ARTICLE

Infections in Pediatric Postdiarrheal Hemolytic Uremic Syndrome

Factors Associated With Identifying Shiga Toxin–Producing Escherichia coli

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Objective: To describe pathogens identified through routine clinical practice and factors associated with identifying Shiga toxin–producing *Escherichia coli* (STEC) infection in patients with postdiarrheal hemolytic uremic syndrome (D+HUS).

Design: Population-based active surveillance.

Setting: Hospitals in the FoodNet surveillance areas from 2000 through 2010.

Participants: Children younger than 18 years with D+HUS.

Main Exposures: Testing for STEC and demographic and clinical characteristics.

Main Outcome Measures: Percentage of patients with evidence of infection with likely HUS-causing agents and associations between exposures and evidence of STEC infection.

Results: Of 617 patients, 436 (70.7%) had evidence of infection with likely HUS-causing agents: STEC O157 (401 patients), non-O157 STEC (21 patients), O157 and non-O157 STEC (1 patient), *Streptococcus pneumoniae* (11 patients), and other pathogens (2 patients). Among patients without microbiological evidence of STEC, 76.9% of those tested had serologic evidence of STEC infection. Children more likely to have evidence of STEC infections included those patients tested for STEC less than 4 days after diarrhea onset, 12 months or older (71.6% vs 27.8% if <12 months of age), with infections as part of an outbreak (94.3% vs 67.3%), with bloody diarrhea (77.2% vs 40.4%), with onset during June through September (76.9% vs 60.1%), with a leukocyte count greater than 18 000/μL (to convert to $\times 10^9$/L, multiply by 0.001) (75.7% vs 65.3%), or with only moderate anemia (hemoglobin >7.0 g/dL [to convert to grams per liter, multiply by 10] or hematocrit greater than 20% [to convert to a proportion of 1, multiply by 0.01]) (75.1% vs 66.3%). However, many of these associations were weaker among children with thorough STEC testing.

Conclusions: Early stool collection for *E coli* O157 culture and Shiga toxin testing of all children with possible bacterial enteric infection will increase detection of STEC strains causing HUS. In the absence of microbiological evidence of STEC, serologic testing should be performed.

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SHIGA TOXIN–PRODUCING *Escherichia coli* (STEC) cause illnesses ranging from mild diarrhea to postdiarrheal hemolytic uremic syndrome (D+HUS). Children have the highest incidence of D+HUS, a potentially fatal thrombotic microangiopathy.[1,2] Although STEC O157 is the most frequently reported serogroup,[3] more than 50 other serogroups are estimated to cause two-thirds of STEC illnesses.[4] Detection of non-O157 STEC infections is increasing as more laboratories use assays to detect Shiga toxins.[5-7]

Population-based descriptions of STEC strains causing D+HUS in the United States are outdated and limited to single states.[8-14] The 1 US study of national scope found that STEC O157 accounted for more than 80% of D+HUS.[15] This study, like many, used a microbiological testing protocol that was more comprehensive than that used in typical clinical practice. Although studies with rigorous microbiological protocols have detected STEC in nearly 90% of cases,[16] routine clinical practice yields a lower percentage, limiting population-based descriptions of the types of STEC causing D+HUS.

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Table 1. Percentage of Postdiarrheal Hemolytic Uremic Syndrome Cases With Stool Testing and Microbiological Evidence of STEC by Year, FoodNet, 2000-2010

Variable	Year											
	2000 (n = 46)	2001 (n = 64)	2002 (n = 50)	2003 (n = 41)	2004 (n = 42)	2005 (n = 49)	2006 (n = 69)	2007 (n = 71)	2008 (n = 60)	2009 (n = 59)	2010 (n = 66)	*P* Value[a]
Cultured for STEC O157	95.7	95.3	94.0	95.1	95.2	91.8	91.3	90.1	91.7	88.1	89.4	.03
Tested for Shiga toxin	45.7	48.4	48.0	43.9	40.5	38.8	58.0	59.2	65.0	67.8	86.4	<.001
With STEC O157 isolated	60.9	50.0	70.0	46.3	47.6	44.9	62.3	47.9	50.0	52.5	60.6	.75
With non-O157 STEC isolated	0	1.6	0	2.4	7.1	2.0	4.4	5.6	1.7	3.4	6.1	.06

Abbreviation: STEC, Shiga toxin–producing *Escherichia coli*.
[a] On the basis of the Cochran-Armitage test.

We analyzed 11 years of population-based D+HUS surveillance data collected by FoodNet to describe infectious agents identified through routine testing and the factors associated with establishing antecedent STEC infection, the aim being identification of practices that increase detection of STEC-related D+HUS.

METHODS

CASE DEFINITION

A confirmed case was defined as an illness diagnosed as D+HUS in a child younger than 18 years with (1) an HUS diagnosis within 21 days after the onset of self-reported diarrhea (or any diarrhea during the 3 weeks before HUS diagnosis if there was evidence of STEC infection), (2) anemia (hemoglobin or hematocrit below age- and sex-specific thresholds),[17] (3) thrombocytopenia (platelet count $<150 \times 10^3/\mu L$ [to convert to $\times 10^9/L$, multiply by 1]), (4) azotemia (serum creatinine level $\geq$1.0 mg/dL [to convert to micromoles per liter, multiply by 88.4] if <13 years old and $\geq$1.5 mg/dL if $\geq$13 years old), and (5) red blood cell fragmentation. A probable case met all criteria except the last.

CASE ASCERTAINMENT

FoodNet sites (Connecticut, Georgia, Maryland, Minnesota, New Mexico, Oregon, Tennessee, and selected counties in California, Colorado, and New York) conducted active hospital-based surveillance for pediatric D+HUS; start year for sites varied from 1997 to 2004. In 2010, the FoodNet catchment included 47.1 million persons (15% of the US population). Each site established a network of pediatric nephrologists and hospital infection control personnel. FoodNet personnel routinely contacted network members to identify cases and completed standardized case report forms by interviewing treating physicians, abstracting medical records, or both. Data collected included demographics, medical history, microbiological and serologic findings, and selected laboratory parameters. Race, as indicated by a patient's caregiver, physician, or medical records, was recorded because it is associated with D+HUS[18]; categories included American Indian/Alaska Native, Asian/Pacific Islander, black, white, and unknown.

To augment case finding, all FoodNet sites, except New Mexico, reviewed hospital discharge data. Records of hospitalizations assigned any of the following *International Classification of Disease, Clinical Modification, Ninth Revision* codes were reviewed: (1) 283.11 (HUS), (2) 584.X and 283.X and 287.X (acute renal failure, hemolytic anemia, and thrombocytopenia), and (3) 446.6 and 008.X or 009.X (thrombotic thrombocytopenic purpura with diarrhea caused by *E coli* or unknown pathogen). Because of delayed availability of records, at the time of analysis, 5 of 9 sites had completed review for patients hospitalized through 2010; 3 had completed reviews through 2009 and 1 through 2008. This surveillance was deemed nonresearch by the Centers for Disease Control and Prevention (CDC).

MICROBIOLOGICAL AND SEROLOGIC TESTING

All testing was conducted at the discretion of attending physicians. Our surveillance protocol did not specify any required testing. Results collected included stool culture for *E coli* O157 (eg, culture on sorbitol-MacConkey or other selective and differential agar), stool assays for Shiga toxins, and positive studies for other pathogens, including non-O157 STEC, in stool and other specimens. Tests may have been performed in a clinical laboratory, a state public health laboratory, or at the CDC. Methods differed among laboratories. For example, to detect evidence of Shiga toxins, most clinical laboratories used enzyme immunoassay, but the CDC and some state laboratories used polymerase chain reaction assays.[5] The CDC tested serum samples received for IgG or IgM to the lipopolysaccharide of *E coli* O157, O111, or both by enzyme immunoassay.[19] We obtained Shiga toxin 1 (Stx1) and 2 (Stx2) profiles for STEC isolates reported from 2007 through 2010. We obtained additional toxin profiles and polymerase chain reaction results for *eae* (intimin) and *ehxA* (enterohemolysin) for non-O157 STEC isolates from 2000 through 2010 from the CDC National *E coli* Reference Laboratory.

Cases were categorized into 3 groups based on evidence of STEC: (1) microbiological evidence through culture (regardless of serologic evidence), (2) serologic evidence only, and (3) no evidence of STEC (either no testing performed or no positive STEC findings). Microbiological evidence was further categorized as confirmed (isolation of an *E coli* that produced Shiga toxin or, for *E coli* O157, presence of the H7 antigen) or probable (isolation of *E coli* O157 without evidence of the H7 antigen or Shiga toxin).

Pathogens we defined as likely HUS-causing agents were STEC, other Shiga toxin–producing Enterobacteriaceae, *Streptococcus pneumoniae*, human immunodeficiency virus, Epstein-Barr virus, varicella-zoster virus, and influenza A.

Table 2. Patients With Postdiarrheal HUS by Evidence of STEC Infection and Other Infectious Causes of HUS, 2000-2010

Cause	No. (%) of Patients
Microbiological evidence of STEC[a]	**353** (57.2)
Confirmed STEC O157 isolated[b]	325 (52.7)
Probable STEC O157 isolated[c]	8 (1.3)
Non-O157 STEC isolated[d]	19 (3.1)
STEC O157:H7 and STEC O26:H11 isolated	1 (0.2)
Shiga toxin detected in stool but STEC not isolated[e]	2 (0.3)
Serologic evidence of STEC in cases with no microbiological evidence[a]	**70** (11.3)
Escherichia coli O157	68 (11.0)
E coli O111[f]	2 (0.3)
No evidence of STEC or other established infectious causes of HUS[g]	**159** (25.8)
Only stool tested for STEC	138 (22.4)
Stool and serum tested for STEC	21 (3.4)
No STEC testing performed	**20** (3.2)
Microbiological evidence of other infectious causes of HUS	**13** (2.1)
Shigella dysenteriae type 1 isolated from blood and Shiga toxin detected in stool[h]	1 (0.2)
Streptococcus pneumoniae isolated[i]	11 (1.8)
Pandemic H1N1 Influenza A	1 (0.2)
Total	**617** (100)

Abbreviations: HUS, hemolytic uremic syndrome; STEC, Shiga toxin–producing *Escherichia coli.*

[a] Evidence of additional pathogens was found in the stool samples of 25 patients with microbiological or serologic evidence of STEC infection: *Clostridium difficile* (7 patients), *Streptococcus pyogenes* (5 patients), nontyphoidal *Salmonella* (3 patients), *Giardia* (3 patients), rotavirus (2 patients), *Campylobacter* spp (2 patients), *Aeromonas* spp (1 patient), *Yersinia entercolitica* (1 patient), enterotoxigenic *Escherichia coli* (1 patient), and *Plesiomonas shigelloides* (1 patient).

[b] A total of 310 isolates were O157:H7, 9 were O157:H-, and 6 had no H antigen information reported.

[c] Only 1 patient was known to have a stool sample tested for Shiga toxin. Two isolates were O157:H-, and the other 6 had no H antigen information reported.

[d] Serotypes were O111:H- (3 patients), O121:H19 (2 patients), O145:H- (2 patients), and 1 patient each with O26:H-, O26:H11, O103:H2, O111:H8, O111 with no reported H antigen information, O121 with no reported H antigen information, O130:H11, O145:H25, O145:H28, and O145 with no reported H antigen information. Two isolates had no reported serogroup. All isolates were from stool specimens, except for the O26:H11 isolate, which was isolated from a urine sample of an 11-month-old girl.

[e] Stool culture for 1 patient did not yield *E coli* O157; the other patient did not undergo *E coli* O157 culture.

[f] Few serum samples received at the Centers for Disease Control and Prevention were tested for immunoglobulins to *E coli* O111 lipopolysaccharide, but the specific number tested is unknown,

[g] Evidence of other pathogens or putative pathogens was found in stool samples of 14 patients with no evidence of STEC: *Clostridium difficile* (3 patients), *Aeromonas* spp (2 patients), *Campylobacter* spp (2 patients), *Giardia* (2 patients), adenovirus (1 patient), enterotoxigenic *E coli* (1 patient), *Pseudomonas* spp (1 patient), rotavirus (1 patient), and *Shigella flexneri* type 2b (1 patient).

[h] In a US-born child of a family that immigrated from Africa 4 years earlier.

[i] Six isolates from blood, 2 from endotracheal aspirate, 2 from pleural fluid, and 1 from sputum. Serotypes of 6 blood and 1 pleural fluid isolates were 19A (4 isolates), 003, 004, and 35B. No serotype information was available for the 4 remaining isolates. Stool samples from 6 of 11 patients with *S pneumoniae* infection were cultured for *E coli* O157 (4 patients), tested for Shiga toxin (1 patient), or both (1 patient).

STATISTICAL ANALYSIS

The number of cases ascertained from 2000 through 2010 determined the sample size. A 2-tailed significance level of .05 was used for all tests. Mean annual incidence rates of D+HUS by year of age were calculated using US Census Bureau data. The Cochran-Armitage test was used to assess trends by year. Univariate associations between evidence of STEC infection and clinical factors were assessed using the Fisher exact test; patients infected with other HUS-causing agents and patients with only evidence of Shiga toxin were excluded. Potential confounding by geography was assessed by change in summary Mantel-Haenszel odds ratios following stratification by the FoodNet site.

We performed post hoc analyses to assess the extent to which factors associated with STEC evidence varied by completeness of STEC testing. We defined thorough STEC testing in 2 ways: complete stool testing (ie, performance of *E coli* O157 culture and Shiga toxin testing) and complete stool testing or serologic testing. We constructed 4×2 tables, with each row representing 1 exposure combination of 2 dichotomous factors: thorough STEC testing and a given clinical factor. These tables contain all data needed for assessment of interaction on additive and multiplicative scales.[20] We calculated the risk of having documented STEC evidence for patients in each exposure category. We calculated risk differences for each clinical factor separately for patients with and without thorough STEC testing.

RESULTS

PATIENTS

We identified 617 (523 confirmed and 94 probable) patients with D+HUS. The median age of patients was 3.7 years (range, 2.9 months to 17.8 years). The mean annual incidence of D+HUS was 0.54 case per 100 000 children. Incidence trended downward with age from a peak in children aged 12 to 36 months. A total of 55.3% of the patients were girls. Of the 532 patients (86.2%) with race reported, more were white (90.4%) and fewer were black (4.3%) than would be expected from the population under surveillance (71.3% white and 19.3% black). Diarrhea began in June through September for 59.3% of patients.

STEC TESTING

Stool was cultured for STEC O157 in 569 patients (92.2%) and tested for Shiga toxin in 348 patients (56.4%). The percentage cultured for STEC O157 decreased and the percentage tested for Shiga toxin increased from 2000 through 2010 (**Table 1**).

Microbiological evidence of STEC infection was found in 353 patients (57.2%): 333 with STEC O157, 19 with non-O157 STEC (serogroups O111 [5 patients], O145 [5 patients], O121 [3 patients], O26 [2 patients], O103 [1 patient], O130 [1], unknown [2 patients]), and 1 with both STEC O157 and STEC O26 (**Table 2**). No significant trend was seen from 2000 through 2010 in the percentage of patients with STEC O157 isolated, but there was a gradual near-significant increase in the percentage of patients with non-O157 STEC isolated (Table 1). Shiga toxin profiles were reported for 103 STEC O157 isolates and 17 non-O157 STEC isolates. Most isolates produced Stx2 (**Table 3**).

Serologic testing was performed for 91 of 264 patients (34.5%) without microbiologic evidence of STEC; 70 (76.9%) had serologic evidence of infection, 68 with O157 and 2 with O111. In total, 423 patients (68.6%) had evidence of STEC infection, including 401 (94.8%)

Table 3. Presence of Virulence Genes in Shiga Toxin–Producing *Escherichia coli* Isolates From Patients With Postdiarrheal Hemolytic Uremic Syndrome by Serogroup, 2000-2010

Serogroup	Shiga Toxin			*eae*[a]	*ehxA*[a]
	*stx*1 Alone	*stx*2 Alone	*stx*1 and *stx*2		
O157 (n = 103)[b]	0/103	78/103 (75.7)	25/103 (24.3)		
O111 (n = 4)[c]	0/4	0/4	4/4 (100)	4/4 (100)	4/4 (100)
O121 (n = 2)[c]	0/2	2/2 (100)	0/2	2/2 (100)	2/2 (100)
O145 (n = 5)	0/5	5/5 (100)	0/5	5/5 (100)	5/5 (100)
O26 (n = 2)[d]	1/2 (50.0)	0/2	1/2 (50.0)	2/2 (100)	1/2 (50.0)
O103 (n = 1)	1/1 (100)	0/1	0/1	1/1 (100)	1/1 (100)
O130 (n = 1)	0/1	1/1 (100)	0/1	0/1	1/1 (100)
Unknown (n = 1)[c]	0/1	0/1	1/1 (100)		
Total	**2/119** (1.7)	**86/119** (72.3)	**31/119** (26.1)	**14/15** (93.3)	**14/15** (93.3)

[a] *eae* encodes intimin, and *ehxA* encodes enterohemolysin; *eae* and *ehxA* data were not available for STEC O157 (test not generally performed because genes almost invariably present) or for isolates of unknown serogroup.
[b] Shiga toxin data collected for 103 of 135 STEC O157 isolates (76.3%) from 2007 through 2010.
[c] Virulence factor information is not available for 1 isolate from each of the following serogroups: O111, O121, and unknown.
[d] Excluded 1 Stx1-only STEC O26 isolate from a patient whose stool sample also yielded a *stx*2-only STEC O157 isolate.

with STEC O157, 21 (5.0%) with non-O157 STEC, and 1 with both.

OTHER PATHOGENS

Evidence of other likely HUS-causing agents was found in 13 patients without evidence of STEC infection: *S pneumoniae* (11 patients), *Shigella dysenteriae* type 1 (1 patient), and pandemic H1N1 influenza A (1 patient). The 11 patients with *S pneumoniae* infection tended to be younger (median age, 1.8 years) than other patients, and all 11 had diarrhea onset during October through March. Blacks were more likely than whites to have *S pneumoniae* isolated (13.0% vs 1.1%) and more likely to become ill during October through March (52.2% vs 25.8%).

Other pathogens were found infrequently in stool (Table 2). *Streptococcus pyogenes* was isolated from stool in 2 of 5 patients (40.0%) with STEC O145 infections and 3 of 401 patients (0.7%) with STEC O157 infections. Children with mixed STEC and *S pyogenes* infections were generally older than other children with STEC infections (median age, 7.2 vs 3.6 years).

FACTORS ASSOCIATED WITH EVIDENCE OF STEC INFECTION

Children were more likely to have evidence of STEC infection if they were 12 months or older, were part of a recognized outbreak, or had bloody diarrhea, diarrhea onset during June through September, a white blood cell count greater than 18 000/μL (to convert to $\times 10^9$/L, multiply by 0.001), only moderate anemia, or stool cultured for *E coli* O157 or tested for Shiga toxin less than 4 days after diarrhea onset (**Table 4**). There was no apparent confounding by geography.

We found evidence that the effect of 5 clinical characteristics (being part of a recognized outbreak, bloody diarrhea, diarrhea onset during June through September, white blood cell count >18 000/μL, and only moderate anemia) on the risk of having *any* evidence of STEC varied by completeness of STEC testing; the increase in risk was larger among patients without thorough STEC testing (**Table 5**). We observed similar effects of these 5 characteristics on the risk of having *microbiological* evidence of STEC infection; the increase in risk was larger among those without complete stool testing (data not shown).

COMMENT

In this first population-based description of pediatric D+HUS of national scope in the United States, most (94.8%) of the two-thirds of children with evidence of STEC infection had STEC O157 infection. The marked increase in the use of Shiga toxin testing from 2000 through 2010 was not accompanied by as significant of an increase in the percentge of D+HUS cases attributable to non-O157 STEC. Our findings reaffirm previously established associations between several clinical factors and antecedent STEC infection. In addition, we found that most of these associations were weaker among children with thorough STEC testing. Thus, our findings support the value of STEC testing of children with possible bacterial enteric infection even in the absence of factors, such as summertime illness and bloody diarrhea, that have traditionally been used to support decisions to conduct STEC testing. Furthermore, we found that 2.1% of D+HUS cases are unlikely caused by STEC infection.

Our finding that 68.6% of children with D+HUS had evidence of STEC infection is comparable to the 60% to 88% found in other studies.[15,16,21-26] However, only 34.5% of patients without microbiological evidence of STEC had serologic testing. Of those tested, 76.9% were seropositive. Applying this percentage to all children without evidence of infection by a likely HUS-causing agent would increase the estimate of cases with STEC infection to 88.5%. In contrast to routine practices in our surveillance area, studies with the highest STEC detection rates used immunomagnetic separation, a stool culture technique that increases STEC detection sensitivity.[16,25-27] Greater use of immunomagnetic separation in the United States, in addition to serologic testing, could increase the percentage of cases with serogroup-specific evidence of STEC infection further.

Table 4. Factors Associated With Evidence of STEC Infection Among 602 Children With Postdiarrheal HUS, FoodNet, 2000-2010[a]

Factor	No.	Microbiological Evidence of STEC		Any Evidence of STEC	
		No. (%)	*P* Value[b]	No. (%)	*P* Value[b]
Age ≥12 mo					
Yes	584	349 (59.8)	.002	418 (71.6)	<.001
No	18	4 (22.2)		5 (27.8)	
Part of recognized outbreak					
Yes	70	60 (85.7)	<.001	66 (94.3)	<.001
No	490	270 (55.1)		330 (67.3)	
Unknown or missing	42	23 (54.8)		27 (64.3)	
Bloody diarrhea					
Yes	487	325 (66.7)	<.001	376 (77.2)	<.001
No	99	23 (23.2)		40 (40.4)	
Unknown or missing	16	5 (31.3)		7 (43.8)	
Diarrhea onset in June-September					
Yes	363	233 (64.2)	<.001	279 (76.9)	<.001
No	238	120 (50.4)		143 (60.1)	
Unknown or missing	1	0		1 (100)	
Highest WBC >18, 000/μL 7 days before to 3 days after HUS diagnosis					
Yes	288	194 (67.4)	<.001	218 (75.7)	.01
No	294	150 (51.0)		192 (65.3)	
Unknown or missing	20	9 (45.0)		13 (65.0)	
Lowest hemoglobin >7.0 g/dL or hematocrit >20% 7 days before to 3 days after HUS diagnosis					
Yes	273	175 (64.1)	.02	205 (75.1)	.02
No	329	178 (54.1)		218 (66.3)	
Escherichia coli O157 culture <4 days after diarrhea onset[c]					
Yes	258	212 (82.2)	<.001	224 (86.8)	<.001
No	295	140 (47.5)		187 (63.4)	
Unknown or missing	9	0		1 (11.1)	
Shiga toxin testing <4 days after diarrhea onset[d]					
Yes	149	133 (89.3)	<.001	137 (91.9)	<.001
No	173	107 (61.8)		133 (76.9)	
Unknown or missing	21	4 (19.0)		10 (47.6)	
Complete stool STEC testing[e]					
Yes	330	244 (73.9)	<.001	276 (83.6)	<.001
No	230	99 (43.0)		130 (56.5)	
Unknown or missing	42	10 (23.8)		17 (40.5)	
Complete stool STEC testing or serologic testing					
Yes	391	259 (66.2)	<.001	329 (84.1)	<.001
No	175	85 (48.6)		85 (48.6)	
Unknown or missing	36	9 (25.0)		9 (25.0)	

Abbreviations: HUS, hemolytic uremic syndrome; STEC, Shiga toxin–producing *Escherichia coli;* WBC, white blood cell count.

SI conversion factors: To convert WBC to $\times 10^9$/L, multiply by 0.001; to convert hemoglobin to grams per liter, multiply by 10; to convert hematocrit to a proportion of 1, multiply by 0.01.

[a] A total of 15 of 617 patients were excluded from this table (11 with *Streptococcus pneumoniae* infection, 1 with *Shigella dysenteriae* type 1 infection, 1 with pandemic H1N1 influenza A infection, and 2 with only evidence of Shiga toxin in stool).

[b] All *P* values reflect 2 × 2 analyses in which only cases with known factor information are used.

[c] Restricted to cases cultured for *E coli* O157.

[d] Restricted to cases tested for Shiga toxin.

[e] Performance of culture for *E coli* O157 and Shiga toxin testing.

All but 1 of the non-O157 serogroups we observed were among the 6 most common serogroups detected in ill persons in the United States.[28] However, our Shiga toxin profile findings suggest that increases in detection of common non-O157 STEC serogroups as causes of diarrheal illness, occurring as a result of increased Shiga toxin testing,[7] has not led to an equivalent increase in the reported incidence of D+HUS attributable to these serogroups. Among 14 D+HUS cases in our series with evidence of infection with only 1 of the 6 most common non-O157 STEC serogroups (5 serogroups represented), 12 isolates (85.7%) produced Stx2, a factor strongly associated with D+HUS.[29] In contrast, the percentage of isolates of these 5 serogroups that produced Stx2 from a convenience sample of isolates from ill persons was 36.2% (217 of 600).[28] The only common serogroup not detected in our D+HUS surveillance was O45, which rarely produces Stx2.[28] Emergence of new strains,

Table 5. Interaction Between Completeness of Testing and Clinical Factors in Risk of Identifying STEC Infection in Children With Postdiarrheal Hemolytic Uremic Syndrome[a]

Factor	Thorough STEC Testing[b]	No. of Patients		Risk	Risk Difference[d]
		Evidence of STEC[c]	No Evidence of STEC		
Part of recognized outbreak					
No	No	65	76	0.46	**Reference**
Yes	No	15	3	0.83	**0.37**
No	Yes	257	58	0.82	Reference
Yes	Yes	51	1	0.98	0.16
Bloody diarrhea					
No	No	2	32	0.06	**Reference**
Yes	No	83	51	0.62	**0.56**
No	Yes	38	15	0.72	Reference
Yes	Yes	285	45	0.86	0.14
Diarrhea onset in June–September					
No	No	23	47	0.33	**Reference**
Yes	No	62	43	0.59	**0.26**
No	Yes	116	29	0.80	Reference
Yes	Yes	212	33	0.87	0.07
Highest WBC >18,000/μL[e]					
No	No	35	53	0.40	**Reference**
Yes	No	50	34	0.60	**0.20**
No	Yes	154	33	0.82	Reference
Yes	Yes	163	26	0.86	0.04
Lowest hemoglobin[f] >7.0 g/dL or hematocrit[e] >20%					
No	No	40	56	0.42	**Reference**
Yes	No	45	34	0.57	**0.15**
No	Yes	172	40	0.81	Reference
Yes	Yes	157	22	0.88	0.07

Abbreviations: STEC, Shiga toxin–producing *Escherichia coli;* WBC, white blood cell count.

SI conversion factors: To convert WBC to $\times 10^9$/L, multiply by 0.001; to convert hemoglobin to grams per liter, multiply by 10; to convert hematocrit to a proportion of 1, multiply by 0.01.

[a] Fifteen of 617 patients were excluded from this table (11 with *Streptococcus pneumoniae* infection, 1 with *Shigella dysenteriae* type 1 infection, 1 with pandemic H1N1 influenza A infection, and 2 with only evidence of Shiga toxin in stool).

[b] Defined as either (1) performance of culture for *E coli* O157 and Shiga toxin testing or (2) serologic testing for common STEC serogroups.

[c] Either isolation of STEC in culture (microbiological evidence) or serologic evidence of recent infection with a common STEC serogroup.

[d] For each factor, 2 risk differences are shown. The differences in boldface refer to patients who did not have thorough STEC testing. The nonboldface differences refer to patients who had thorough STEC testing.

[e] By study design, values were obtained from 7 days before to 3 days after diagnosis of hemolytic uremic syndrome.

[f] For 35 patients with no reported hemoglobin, we used reported hematocrit values.

such as the Stx2-producing enteroaggregative *E coli* O104 that caused D+HUS in Europe in 2011, can occur.[30] Further study is needed to understand the role of other virulence factors and the subtypes of Stx2 in STEC strains causing D+HUS in the United States.

We, similar to others, found that children with stool cultured for STEC O157 within 4 days of diarrhea onset were more likely to have STEC detected.[31] Identifying an STEC infection early allows for measures to minimize transmission to others[32] and to improve outcomes.[33,34] Although serologic testing is less constrained by timing, microbiological evidence should always be sought because it yields an isolate that can be subtyped for outbreak detection and characterized by virulence factors.

Like others, we found that bloody diarrhea, diarrhea in summer months, and being part of an outbreak were associated with evidence of STEC infection.[15,24,35] We showed that these associations are weaker in the presence of thorough STEC testing. For example, whereas overall 40.4% of patients with nonbloody diarrhea (vs 77.2% with bloody diarrhea) had evidence of STEC infection (Table 4), the percentage increased to 71.7% (vs 86.4% with bloody diarrhea) among patients with thorough testing (Table 5). Our findings argue against the practice of some laboratories to limit STEC testing to patients with bloody stools or to summer months.[6]

Routine laboratory parameters should not overly influence STEC testing decisions. Although, as noted by others,[25] children with D+HUS and marked leukocytosis were more likely to have evidence of STEC, 46.8% of children with evidence of STEC infection had a white blood cell count less than 18 000/μL. Our finding that children whose hemoglobin level remained greater than 7.0 g/dL (or whose hematocrit was >20%) were more likely to have evidence of STEC might relate to earlier presentation for care, before the hemoglobin level could decrease lower. Alternatively, a higher hemoglobin level could be a marker of dehydration, which, in turn, could be associated with other signs and symptoms that prompt testing for STEC.[36]

We found that increasing use of Shiga toxin testing among patients with D+HUS from 2000 through 2010 coincided with a decrease in stool culturing for *E coli* O157. This finding is concerning because the fastest method to identify *E coli* O157 infections remains cul-

ture.[5] To maximize the number of STEC infections detected, all stool specimens submitted for bacterial testing to clinical laboratories from patients with community-acquired diarrhea or suspected HUS, even in the absence of diarrhea, should be promptly cultured for *E coli* O157 and assayed for Shiga toxins.[5,37] Because 1.4% of clinical laboratories surveyed in 2007 followed these practices,[6] it is important that physicians know what tests their laboratories perform and request guideline-based testing. Isolates and Shiga toxin–positive samples should be sent to public health laboratories for characterization and isolation. To diagnose the origin of D+HUS cases without microbiological evidence of STEC in the clinical laboratory, stool samples should be sent to public health laboratories able to perform immunomagnetic separation–assisted culture and polymerase chain reaction testing for Shiga toxin, and serum samples should be sent through a public health laboratory to the CDC. The CDC has validated tests to detect anti-O157 and anti-O111 serum antibodies and, in some cases, can use unvalidated tests to detect antibodies to additional serogroups.

Analyses of data collected in nonresearch contexts are more prone to introduction of nonstatistical sources of uncertainty through measurement error, variability in testing procedures, missing data, and other factors. We did not adjust our findings to address statistical uncertainty arising from multiple comparisons because doing so may imply unjustified confidence in reported precision. Nevertheless, the factors we identified agree with other studies.

We show that a portion of D+HUS is likely to be caused by *S pneumoniae*, a known cause of nondiarrheal HUS. The suspected mechanism is bacterial neuraminidase-mediated exposure of antigens on erythrocytes, platelets, and glomerular endothelium, followed by binding of host antibodies.[38] Eleven patients (5.7%) without evidence of STEC infection had *S pneumoniae* isolated. The illness seasonality and young age of these patients fit the epidemiology of pneumococcal HUS.[39] Our findings that children with no evidence of STEC infection were more likely to have illness in nonsummer months and to be younger suggest that *S pneumoniae* may account for more D+HUS cases than we identified, perhaps especially in black children. Although our surveillance may have captured pneumococcal HUS cases because of coincidental presence of mild diarrhea unrelated to *S pneumoniae*, 4 of 9 children with *S pneumoniae* infection with data had bloody diarrhea; *S pneumoniae* infection may be an underrecognized cause of bloody diarrhea.[40] One additional patient had infection with pandemic H1N1 influenza A, a potential trigger for HUS, potentially through its neuraminidase activity or its association with *S pneumoniae* infection.[41]

Some of the enteric pathogens found in children without evidence of STEC infection have been noted as possible causes of D+HUS.[42-45] However, these reports are more than 20 years old, and limited assessments for simultaneous STEC infection were conducted. Patients with STEC-related D+HUS may have simultaneous evidence of other infections.[46] Although many of the non-STEC enteric infections we observed likely occurred in children in whom STEC infection was missed, D+HUS surveillance should continue collecting information on other pathogens to generate testable hypotheses. For example, it is uncertain whether the more frequent isolation of *S pyogenes* in the stool of slightly older children with concurrent non-O157 STEC infection is of biological importance. *Streptococcus pyogenes* has been isolated in stool, either alone or in the presence of STEC from children with D+HUS, and it can cause acute poststreptococcal glomerulonephritis.[46-48] Others have reported simultaneous occurrence of HUS and acute poststreptococcal glomerulonephritis.[48]

In summary, STEC O157 continues to account for most pediatric D+HUS cases in the United States. However, one-third of children had no evidence of STEC infection. Although 5.7% of these children had documented *S pneumoniae* infection, most likely had undiagnosed STEC infections. Early and complete testing for all STEC in children with diarrheal illness is needed for more complete description of the infectious causes of D+HUS.

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