Risk Factors for the Hemolytic Uremic Syndrome in Children Infected With *Escherichia coli* O157:H7: A Multivariable Analysis

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Background. *Escherichia coli* O157:H7 is the leading cause of hemolytic uremic syndrome (HUS). Risk factors for development of this complication warrant identification.

Methods. We enrolled children infected with *E. coli* O157:H7 within 1 week of the onset of diarrhea in this prospective cohort study. The study was conducted in 5 states over 9.5 years . The primary and secondary outcomes were HUS (hematocrit <30% with smear evidence of hemolysis, platelet count $<150 \times 10^3/\mu L$, and serum creatinine concentration > upper limit of normal for age) and oligoanuric HUS. Univariate and multivariable and ordinal multinomial regression analyses were used to test associations between factors apparent during the first week of illness and outcomes.

Results. Of the 259 children analyzed, 36 (14%) developed HUS. Univariate analysis demonstrated that children who received antibiotics during the diarrhea phase more frequently developed HUS than those who did not (36% vs 12%; $P = .001$). The higher rate of HUS was observed across all antibiotic classes used. In multivariable analysis, a higher leukocyte count (adjusted odds ratios [aOR] 1.10; 95% CI, 1.03–1.19), vomiting (aOR 3.05; 95% CI, 1.23–7.56), and exposure to antibiotics (aOR 3.62; 95% CI, 1.23–10.6) during the first week of onset of illness were each independently associated with development of HUS. Multinomial ordinal logistic regression confirmed that initial leukocyte count and antibiotic use were independently associated with HUS and, additionally, these variables were each associated with the development of oligoanuric HUS.

Conclusions. Antibiotic use during *E. coli* O157:H7 infections is associated with a higher rate of subsequent HUS and should be avoided.

INTRODUCTION

Gastrointestinal infection with *Escherichia coli* O157:H7 is the leading cause of the hemolytic uremic syndrome (HUS) [1], a thrombotic microangiopathy that ensues approximately 1 week after diarrhea onset in approximately 15% of infected children [2, 3]. HUS is most likely caused by *E. coli* Shiga toxin (Stx) absorbed from the gut [1]. Because interventions cannot hasten recovery once HUS is established, averting this complication is highly desirable.

The painful bloody diarrhea that frequently accompanies *E. coli* O157:H7 infections prompts consideration for antibiotic treatment even before knowing stool culture results. However, the possibility that antibiotics could precipitate HUS has been a concern since the 1980s [4]. Antibiotics promote Stx release from *E. coli* [5–7], and if this process occurs in humans, antibiotic administration might increase HUS risk. Indeed, in several outbreaks [4, 8–10] from which data were necessarily extracted after illnesses resolved, HUS

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rates were higher among antibiotic-treated patients, but differences were variably statistically significant. In partial contrast, children who received fosfomycin in a Japanese outbreak had lower rates of HUS than those given other antibiotics [11], but only when fosfomycin was started on the second, but not on any other, day of illness. Moreover, almost all children in that outbreak received antimicrobials, so comparison to nontreatment was impossible.

Sporadic infections better reflect the diversity of *E. coli* O157:H7 strains encountered by humans than do outbreak infections. Among relevant studies of sporadic infections, a randomized controlled antibiotic trial in infected children failed to demonstrate statistically significant harm or benefit from trimethoprim-sulfamethoxazole [12], but randomization was late in illness. By amalgamating all bactericidal agents used, Smith et al [13] associated antibiotic use with HUS development among infected children in Minnesota. However, the sensitivity and precision of this comprehensive study were reduced by retrospective data extraction, wide confidence intervals, and inability to consider leukocyte counts (leukocytosis is often associated with HUS development [9, 14, 15]). We demonstrated that antibiotic use in early *E. coli* O157:H7 infections was associated with risk of developing HUS [2], but the small size of the study cohort (n = 71) produced wide 95% confidence intervals (CIs), precluding precise estimates of risk magnitude. Here, we report our now completed 9.5-year observational, multistate, prospective cohort study of 259 children infected with *E. coli* O157:H7 where we analyzed variables associated with HUS, with particular emphasis on antibiotic use.

METHODS

Study Design

The protocols for this prospective cohort study have been detailed [2]. Each participating hospital's institutional review board approved this research. Written informed consent was obtained from subjects' parents or guardians. If appropriate, assent was obtained from subjects.

Study Participants

Between April 1997 and October 2006, we enrolled 259 children infected with *E. coli* O157:H7 under age 10 (the decade of life with the highest incidence of HUS [16]). We restricted this analysis to subjects enrolled within the first 7 days of illness, the interval during which patients who subsequently develop HUS customarily seek care [17, 18], and to those who had not yet developed HUS. Original source documents were re-reviewed by the senior author to confirm all subjects met entry criteria.

Outcomes, Timing, and Interval Definitions

The primary and secondary outcomes were HUS (hematocrit <30% with fragmented erythrocytes on peripheral blood smear, platelets $<150 \times 10^3/\mu L$, and serum creatinine concentration > upper limit of normal for age [19]) developing by day 14 of illness and oligoanuric HUS (urine output <0.5 mL/kg/h for ≥1 calendar days after HUS onset [17]), respectively. Day 1 of illness was defined as day 1 of diarrhea.

Data Collected

A standardized questionnaire was administered to each subject's caregiver(s) within 2 days of enrollment to record demographic data, presence and timing of symptoms and signs, and all prescription and nonprescription medications (classified as antibiotics, antimotility drugs [if they inhibit intestinal peristalsis, including opioids], acetaminophen, and nonsteroidal anti-inflammatory drugs) taken during illness. All prescription drugs were verified by the ordering provider or medical records. Only medications taken during week 1 of illness and before HUS ensued were analyzed.

Hematologic and renal function tests were obtained until HUS developed or until diarrhea resolved and HUS had clearly not developed. The leukocyte count chosen for analysis was the initial determination obtained.

If available, *E. coli* O157:H7 from the children treated with antibiotics were tested for susceptibilities to ampicillin, azithromycin, cefotaxime, and trimethoprim-sulfamethoxazole to confirm that antibiotics were not conferring a selective advantage on resistant pathogens in the gut. These agents, plus metronidazole, represented the antibiotic classes used in this cohort. We assigned susceptibilities using standard cut-points [20], except for azithromycin for which we used published data [5, 21, 22].

Statistical Analysis

Differences between groups (HUS vs no HUS) were compared using independent sample *t* tests for continuous variables and χ^2 or Fisher exact test (if a cell contained <5 members) for categorical variables. We used univariate logistic regression to examine associations between each variable and development of HUS, followed by multivariable regression, including all single variables associated with HUS ($\alpha < 0.05$). Although the interval from diarrhea onset to stool culture did not retain statistical significance for development of HUS, we included this variable as an a priori [2] risk factor for the current analysis. The nested multivariable models and goodness-of-fit were assessed using the likelihood ratio and the Hosmer–Lemeshow test, respectively.

To analyze risk factors for the secondary outcome, we performed ordinal logistic regression to model the outcome (no HUS → nonoligoanuric HUS → oligoanuric HUS) to preserve the hierarchy of complications from least to most serious. The statistically significant risk factors from the initial multivariable logistic regression model were used in the ordinal

Table 1. Characteristics of the Cohort

Characteristic[a]	Frequency of HUS (%)	No. of HUS Cases/Total No. of Cases With Each Characteristic	*P* Value
All patients	14	36/259	…
Age in years (months)			.11[b]
0–1 (0–24 months)	13	8/64	…
2–3 (24–48 months)	22	16/73	…
4–6 (48–84 months)	14	7/49	…
7–10 (older than 84 months)	7	5/73	…
Sex			.67[c]
Male	13	18/138	…
Female	15	18/121	…
Race			.84[d]
White	14	33/229	…
African American	0	0/5	…
Asian	8	1/13	…
American Indian/Alaska Native	0	0/2	…
More than 1 race	20	2/10	…
Hispanic ethnicity			1.00[d]
No	14	32/226	…
Yes	12	4/33	…
Fever before visit[e]			.15[d]
Not provided	12	3/25	…
No	11	15/142	…
Yes	20	18/92	…
Vomiting before enrollment			.004[c]
No	7	7/107	…
Yes	20	29/152	…
Bloody stool			.22[d]
No	4	1/23	…
Yes	15	35/236	…
Initial leukocyte count			<.001[b]
$3.2–8.9 \times 10^3/\mu L$	5	3/65	…
$9.0–11.4 \times 10^3/\mu L$	12	8/65	…
$11.5–14.1 \times 10^3/\mu L$	6	4/65	…
$14.2–35.6 \times 10^3/\mu L$	33	21/64	…
Day of illness of first stool culture			
1–4	16	33/218	.17[b]
≥5	7	3/41	…
Day of illness of initial leukocyte count			
1–4	16	16/102	.13[b]
≥5	13	20/157	…

Abbreviation: HUS, hemolytic uremic syndrome.

[a] Characteristics of all subjects are provided and corresponding rates of HUS. HUS groups both with and without oligoanuria. *P* values determined by statistical tests, as noted.

[b] *P* value for *t* test, HUS versus no HUS.

[c] *P* value for chi-square test, HUS versus no HUS.

[d] *P* value for Fisher exact test, HUS versus no HUS.

[e] Caregiver report of whether or not the child had a fever prior to their presentation to care. Answers not provided for 25 children, 3 of whom developed HUS.

logistic regression model. To test the proportionality of odds for the outcomes, we used the likelihood ratio and Brant tests. The final model, which included only leukocyte count and antibiotics, did not violate the proportionality of odds assumption. The mean probabilities for ordered outcome were generated from the ordinal model using leukocyte count dichotomized around the median of 11.5×10^3/μL and use of antibiotics.

Multivariable logistic regression results are reported as adjusted odds ratios (aORs) with 95% CI and 2-tailed *P* values. Computations used Intercooled STATA 12.0 (StataCorp LP). Absolute risk difference, ratios, and number needed to harm were calculated by standard methods [23].

RESULTS

Participants and Outcomes

The 259 subjects (Table 1) represent 231 putatively different strains, accounting for 16 sib or household pairs and 4 outbreaks involving 16 subjects. Of the 259 subjects, 36 (14%) developed HUS and 11 (31% of the HUS cases) were oligoanuric, each of whom was dialyzed. All patients survived.

Risk Factors for HUS

Exposure to antibiotics was associated with greater HUS rates (36% vs 12%, *P* = .001; Table 2) and, by extension, greater oligoanuric HUS rates (12% vs 3%, *P* = .005; Table 3). Each antimicrobial class prescribed was associated with higher rates of subsequent HUS compared to nonuse of any such agents; exposure to trimethoprim-sulfamethoxazole or metronidazole was each statistically significantly associated with HUS. Antibiotic administration rates were 12 (12%) of 101 and 13 (8%) of 158 subjects enrolled before and after publication of our interim results on 29 June 2000 (*P* = .33), respectively. The rates of HUS among infected patients before and after this date were, respectively, 13% and 15% (*P* = .70). Antimotility agent use was not associated with increased HUS risk.

Subjects who received antibiotics had higher mean (standard deviation) initial leukocyte counts (15.1 (6.0) $\times 10^3$/μL vs 11.7 (4.5) $\times 10^3$/μL; *P* < .001) and more frequently received acetaminophen (*P* = .04) than those who did not, but the 2 groups had similar rates of bloody diarrhea, vomiting, and fever (Table 3). The subjects who received antibiotics were also more likely to develop HUS (24% vs 8%) and oligoanuric HUS (12% vs 3%; *P* = .005) than those not receiving antibiotics.

After adjusting for age, vomiting, initial leukocyte count, acetaminophen and antibiotic use, and intervals between onset of diarrhea to initial leukocyte determination and stool culture submission, only leukocyte count, vomiting history, and antibiotic exposure during the first 7 days of illness were independently associated with the development of HUS. According to the multivariable model, each 1×10^3/μL increase in leukocyte count above 11.5×10^3/μL was associated with a 10% increased risk of subsequent HUS (aOR 1.10; 95% CI, 1.03–1.19; *P* = .008). Furthermore, vomiting among children infected with *E. coli* O157:H7 was associated with a 3-fold higher risk for developing HUS compared to those with no vomiting (aOR 3.05; 95% CI, 1.23–7.56; *P* = .02). Exposures to antibiotics within the first week of illness also tripled the risk of developing HUS (aOR 3.62; 95% CI, 1.23–10.6; *P* = .02; Table 4).

The full model (adjusting for age, intervals from onset to initial leukocyte count determination and stool culture submission, and acetaminophen) was compared to a simplified model of only the 3 significant variables. The simpler multivariable regression model using initial leukocyte count (aOR 1.11; 95% CI, 1.03–1.19; *P* = .005), vomiting (aOR 3.16; 95% CI, 1.28–7.82; *P* = .01), and antibiotics (aOR 3.53; 95% CI, 1.29–9.66; *P* = .01) retained statistical significance and did not

Table 2. Medication Use and Frequency of Hemolytic Uremic Syndrome

Medication Use[a]	Frequency of HUS[a] (%)	No. of Cases of HUS/Total With Exposure	*P* Value
All patients	14	36/259	…
Acetaminophen[b]			.09[c]
No	12	25/206	…
Yes	21	11/52	…
Nonsteroidal anti-inflammatory drugs[b]			1.00[d]
No	14	33/235	…
Yes	13	3/23	…
Antimotility drugs[b]			.71[c]
No	14	31/227	…
Yes	16	5/31	…
Antibiotics			.001[c]
No	12	27/234	…
Yes	36	9/25	…
Specific antibiotics			
Trimethoprim-sulfamethoxazole (n = 9)	44	4/9	.02[e]
β-lactams (n = 9)	22	2/9	.29[e]
Metronidazole (n = 3)	67	2/3	.04[e]
Azithromycin (n = 4)	25	1/4	.40[e]

Abbreviation: HUS,hemolytic uremic syndrome.

[a] Medications used and corresponding numbers and percentages of HUS by each medication. *P* values determined by statistical tests, as noted.

[b] Total number equals 258 because the caregiver of 1 subject (who did not develop HUS) reported giving a nonprescription medication, but we could not determine its identity.

[c] *P* value for χ^2 test, HUS versus no HUS.

[d] *P* value for Fisher exact test, HUS versus no HUS.

[e] *P* value for Fisher exact test comparing individual class of antibiotic and HUS, compared to patients not treated with antibiotics.

Table 3. Characteristics Associated With Antibiotic Administration[a]

All Patients	Antibiotic Use: Yes N = 25	Antibiotic Use: No N = 234	*P* Value
Age in years, mean (SD)	4.8 (2.7)	4.4 (2.7)	.44[b]
Sex female, No. (%)	15 (60)	106 (45)	.16[c]
Bloody stool noted, No. (%)	23 (92)	213 (91)	1.00[d]
Fever reported before visit[e], No. (%)	…	…	.54[d]
Not reported, No. (%)	1 (4)	24 (10)	…
Yes, No. (%)	11 (44)	81 (35)	…
No, No. (%)	13 (52)	129 (55)	…
Vomiting before enrollment, No. (%)	14 (56)	138 (59)	.77[c]
Initial leukocyte count, mean (SD) $\times 10^3/\mu L$	15.1 (6.0)	11.7 (4.5)	<.001[b]
Onset to first stool culture (days), mean (SD)	2.6 (1.3)	3.1 (1.5)	.12[b]
Onset to initial leukocyte count (days), mean (SD)	4.2 (1.6)	4.2 (1.9)	.82[b]
Medications administered before onset of HUS (antibiotics n = 25, no antibiotics n = 233)[f]			
Acetaminophen	9 (36%)	43 (18%)	.04[c]
Nonsteroidal anti-inflammatory drugs	3 (12%)	20 (9%)	.48[d]
Antimotility agents	1 (4%)	30 (13%)	.33[d]
Progression to HUS			.005[d]
No HUS, No. (%)	16 (64)	207 (88)	…
Nonoligoanuric HUS, No. (%)	6 (24)	19 (8)	…
Oligoanuric HUS, No. (%)[g]	3 (12)	8 (3)	…
Onset to HUS in days, mean (SD)[h]	7.5 (1.9)	7.6 (2.0)	.93[b]

Abbreviations: HUS, hemolytic uremic syndrome; SD, standard deviation.

[a] Characteristics of subjects who did and did not receive antibiotics, expressed as corresponding numbers and percentages. *P* values determined by statistical tests, as noted.

[b] *P* value for *t* test, antibiotics versus no antibiotics.

[c] *P* value for χ^2 test, antibiotics versus no antibiotics.

[d] *P* value for Fisher exact test, antibiotics versus no antibiotics.

[e] Twenty-five subjects did not respond to this question, of whom 1 received an antibiotic.

[f] Excludes the 1 subject for whom we could not determine if these medications were administered and who did not receive antibiotics. Percentages relate to the included subjects only; denominators for these variables are provided.

[g] This value represents the ordered categories of complication from no HUS to nonoligoanuric HUS to oligoanuric HUS; prior tables group oligoanuric and nonoligoanuric together.

[h] The interval pertains only to the 36 children who developed HUS.

differ significantly from the full model with the additional adjustment factors (likelihood ratio test 3-variable vs full-model; $P = 1.00$).

The absolute antibiotic-attributable risk increase for HUS was 24.46% (95% CI, 5.21%–43.72%) above baseline. This corresponds to 1 additional case of HUS for every 4 (95% CI, 2–19) infected children treated with antibiotics.

Risk Factors for Oligoanuria

Ordinal logistic regression was used to analyze the hierarchy of infection complications associated with the statistically significant risk factors listed previously. Because of the zero in the cell comparing vomiting and oligoanuria (Table 5), only initial leukocyte count and antibiotics were used in the multinomial model, each of which was statistically significant (respective multinomial odds ratios [ORs] are OR 1.12; 95% CI, 1.04–1.19; $P = .001$; and OR 3.0; 95% CI, 1.15–7.64; $P = .02$). The test of the proportionality of odds via the likelihood ratio ($P = .97$) and Brant tests ($P = .94$) demonstrated no violation of the proportionality of odds assumption. The ordinal logistic regression model estimated the mean probability of each category of illness (no HUS, nonoligoanuric HUS, oligoanuric HUS) combining 2 exposure variables: whether or not there was early exposure to antibiotics and whether or not the initial leukocyte count was above the median of $11.5 \times 10^3/\mu L$. As demonstrated in Table 6, groups with high leukocyte counts had a 4.7% rate of nonoligoanuric HUS if they did not receive antibiotics and a 14.7% rate if they did.

Table 4. Multivariable Analysis for Risk Factors Associated With Hemolytic Uremic Syndrome

Risk Factor[a]	Multivariable OR (95% CI)[b]	*P* Value
Age[c]	0.89 (0.77–1.04)	.15
Vomiting before enrollment	3.05 (1.23–7.56)	.02
Initial leukocyte count[c]	1.10 (1.03–1.19)	.008
Days from onset of diarrhea to first leukocyte count determination	0.87 (0.63–1.20)	.40
Days from onset of diarrhea to stool culture	0.98 (0.65–1.48)	.94
Acetaminophen	1.39 (0.58–3.34)	.46
Antibiotics	3.62 (1.23–10.6)	.02

Abbreviation: CI, confidence interval; HUS, hemolytic uremic syndrome; OR, odds ratio.

[a] Multivariable analysis adjusted for risk factors as listed.

[b] 95% CI.

[c] Modeled as a continuous variable.

Isolate Susceptibilities

Infecting isolates were available for 7 of the 9, 7 of the 8, and 3 of the 4 children treated with trimethoprim-sulfamethoxazole, β-lactam antibiotics, or azithromycin, respectively. Each of these 17 isolates was susceptible to these antimicrobials as well as to azithromycin (inhibited by 1.5 μg/mL, a range generally considered to be inhibitory [5, 21, 22]).

DISCUSSION

Our now-completed multistate, multicenter, multiyear prospective cohort study demonstrates that in the first 7 days after onset of diarrhea caused by *E. coli* O157:H7, higher initial leukocyte count, vomiting, and use of antibiotics are independently associated with subsequent development of HUS. Our larger cohort enabled us to more precisely estimate the risk of HUS attributable to antibiotics and to extend the more restricted associations between antibiotic treatment of these infections and development of HUS reported by Smith et al [13], probably because we extracted data prospectively. We also observed higher rates of HUS associated with metronidazole and, to a lesser extent, azithromycin, neither of which were taken by children in the initial report from this cohort. Nitschke, et al, recently reported that azithromycin use late in illness was associated with more rapid decolonization when given to adults infected with Shiga toxin-producing *E. coli* O104:H4 [24]. All 22 subjects in their treatment group had developed HUS. In our cohort, all subjects were exposed within the first seven days of illness. Though the numbers of children treated with azithromycin in our study are small, our data provide no support for the safety or efficacy of this antibiotic early in *E. coli* O157:H7 infections. There has been sentiment in favor of conducting a randomized controlled trial of antibiotics in patients infected with Stx-producing *E. coli* [25, 26], but our

Table 5. Characteristics of Patients With Hemolytic Uremic Syndrome and Risk for Oligoanuria

		Oligoanuria		
Characteristic[a]	Total HUS	Yes	No	*P* Value
Patients with HUS	36	11	25	
Age in years, mean (SD)	3.7 (2.3)	4.5 (2.7)	3.4 (2.1)	.17[b]
Male/female sex	18/18	4/7	14/11	.47[c]
Bloody stool noted, yes/no	35/1	11/0	24/1	1.00[c]
Fever reported before visit, unknown/yes/no[d]	3/18/15	1/7/4	2/11/11	.87[c]
Vomiting before enrollment, yes/no	29/7	11/0	18/7	.08[c]
Initial leukocyte count, mean (SD) × 10^3/μL	15 (5.7)	16 (5.7)	14.6 (5.9)	.52[b]
Onset to first stool culture in days, mean (SD)	2.7 (1.1)	3.2 (1.4)	2.5 (1.0)	.11[b]
Onset to initial leukocyte count in days, mean (SD)	3.8 (1.6)	3.9 (1.8)	3.8 (1.5)	.80[b]
Medications administered early in illness				
Acetaminophen, yes/no	11/25	2/9	9/16	.44[c]
Nonsteroidal anti-inflammatory drugs, yes/no	3/33	1/10	2/23	1.00[c]
Antimotility agents, yes/no	5/31	3/8	2/23	.15[c]
Antibiotics, yes/no	9/27	3/8	6/19	1.00[c]

Abbreviations: HUS, hemolytic uremic syndrome; SD, standard deviation.

[a] Characteristics of subjects with HUS *P* values determined by statistical tests, as noted by individual superscripts, and test significance of differences in the oligoanuria versus no oligoanuria groups.

[b] *P* value for *t* test.

[c] *P* value for Fisher exact test.

[d] Three subjects did not respond to this question, of whom 1 developed oligoanuria.

Table 6. Probabilities of Hemolytic Uremic Syndrome (HUS) by no HUS → Nonoligoanuric HUS → Oligoanuric HUS

Grouping	No HUS[a] (%)	Nonoligoanuric HUS[a] (%)	Oligoanuric HUS[a] (%)
No antibiotics + low leukocyte count	92.3	5.6	2.1
No antibiotics + high leukocyte count	84.0	11.3	4.7
Antibiotics + low leukocyte count	77.4	15.5	7.0
Antibiotics + high leukocyte count	60.0	25.3	14.7

Abbreviation: HUS, hemolytic uremic syndrome.

[a] Probabilities are given as percentages by the ordinal logistic regression model of initial leukocyte count dichotomized into low and high leukocyte count by the median point ($11.5 \times 10^3/\mu L$) and antibiotic exposure. The likelihood ratio and Brant tests were, respectively, $P = .93$ and $P = .89$. There is no violation of the proportionality of odds assumption.

data suggest that in view of the chance of harm from these medications, such a trial would be ill considered, even if it were feasible.

Our study has other important findings and implications. First, the frequencies of antibiotic treatment of infected patients and of HUS did not diminish appreciably after publication of our report in 2000. Indeed, antibiotic use in these infections appears common: 36% of HUS patients in an 11-center study conducted in 2007 and 2008 in the United States and Scotland received antibiotics before HUS was diagnosed [18], as did 23% and 44% of children infected with *E. coli* O157:H7 in Minnesota (1996–2002) [13] and in a multistate surveillance project (FoodNet; 1990s) [27]. These rates of antibiotic use actually exceeded the rates we observed. There are only 9 diagnosed *E. coli* O157:H7 infections per million general population in the United States [28], and conveying management recommendations for infrequently encountered illnesses poses challenges. Nevertheless, it is very concerning that patients seeking care for diarrhea are more likely to receive antimicrobials than to submit their stools for culture [29]. Second, the enumeration of the complications from *E. coli* O157:H7 infections has been hindered by varying definitions of HUS and of severe renal injury across studies. Using our definition of HUS, which employs stringent but clearly defined and clinically relevant criteria, and our definition of oligoanuria, we demonstrated that antibiotics and elevated leukocyte count are associated not only with HUS but with severe HUS as well. Other studies have not reported that antibiotics are associated with increased HUS severity [17, 18, 30], but the same degree of analysis, with study entry of all subjects during the diarrhea phase, was not employed. However, by averting a case of HUS, our ordinal regression model suggests that severe HUS is averted in parallel, and it would be interesting to determine if use of antibiotics prior to HUS has any additional deleterious effects on the rates of chronic sequelae. Third, metronidazole, which does not inhibit *E. coli*, was associated with development of HUS, as was also observed in Minnesota [13]. Metronidazole might provide a competitive advantage for *E. coli* O157:H7 in the gut by suppressing competing enteric microbiota. Fourth, our cohort's size enabled us to more confidently quantify the association between leukocyte count and HUS in multivariable analysis, which might help stratify risk for complicated outcomes of *E. coli* O157:H7 infections.

The strengths of our study include the large number of children analyzed, the wide diversity of strains that infected patients, and, most importantly, the time frame during which subjects were recruited, with interviews performed and data obtained while subjects were still ill. Nonetheless, symptomatic patients occasionally are identified later in illness, usually when a culture is reported positive but before HUS ensues, and we found a similar risk of subsequently developing HUS in patients treated later than day 7 of illness (data not shown). We do wish to note, however, that if a justifiable reason to use antibiotics emerges after HUS is established, our data do not suggest that at that stage of illness such usage is harmful.

Though we did not associate their use with HUS, we do not condone using antimotility agents in *E. coli* O157:H7 infections. Antimotility drugs offer no therapeutic benefit in these illnesses and have been associated with prolongation of bloody diarrhea [9] and worse renal and neurologic outcomes [9, 31].

We acknowledge that as with any observational study, unmeasured factors might have influenced the observed association. We attempted to minimize recall biases by gathering critical information prospectively, before symptoms resolved. Also, we cannot discount the possibility that antibiotics were used because of the severity of the illness in children who were destined to develop HUS. However, we found no correlation between antibiotic use and several indices of severity, especially bloody diarrhea, vomiting, and caregiver report of fever. Moreover, after incorporating physiologically relevant potential confounders (initial leukocyte count, age, vomiting, coadministration of acetaminophen, and interval from disease onset to first presentation), exposure to antibiotics remained significantly associated with developing HUS. Additionally, this analysis was necessarily limited to the classes of antibiotics prescribed by treating physicians, and though the panel was broad, it did not include either rifamycins or carbapenems. Finally, we continued enrolling subjects 6 years after our interim report of antibiotic association with HUS in 2000 to provide a complete and stronger dataset. Secular changes in patient characteristics could have altered our findings, but there were no statistically significant differences in

such characteristics in the children enrolled before and after publication of our interim results.

The members of this cohort and their families have taught us much about how *E. coli* O157:H7 acts in humans and how hosts respond to these pathogens. At initial presentation, most commonly soon after the first bloody stool is noted, patients have elevated plasma fragment 1 + 2 and D-dimer concentrations, suggesting thrombin generation and intravascular fibrin accretion, respectively [3]. Within the first 4 days of illness, plasma platelet activating factor is elevated [32] and circulating von Willebrand factor multimers are sheared [33], probably from nascent intravascular thrombi. The prothrombotic process that plausibly causes renal insufficiency in HUS appears, therefore, well underway early in illness, even though platelet counts and hematocrits are still normal at that point. Fecal *E. coli* O157:H7 and Stx concentrations diminish soon after presentation; by the time HUS ensues, this pathogen is usually cleared [34, 35] and fecal toxin is undetectable [34]. Paradoxically, children who subsequently develop HUS have lower fecal-free toxin concentrations than those who do not [34]. Such bacterial clearance and toxin expression kinetics present challenges to attempts to treat infected individuals with antitoxins.

Despite these pathophysiologic and microbial realities, some actions can mitigate the damage caused by Stx-producing *E. coli* after they gain entry to human populations. Clinical profiling on presentation and good microbiology (which must include plating stool on sorbitol MacConkey agar) are critical for effective early illness management [36]. Hospitalization of infected patients prevents secondary infections in the community [37], and use of intravenous volume expansion provides relative nephroprotection [17, 38]. Finally, based on previous studies [2, 4, 8, 10, 13] and this report, which is the largest cohort of infected children prospectively assembled and studied, antibiotics should not be given to patients definitely or possibly infected with *E. coli* O157:H7. However, even when such logical and simple practices are implemented, a subset of infected children will still develop HUS, and in a smaller subset, the resulting HUS will be severe (ie, oligoanuric). For these reasons, we reiterate that the best way to prevent HUS is to prevent primary human infection with *E. coli* O157:H7.

Notes

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