators) we believe to be the driving force in the etiology of STEC-associated illness. When serotype was included in the multivariate models, we were not surprised to find a statistically significant

association between HUS and STEC O111 (OR 2.89 [95% CI 1.15–7.23]; *P* = .024); however, the association between HUS and *stx*₂ remained robust and was more significant (OR, 27.2 [95% CI, 3.60–205]; *P* = .001).

We did not receive submissions from cases of illness yielding 2 or more different STEC isolates. The lack of these cases in our collection is likely due, in large part, to common culturing practices that are not sensitive enough to recover >1 different STEC isolate from a stool specimen. Studies are needed to evaluate specimens for mixed STEC infections and to assess the prevalence of such infections, particularly their association with severe illness.

Our survey was limited to isolates sent to the CDC reference laboratory and does not represent the results of formal surveillance. Nonetheless, this large convenience sample provides the most informative data on non-O157 STEC infections in the United States. A convenience sample is subject to a number of biases. Most infections are not detected, because clinical laboratories do not test specimens for non-O157 STEC. Conversely, laboratories equipped to identify STEC may have isolated strains and not sent them to the CDC. However, the number of non-O157 STEC isolates not submitted to the CDC was likely small, because most US clinical and public health laboratories lacked the resources for serotyping *E. coli* during the survey period. It is likely that some clinical laboratories identified Shiga toxin–positive specimens but did not submit an isolate for serotyping. Although we cannot assess the extent

Table 2. Prevalence of virulence genes among 21 human non-O157 Shiga toxin-producing *Escherichia coli* (STEC) isolates associated with hemolytic-uremic syndrome (HUS) and 75 non-O157 STEC isolates associated with bloody diarrhea, 1983–2002.

| Disease, serogroup | Shiga toxin | | | <i>eae</i> | <i>E-hly</i> |
|-------------------------------------|-------------------------------|-------------------------------|---|-------------|--------------|
| | <i>stx</i> ₁ alone | <i>stx</i> ₂ alone | <i>stx</i> ₁ and <i>stx</i> ₂ | | |
| HUS | | | | | |
| O111 (<i>n</i> = 10) | 1/10 (10) | 0/10 (0) | 9/10 (90) | 9/9 (100) | 8/9 (89) |
| Other ^a (<i>n</i> = 11) | 0/11 (0) | 8/11 (73) | 3/11 (27) | 7/11 (64) | 8/11 (73) |
| Total (<i>n</i> = 21) | 1/21 (5) | 8/21 (38) | 12/21 (57) | 16/20 (80) | 16/20 (80) |
| Bloody diarrhea | | | | | |
| O26 (<i>n</i> = 12) | 10/12 (83) | 0/12 (0) | 2/12 (17) | 12/12 (100) | 12/12 (89) |
| O111 (<i>n</i> = 8) | 4/8 (50) | 0/8 (0) | 4/8 (50) | 7/8 (88) | 8/8 (100) |
| O103 (<i>n</i> = 12) | 12/12 (100) | 0/12 (0) | 0/12 (0) | 12/12 (100) | 12/12 (100) |
| O121 (<i>n</i> = 13) | 0/13 (0) | 13/13 (100) | 0/13 (0) | 12/13 (92) | 12/13 (92) |
| O45 (<i>n</i> = 10) | 10/10 (100) | 0/10 (0) | 0/10 (0) | 10/10 (100) | 9/10 (90) |
| O145 (<i>n</i> = 4) | 1/4 (25) | 2/4 (50) | 1/4 (25) | 4/4 (100) | 4/4 (100) |
| Other ^b (<i>n</i> = 9) | 3/9 (33) | 5/9 (56) | 1/9 (11) | 6/9 (67) | 6/9 (67) |
| Undetermined (<i>n</i> = 7) | 6/7 (86) | 0/7 (0) | 1/7 (14) | 7/7 (100) | 7/7 (100) |
| Total (<i>n</i> = 75) | 46/75 (61) | 20/75 (27) | 9/75 (12) | 70/75 (93) | 70/75 (93) |

NOTE. Data are proportion (%) of isolates.

^a For O14, O22, O26, O50, O79, O113, O121, O137, O145, O165, and O172, 1 isolate each.

^b For O8, O49, O79, O117, O118, O137, O140, O143, and O165, 1 isolate each.

of this activity, we have no reason to believe that there was a systematic exclusion bias that would have altered the distribution of serogroups we describe here. Formal surveillance could ameliorate these biases and provide more-informative data by better describing the spectrum of STEC serotypes that cause disease, their frequency and geographic distribution, and the clinical illness they cause.

We received clinical data for only one-third of specimens; we assumed that the isolates we received were forwarded to public health laboratories by clinical facilities for the diagnostic evaluation of illness. Although both diarrhea and HUS are well-characterized conditions, use of a standardized diagnosis on the specimen submission form was not required; the diagnoses included on the forms were assigned at the discretion of the submitter on the basis of available clinical information,

which may have been limited and led to underreporting. Diagnosis of non-O157 STEC infection may have been pursued more aggressively in very ill persons, particularly those with bloody diarrhea or HUS. A high proportion of isolates that were included in small, previous series were from patients with more-severe illness [34], suggesting that some of the patients with missing clinical data also had severe illness. This classification error would alter the statistical associations of HUS and bloody diarrhea with non-O157 serogroups and virulence factors we observed; however, without better clinical data, we cannot speculate reliably to what degree. The associations we found are consistent with the findings of previous reports and are biologically plausible. The present study was unable to analyze important questions regarding the clinical care of persons with STEC infection, such as the benefit or harm resulting from

Table 3. Reported outbreaks of human non-O157 Shiga toxin-producing *Escherichia coli* infections in the United States, 1983–2002.

| Year | Serogroup | State | No. of ill persons | Serologically confirmed | Suspected exposure/vehicle | HUS reported | Exposure/vehicle confirmed | Reference |
|------|-----------|--------------|--------------------|-------------------------|----------------------------|--------------|----------------------------|--------------------------|
| 1990 | O111 | Ohio | 5 | Yes | Undetermined | Yes | No | [7] |
| 1994 | O104 | Montana | 18 | Yes | Milk | No | No | [6] |
| 1999 | O121 | Connecticut | 11 | Yes | Lake water | Yes | No | [60] |
| 1999 | O111 | Texas | 56 | Yes | Salad bar | Yes | No | [10] |
| 2000 | O103 | Washington | 18 | Yes | Punch | Yes | No | Unpublished ^a |
| 2001 | O111 | South Dakota | 3 | No | Day care | No | No | [61] |
| 2001 | O26 | Minnesota | 4 | No | Lake water | No | No | Unpublished ^a |

NOTE. HUS, hemolytic-uremic syndrome.

^a Unpublished data reported to the Centers for Disease Control and Prevention (presented with permission from the Washington State Department of Health and the Minnesota Department of Public Health).

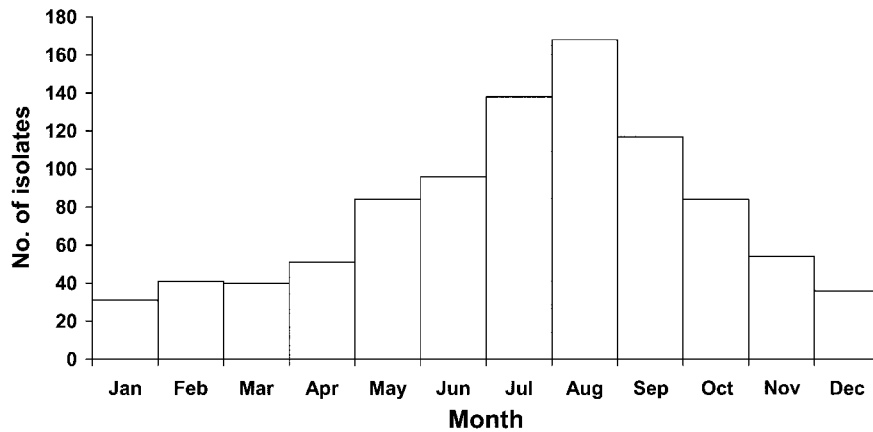


Figure 2. Human non-O157 Shiga toxin-producing *Escherichia coli* isolates submitted to the Centers for Disease Control and Prevention for confirmation, by month of illness onset or specimen collection, 1983–2002 ($n = 940$).

the use of antibiotics in the treatment of non-O157 STEC infection; clinical complications of these infections other than HUS (e.g., stroke); length of illness; and period of bacterial shedding. At a minimum, future surveillance-based case reporting of non-O157 STEC infections should indicate definitively whether bloody diarrhea and HUS were present or absent by use of standardized definitions.

The present study included only sporadic infections with non-O157 STEC. From 1992 to 2002, 7 outbreaks of non-O157 STEC infections were reported in the United States (table 3); 5 were serologically confirmed. Three were caused by STEC O111, and 1 each was caused by STEC O26, O103, O104, and O121. Epidemiologic investigation associated 6 outbreaks with specific exposures (3 foodborne and 3 environmental); however, a vehicle was microbiologically confirmed in none. Improved surveillance and awareness of non-O157 STEC as pathogens will improve the identification and reporting of outbreaks, which may be currently underrecognized.

Most non-O157 STEC cannot be visually distinguished from nonpathogenic *E. coli* on differential plating media (e.g., sorbitol MacConkey's agar [SMAC]), which until recently was the principal method used to screen bloody diarrhea and specimens from persons with HUS for STEC O157:H7. O157:H7 colonies appear pale, whereas other *E. coli*, including most non-O157 STEC, appear pink. Screening for non-O157 STEC has been facilitated by new assays that detect Shiga toxin; submission of non-O157 STEC isolates to the CDC for confirmation increased substantially after the commercial introduction of Shiga toxin EIAs (figure 1). Notably, simultaneous culture of stools on SMAC and screening for Shiga toxin by EIA has identified more STEC than has either procedure alone [33, 62].

The advent of Shiga toxin EIAs has substantially increased the capacity of community-based laboratories to diagnose non-O157 STEC infections. Before the mid-1990s, diagnosis was limited to a few academic and public health laboratories with

specialized capacity. Although the 74 isolates received before the mid-1990s may be less representative of community-based illness, we have presented data from both before and after the development of Shiga toxin EIAs to illustrate how these assays can enhance routine surveillance for non-O157 STEC. The proportional distribution of serotypes among the 866 isolates received during the period 1997–2002 was not significantly different from that among all 940 isolates. Inclusion of isolates from both periods would not affect our analysis of the associations between clinical syndromes and virulence factors.

Although direct testing of stool by use of these EIAs to detect the presence of Shiga toxin-producing bacteria might seem to obviate the clinical need to culture stool, the importance of obtaining STEC isolates for public health surveillance cannot be overemphasized. Non-O157 STEC may be as common a cause of diarrhea as other, better recognized bacterial agents. Notably, most of the isolates we received from persons described as having diarrhea were not described as having either bloody diarrhea or HUS, and these persons may have presented with clinical symptoms similar to those of infections with other enteric pathogens. The low infectious dose of some non-O157 STEC genotypes and their potential to cause severe life-threatening illness, particularly among children, make these agents an important public health concern. For this reason, it is essential that Shiga toxin-positive specimens or isolates be forwarded to public health laboratories for serological and molecular characterization of the Shiga toxin-producing organisms; identification of the toxin alone is inadequate and should not be used to replace culture and serotyping.

Improved national surveillance will advance our understanding of the epidemiology of non-O157 STEC infections and allow us to monitor changes in the frequency with which major serogroups cause illness over time. We strongly encourage clinicians to consider STEC infection when diagnosing illnesses and clinical laboratories to consider screening all specimens

from persons with diarrhea for STEC, both O157 and non-O157. If screening all specimens for non-O157 STEC is impractical, then we advise, at a minimum, evaluating specimens from persons with bloody diarrhea or HUS; this practice could be reserved for specimens that do not yield STEC O157. Molecular methods, such as Shiga toxin EIAs, should be used in tandem with culture and serotyping and never alone. At this time, isolating and serotyping *E. coli* isolates that are positive for Shiga toxin is the only way to monitor trends, to detect emerging STEC serotypes, and to define their epidemiology. Providing public health laboratories with the expertise and antisera needed to identify STEC that belong to the 6 major non-O157 serogroups is a first step that could speed the identification of outbreaks and improve our overall understanding of STEC.

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