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Long-Term Sentinel Surveillance for Enterotoxigenic *Escherichia coli* and Non-O157 Shiga Toxin-Producing *E. coli* in Minnesota

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Background. Enterotoxigenic *Escherichia coli* (ETEC) and non-O157 Shiga toxin-producing *E. coli* (STEC) are not detected by conventional culture methods. The prevalence of ETEC infections in the United States is unknown, and recognized cases are primarily associated with foreign travel. Gaps remain in our understanding of STEC epidemiology.

Methods. Two sentinel surveillance sites were enrolled: an urban health maintenance organization laboratory (Laboratory A) and a rural hospital laboratory (Laboratory B). Residual sorbitol MacConkey (SMAC) plates from stool cultures performed at Laboratory A (1996–2006) and Laboratory B (2000–2008) were collected. Colony sweeps from SMAC plates were tested for genes encoding STEC toxins *stx1* and *stx2* (1996–2008) and ETEC heat-labile and heat-stable toxins *eltB*, *estA* 1, 2 and 3 (2000–2008) by polymerase chain reaction (PCR)-based assays.

Results. In Laboratory A, a bacterial pathogen was identified in 7.0% of 21 970 specimens. During 1996–2006, *Campylobacter* was the most common bacterial pathogen (2.7% of cultures), followed by *Salmonella* (1.2%), *Shigella* (1.0%), and STEC (0.9%). Among STEC (n = 196), O157 was the most common serogroup (31%). During 2000–2006, ETEC (1.9%) was the second most common bacterial pathogen after *Campylobacter* (2.6%). In Laboratory B, of 19 293 specimens tested, a bacterial pathogen was identified for 5.5%, including *Campylobacter* (2.1%), STEC (1.3%), *Salmonella* (1.0%), and ETEC (0.8%). Among STEC (n = 253), O157 was the leading serogroup (35%). Among ETEC cases, 61% traveled internationally.

Conclusions. Enterotoxigenic *E. coli* and STEC infections were as common as most other enteric bacterial pathogens, and ETEC may be detected more frequently by culture-independent multiplex PCR diagnostic methods. A high proportion of ETEC cases were domestically acquired.

Keywords. enterotoxigenic *Escherichia coli*; pathogenic *Escherichia coli*; Shiga toxin-producing *Escherichia coli*.

Enterotoxigenic *Escherichia coli* (ETEC) is considered to be the leading cause of traveler's diarrhea [1–3]. Although Shiga toxin-producing *E. coli* (STEC) O157 is a well documented cause of gastroenteritis and hemolytic uremic syndrome (HUS) in the United States, non-O157 STEC also have been recognized as important pathogens, with a wide range of clinical presentations, from mild illness to HUS [4, 5]. Both ETEC and non-O157 STEC have been implicated in outbreaks in the United States [6, 7]. In contrast to STEC O157, ETEC and non-O157 STEC are not detected by conventional stool culture methods in clinical laboratories. Culture-independent diagnostic tests

(CIDTs), including enzyme-linked immunoassay tests to detect Shiga toxins, became commercially available to clinical laboratories around 2000. The adoption of these tests has been increasing since [8, 9], leading to a corresponding increase in the recognition of non-O157 STEC as an important cause of illness [10–13]. However, because commercially available assays for ETEC have been lacking, and the adoption of Shiga toxin assays by clinical laboratories has been neither universal nor uniform, the true importance of these types of diarrheagenic *E. coli* as enteric pathogens in the United States is unknown.

The objective of our study was to use long-term sentinel surveillance to determine the frequency with which ETEC and non-O157 STEC infections occur in a rural and an urban setting in Minnesota, and the relative frequency of these pathogens compared with other bacterial enteric pathogens.

METHODS

Surveillance

Two sentinel sites were enrolled in this study: Laboratory A, a large health maintenance organization laboratory that served the Minneapolis-St. Paul metropolitan area; and Laboratory B, a hospital laboratory that served a small city and surrounding

rural area that is rich in animal agriculture, particularly dairy production. The exact population size served by these 2 laboratories is unknown. Every stool submitted for bacterial culture at both laboratories was plated on a sorbitol MacConkey agar (SMAC) plate. Residual SMAC plates from every stool culture were sent to the Minnesota Department of Health (MDH) Public Health Laboratory (PHL) for STEC and ETEC polymerase chain reaction (PCR) testing regardless of the culture results at the clinical laboratory [5]. Only 1 isolate per person was included.

For Laboratory A, STEC testing was performed from 1996 to 2006, and ETEC testing was performed from 2000 to 2006. For Laboratory B, testing both for STEC and ETEC was performed from 2000 to 2008.

During the entire study period, active laboratory-based surveillance for reportable bacterial pathogens was conducted at both laboratories; all *Campylobacter*, STEC O157, *Salmonella*, *Shigella*, *Vibrio*, and *Yersinia* isolates cultured at both laboratories were submitted to the MDH PHL for confirmation. During the study period, CIDTs for the detection of *Campylobacter* and/or STEC were not being used by these sentinel site laboratories.

Laboratory Methods

Nucleic acid extraction was initiated upon receiving a SMAC plate from a sentinel laboratory. Template DNA was prepared from colony sweeps. Six sweeps were made through representative areas of growth, which included all visible colony morphologies, avoiding the primary inoculation area, and mixed using a 1.0 μL disposable loop. One loopful of the mixed sweep material and 200 μL molecular grade water (Sigma) were heated for 15 minutes in boiling water and centrifuged at 16 000 *g* for 2 minutes. Clear supernatants containing bacterial DNA were withdrawn for PCR analysis.

From January 2000 through July 2005, Shiga toxin genes *stx1* and *stx2* were detected by PCR using previously described primers and amplification methods [14]. In July 2005, the PCR method of Paton and Paton [15] was implemented to increase Shiga toxin gene detection sensitivity, to detect the intimin-encoding gene *eae* to identify potential enteropathogenic *E. coli*, and to detect the alpha-hemolysin-encoding gene *hlyA*, another marker for STEC.

Specimen sweeps testing positive for *stx* had up to 24 individual colonies tested for *stx* by PCR. Shiga toxin gene-positive isolates were identified by standard biochemical methods [16]. Somatic and flagellar antigens were determined using Denka Seiken antisera. If a sample sweep was positive for *stx* by PCR but a *stx*-positive colony could not be isolated, the sample was classified as PCR-positive STEC, not isolated. If an individual *E. coli* colony was Shiga toxin gene-positive and tested negative for O157, it was classified as STEC even if the serogroup could not be determined (e.g., O rough or undetermined).

For ETEC, the plate sweep was tested for *estA1*, *estA2*, *estA3* (*E. coli* heat-stable toxin or LT1-encoding genes), and *eltB* (heat labile or ST1-encoding gene) by Multiplex SYBR Green PCR. Single gene primers and probes were then used to confirm positives by TaqMan PCR. Primer and probe sequences for LT1 and ST1 genes are listed in Table 1. SYBR Green detection assay was 25 μL volume with final concentrations of 1× SYBR Green buffer (SYBR Green Kit, Applied Biosystems), 3.5 mM MgCl₂, 1.0 mM dNTPs with dUTP (Applied Biosystems, Foster City, CA), 10 nM fluorescein (Bio-Rad, Hercules, CA), 400 nM *estA1* primers, 400 nM *estA2,3* primers, 200 nM *eltB* primers, 0.625 units AmpliTaq Gold (Applied Biosystems), and 1.0 μL sample supernatant DNA. Amplification conditions were as follows: 50°C for 2 minutes, 95°C for 10 minutes, then 40 cycles of 15 seconds at 95°C and 1 minute at 60°C. Samples were positive if the cycle threshold (Ct) value exceeded background level in <40 cycles and the melt curve temperature (Tm) matched those of the controls; *estA* Tm = 77–77.5°C and *eltB* Tm = 80.5–81°C. Samples that crossed threshold having incorrect Tm were considered negative. TaqMan probe confirmation has each gene primer and probe PCR assay in 25 μL volume with final concentrations of 1× TaqMan buffer (TaqMan Kit, Applied Biosystems), 5.0 mM MgCl₂, 1.0 mM dNTPs with dUTP (Applied Biosystems), 1000 nM specified primer set (*estA1*, *estA2,3*, or *eltB*), 250 nM specific probe (*estA1*, *estA2*, *estA3*, or *eltB*), 0.625 units Hot Star Taq (QIAGEN), and 2.0 μL

Table 1. Oligonucleotide Primers and TaqMan Hybridization Probes Used in PCR Assays for Identifying *Escherichia coli* Heat-Labile (LT1) and Heat-Stable (ST1) Genes Indicative of Enterotoxigenic *E. coli*[a]

Gene[b,c]	Sequence	Product Size
eltB		73 bp
eltB-205	5′ TAA GAG CGG CGC AAC ATT T 3′	
eltB-277	5′ TTC AAT GGC TTT TTT TTG GGA 3′	
eltB probe	5′ TTG ACT GCC CGG GAC TTC GAC CT 3′	
estA1		151 bp
estA1-353	5′ AGT CAA CTG AAT CAC TTG ACT CTT CA 3′	
estA1-503	5′ CCA GCA CAG GCA GGA TTA CA 3′	
estA1 probe	5′ AAT CAG AAA ATA TGA ACA ACA CAT TTT ACT GCT GTG AA 3′	
estA2,3		139 bp
estA2,3-197	5′ CCT TTC GCT CAG GAT GCT AAA C 3′	
estA2,3-335	5′ ACA ATT CAC AGC AGT AAT TGC TAC TAT TC 3′	
estA2 probe	5′ CGA TTC TAG TGT AAT TTT TTC TTT TGA AGA CCC TGC T 3′	
estA3 probe	5′ AGT AGA GTC TTC AAA AGA AAA AAT CAC ACT AGA ATC A 3′	

Abbreviations: bp, base pairs; PCR, polymerase chain reaction.

[a] The SYBR Green and TaqMan primers and probes were developed by Minnesota Department of Health (unpublished data).

[b] DNA oligonucleotide primers were synthesized by Integrated DNA Technologies, Inc. (Coralville, IA).

[c] Hybridization probes (5′ FAM/TAMRA-Q 3′) were synthesized by Operon Biotechnologies, Inc. (Huntsville, AL).

sample supernatant DNA. Amplification conditions were as follows: 50°C for 2 minutes, 95°C for 10 minutes, then 45 cycles of 15 seconds at 95°C and 1 minute at 60°C. A specimen was positive if the Ct value exceeded background level <45 cycles. Specimens positive for SYBR Green but negative on TaqMan assay were subcultured for colony isolation. Up to 24 individual colonies were retested by SYBR Green PCR. Specimens positive only for SYBR Green and without identified positive isolates were classified as negative for ETEC. Isolate identity for selected STEC and ETEC isolates was further confirmed at the Centers for Disease Control and Prevention (Atlanta, GA). Starting in 2005, testing for *E. coli* virulence factor *bfp* (bundle-forming pilus encoding gene), indicative of enteropathogenic *E. coli* (EPEC), was also done by PCR following the methods of Gunzberg [17].

Descriptive and Statistical Analyses

Descriptive analyses were conducted for the entire study period. However, because study time periods differed between the 2 sentinel sites, and ETEC surveillance was initiated in 2000, statistical comparisons of the 2 sentinel sites were restricted to 2000–2006, when surveillance was conducted at both sites. Analyses were conducted using Epi Info 7.1.3.10 (Centers for Disease Control and Prevention, Atlanta, GA).

After the sentinel surveillance study period ended, both laboratories adopted ImmunoCard STAT! EHEC (Meridian Bioscience, Cincinnati, OH) to detect Shiga toxin and sent the enrichment broths to the MDH PHL for confirmation by real-time PCR. Both laboratories still used culture to detect *Campylobacter*, *Salmonella*, STEC O157, and *Shigella* and sent isolates for confirmation at the PHL. To understand how our sentinel surveillance data compared with current surveillance data that include the use of CIDTs for STEC, the frequency of pathogens using sentinel surveillance data was compared to the 2013–2014 surveillance data at each site.

Starting in 2000 until the end of the enrollment period for each site (2006 for site A, and 2008 for site B), ETEC and STEC cases were interviewed about international travel in the 7 days prior to illness onset. The number and proportion of ETEC and STEC cases that reported international travel were described.

RESULTS

Laboratory A (Urban Laboratory)

From Laboratory A, 21 970 SMAC culture plates (cultures) were tested during 1996–2006, with a median of 1997 cultures per year (range, 1589 to 2432) (Table 2, Figure 1). A bacterial pathogen was identified from 1540 (7.0%) cultures overall, with a range of 4.9% to 11% per year. Excluding ETEC, because it was not tested for during the entire study period, *Campylobacter* was the most common bacterial pathogen isolated, accounting for 586 (2.7%) cultures, followed by *Salmonella* at 264 (1.2%), *Shigella* at 223 (1.0%), STEC (O157, non-O157, and not isolated

combined) at 196 (0.9%), *Vibrio* at 6 (0.03%), and *Yersinia* at 4 (0.02%) (Table 2, Figure 1).

Among the 13 802 cultures tested during 2000–2006, the period when ETEC testing was performed for Laboratory A, *Campylobacter* remained the most common bacterial pathogen, accounting for 356 (2.6%) cultures. The second most commonly identified pathogen was ETEC, accounting for 256 (1.9%) cultures, followed by *Shigella* at 165 (1.2%), *Salmonella* at 151 (1.1%), STEC at 129 (0.9%), *Yersinia* at 3 (0.02%), *Vibrio* at 2 (0.01%), and EPEC at 2 (0.01%) (Table 2, Figure 1).

Among the 196 STEC detected over the entire study period, 60 (31%) were O157:H7 or O157:non-motile, 88 (45%) were serogroups other than O157, and 48 (25%) were *stx* positive but were not isolated (Table 2).

The median annual number of cases for *Campylobacter* during the study period was 52 compared with a median of 52 during 2013–2014; *Salmonella* was 23 during the study period vs 25 during 2013–2014; *Shigella* was 17 vs 8; STEC O157 was 6 vs 5; STEC non-O157 was 7 vs 5; and STEC not isolated was 3 vs 0.

Laboratory B (Rural Laboratory)

For Laboratory B, 19 293 cultures were tested during 2000–2008, with a median of 2044 plates per year (range, 1959 to 2561). A bacterial pathogen was identified in 1069 (5.5%) cultures tested overall, with a range of 4.8% to 7.2% per year. *Campylobacter* was again the most common bacterial pathogen, accounting for 401 (2.1%) plates tested. Shiga toxin-producing *E. coli* (O157, non-O157, and not isolated combined) was the second most common bacterial pathogen isolated at 253 (1.3%), followed by *Salmonella* at 196 (1.0%), ETEC at 153 (0.8%), *Shigella* at 41 (0.2%), *Yersinia* at 16 (0.08%), and EPEC at 9 (0.05%) (Table 3, Figure 1).

Among the 253 STEC detected, 88 (35%) were O157:H7 or O157:nonmotile, 109 (43%) were non-O157, and 56 (22%) were *stx* positive but were not isolated (Table 3).

The median annual number of cases for *Campylobacter* during the study period was 40 compared to a median of 42 during 2013–2014; *Salmonella* was 21 during the study period vs 35 during 2013–2014; STEC O157 was 9 vs 8; STEC non-O157 was 9 vs 10; STEC not isolated was 5 vs 1; *Shigella* was 4 vs 4.

Site Comparisons

To evaluate differences between the urban and rural populations, the proportion of positives for each pathogen from 2000 through 2006 (the time period when surveillance was conducted simultaneously at both sites) was compared. Statistically significant differences were found, with a higher proportion of STEC O157 in the rural site and a higher proportion of ETEC, *Shigella*, and *Campylobacter* in the urban site (Table 4). No significant differences in the proportion of *Salmonella*, non-O157 STEC, or *Yersinia* were found between the sites.

Six hundred thirty-four ETEC and STEC cases were interviewed about illness and exposures, including 119 STEC and

Table 2. Enteric Bacterial Pathogens Isolated From Patients at an HMO Serving an Urban Geographical Area (Laboratory A), Minnesota, 1996–2006

Pathogen	1996 (n = 1905) No. (%)	1997 (n = 2048) No. (%)	1998 (n = 2243) No. (%)	1999 (n = 1972) No. (%)	2000 (n = 2432) No. (%)	2001 (n = 2114) No. (%)	2002 (n = 1997) No. (%)	2003 (n = 1667) No. (%)	2004 (n = 1589) No. (%)	2005 (n = 1722) No. (%)	2006 (n = 2281) No. (%)
Campylobacter	52 (2.7)	70 (3.4)	74 (3.3)	34 (1.7)	79 (3.2)	60 (2.8)	58 (2.9)	39 (2.3)	34 (2.1)	45 (2.6)	41 (1.8)
Salmonella[a]	28 (1.5)	38 (1.9)	27 (1.2)	20 (1.0)	22 (0.9)	27 (1.3)	12 (0.6)	16 (1.0)	23 (1.4)	17 (1.0)	34 (1.5)
Shigella	7 (0.4)	11 (0.5)	17 (0.8)	23 (1.2)	84 (3.5)	19 (0.9)	24 (1.2)	2 (0.1)	6 (0.4)	3 (0.2)	27 (1.2)
Escherichia coli O157:H7	8 (0.4)	8 (0.4)	3 (0.1)	9 (0.5)	10 (0.4)	7 (0.3)	2 (0.1)	1 (0.06)	0 (0)	6 (0.3)	3 (0.1)
E. coli O157:NM	–	1 (0.05)	–	–	1 (0.04)	–	–	–	–	–	–
Other STEC[b]	5 (0.3)	2 (0.1)	11 (0.5)	4 (0.2)	19 (0.8)	7 (0.3)	10 (0.5)	4 (0.2)	2 (0.1)	11 (0.6)	13 (0.6)
Stx-positive; not isolated	5 (0.3)	1 (0.05)	5 (0.2)	4 (0.2)	3 (0.1)	3 (0.1)	3 (0.2)	3 (0.2)	2 (0.1)	11 (0.6)	8 (0.4)
Enterotoxigenic *E. coli*[c]	–	–	–	–	40 (1.6)	42 (2.0)	36 (1.8)	32 (1.9)	31 (2.0)	39 (2.3)	36 (1.6)
Aeromonas spp	1	–	–	–	–	–	–	–	–	–	–
Plesiomonas shigelloides	–	1	–	–	–	–	–	–	–	–	–
Vibrio hollisae	–	–	2	–	–	–	–	–	–	–	–
Vibrio parahaemolyticus	–	1	–	–	–	–	–	1	–	1 (0.06)	0 (0.0)
Enteropathogenic *E. coli*	–	–	–	–	–	–	–	–	–	2 (0.1)	–
Yersinia	–	–	–	–	1	–	–	–	1 (0.1)	1 (0.06)	–
No bacterial pathogens	1799 (94.4)	1915 (93.5)	2104 (93.8)	1978 (95.2)	2173 (89.4)	1949 (92.2)	1852 (92.7)	1569 (94.1)	1490 (93.8)	1586 (92.1)	2119 (92.9)
Total	1905 (100)	2048 (100)	2243 (100)	1972 (100)	2432 (100)	2114 (100)	1997 (100)	1667 (100)	1589 (100)	1722 (100)	2281 (100)

Abbreviations: ETEC, enterotoxigenic *E. coli*; HMO, health maintenance organization; STEC, Shiga toxin-producing *E. coli*.

[a] *Salmonella* serotypes: Typhimurium, 68 (26%); Enteritidis, 47 (18%); Newport, 16 (6%); Typhimurium var. Copenhagen, 13 (5%); Heidelberg, 13 (5%); other, 107 (41%).

[b] STEC serogroups: O26, 18 (20%); O103, 18 (20%); O111, 10 (11%); O145, 4 (5%); 20 other serogroups, rough or undetermined, 37 (43%).

[c] ETEC serogroups: O25, 22 (9%); O6, 18 (7%); O27, 14 (5%); other, 60 (23%); not serotyped, 83 (32%); not isolated, 59 (23%). ETEC testing began in 2000.
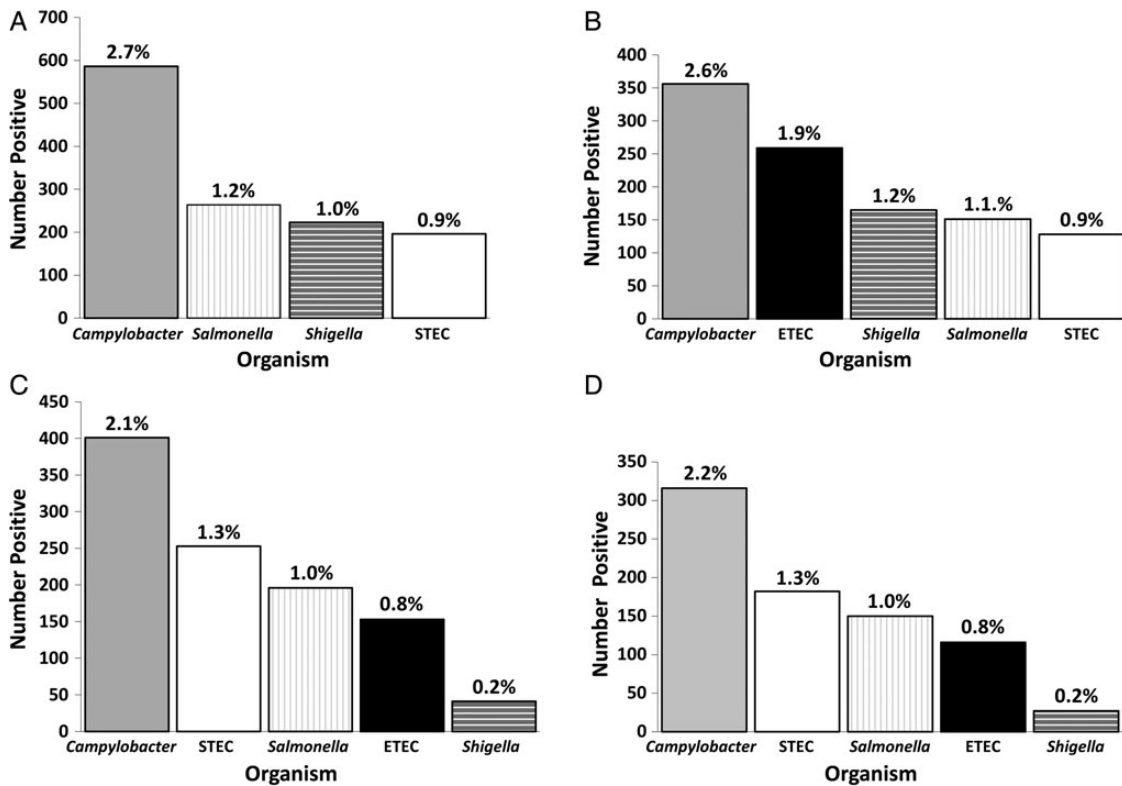
Figure 1. Number and proportion of bacterial pathogens isolated from patients at a health maintenance organization serving an urban geographical area (Laboratory A) and at a laboratory serving a rural agricultural area of Minnesota (Laboratory B). Time frames reported include the entire study period at each laboratory as well as the years when testing was conducted simultaneously at both laboratories. (a) 1996–2006 (n = 21 940 sorbitol MacConkey agar [SMAC] plates tested), Laboratory A. (b) 2000–2006 (n = 13 802 SMAC plates tested), Laboratory A. (c) 2000–2008 (n = 19 293 SMAC plates tested), Laboratory B. (d) 2000–2006 (n = 14 578 SMAC plates tested), Laboratory B. Abbreviations: ETEC, enterotoxigenic *E. coli*; STEC, Shiga toxin-producing *E. coli*.

194 ETEC cases from Laboratory A and 200 STEC and 121 ETEC cases from Laboratory B. Among Laboratory A cases, 125 of 192 (65%) ETEC cases that answered the international travel question reported traveling internationally in the 7 days prior to onset. Travel was evaluated by month of specimen collection and ranged from 3 of 8 (38%) international travelers in May to 20 of 25 (80%) in August. In 3 months (May, September, and November), the proportion of cases that traveled internationally was 50% or lower (38%, 50%, and 40%, respectively). Nineteen of 111 (17%) STEC cases reported traveling internationally; all were non-O157. Among Laboratory B cases, 66 of 119 (55%) ETEC cases reported traveling internationally. By month of specimen collection date, travel ranged from 1 of 5 (20%) international travelers in December to 12 of 15 (80%) in March. In 5 months (February and September through December), the proportion of cases that traveled internationally was 50% or lower (50%, 30%, 42%, 40%, and 20%, respectively). Eleven of 184 (6%) STEC cases reported traveling internationally; 8 were non-O157.

DISCUSSION

This long-term, multisite, sentinel surveillance study in Minnesota is the first to provide extensive data on the frequency of occurrence of ETEC infections compared with other bacterial enteric pathogens. In the urban population, ETEC was the second most common bacterial enteric pathogen behind *Campylobacter*, and it was more common than other common enteric bacterial pathogens such as *Salmonella*, *Shigella*, and STEC. In the rural population, ETEC was the fourth most common bacterial enteric pathogen, but it approached *Salmonella* in frequency.

The documentation of ETEC as a common cause of gastroenteritis on par with other common enteric bacterial pathogens has important implications for public health. Enterotoxigenic *E. coli* are not distinguishable from normal flora strains of *E. coli* by culture, and until recently there has not been a test for ETEC available to clinical laboratories. However, multiplex PCR panels that include ETEC as a target are starting to be adopted by clinical laboratories; it is anticipated that they will become widely used in the near future [18, 19]. As a result, public health agencies that require reporting or submission of clinical materials for ETEC (as is the case in Minnesota) will be confronted with issues related to the following: (1) receiving frequent ETEC reports and interviewing case-patients; (2) confirming, isolating, and serotyping ETEC strains when clinical laboratories

Table 3. Enteric Bacterial Pathogens Isolated From Patients at a Hospital Laboratory Serving a Rural Agricultural Area of Minnesota (Laboratory B), 2000–2008

Pathogen	2000 (n = 1959) No. (%)	2001 (n = 1959) No. (%)	2002 (n = 1987) No. (%)	2003 (n = 2045) No. (%)	2004 (n = 2023) No. (%)	2005 (n = 2044) No. (%)	2006 (n = 2561) No. (%)	2007 (n = 2364) No. (%)	2008 (n = 2351) No. (%)
Campylobacter	40 (2.0)	58 (3.0)	40 (2.0)	55 (2.7)	48 (2.4)	37 (1.8)	38 (1.5)	44 (1.9)	41 (1.7)
Salmonella[a]	20 (1.0)	25 (1.3)	25 (1.3)	20 (1.0)	20 (1.0)	19 (0.9)	21 (0.8)	21 (0.9)	25 (1.1)
Shigella	6 (0.3)	5 (0.2)	0 (0.0)	8 (0.4)	2 (0.1)	4 (0.2)	2 (0.08)	2 (0.08)	12 (0.5)
Escherichia coli O157:H7	12 (0.6)	16 (0.8)	12 (0.6)	8 (0.4)	5 (0.2)	8 (0.4)	11 (0.4)	9 (0.4)	6 (0.3)
E. coli O157:motile not H7	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.05)	0 (0.0)	0 (0.0)	0 (0.0)
Other STEC[b]	5 (0.3)	12 (0.6)	7 (0.4)	5 (0.2)	9 (0.4)	9 (0.4)	16 (0.6)	21 (0.9)	25 (1.1)
Stx-positive; not isolated	4 (0.2)	8 (0.4)	4 (0.2)	5 (0.2)	4 (0.2)	6 (0.3)	15 (0.6)	6 (0.3)	4 (0.2)
Enterotoxigenic *E. coli*[c]	11 (0.6)	16 (0.8)	17 (0.9)	14 (0.7)	16 (0.8)	25 (1.2)	17 (0.7)	26 (1.1)	11 (0.5)
Enteropathogenic *E. coli*	–	–	–	–	–	2 (0.1)	2 (0.08)	3 (0.1)	2 (0.09)
Yersinia	1 (0.05)	1 (0.05)	1 (0.05)	1 (0.05)	1 (0.05)	4 (0.2)	1 (0.04)	4 (0.2)	2 (0.09)
No bacterial pathogens	1860 (94.9)	1818 (92.8)	1881 (94.7)	1929 (94.3)	1918 (94.8)	1929 (94.4)	2438 (95.2)	2228 (94.2)	2223 (94.6)
Total	1959 (100)	1959 (100)	1987 (100)	2045 (100)	2023 (100)	2044 (100)	2561 (100)	2364 (100)	2351 (100)

Abbreviations: ETEC, enterotoxigenic *E. coli*; STEC, Shiga toxin-producing *E. coli*.

[a] *Salmonella* serotypes: Typhimurium, 39 (20%); Enteritidis, 23 (12%); Newport, 16 (8%); Montevideo, 9 (5%); other, 109 (56%).

[b] STEC serogroups: O111, 29 (27%); O103, 28 (26%); O26, 23 (21%); O145, 6 (6%); 9 other serogroups, rough, or undetermined, 23 (21%).

[c] ETEC serogroups: O6, 12 (8%); O169, 8 (5%); other, 66 (43%); not serotyped, 22 (14%); not isolated, 45 (29%).

submit PCR-positive specimens; and (3) subtyping ETEC isolates for the purpose of more efficient outbreak detection and investigation. The burden of all of these activities will be substantial.

Among the ETEC-positive patients in Minnesota, 61% of those interviewed reported foreign travel. A high percentage was expected, because ETEC is endemic in many developing countries visited by Minnesota travelers and is widely appreciated as the leading cause of traveler's diarrhea [1–3]. However, 39% of ETEC-positive patients in Minnesota who were interviewed did not report foreign travel and thus were presumably domestically acquired. Since a recent history of travel is more likely to lead to testing [20, 21], the proportion of ETEC that are domestically acquired is likely higher than what was found in this study. The sources of these infections were not

Table 4. Comparison of Positive Results in the Urban (Laboratory A) vs Rural (Laboratory B) Laboratories, 2000–2006

Agent	Urban No. (%)	Rural No. (%)	RR (95% CI)	P Value
Campylobacter	356 (2.6%)	316 (2.2%)	1.2 (1.0–1.4)	.02
Enterotoxigenic *Escherichia coli*	256 (1.9%)	116 (0.8%)	2.3 (1.9–2.9)	<.001
Salmonella	151 (1.1%)	150 (1.0%)	1.1 (0.8–1.3)	NS
Shigella spp	165 (1.2%)	27 (0.2%)	6.5 (4.3–10)	<.001
Shigella sonnei	147 (1.1%)	23 (0.1%)	6.8 (4.4–10)	<.001
Shigella flexneri	13 (0.1%)	4 (<0.1%)	3.4 (1.0–11)	.03
Non-O157 Shiga toxin-producing *E. coli*	65 (0.5%)	63 (0.4%)	1.1 (0.8–1.5)	NS
Shiga toxin-producing *E. coli* O157	30 (0.2%)	73 (0.5%)	0.4 (0.3–0.7)	<.001
Total Stool Cultures	13 802	14 578		

Abbreviations: CI, confidence interval; NS, not significant; RR, relative risk.

determined, but produce imported from countries where ETEC is endemic is a likely source. Enterotoxigenic *E. coli* outbreaks due to imported produce are occasionally identified in Minnesota, other areas of the United States, and other countries [22–25]. These outbreaks are identified because they manifest as groups of ill people associated with discrete events or establishments that are reported to public health; when these groups are interviewed, the symptom and incubation profile that is relatively specific for ETEC becomes apparent, and patients are tested for ETEC. However, the outbreaks of ETEC infections that are identified almost certainly represent the proverbial tip of the iceberg. Because multiplex tests that include ETEC are widely used, pathogen-specific surveillance for ETEC may make it possible to detect ETEC outbreaks associated with produce or other foods distributed through retails settings (ie, grocery stores), in the same way that pathogen-specific surveillance for *Salmonella* and other pathogens has been so successful in detecting outbreaks associated with imported produce sold at retail [22, 26–28]. Therefore, along with the burden associated with an anticipated increase in ETEC reports comes greater opportunity to detect and control outbreaks.

These findings are also important for clinicians, because they should consider ETEC as a possible etiology of their patients' gastroenteritis and should not simply discount ETEC-positive results if the patient does not have a travel history.

In contrast to ETEC, our study suggested that EPEC (as defined by the presence of *bfp* and *eae*) is not a common pathogen in Minnesota. Our study also provided further data on the frequency of occurrence of STEC infections compared with other bacterial enteric pathogens. In the rural population, STEC was the second most common bacterial enteric pathogen in the rural

population, behind *Campylobacter*. In the urban population, STEC was the fifth most common bacterial enteric pathogen. Culture-independent diagnostic tests for Shiga toxin, which enable the subsequent identification of non-O157 STEC serogroups, have been in use for a number of years, and their use is continuing to increase rapidly [8, 9]. As has been reported in previous studies based on testing of Shiga toxin-positive specimens submitted by clinical laboratories, in our study non-O157 serogroups were detected more commonly than O157. Because of the increasing use of Shiga toxin tests, the same burdens and opportunities for public health as discussed for ETEC are already in play. However, should culture-independent testing for STEC (e.g., immunoassays or multiplex PCRs that detect Shiga toxin or Shiga toxin genes, respectively) become universal, these burdens and opportunities will increase even further. Furthermore, as multiplex PCRs become more widely adopted, many clinical laboratories will likely stop culture for all pathogens, thus delaying the identification of STEC O157 until received at the PHL. This delay could have negative impacts on patient care [29].

Although the order of non-O157 serogroups differed slightly between the urban and rural sites, the top 3 in both sites included serogroups O111, O26, and O103. This result is consistent with previous studies [9–11]. The relative frequency of STEC, including non-O157 cases, compared with other common enteric bacterial pathogens in the study was comparable to that observed in 2013–2014 at both sites despite the differences in identification methods, with 1 minor exception; *Salmonella* was more common than STEC in the rural site in 2013–2014. This finding supports the hypothesis that increases in the identification of non-O157 STEC in the United States are likely due to detection methods and not an actual increase in incidence [9].

When the urban and rural monitoring sites were compared directly over the same time period, the relative abundance of several pathogens differed between the sites. In the urban population, ETEC and *Shigella* ranked higher than in the rural population. A higher proportion of STEC O157 in rural areas could be due to a higher likelihood of direct or indirect contact with cattle, cattle run-off, or other agricultural exposures known to be associated with STEC O157 infections [30]. Further study is needed to identify sources for these differences.

This study has some potential limitations. Testing methodology changed over time, and we had no way to verify that all SMAC cultures were in fact submitted. Nonetheless, these data collected over an extended period of time from 2 different sites are a good indication of the impact of the adoption of new CIDTs, including multiplex PCR assays. The identification of STEC other than O157 will continue to increase due to broader adoption of CIDTs. As a result, we will be able to identify a large number of ETEC infections, and we will likely identify a few typical EPEC infections. This strategy will provide new opportunities for outbreak identification and may be useful in better understanding the epidemiology of these pathogens (e.g., proportion of domestically acquired ETEC infections). Unfortunately, the burden on public health laboratories could be quite large. With the adoption of CIDTs, many clinical laboratories will (1) stop bacterial cultures and (2) submit stool or enrichment broths from CIDT-positive clinical tests to public health laboratories for confirmation. In addition, ETEC appears to be as common as other pathogens already under surveillance, and they are burdensome to confirm by culture, which will also add a new burden to the already strained public health laboratories.

CONCLUSIONS

In summary, this study found that when testing all stools submitted to 2 laboratories, the prevalence of ETEC and STEC infections were comparable with other enteric bacterial pathogens. Enterotoxigenic *E. coli* were the second most common enteric pathogen in the urban laboratory, and STEC were more common in the rural laboratory than the urban one and the second most common enteric pathogen identified in the rural laboratory. Findings of importance for clinicians include that a higher proportion of STEC O157 was found in rural areas, and a high proportion of ETEC (39%) patients did not travel internationally in the 7 days prior to their illness.

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