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Shiga Toxin-Producing *Escherichia coli*: a Single-Center, 11-Year Pediatric Experience

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The aim of this study was to identify the best practices for the detection of Shiga toxin-producing *Escherichia coli* (STEC) in children with diarrheal illness treated at a tertiary care center, i.e., sorbitol-MacConkey (SMAC) agar culture, enzyme immunoassay (EIA) for Shiga toxin, or the simultaneous use of both methods. STEC was detected in 100 of 14,997 stool specimens submitted for enteric culture (0.7%), with 65 cases of *E. coli* O157. Among *E. coli* O157 isolates, 57 (88%) were identified by both SMAC agar culture and EIA, 6 (9%) by SMAC agar culture alone, and 2 (3%) by EIA alone. Of the 62 individuals with diarrheal hemolytic uremic syndrome (HUS) seen at our institution during the study period, 16 (26%) had STEC isolated from cultures at our institution and 15 (24%) had STEC isolated at other institutions. No STEC was recovered in 31 cases (50%). Of the HUS cases in which STEC was isolated, 28 (90%) were attributable to *E. coli* O157 and 3 (10%) were attributable to non-O157 STEC. Consistent with previous studies, we have determined that a subset of *E. coli* O157 infections will not be detected if an agar-based method is excluded from the enteric culture workup; this has both clinical and public health implications. The best practice would be concomitant use of an agar-based method and a Shiga toxin EIA, but a Shiga toxin EIA should not be considered to be an adequate stand-alone test for detection of *E. coli* O157 in clinical samples.

Shiga toxin-producing *Escherichia coli* (STEC) causes a spectrum of disease, with manifestations ranging from mild self-limited diarrhea to the life-threatening hemolytic uremic syndrome (HUS) (1–7). The incidence and severity of STEC infections are highest in children, with disproportionate numbers of HUS cases being reported for this group (7–10). *E. coli* O157:H7 is the serotype most often implicated in HUS worldwide (1, 9, 11–15).

In 2009, the US Centers for Disease Control and Prevention (CDC) published guidelines indicating that laboratories should simultaneously perform a selective and differential agar-based test to detect *E. coli* O157 and a test to detect Shiga toxins or Shiga toxin genes for all stool samples submitted for bacterial culture (16). These complementary methods are recommended because of the increasing recognition that non-O157:H7 STEC strains cause disease and the lack of evidence that STEC enzyme immunoassays (EIAs) are as good as or better than sorbitol-MacConkey (SMAC) agar screening for the detection of *E. coli* O157, which remains the most clinically actionable STEC serotype. Furthermore, some studies suggest that a toxin EIA is inferior to SMAC agar screening for detecting *E. coli* O157:H7 (14, 17–24).

Infections with STEC are rare (FoodNet data suggest that approximately 4,000 cases of *E. coli* O157:H7 infections per year occur in the United States) (25) but, for the individuals infected, there are considerable benefits to making a timely diagnosis. Specifically, the earlier a microbiological diagnosis is attained, the better the clinical outcome is (26). Moreover, by identifying STEC as the probable cause of illness that is often quite severe, clinical care can be focused. However, concern remains that uniform screening of all stool samples for these pathogens has little public health or clinical value. Advocates for limiting STEC testing to "request-only" status encourage laboratories to consider local disease prevalence, seasonal incidence, and costs before implementing the CDC guidance (24, 27, 28). The counterpoint to this argument is that broad STEC screening prevents severe clinical outcomes through early detection and optimization of care. Best practices dictate that intravenous hydration and conservative management of severe diarrhea be instituted as expeditiously as possible, and laboratory detection of a STEC strain often prompts such interventions (2, 26, 29).

Considering the costs of parallel testing and the paucity of data on which to judge the merits of the CDC guidelines, many laboratories do not perform STEC testing unless it is specifically requested, and nearly 10% of laboratories have abandoned agar-based testing in favor of exclusively performing EIAs for Shiga toxin detection (30). The St. Louis Children's Hospital (SLCH) microbiology laboratory has followed the practice of performing simultaneous SMAC agar cultures and STEC EIAs for all stool specimens since 2001; this practice was recommended by the CDC in 2009. Our objectives in this study were to report our microbiological and clinical experiences with these pathogens between 2001 and 2011 and to provide data to evaluate the relative value of each component of the recommended testing methodology.

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TABLE 1 Isolates recovered from all routine stool cultures from 2001 to 2011

Species isolated	No. (%) of isolates in:											Total no. (%)
	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	
Total	1,345	1,227	1,283	1,122	1,178	1,179	2,185	1,248	1,585	1,437	1,188	14,977
Shigella spp.	18	24	37	4	2	27	166	17	184	102	1	582 (3.9)
Salmonella spp.	26	24	17	11	17	18	25	17	12	15	18	200 (1.3)
Aeromonas spp.	14	26	16	6	7	9	19	16	12	12	7	144 (1.0)
Campylobacter jejuni	10	11	7	13	11	13	20	21	11	11	15	143 (1.0)
E. coli O157	2	3	4	6	14	8	7	6	8	0	7	65 (0.4)
Yersinia enterocolitica	12	8	6	10	5	7	2	3	3	1	2	59 (0.4)
Non-O157 STEC[a]	1	1	3	2	3	6	3	4	5	5	2	35 (0.2)
Plesiomonas shigelloides	0	0	2	0	2	0	1	1	1	0	1	8 (0.05)
Edwardsiella tarda	0	0	1	0	0	0	0	0	0	1	0	2 (0.01)

[a] STEC, Shiga toxin-producing *E. coli*.

MATERIALS AND METHODS

Population. All stool and rectal swab specimens submitted to the microbiology laboratory at SLCH for enteric bacterial culture between 1 January 2001 and 31 December 2011 were included in this analysis. Clinical and laboratory data pertaining to patients with positive SMAC agar culture and/or positive EIA results were extracted from electronic medical records. Cases of diarrhea-associated HUS were identified by reviewing all electronic medical records from the study period for patients with a primary or secondary diagnosis of HUS (International Classification of Diseases 9 [ICD-9] code 283.11). HUS was defined stringently using the criteria of anemia (hematocrit level of <30%), thrombocytopenia (platelet count of <150,000 cells/mm^3), and azotemia (creatinine level above the upper limit of the reference range for age) (2, 31, 32).

Human study approval. This study was approved by the Human Research Protection Office of Washington University School of Medicine.

Laboratory methods. All stool and rectal swab samples were cultured on blood agar with ampicillin, Hektoen enteric agar, MacConkey agar, SMAC agar, *Campylobacter* blood agar, *Yersinia* selective agar, and Gram-negative (GN) broth for the detection of enteric pathogens (all media were from Remel, Lenexa, KS), according to standard laboratory procedures. Cultures were inoculated upon specimen receipt (24 h per day and 7 days per week). SMAC agar cultures were examined for non-sorbitol-fermenting colonies, which were then tested with an *E. coli* O157 latex agglutination test and autoagglutination control (Remel, Lenexa, KS) to presumptively identify *E. coli* O157. The identification of latex-positive colonies was confirmed according to standard laboratory procedures. Following incubation, the Premier EIA was performed on the overnight GN broth culture for detection of Shiga toxins 1 and 2 (Meridian Bioscience Inc., Cincinnati, OH). All isolates of *E. coli* O157, as well as an aliquot of each positive GN broth culture (if EIA results were positive and SMAC agar culture results were negative), were forwarded to the Missouri State Public Health Laboratory (Jefferson City, MO) for confirmation by PCR and serotyping. As we had incomplete H typing reports for many *E. coli* O157 isolates, we do not specify serotypes and indicate only serogroups.

Statistical analysis. Student's *t* test, Fischer's exact test, and analysis of variance (ANOVA) were used to evaluate the data using SPSS v20 (IBM, Chicago, IL). Two-tailed *P* values of <0.05 were considered significant.

RESULTS

Enteric pathogens detected. During the 11-year study period, 14,977 enteric cultures were performed with stool or rectal swab specimens, and a bacterial pathogen was isolated in 1,238 cases (8.3%). Pathogens detected included *Shigella* spp. ($n = 582$ [3.9% of total cultures]), *Salmonella* spp. ($n = 200$ [1.3%]), *Aeromonas* spp. ($n = 144$ [1%]), *Campylobacter jejuni* ($n = 143$ [1%]), *E. coli* O157 ($n = 65$ [0.4%]), *Yersinia enterocolitica* ($n = 59$ [0.4%]), non-O157 STEC ($n = 35$ [0.2%]), *Plesiomonas shigelloides* ($n = 8$ [0.05%]), and *Edwardsiella tarda* ($n = 2$ [0.01%]) (Table 1).

Recovery of STEC. Of the specimens that screened positive for STEC by one or both methods ($n = 107$), STEC strains were isolated from 100. While the majority of STEC isolates belonged to serogroup O157 ($n = 65$), 35 isolates, representing 12 additional STEC serotypes, were identified by the Missouri State Laboratory (Table 2). Among the 65 *E. coli* O157 isolates, 57 (88%) were initially detected by both screening methods, two (3%) by EIA alone, and six (9%) by SMAC agar culture alone (Table 3). All 35 non-O157 STEC isolates were initially detected by EIA alone. STEC was isolated from samples collected in all seasons, with both O157 STEC and non-O157 STEC infections being detected throughout the year over the entire study period (Fig. 1).

Clinical features of STEC infections. The clinical features of patients with O157 and non-O157 STEC infections are provided in Table 4. The HUS rate among the 100 subjects whose stool specimens contained an STEC strain isolated at SLCH was 16%, including 13 (20%) of 65 patients infected with *E. coli* O157 and 3 (9%) of 35 patients infected with non-O157 STEC ($P = 0.14$). Grossly bloody stools, as established by patient history or direct observation by a clinician, were reported for 59 (94%) of 63 subjects from whom *E. coli* O157 was cultured, with data not being available for two subjects. In comparison, grossly bloody stools were reported for 19 (66%) of 29 subjects from whom non-O157

TABLE 2 Serotype identification of STEC isolates from 2001 to 2011

Serotype	No. of isolates
Total (serotype-confirmed STEC)	100
E. coli O157[a]	65
E. coli O103	9
E. coli O145	6
E. coli O111:nonmotile	6
E. coli O26	3
E. coli O121	3
E. coli O45:H2	2
All others[b]	6

[a] Includes 60 serotype-confirmed *E. coli* O157:H7 isolates and 5 serogroup-confirmed *E. coli* O157 isolates, not further specified.
[b] Includes one isolate each of the following *E. coli* strains: O91:nonmotile, O128:H2, O165:nonmotile, O174:H21, O177:nonmotile, and O-rough:nonmotile.

TABLE 3 Laboratory detection of STEC, according to method, from 2001 to 2011

Yr	No. of stool cultures	No. of cases detected by[a]: SMAC agar culture alone			EIA alone			SMAC agar culture and EIA		
		E. coli O157	Non-O157 STEC	No organism recovered	*E. coli* O157	Non-O157 STEC	No organism recovered	*E. coli* O157	Non-O157 STEC	No organism recovered
Total	14,977	6	0	0	2	35	6	57	0	1
2001	1,345	2	0	0	0	1	1	0	0	0
2002	1,227	0	0	0	0	1	2	3	0	0
2003	1,283	0	0	0	0	3	0	4	0	0
2004	1,122	0	0	0	0	2	0	6	0	0
2005	1,178	0	0	0	0	3	1	14	0	0
2006	1,179	1	0	0	0	6	1	7	0	0
2007	2,185	2	0	0	1	3	1	4	0	0
2008	1,248	0	0	0	0	4	0	6	0	1
2009	1,585	0	0	0	1	5	0	7	0	0
2010	1,437	0	0	0	0	5	0	0	0	0
2011	1,188	1	0	0	0	2	0	6	0	0

[a] EIA, enzyme immunoassay; SMAC, sorbitol-MacConkey; STEC, Shiga toxin-producing *E. coli*.

E. coli was cultured ($P < 0.001$). The median age, gender distribution, presence of fever (patient or physician report), need for dialysis if HUS ensued, and mode of specimen collection (rectal swab versus diaper or cup specimen) did not differ significantly between O157 and non-O157 STEC infections, although the rate of hospitalization was greater for patients infected with *E. coli* O157 (91% versus 66%; $P = 0.005$). Also, among hospitalized patients, those infected with *E. coli* O157 had significantly longer lengths of stay than did those infected with non-O157 STEC (median of 4 days [range, 0 to 35 days] versus 1 day [range, 0 to 12 days]; $P = 0.003$). Patients infected with *E. coli* O157 did not significantly differ from those infected with non-O157 STEC with respect to laboratory values at presentation, including white blood cell counts, hemoglobin levels, hematocrit values, platelet counts, and creatinine levels.

Microbiological findings for patients with diarrheal HUS. During the study period, 92 children had HUS as a primary or secondary diagnosis (Fig. 2). Of these, 62 met the diagnostic criteria for diarrheal HUS at the time of presentation, although only 16 (26%) of these individuals had STEC isolated from stool specimens at our institution. Of the HUS cases in which STEC was isolated either at our institution or at another hospital ($n = 31$), 28 (90%) were attributed to *E. coli* O157. Among the cases in which STEC was not isolated, Shiga toxin was detected at another hospital in two cases, one in which the patient had negative culture results at our institution and one in which cultures were not performed at our institution. Non-O157 STEC was isolated from three HUS cases. Notably, the sister of one of those patients was hospitalized at the same time as the patient and was infected with serotype-confirmed *E. coli* O157.

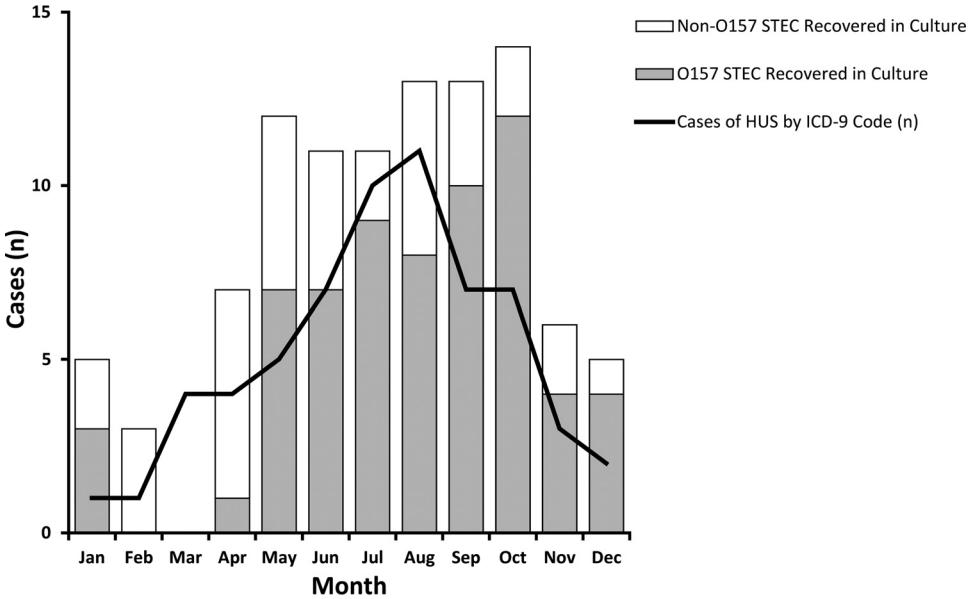

FIG 1 Shiga toxin-producing *E. coli* (STEC) and hemolytic uremic syndrome (HUS) cases by month from 2001 to 2011. The numbers of cases of O157 STEC and non-O157 STEC recovered in culture and the number of cases of HUS are shown according to the month of presentation during the 11-year study period. Cases of HUS (established by strict diagnostic criteria) were ascertained through ICD-9 codes and chart review.

TABLE 4 Clinical features of patients with STEC isolated from stool samples, according to serotype

Parameter	*E. coli* O157 ($n = 65$)	Non-O157 STEC ($n = 35$)	*P*
Age (median [range]) (yr)	7.5 (0.9–17.1)	8.6 (1.0–18.4)	0.26
Male (no. [%])	34 (52)	21 (60)	0.46
Admitted to hospital (no./total no. [%])	59/65 (91)	23/35 (66)	0.005
Length of hospital stay (median [range]) (days)	4.0 (0–35)	1.0 (0–12)	0.003
STEC reported prior to discharge from hospital (no./total no. [%])	52/65 (80)	16/35 (46)	<0.001
Fever by report or examination (no./total no. [%])	17/57 (30)	3/26 (12)	0.07
Met clinical criteria for HUS (no./total no. [%])	13/65 (20)	3/35 (9)	0.14
Received dialysis (no./total no. [%])	8/65 (12)	1/35 (3)	0.12
Grossly bloody stool by report or examination (no./total no. [%])	59/63 (94)	19/29 (66)	<0.001
Culture submitted on rectal swab (no./total no. [%])[a]	8/65 (12)	9/35 (26)	0.09
White blood cell count at presentation (median [range]) (1,000 cells/mm^3) (no.)	11.3 (5.0–30.0) (61)	9.1 (6.0–42.0) (28)	0.26
Hemoglobin level at presentation (median [range]) (g/dl) (no.)	13.2 (6.2–16.9) (61)	13.1 (6.9–15.9) (28)	0.65
Hematocrit at presentation (median [range]) (%) (no.)	38.0 (18.0–49.3) (61)	34.8 (19.3–44.9) (28)	0.68
Hematocrit nadir (median [range]) (%) (no.)	32.9 (13.6–45.8) (62)	33.2 (15.0–40.5) (29)	0.12
Platelet count at presentation (median [range]) (1,000 cells/mm^3) (no.)	246 (18–452) (60)	239 (27–409) (28)	0.47
Platelet count nadir (median [range]) (1,000 cells/mm^3) (no.)	199 (8–406) (62)	194 (14–373) (29)	0.46
Creatinine level at presentation (median [range]) (mg/dl) (no.)	0.4 (0.2–10.5) (60)	0.6 (0.2–3.7) (29)	0.97
Peak creatinine level (median [range]) (mg/dl) (no.)	0.6 (0.2–10.4) (61)	0.6 (0.2–5.0) (29)	0.16
Creatinine level outside reference range for age and gender (no./total no. [%])	13/61 (21)	4/29 (14)	0.57

[a] All other cultures were stool specimens received in a cup or diaper.

Cost analysis. We have estimated the cost of performing a SMAC agar culture as $1 per culture, including consumable materials and technician time, totaling $14,977 over the study period. The cost of performing a STEC EIA at our institution, including consumable materials and technician time, is considerably higher, estimated as $15 per test, totaling $224,655 in testing over the 11-year study period. During this period, 94 cases of STEC (including both *E. coli* O157 and non-O157 STEC) were detected by EIA, resulting in an approximate cost of $2,390 per positive result. All except two of the *E. coli* O157 infections were also detected by SMAC agar culture. The cost of EIA detection of non-O157 STEC, which would have gone undetected if only SMAC agar cultures had been performed, was $6,419 per positive result.

DISCUSSION

To our knowledge, this study represents the largest evaluation of the diagnostic yield of unbiased simultaneous testing of stool samples with an agar-based method for *E. coli* O157 detection and an EIA for Shiga toxin detection. Non-O157 STEC represented approximately one-third of the cases, and *E. coli* O157 and non-O157 STEC were detected throughout the year. One-quarter of the infections would have been missed if STEC screening had been restricted to the summer months, a strategy proposed by some groups (33). Importantly, the EIA failed to detect six cases of *E. coli* O157, the most clinically actionable serotype of STEC, emphasizing the shortcomings of using this assay as a stand-alone test (14, 17–21, 34).

Our institutional recovery rates of 0.6% for all STEC strains and 0.4% for *E. coli* O157 from all stool cultures over the 11-year study period resemble rates reported for a Boston pediatric institution from 2004 to 2009 (35) but are lower than rates reported for Seattle Children's Hospital (3.5% STEC and 2.0% *E. coli* O157) (21). This discrepancy may be attributable to smaller numbers of

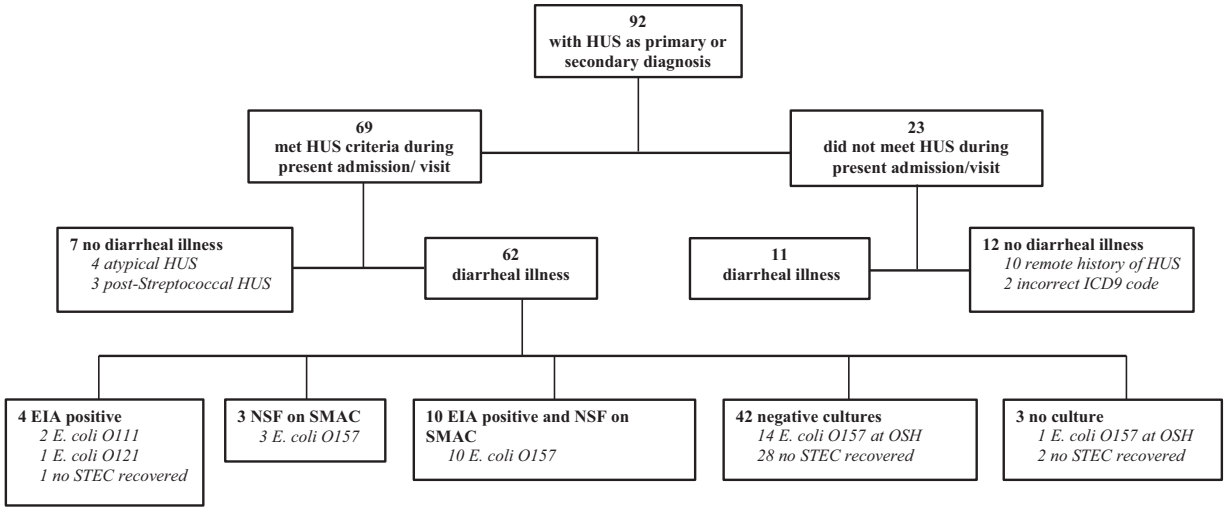

FIG 2 Features of patients with HUS as a primary or secondary diagnosis from 2001 to 2011. The chart diagrams the pertinent clinical and laboratory features of all patients with an ICD-9 code of 283.11 (hemolytic uremic syndrome) for a primary or secondary diagnosis during the 11-year study period. NSF, non-sorbitol fermenter; OSH, outside hospital; SMAC, sorbitol-MacConkey agar.

subjects in the Seattle study ($n = 254$) and restriction to patients in the emergency department in Seattle and/or to regional variability in STEC prevalence. It would be inappropriate to compare our institutional STEC recovery rates to the STEC recovery rates reported by the CDC during the same time period, given the preanalytic bias introduced into the CDC data by the variability of STEC detection methods used by the reporting laboratories. Of note, the majority of non-O157 STEC serotypes reported by the CDC FoodNet for 2011 included O26, O103, O111, O121, O45, and O145, which were also the most frequently isolated serotypes in our study (36).

Our data demonstrate the severity of STEC infections inclusive of and beyond *E. coli* O157, with over 80% of all patients with STEC isolated from their stool specimens during the study period being admitted to the hospital. Our demonstration of higher admission rates and longer lengths of stay among children infected with *E. coli* O157 than among those infected with non-O157 STEC provides additional evidence in support of the concept that *E. coli* O157 causes more severe illness than does non-O157 STEC. The finding that grossly bloody stools were reported more frequently for subjects from whom *E. coli* O157 was isolated underscores the significance of specifically asking patients and patients' parents about the presence of blood in the stools, as well as examining the appearance of fresh stools in the clinical setting. While our data emphasize the clinical significance of blood in the stools, we think it would be unwise to task receiving laboratory technicians with triaging STEC testing on the basis of the presence of blood in the stools. Slutsker and colleagues assessed the utility of laboratory evaluation for the presence of blood in stool specimens from which *E. coli* O157 was isolated (37). They found that blood was visible in 63% of specimens at the time of laboratory receipt, suggesting that nearly one-half of all cases of *E. coli* O157 would have been missed if screening had been restricted to specimens with visible blood. It is not uncommon for the blood in stool specimens to be oxidized and thus not readily visible in the samples by the time the specimens arrive in the laboratory; for rectal swab specimens or fecal specimens in transport medium, it is nearly impossible to assess the absence or presence of blood in most cases.

The overall percentage of HUS cases that were culture negative (50%) is consistent with a report from over 2 decades ago in the Pacific Northwest (9) and from a more recent multicenter study of children with diarrhea-associated HUS (38). Of note, the classification of stool specimens received for culture as formed or unformed was not captured or reported in any of these studies, including ours. The failure to isolate STEC from the stool may be explained by the natural history of STEC infections. In many cases, patients become ill with diarrhea, which variably progresses to bloody diarrhea, followed by resolution of the enteric symptoms; hemolysis, thrombocytopenia, and kidney injury do not occur until late in the disease, often after the diarrhea has resolved. In one prospective study, the likelihood of isolating *E. coli* O157 was shown to be highest earlier in the course of illness, with the pathogen being detected in 96% of HUS cases with cultures obtained within 6 days after the onset of diarrhea and in 33% of stool cultures obtained more than 7 days after the onset of diarrhea (9). Indeed, of 42 patients with HUS who had negative stool cultures in our laboratory, 14 had *E. coli* O157 isolated prior to transfer to our institution. These findings are consistent with previously published reports demonstrating that the ability to isolate *E. coli* O157 decreases if cultures are performed after HUS is diagnosed, although serological evidence of recent infection with *E. coli* O157 has been reported for a subset of patients whose stools do not yield STEC (13, 39–41).

The failure to detect STEC in the stool at the time of HUS diagnosis may reflect the clearance of *E. coli* O157 prior to diagnosis. Even when non-O157 STEC is isolated from patients with diarrhea-associated HUS, the significance of this finding is not always clear, with multiple examples of coinfection with *E. coli* O157 and non-O157 STEC (5, 13, 40, 42, 43). Although we did not perform serological testing in this study, there was circumstantial evidence for a dual STEC infection in one patient with HUS from whom only STEC O111 was isolated, as that patient's sister was infected with *E. coli* O157 at the same time.

Isolated Shiga toxin EIA testing without concomitant SMAC agar culturing has clear utility in selected situations, such as very rare outbreaks of infections caused by non-O157 STEC (44–47). However, Shiga toxin EIA testing is frequently used in situations in which these circumstances do not apply, as the lone methodology for detecting STEC, including *E. coli* O157 (30). Our data now present a basis on which to measure the value of blanket screening using an EIA. The large discrepancy between the cost of adding an SMAC agar plate to each routine culture ($1 per culture) and the cost of adding an EIA to each culture ($15 per culture) is a practical consideration for many laboratories that perform STEC screening. In this study, the chief value of STEC detection by EIA was in the identification of *E. coli* O157 that was not detected on SMAC agar in two cases (a situation that has been reported previously [48]) and the identification of three cases of HUS associated with non-O157 STEC. However, we do not wish to minimize the value of finding a cause of illness among the 35 patients with non-O157 STEC infections, many of whom were treated by one of the authors (P.I.T.), even if they did not develop HUS. Most of these patients were sufficiently ill to be hospitalized, and the identification of an etiology provided assurance that this event would be self-limiting.

Our data also demonstrate pitfalls in evaluating the role of non-O157 STEC in HUS solely by examining laboratory data. By concurrently performing an analysis of all culture results from patients meeting the diagnostic criteria for HUS, as ascertained by reviewing all electronic medical records associated with the ICD-9 code for HUS, we were able to capture culture-negative cases. Without taking into account culture-negative cases or examining records for microbiology results obtained before transfer to our institution, we might have concluded that 19% of HUS cases are caused by non-O157 STEC. Other laboratory-based data suggest that non-O157 STEC causes, overall, a similar number of cases of HUS as does *E. coli* O157 (49) but, as this study demonstrates, the proportion of culture-negative HUS cases is appreciable. If non-O157 STEC is cleared more slowly than *E. coli* O157, then laboratory-based series might have an additional ascertainment bias in favor of associating non-O157 STEC with HUS.

Early recognition of children at risk for HUS is strongly associated with good outcomes (i.e., avoidance of anuria if HUS ensues) (26), and it would be worthwhile to determine why cases are not being detected earlier. In our experience, positive culture results prompt recognition that patients are at risk for HUS, leading providers to seek consultation and/or to provide aggressive intravenous volume expansion, which is associated with milder HUS courses if this complication develops (26, 38, 50, 51). When patients are admitted with HUS, it is important to attempt to isolate

STEC, and particularly *E. coli* O157, from the earliest possible specimens available. This involves identifying the source of the original culture and confirming that the appropriate microbiological methods were applied if testing was performed and the results were negative.

The major strengths of the present study are the large number of samples evaluated, which encompass more than a decade's worth of data, and the systematic ascertainment and review of all cases with a diagnosis code for HUS, irrespective of culture results. However, our study has several limitations, chiefly its retrospective format and the study site, i.e., a large, urban, tertiary care hospital. Our observations may not be directly applicable to community hospitals or outpatient settings, which are often the first point of contact with the medical system for children with diarrhea.

In conclusion, our retrospective review demonstrates that optimal detection of STEC is achieved by the simultaneous use of an agar-based method to detect *E. coli* O157 and a test to detect Shiga toxin. Consistent with previous studies, we have determined that a subset of *E. coli* O157 infections will not be detected if an agar-based method is excluded from the enteric culture workup; this has both clinical and public health implications. The best practice would be concomitant use of an agar-based method and a Shiga toxin EIA, but a Shiga toxin EIA should not be considered to be an adequate stand-alone test for detection of *E. coli* O157 in clinical samples.

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