Impact of infection by verotoxigenic *Escherichia coli* O157:H7 on the use of surgical services in a children's hospital

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Objectives: To determine the impact of *Escherichia coli* O157:H7 infection in children on the need for surgical assessment in a pediatric surgical practice and whether clinical and bacteriologic variables might contribute to that need.

Design: Examination of a case series.

Setting: A tertiary-care pediatric hospital.

Patients: Between 1990 and 1994, *E. coli* O157:H7 gastrointestinal infections were documented among 85 children, 29 of whom suffered from hemolytic-uremic syndrome.

Intervention: Surgical consultation for presumed or proven complications of the infection.

Main Outcome Measures: The frequency of and reasons for surgical consultation, clinical and bacteriologic variables between patients who did or did not require surgical assessment.

Results: Of the 85 children, 17 (20%) were assessed by the surgical service. The majority of these children were inpatients. Two required abdominal surgery. Female gender, older age and progression to hemolytic-uremic syndrome were factors associated in univariate analyses with a likelihood of need for surgical assessment; variation in bacterial genotype was not.

Conclusion: There is the potential for verotoxigenic *E. coli* O157:H7 infection to have a considerable impact on the utilization of pediatric surgical services.

Objectifs : Déterminer l'impact d'une infection par *Escherichia coli* O157:H7 chez les enfants sur le besoin d'une évaluation chirurgicale en pratique chirurgicale pédiatrique et si des variables cliniques et bactériologiques pourraient contribuer à ce besoin.

Conception : Étude d'une série de cas.

Contexte : Hôpital pédiatrique de soins tertiaires.

Patients : Entre 1990 et 1994, on a documenté des infections gastro-intestinales par *E. coli* O157:H7 chez 85 enfants, dont 29 étaient atteints du syndrome urémique-hémolytique.

Intervention : Consultation d'un chirurgien pour complications présumées ou démontrées de l'infection.

Principales mesures des résultats : Fréquence et raisons des consultations chirurgicales, variables cliniques et bactériologiques entre des patients qui ont eu ou non besoin d'une évaluation chirurgicale.

Résultats : Sur les 85 enfants, 17 (20 %) ont été évalués par le service de chirurgie. La plupart de ces enfants étaient des patients hospitalisés. Deux ont dû subir une chirurgie abdominale. Le sexe féminin, l'âge plus avancé et l'évolution jusqu'au syndrome urémique-hémolytique étaient des facteurs des analyses à une variable liés au besoin probable d'une évaluation chirurgicale. La variation du génotype bactérien n'était pas un facteur.

Conclusion : L'infection par *E. coli* O157:H7 vérotoxigène peut avoir une incidence considérable sur l'utilisation des services de chirurgie pédiatrique.

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Verotoxigenic types of *Escherichia coli* have been recognized as important causes of gastroenteritis among all age groups, although, numerically, the majority of laboratory diagnoses are made with enteric specimens from the pediatric age group. This subset of *E. coli* bacteria produce single or multiple toxins (variably termed verotoxins or Shiga-like toxins), which have potent cytotoxic properties.[1] The majority of clinical disease in children occurs within the gastrointestinal tract. It may be manifest as transient watery diarrhea or may progress to bacterial dysentery (hemorrhagic colitis or "hamburger disease"). Whereas bacteremic disease, which is associated with the gastrointestinal symptoms, is practically not recognized, systemic disease in the form of the hemolytic-uremic syndrome (HUS) is. It is believed that toxin absorption and systemic spread of the same toxin, along with subsequent microangiopathic changes, lead to the classic triad of anemia, uremia and thrombocytopenia.

In Canada, the most common verotoxigenic *E. coli* is the serotype O157:H7. It is also now recognized that most cases of childhood HUS are associated with verotoxigenic *E. coli* gastrointestinal infection. Again, the O157:H7 serotype predominates as the etiologic agent.[2] Surgical complications of HUS have been detailed, although much of this work predates the science that was able to link bacterium with disease. Given the recognized common association of HUS with verotoxigenic *E. coli*, it is rational to deduce that these surgical complications were in large part related to the manifestation of an initial gastrointestinal infection.

Herein, we examine the impact of verotoxigenic *E. coli* O157:H7 infection on a pediatric surgical practice. Since infection is dependent upon a strict laboratory definition, which then serves as the standard for the identification of infected patients, this review encompasses a full spectrum of disease, including HUS. In addition we examined whether bacterial genotypic traits could be associated with the greater likelihood for a patient to require assessment by the surgical service.

Methods

Patients

Data were obtained for patients who were admitted to our children's hospital between 1990 and 1994. These children were either admitted as inpatients or seen through requests for consultation in the ambulatory care areas of the hospital. All children were found to have *E. coli* O157:H7 in stool specimens. A diagnosis of HUS was made by the finding of the classic triad: microangiopathic hemolytic anemia, thrombocytopenia and uremia. Patient data were obtained retrospectively from medical records.

Bacteriologic method

Bacteria were initially obtained by screening for sorbitol-negative *E. coli* from sorbitol-MacConkey media. Suspect bacterial colonies were serogrouped as O157 with commercial polyclonal antisera and subsequently confirmed as *E. coli* O157:H7. All such isolates subsequently proved to be toxigenic.

Toxin typing

Isolates were assessed for specific forms of verotoxin type by genetic amplification with polymerase-chain-reaction technology.[3] Genetic sequences from verotoxin 1 and 2 genes were amplified, and the products of amplification were detected by ethidium bromide staining of conventional agarose gels. The verotoxin 1 gene product of 130 base-pairs and the verotoxin 2 gene product of 346 base-pairs were sought.

Verotoxin 2 subtyping

Given the proposed heterogeneity of verotoxin 2,[4] we sought to determine whether such variation in amino acid sequences and then perhaps biologic activity might be associated with a greater severity of illness, which would then prompt surgical consultation. Verotoxin 2 subtyping was generally accomplished according to previously published methods.[4,5] Primers GK5 and GK6 were used to amplify a portion of the verotoxin 2 B subunit gene. After amplification, the product is digested with restriction endonucleases Hae III and Fok I; the resulting variable products are then determinants for whether the verotoxin 2 is of two major variants: VT2 or VT2v.

Plasmid profiles

Small-scale plasmid extractions were performed by modifying the alkaline lysis method as previously detailed.[6] Because we have found evidence for the association of a 4 kb plasmid with lesser progression to HUS, we sought to determine whether the same plasmid or any other might be associated with disease that would prompt surgical consultation.

eaeA gene detection

The *eaeA* gene and the *eae*-gene complex have been proposed as candidate genes, which specify products that may be integral to host attachment for enteropathogenic and enterohemorrhagic *E. coli*.[7] Although verotoxigenic *E. coli* attachment is likely to involve several factors,[8] we sought to determine whether *eaeA*-gene variability (and thus perhaps attachment) among strains would lead to variable severities

of illness with or without surgical consultation. EaeA presence was also determined by use of polymerase-chain-reaction technology. An amplification product of 854 base-pairs was obtained if the *eaeA* gene was present.[7]

Statistical analysis

The χ^2 and *t*-tests were used to assess the probability of a significant difference between patient groups for both clinical and bacteriologic variables.

Results

Eighty-five children with culture-proven *E. coli* O157:H7 infection were recorded for the 5-year period; 29 (34%) of these patients suffered from HUS. In total, there were 46 males (5 surgical, 41 nonsurgical) and 39 females (12 surgical, 27 nonsurgical). A significantly ($p < 0.05$) greater proportion of females was assessed by the surgical service. Overall, 17 (20%) patients (15 inpatients, 2 outpatients) were seen in consultation by the surgical service.

Table I provides highlights of demographic and clinical data for children who were assessed by the surgical service. These children were significantly older than those who were not seen (mean age 99.5 months v. 71.0 months, $p < 0.05$). Nine of these 17 patients had HUS, a frequency significantly greater than that among the nonsurgical group (53% v. 29%, $p < 0.05$). A majority of these patients had received pharmacologic agents of various types, and these were commonly prescribed as antibiotics, antidiarrheal agents, antiemetics and analgesics. There was no significant difference between the 2 groups for any prior exposure to antibiotics ($p > 0.10$) or antimotility agents ($p > 0.10$). In addition, there was no significant difference for prolonged (longer than 24 hours) use of antimotility agents ($p > 0.10$). Requests for consultation were more likely to be owing to the need for assessment of a surgical abdomen. Surgical intervention was required for only 2 of these patients; large-bowel perforation had occurred as a late complication in 1 child, and the other child underwent laparotomy for suspected appendicitis (cecal inflammation was visually evident and histopathological examination confirmed mild appendicitis).

Of the 85 children documented as having *E. coli* O157:H7 infection, bacterial isolates were available for analysis from 78. The majority (95%) of these bacteria possessed the verotoxin 1/2 genotype. No significant differences between strains from surgical or nonsurgical groups were seen for verotoxin type (surgical group 100% verotoxin 1/2; nonsurgical group 94% verotoxin 1/2 and 6% verotoxin 2 only), VT2 subtype (all verotoxin 2 rather than verotoxin 2v subtype except for one isolate) or *eaeA* gene detection (all positive) ($p > 0.10$ for each comparison). The single VT2v subtype strain was likely to have been imported from Europe. In addition, the 4 kb plasmid was equally present among strains from either patient group (19% v. 21%, $p > 0.10$). Varied plasmid profiles were observed among these bacteria, analogous to the diversity that were detailed previously.[6] Strains that possessed the 100 kb plasmid only were most common, but there was no significant difference in the frequency of such strains between either patient group (surgical 69%, nonsurgical 48%, $p > 0.10$).

Discussion

For decades it was recognized that acute colitis often preceded or coincided with the development of HUS.[9–12] Prior to the discovery of verotoxigenic *E. coli*, it was unclear why a multisystem illness, which included a specific nephropathy, would have an associated large-bowel disease. Although many patients with these complications were thought to have rectal bleeding, others suffered from pancolitides, which clinically and microscopically simulated ulcerative colitis or classic bacterial dysentery. More serious complications of colonic involvement included toxic megacolon, rectal prolapse, perforation and intussusception. The demonstration that bacterial enteric pathogens were associated with both gastroenteritis and subsequent HUS allowed clinicians and scientists to propose a unifying hypothesis for disease causation: bacterial colonization and toxin production are responsible for the local gastrointestinal disorder, and systemic toxin absorption is responsible for HUS. Although there are several variants of HUS (other infectious agents and noninfectious causes), the most common form in children is associated with gastroenteritis and verotoxigenic *E. coli* (more than 90% in Canada).[2]

Now that these common etiologic agents are defined, it has also been possible to further our understanding of the spectrum of gastrointestinal illness. Although rare, some patients may transiently carry verotoxigenic *E. coli* immediately after acquisition, but shedding (asymptomatic carriage) is not prolonged. Others may suffer from a transient illness that is manifest by watery diarrhea only. Vomiting and abdominal pain are common accompaniments. From the perspective of a pediatric hospital, many patients will have evidence of hemorrhagic colitis, the complications of which have been detailed as those complicating the colitis that is associated with HUS. Such clinical heterogeneity is also reflected by the variation in the quantity of fecal leukocytosis[13] and by the variation in the appearance of colonic biopsies.[14] In addition to the aforementioned gastrointestinal complications, we have previously described a

patient with an *E. coli* O157:H7-associated appendicitis and rupture.[15] A child with mild appendicitis is presented in this study (Table I). Tapper and colleagues[16] have detailed the complications of HUS from a recent large outbreak in the United States.

Female gender, older patient age and a concomitant diagnosis of HUS were more likely to be associated with the need for surgical assessment. Although a few patients were assessed for the possible placement of a hemodialysis line, the majority suffered from an apparently acute abdominal crisis that may have warranted surgical intervention. Apart from the diagnosis of HUS, we did not attempt to measure the severity of illness before a request for consultation. Nevertheless, the majority of patients who were subsequently assessed by the surgical service had already received various drugs. We have previously shown that young age is a

Table I

Demographic and Clinical Features for 17 Children With *Escherichia coli* O157:H7 Infection Who Were Seen by the Surgical Service

Age, mo	Sex	Site	Illness	Concern	Days ill	Drugs used
14	F	I	HUS	? intussusception	4	Activated attapulgite
24	F	I	HUS	Diarrhea and emesis; ? intussusception	4	None
38	F	I	G	Diarrhea; intussusception	3	Metronidazole, kaolin, pectin, paregoric
38	F	I	HUS	Diarrhea and abdominal pain; ? intussusception	3	Ampicillin, bismuth subsalicylate
45	M	I	HUS	Diarrhea; bowel perforation; laparotomy and hemicolectomy; insertion of dialysis line	17	Dimenhydrinate
53	F	I	G	Diarrhea/rectal prolapse; ? bowel obstruction	2	None
61	F	I	HUS	Insertion of hemodialysis line and chest tube	5	Metronidazole
68	M	I	HUS	Diarrhea; ? rectal bleeding	3	None
119	F	I	HUS	Severe colitis; insertion of hemodialysis line	3	Cotrimoxazole, bismuth subsalicylate, loperamide, meperidine, hydroxyzine, prochlorperazine, metoclopramide
137	F	O	G	Diarrhea and abdominal pain; ? appendicitis	1	None
140	F	O	G	Diarrhea and abdominal pain; ? appendicitis	2	None
140	F	I	G	Diarrhea and abdominal pain; laparotomy and appendectomy	3	Ampicillin, dimenhydrinate, acetominophen, dicyclomine, morphine, codeine
145	F	I	HUS	Insertion of hemodialysis line	7	Amoxicillin
146	M	I	G	Diarrhea and emesis; severe abdominal pain	2	Ampicillin, metronidazole, gentamicin
160	M	I	G	Diarrhea and abdominal pain; ? appendicitis	2	Dimenhydrinate, neomycin, ampicillin
182	F	I	G	Severe abdominal pain; ? appendicitis	2	Hyoscine
182	M	I	HUS	Diarrhea; ? rectal bleeding	5	Ampicillin, dimenhydrinate

I = inpatient, O = outpatient, G = gastroenteritis, HUS = hemolytic-uremic syndrome

risk factor for progression to HUS.[17] It is unclear in this study why older age would be associated with an increased likelihood of surgical consultation, but the ability of a child to articulate symptoms must be considered. Furthermore, the manifestations of verotoxigenic *E. coli* infection in older children might have led some to believe that noninfectious and possible surgical causes of hematochezia or colitis (e.g., ulcerative colitis) required assessment. Although preliminary data had indicated that female gender might also be a risk factor for HUS,[17] we were not able to confirm this.[6,18] Rowe and colleagues[19] have proposed that female gender is associated with increased risk of hemolytic anemia. Overall, however, it is evident that the requirement for an active surgical procedure is quite uncommon among patients with *E. coli* O157:H7 infection today.

We did not find any association between bacterial genotype and the need for surgical assessment. Many of the currrently proposed risk factors for progression to HUS are clinical or patient-based, but some bacterial traits have also been linked (e.g., 4 kb plasmid,[6] verotoxin 2 only genotype[20]). Essentially, the progression to HUS depends on both bacterial virulence and characteristics of the individual patient, although similar factors do not necessarily contribute to the determination of whether an illness requiring surgical assessment will develop.

Although active surgical intervention occurred in only a small proportion of patients with *E. coli* O157:H7 infection, surgical consultation was required for a sizeable proportion. Accordingly, there is the potential for such an infection to have a considerable impact on the pediatric surgical service, depending upon the frequency of infection in the general population. The ability to reduce infection by interrupting foodborne transmission should not be underestimated. In parallel, a reduction in disease incidence will likely be associated with a decrease in the periodic requirement for surgical intervention.

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