Shiga Toxin-Producing *Escherichia coli* in Children: Diagnosis and Clinical Manifestations of O157:H7 and Non-O157:H7 Infection[▽]

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Shiga toxin-producing *Escherichia coli* (STEC), a cause of food-borne colitis and hemolytic-uremic syndrome in children, can be serotype O157:H7 (O157) or other serotypes (non-O157). *E. coli* O157 can be detected by culture with sorbitol-MacConkey agar (SMAC), but non-O157 STEC cannot be detected with this medium. Both O157 and non-O157 STEC can be detected by immunoassay for Shiga toxins 1 and 2. The objectives of this study were first to compare the diagnostic utility of SMAC to that of the Premier EHEC enzyme immunoassay (Meridian Diagnostics) for detection of STEC in children and second to compare the clinical and laboratory characteristics of children with serotype O157:H7 STEC and non-O157:H7 STEC infections. Stool samples submitted for testing for STEC between April 2004 and September 2009 were tested by both SMAC culture and the Premier EHEC assay at Children's Hospital Boston. Samples positive by either test were sent for confirmatory testing and serotyping at the Hinton State Laboratory Institute (HSLI). Chart review was performed on children with confirmed STEC infection. Of 5,110 children tested for STEC, 50 (0.9%) had STEC infection confirmed by culture; 33 were O157:H7 and 17 were non-O157:H7. The Premier EHEC assay and SMAC culture detected 96.0% and 58.0% of culture-confirmed STEC isolates (any serotype), respectively, and 93.9% and 87.9% of STEC O157:H7 isolates, respectively. There were no significant differences in disease severity or laboratory manifestations of STEC infection between children with O157:H7 and those with non-O157 STEC. The Premier EHEC assay was significantly more sensitive than SMAC culture for diagnosis of STEC, and O157:H7 and non-O157:H7 STEC caused infections of similar severity in children.

Shiga toxin-producing *Escherichia coli* (STEC) causes cases of diarrhea, bloody diarrhea, and hemorrhagic colitis. STEC infection also causes hemolytic-uremic syndrome (HUS), a life-threatening condition characterized by hemolytic anemia, thrombocytopenia, and renal failure (3, 31). Transmission of STEC occurs through contaminated foods, such as ground beef, through contaminated water, and by person-to-person spread (2, 6, 7, 15, 26–28). In the United States, O157:H7 (O157) is the most common serotype of STEC (24, 27, 29, 30) and is the serotype most often associated with HUS (1, 3, 31). Approximately 150 non-O157 STEC serotypes also cause diarrheal disease (12), and in the United States, 33 to 61% of STEC infections are caused by non-O157 serotypes (4, 5, 10, 14, 16, 17, 20, 21, 23–25). Some studies suggest that non-O157 STEC infection causes milder disease than O157 infection (5, 14, 20, 25).

E. coli O157 infection and HUS are largely pediatric illnesses, although they can occur at any age. A recent 6-year review of active and passive surveillance data collected from multiple sites in the United States showed that the highest incidence of both *E. coli* O157 infection and HUS occurred among children of <5 years old and that the median age of patients with HUS was 4 years old (13). Most studies of O157 and non-O157 STEC have included mixed populations of

adults and children (4, 5, 10, 14, 16, 17, 21, 23, 25). While outbreaks of non-O157 STEC have been reported in children (9) and Klein et al. report that 38% of STEC infections were non-O157 in their pediatric cohort (20), the incidence of non-O157 STEC infection in the United States and the clinical sequelae in the pediatric population need to be more clearly defined.

The Centers for Disease Control (CDC) recommends that all stool samples from patients with acute community-acquired diarrhea or possible HUS be tested for the presence of STEC (12). Testing for O157 STEC is frequently performed by stool culture with sorbitol-MacConkey agar (SMAC). Most O157 STEC strains do not rapidly ferment sorbitol, so sorbitol-negative colonies on SMAC at 24 h are potential O157 STEC strains which can be identified further by serotype. Non-O157 STEC cannot be detected by SMAC culture. Immunoassays for Shiga toxins are a serotype-independent method for detection of STEC directly from stool or from broth cultures (18). A CDC guideline issued in 2009 recommends that stool samples be tested using both a selective culture for O157 STEC and a method to detect non-O157 STEC serotypes by the presence of Shiga toxins or Shiga toxin genes (12). At Children's Hospital Boston (CHB), we have used both SMAC culture and the Premier EHEC enzyme immunoassay (Meridian Diagnostics) for Shiga toxins 1 and 2 on broth culture for detection of STEC since April 2004. In this study, we reviewed results of STEC testing of all stool samples during a 65-month period. Our objectives were to compare the test characteristics of SMAC culture and the Premier EHEC assay for the diagnosis of STEC, to determine the proportion of non-O157 STEC infec-

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tion in our pediatric population, and to compare the manifestations of O157 STEC and non-O157 STEC infections in a pediatric population.

MATERIALS AND METHODS

Patient selection and data collection. Data were collected from CHB records from 1 April 2004 to 1 September 2009. CHB is a 390-bed tertiary-care hospital that serves a large metropolitan area. All stool samples submitted for STEC detection were reviewed. One sample per patient was included in the analysis, including the first positive sample from all children with at least one positive test and the first negative sample from children with multiple negative samples.

Chart reviews were performed on all subjects with culture-confirmed STEC (defined below). The presence of fever was determined by a history of fever in the Emergency Department (ED), clinic, or admission record or a documented temperature of $>38.0^{\circ}\text{C}$. Presence of bloody stool was determined from the ED, clinic, or admission record or a guaiac-positive stool sample. Patients with STEC who had anemia with microangiopathic hemolytic changes and evidence of renal injury (hematuria, proteinuria, or elevated creatinine) and history of acute or bloody diarrhea within 3 weeks prior to presentation were considered to have HUS, based on the CDC case definition for confirmed HUS (<http://www.cdc.gov/ncphi/od/ai/casedef/hemolyticcurrent.htm>, accessed 5 October 2010). Laboratory values were collected from the date of stool sample collection and the associated hospital admission, when available. Elevated white blood cell (WBC) count, low hematocrit, low platelet count, and elevated creatinine were defined using the laboratory-defined reference ranges for the subject's age and sex. Hematuria and proteinuria were defined by any heme or protein detected by urinalysis. Hemolysis was defined as the presence of schistocytes, burr cells, or helmet cells on a manual smear.

Laboratory testing for STEC. Stool samples submitted for STEC testing were plated on SMAC (Remel, Lenexa, KS) and cultured in GN broth (Becton, Dickinson and Co., Franklin Lakes, NJ) for Shiga toxin testing at CHB. SMAC plates were incubated at 35°C for 16 to 24 h and then examined for non-sorbitol-fermenting colonies. Two colonies of each non-sorbitol-fermenting morphotype were tested for O157 using an Ecolux latex agglutination test (Oxoid, Basingstoke, United Kingdom). Colonies positive for O157 were subcultured and identified by conventional biochemical testing. If no non-sorbitol-fermenting colonies were seen on SMAC, a sweep from the heavy area of the plate was tested for O157, and subculture to SMAC was performed if O157 was detected. Cultures with non-sorbitol-fermenting *E. coli* positive for O157 antigen were considered positive and sent to the Hinton State Laboratory Institute (HSLI).

Stool was cultured in GN broth (Becton, Dickinson and Co., Franklin Lakes, NJ) at 35°C for 16 to 24 h, and the broth was tested for Shiga toxins 1 and 2 by the Premier EHEC assay (Meridian Diagnostics Inc., Cincinnati, OH) at CHB. GN broth samples which were positive in the Premier EHEC assay at CHB were sent to the HSLI. Optical density (OD) values for positive results were collected from microbiology records; a result of "over" was assigned a value of 3, the maximal OD value.

Non-sorbitol-fermenting *E. coli* strains that were positive for O157 antigen and GN broth samples that were positive in the Premier EHEC test were sent to the HSLI for further testing. At the HSLI, putative STEC strains from SMAC were tested for Shiga toxins 1 and 2 with the Premier EHEC assay and identified and serotyped as described below. GN broth samples positive in the Premier EHEC assay at CHB were sent to the HSLI, where the original broth and a subculture in MacConkey's broth were both tested for Shiga toxins with the Premier EHEC assay. Broths positive for Shiga toxin were subcultured to SMAC, eosin methylene blue agar, and CHROMagar O157 plates. Colonies from the subcultured plates were tested using the Premier EHEC assay and confirmed as *E. coli* by use of lysine iron agar and motility agar. Sorbitol fermentation was tested using purple agar base with sorbitol. STEC isolates were serotyped by use of a RIM *E. coli* O157:H7 test (Remel, Lenexa, KS) and by agglutination with antiserum pools containing O26, O45, O103, O111, O121, O126, O145, and O157 (Statens Serum Institut, Hillerød, Denmark). Typing for H antigens and other O antigens was performed at the Centers for Disease Control and Prevention, Atlanta, GA. The HSLI reported positive Premier EHEC assay results on the initial GN or MacConkey's broth regardless of whether STEC was detected in subcultures to agar plates. If STEC was detected from subcultures to agar plates, it was serotyped and reported as well.

Statistical methods. Sensitivity, specificity, positive and negative predictive values, and 95% confidence intervals were calculated for SMAC culture and the Premier EHEC assay performed at CHB by using SAS version 9.1. Specimens positive for STEC were defined as those specimens from which STEC was

TABLE 1. Results of testing for Shiga toxin-producing *E. coli*

No. of isolates	CHB laboratory result		HSLI result	
	Shiga toxin production	<i>E. coli</i> O157 culture	Shiga toxin production	Culture
27	Positive	Positive	Positive	O157:H7 <i>E. coli</i>
4	Positive	Negative	Positive	O157:H7 <i>E. coli</i>
2	Negative	Positive	Positive	O157:H7 <i>E. coli</i>
17	Positive	Negative	Positive	Non-O157:H7 <i>E. coli</i>
8	Positive	Negative	Positive ^a	Negative
6	Positive	Negative	Negative	Negative
1	Negative	Positive	Negative	O157:non-H7 <i>E. coli</i>
1	Positive	Negative ^b	Positive	<i>Shigella flexneri</i>
5,044	Negative	Negative	ND ^c	ND

^a Shiga toxin was detected in broth cultures of stool at the HSLI, but STEC could not be identified upon subculturing of the broth to multiple agar plates.

^b *Shigella flexneri* detected in *Salmonella-Shigella* culture at CHB and confirmed at HSLI.

^c ND, not determined (because sample was not sent to HSLI).

detected by culture at the HSLI. Specimens negative for STEC were defined as specimens which were negative in both the SMAC culture and the Premier EHEC assay at CHB (these were not sent to the HSLI for additional testing) or specimens which were positive by either of these tests at CHB but which did not grow detectable STEC upon subculture at the HSLI. Clinical and laboratory manifestations in children with culture-confirmed O157 STEC and non-O157 STEC were compared. Categorical variables were analyzed using Fisher's exact test. Continuous variables were analyzed using pooled (age) or Satterthwaite (admission duration) *t* tests. Results comparing test characteristics and clinical and laboratory manifestations were calculated using SAS version 9.1. Premier EHEC assay ODs were compared between groups defined in Results by using a Kruskal-Wallis test and Dunn's multiple-comparison test comparing all group pairs. Statistical results comparing OD values were calculated using Prism4 software. Two-sided *P* values of ≤ 0.05 were considered statistically significant.

RESULTS

Performance of SMAC culture and Premier EHEC assay.

Of 5,110 patients tested at CHB, 5,044 (98.7%) had specimens which were negative for STEC by both SMAC culture and the Premier EHEC assay (Table 1). Sixty-six patients (1.3%) had specimens which were positive by SMAC culture, the Premier EHEC assay, or both. A total of 50 (0.9%) of these patients had culture-confirmed STEC. Thirty-three (0.6%) culture-confirmed STEC isolates were O157:H7, and 17 (0.3%) were other serotypes. Non-O157 STEC isolates included 4 isolates of O121:H19, 2 isolates each of O103:H2 and O69:H11, and 1 isolate each of O25:H11, O26:H11, O26:H28, O111:nonmotile, O118:H16, O126:H1, O130:H11, O145:nonmotile, and O146:H28. Both O157:H7 and non-O157 STEC infections occurred year round, with peak incidence in the late summer (Fig. 1).

The sensitivities of the Premier EHEC assay and SMAC culture for culture-confirmed STEC isolates of any serotype were 96.0% (95% confidence interval, 86.3 to 99.5%) and 58.0% (43.2 to 71.8%), respectively. The sensitivities of the Premier EHEC assay and SMAC culture for culture-confirmed STEC O157:H7 were 93.9% (79.8 to 99.3%) and 87.9% (71.8 to 96.6%), respectively. The specificities of the Premier EHEC assay and SMAC culture for culture-confirmed STEC isolates of any serotype were 99.7% (99.5 to 99.8%) and 99.9% (99.9 to 100%), respectively. The positive predictive values of the Pre-

FIG. 1. Number of culture-confirmed O157 and non-O157 Shiga toxin-producing *Escherichia coli* cases by month of infection, from 1 April 2005 to 1 September 2009.

mier EHEC assay and SMAC culture for culture-confirmed STEC isolates of any serotype were 76.2% (63.8 to 86.0%) and 96.7% (82.8 to 99.9%), respectively. The negative predictive values of the Premier EHEC assay and SMAC culture for culture-confirmed STEC isolates of any serotype were 99.9% (99.9 to 100%) and 99.6% (99.4 to 99.7%), respectively.

Sixteen samples gave false positives in either the Premier EHEC assay or SMAC culture. Fifteen false positives occurred with the Premier EHEC test and one occurred with SMAC culture. Of the 15 false-positive specimens by the Premier EHEC assay, 6 of these were negative and 9 were positive by repeat Premier EHEC assay at the HSLI. One sample positive by repeat Premier EHEC assay grew *Shigella flexneri*. The single false-positive SMAC result was a non-sorbitol-fermenting *E. coli* isolate that was positive for O157 antigen at CHB but that was determined not to express H7 antigen or produce Shiga toxins at the HSLI.

We reviewed the ODs of the Premier EHEC assay results obtained at CHB. Samples were categorized as those specimens positive for O157:H7 STEC by culture (group 1), specimens positive for non-O157 STEC by culture (group 2), specimens positive by the Premier EHEC assay at the HSLI but negative for STEC by culture (group 3), and specimens negative by the Premier EHEC assay at the HSLI and for STEC by culture (group 4) (Fig. 2). Group 3 includes the specimen that was positive for *Shigella flexneri* (OD of 0.35). The ODs for group 4, which were false positive for STEC in the Premier EHEC assay, were significantly lower than those in groups 1 and 2, which were true positives in the Premier EHEC assay. Based on culture results, specimens in group 3 were also false positives, although several of these had ODs well above the cutoff value for positivity.

Comparison of children with O157:H7 and non-O157:H7 STEC. Thirty-three children with O157:H7 STEC and 17 children with non-O157:H7 STEC were analyzed. Subjects in both groups had similar age and sex distributions (Table 2). The incidence of bloody stool or fever, hospital and intensive care unit (ICU) admission, and diagnosis of HUS did not differ significantly between the two groups (Table 2). The duration of hospital admission was longer among children with O157:H7 STEC infection than children with non-O157:H7 STEC, but the difference did not reach statistical significance ($P = 0.06$).

FIG. 2. ODs of the positive Premier EHEC assay results from CHB. Group 1 includes specimens positive for Shiga toxin-producing *Escherichia coli* (STEC) serotype O157:H7 by culture, group 2 includes specimens positive for non-O157 STEC by culture, group 3 includes specimens confirmed positive by the Premier EHEC assay at the HSLI but negative for STEC by culture, and group 4 includes specimens determined to be negative by the Premier EHEC assay on retesting and for STEC by culture at the HSLI. Bars represent group means. The cutoff for a positive Premier EHEC assay is shown by the dashed line at an OD of 0.15. P values were obtained using the Kruskal-Wallis test comparing all group pairs (*, $P < 0.01$).

Both groups had similar incidences of elevated WBCs, low hematocrit, low platelet count, elevated creatinine, hematuria, and proteinuria (Table 3). All children with STEC infection and a low hematocrit had hemolysis on manual smear.

DISCUSSION

The Premier EHEC assay was more sensitive than SMAC culture for detection of STEC of any serotype (96.0% versus 58.0%, respectively), while both tests had >99% specificity. Seventeen non-O157:H7 STEC isolates and four O157:H7 isolates were detected in the Premier EHEC assay but not by

TABLE 2. Demographic and clinical characteristics of patients with Shiga toxin-producing *E. coli*

Variable	Result ^a for patients with:		P value (Fisher's exact test)
	O157:H7 STEC ($n = 33$)	Non-O157:H7 STEC ($n = 17$)	
Sex (% male)	48.5	35.2	0.55
Mean age, yr (range)	8.3 (0.4–18)	8.8 (0.6–18)	0.79 ^b
Blood in stool (no. of patients)	26/31 (83.9)	13/16 (81.3)	1.00
Fever (no. of patients)	14/31 (45.2)	5/16 (31.3)	0.53
Admission (no. of patients)	24/33 (72.3)	10/17 (58.8)	0.35
Mean duration, days (range)	5.4 (0–29)	1.6 (0–9)	0.06 ^c
ICU admission (no. of patients)	2/24 (8.3)	0/10 (0.0)	1.00
Hemolytic-uremic syndrome (no. of patients)	7/33 (21.2)	3/17 (17.7)	1.00

^a Results expressed as number of patients indicate the number of patients with the given characteristic over the total number of patients for whom that characteristic was evaluated.

^b t test with equal variances.

^c t test with unequal variances.

TABLE 3. Laboratory characteristics of patients with Shiga toxin-producing *E. coli*

Variable	No. (%) ^a of patients with:		<i>P</i> value (Fisher's exact test)
	O157:H7 STEC (<i>n</i> = 33)	Non-O157:H7 STEC (<i>n</i> = 17)	
Elevated white blood cell count	21/32 (65.6)	8/14 (57.1)	0.74
Low hematocrit	7/32 (21.9)	3/14 (21.4)	1.00
Evidence of hemolysis ^b	7/7 (100)	3/3 (100)	1.00
Elevated creatinine	5/32 (15.6)	4/16 (25.0)	0.44
Low platelet count	7/32 (21.9)	3/14 (21.4)	1.00
Hematuria	10/26 (38.5)	5/10 (50.0)	0.71
Proteinuria	8/26 (30.8)	4/10 (40.0)	0.70

^a Results indicate the number of patients with the given characteristic over the total number of patients for whom that characteristic was evaluated.

^b Determined for patients with low hematocrit and defined as the presence of schistocytes, helmet cells, or burr cells.

SMAC culture. The Premier EHEC assay did not detect two O157:H7 STEC isolates that were identified by SMAC culture. Prior studies have also found that immunoassays for Shiga toxins are sensitive for all STEC isolates (89 to 100%) (11, 18, 22). The Premier EHEC assay and SMAC culture had similar sensitivities for *E. coli* O157:H7 (93.9 and 87.9%, respectively). Non-O157:H7 *E. coli* isolates accounted for 43% of STEC cases in this pediatric population. This large proportion of non-O157:H7 STEC isolates is consistent with reports of other U.S. populations (5, 10, 14, 16, 20, 21) and demonstrates the importance of using a test which will detect all serotypes of STEC.

The Premier EHEC assay resulted in more false-positive results than SMAC culture and therefore had a lower positive predictive value (76.2% versus 96.7%, respectively). We used detection of STEC in culture to define a true-positive result. Of the 15 specimens which were false positive in the Premier EHEC assay at CHB, 9 were positive in the Premier EHEC assay performed at the HSLI (group 3 in Fig. 2), and 6 were not (group 4 in Fig. 2). The ODs of these specimens, particularly those in group 4, were significantly lower than the ODs of the true-positive Premier EHEC assay results. However, several samples with confirmed STEC had low ODs with the Premier EHEC assay, so a higher OD cutoff would have reduced the sensitivity of the Premier EHEC assay. A low positive OD in the Premier EHEC assay might be considered with caution given the potential for a false-positive result, but the result might indicate a true STEC infection even with a negative SMAC culture. Of note, it is possible that the specimens that were positive in the Premier EHEC assay but did not yield STEC in culture contained an STEC isolate that was not detected in culture but which might have been detected by an alternative method, such as a nucleic acid amplified test.

We did not find a difference in severity of disease between children with O157:H7 and non-O157:H7 STEC infection. Non-O157:H7 STEC caused severe illness in a substantial proportion of patients; bloody stool was reported in 81.3%, admission was required for 58.8%, and HUS was diagnosed in 17.7%. Laboratory values indicated similar rates of hemolytic

anemia, leukocytosis, thrombocytopenia, and renal insufficiency among patients with O157:H7 and non-O157:H7 STEC infections. While fewer non-O157:H7-infected patients than O157-infected patients required an ICU admission (0 of 10, versus 2 of 24) and hospital admissions were shorter (means of 1.6 versus 5.4 days), neither of these results reached statistical significance.

Our results differ from other studies, which suggest that O157:H7 STEC causes more-severe disease than non-O157:H7 STEC and that there is a lower risk of HUS among patients with non-O157 STEC infection (5, 14, 20, 25). There are several possible reasons why we did not find a difference in disease severity between children with O157:H7 and non-O157:H7 STEC. Unlike many studies which compare O157:H7 and non-O157:H7 STEC disease (5, 14, 16), our patient cohort was limited to children. Non-O157:H7 STEC infection may be more severe in children, explaining the similarity in disease severity to that of O157 STEC infection in our cohort. Alternatively, different non-O157:H7 serotypes used in our cohort versus other studies may be the cause of more-severe STEC infection. The severity of non-O157 STEC infections in our pediatric patients further supports the importance of a sensitive and rapid diagnostic method to detect all serotypes of STEC.

Recent recommendations from the CDC for diagnosis of STEC are that laboratories perform both a culture for specific detection of O157:H7 and an assay for Shiga toxins (12). This is advocated for optimal sensitivity and speed for detection of STEC. A review of laboratory practices up to the year 2000 in the United States reported that while 95% of 388 laboratories tested stool for *E. coli* O157:H7 by culture, only 3% used a Shiga toxin immunoassay capable of identifying non-O157:H7 serotypes (32). Data from 2008 show that 35% of laboratories participating in a proficiency testing program used a test for Shiga toxins (8). Our results demonstrate that while neither SMAC culture nor the Premier EHEC assay alone will detect all cases of STEC, the Premier EHEC assay is the more sensitive test. Some laboratories may not have the resources to perform both SMAC culture and an assay for Shiga toxins. If only one of the two screening tests can be done, our data support the proposal that use of a Shiga toxin assay should be considered rather than use of an O157:H7 culture (19). If only a Shiga toxin assay is used, it is important that positive samples be subcultured for STEC detection and serotyping, with the goal of identifying and curtailing potential outbreaks of STEC infection.

Our study demonstrates that non-O157 STEC infections compose a significant proportion of all STEC infections in the pediatric patient population and that these infections are associated with severe disease, including HUS. The Premier EHEC assay performed in our microbiology laboratory exhibited excellent sensitivity and specificity for the diagnosis of both O157 and non-O157 STEC infections and provided an accurate diagnosis for many children who tested negative by SMAC culture. An accurate and timely diagnosis of STEC infection provides the potential benefit of guiding the clinician's care of these children and increasing the identification of non-O157 isolates for purposes of infection control and public health disease surveillance.

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