The isolation rate of *Escherichia coli* 0157:H7 in Toronto and surrounding communities

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ABSTRACT: Verocytotoxin-producing strains of *Escherichia coli*, most often serotype 0157:H7, have been associated with both sporadic and epidemic diarrheal disease in Canada. In order to determine the isolation rate of *E coli* 0157:H7 in outpatients with diarrhea, all stool specimens submitted for culture to Med-Chem Laboratories in Metropolitan Toronto between June 1988 and September 1989 were cultured on MacConkey-Sorbitol agar in addition to standard enteric media. A total of 46 (0.3%) of 16,125 stool specimens yielded *E coli* 0157:H7 or verotoxin-producing *E coli* 0157:H⁻. These isolates came from 31 patients with

diarrhea; only 16 (52%) had a history of hemorrhagic colitis and one patient developed hemolytic uremic syndrome. Although MacConkey-Sorbitol agar was useful as a differential medium for detecting *E coli* 0157:H7, 14.5% of all specimens yielded nonsorbitol-fermenting isolates. It is not certain whether the routine use of MacConkey-Sorbitol agar is justified when isolation rates of *E coli* 0157:H7 are very low. **Can J Infect Dis 1990;1(1):23-27**

Key Words: *Escherichia coli* 0157:H7; Hemorrhagic colitis

VEROCYTOTOXIN-PRODUCING STRAINS OF *ESCHERICHIA coli* have been associated with a spectrum of clinical disease including nonbloody diarrhea, hemorrhagic colitis, hemolytic uremic syndrome, and possibly thrombotic thrombocytopenic purpura (1-6). Sporadic community-ac-

quired cases (1,7,8) as well as institutionally acquired outbreaks (9-11) have been described. The serotype that has been identified most often in these cases is *E coli* 0157:H7. Detection of this organism in fecal specimens has been facilitated by the recognition that unlike most other *E coli* serotypes, *E coli* 0157:H7 isolates are late sorbitol fermenters that do not ferment sorbitol within the first 24 h of incubation (12,13). Therefore, MacConkey agar containing sorbitol instead of lactose has been developed for screening fecal specimens for *E coli* 0157:H7. With increasing use of this medium by clinical laboratories, there was an

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exponential increase in the number of isolations of *E coli* 0157:H7 reported in Canada between 1982 and 1987 (11). This organism accounted for 3.6% of all human enteric pathogens referred or reported to the Enteric Bacteriology Division of the Laboratory Centre for Disease Control in Ottawa in 1986.

As with other enteric pathogens, the isolation rate of *E coli* 0157:H7 may vary geographically. Therefore, the purpose of this study was to determine the prevalence of *E coli* 0157:H7 as a cause of diarrheal illness in an outpatient-based population submitting stool specimens for culture to a diagnostic microbiology laboratory in Metropolitan Toronto and surrounding communities. The study also provided an opportunity to describe the spectrum of illness associated with this infection in symptomatic subjects and to assess the usefulness of routinely screening stool cultures with sorbitol-containing MacConkey agar.

PATIENTS AND METHODS

Med-Chem Laboratories is a privately owned, licensed laboratory offering diagnostic biochemistry, hematology, pathology and microbiology services to family physicians, primary care medical clinics and nursing homes in Metropolitan Toronto and the surrounding communities within a 40 mile radius. Between June 1, 1988 and September 30, 1989 all stool specimens submitted to Med-Chem Laboratories for culture were screened on MacConkey-Sorbitol agar containing 1% D-sorbitol (PML Microbiologicals, Mississauga, Ontario), incubated at 35°C for 18 to 24 h. Five nonsorbitol-fermenting colonies were picked from each plate for serogrouping by slide agglutination with 0157 antiserum (Difco Laboratories, Detroit, Michigan) (14). Positive isolates were identified and antimicrobial susceptibility testing done using a commercially available microdilution system, MicroScan (Travenol Laboratories, Mahwah, New Jersey). All *E coli* 0157 isolates were sent to the Central Public Health Laboratory, Ontario Ministry of Health for O and H serotype confirmation by tube agglutination and determination of verocytotoxin production. Standard enteric media were used for the isolation of *Salmonella*, *Shigella*, *Yersinia* and *Campylobacter* species. Examination for ova and parasites or *Clostridium difficile* culture and cytotoxin assay were done only on request.

The family physicians of patients from whom *E coli* 0157:H7 or nonmotile, verotoxin-producing *E coli* 0157 was isolated were contacted by telephone within one month for clinical and demographic data.

TABLE 1
Enteric pathogens isolated from 16,125 fecal specimens processed between June 1988 and September 1989

Pathogen	Number of isolates (%)	Number of patients
<i>Salmonella</i> species	600 (3.7)	460
<i>Campylobacter</i> species	506 (3.1)	385
<i>Shigella</i> species	91 (0.6)	67
<i>Yersinia</i> species	89 (0.5)	67
<i>Escherichia coli</i> 0157	46 (0.3)	31

RESULTS

During the 16 month survey, verotoxin-producing *E coli* 0157:H7 or *E coli* 0157:H⁻ was isolated from 46 (0.3%; confidence interval 0.008%) of 16,125 stool specimens. As shown in Table 1, these were the least frequently isolated bacterial enteric pathogens. For the last two months of the survey, the average number of stool specimens per patient was 1.39; this is probably a reasonable estimate for the entire study period. The *E coli* 0157 isolates came from 31 patients (18 males and 13 females) who ranged in age from six months to 70 years. Eighteen patients (58%) were less than 10 years of age. None of the cases appeared to be epidemiologically related. The majority (87%) of infections occurred in the summer months (Figure 1); none was acquired during foreign travel. One patient was coinfecting with *Salmonella enteritidis*. Of all patients from whom verotoxin-producing *E coli* was isolated, 74% submitted stool specimens within four days of the onset of symptoms, and 84% submitted within five days.

Figure 1 The distribution of verocytotoxin-producing strains of *Escherichia coli* 0157 by month from June 1988 to September 1989

TABLE 2
Clinical manifestations of infection due to verocytotoxin-producing *Escherichia coli* 0157 in 31 patients

Symptom	Number of patients (%)
Diarrhea	31 (100)
Bloody diarrhea	16 (52)
Abdominal pain	22 (71)
Fever ($\geq 37.8^{\circ}\text{C}$)	7 (23)
Nausea/vomiting	5 (16)
Hemolytic uremic syndrome	1 (3)

All patients with *E coli* 0157 had generally watery diarrhea, but only 16 (52%) had clinical manifestations typical of hemorrhagic colitis – bloody diarrhea, abdominal pain and little or no fever (Table 2). The mean duration of symptoms was eight days (range one to 21). Nine patients were treated empirically with antimicrobial agents (erythromycin, metronidazole, ciprofloxacin or trimethoprim-sulfamethoxazole). Two patients were hospitalized: a two-year-old child with hemolytic uremic syndrome, and a 25-year-old woman who underwent a laparotomy for presumed appendicitis but was found to have severe colitis.

A total of 2352 (14.5%) of 16,125 stool specimens yielded nonsorbitol-fermenting colonies on MacConkey-Sorbitol agar; 46 (2.0%) of these were verocytotoxin-producing strains of *E coli* 0157 (40 *E coli* 0157:H7 and six *E coli* 0157:H⁻). These strains represented eight different biotypes determined by the numeric MicroScan profile. All isolates were susceptible to ampicillin, cephalothin, trimethoprim-sulfamethoxazole, ciprofloxacin and gentamicin; two isolates were resistant to tetracycline.

Eighty-four grossly bloody stool specimens were received by the laboratory during the 16 month study. Only two (2.4%) of them yielded *E coli* 0157:H7 despite a history of bloody diarrhea from 16 patients with this infection. In contrast, grossly bloody stool specimens yielded *Campylobacter jejuni* from seven patients, *Salmonella* species from six, and *Shigella* species from three.

DISCUSSION

The association of *E coli* 0157:H7 with the syndrome of hemorrhagic colitis was first recognized in 1983 (12,15). In the same year, Karmali et al (3) reported that these organisms and other verocytotoxin-producing strains of *E coli* were associated with cases of hemolytic uremic syndrome. Since then, it has become apparent that verocytotoxin-producing *E coli*, most com-

monly *E coli* 0157:H7, are associated with a spectrum of enteric illness, including both bloody and nonbloody diarrhea (6,7,16). Recommendations for screening of fecal specimens with a MacConkey-Sorbitol agar in order to facilitate detection of sorbitol-negative *E coli* 0157:H7 have been made (13,17,18). Whether routine screening of all specimens is warranted has not been established. In 1989, 796 isolates of verotoxin-producing *E coli* were referred to the Enteric Reference Laboratory of the Central Public Health Laboratory, Ontario Ministry of Health (personal communication). All but a few of these isolates were *E coli* 0157:H7, and they were the third most common bacterial enteric pathogen referred to the laboratory, after campylobacter (5998 isolates) and salmonella (4868 isolates). However, few studies have determined the isolation rate of *E coli* 0157:H7 in stool specimens submitted to diagnostic laboratories. The recovery of verocytotoxin-producing *E coli* 0157 from only 0.3% of 16,125 specimens processed by Med-Chem Laboratories in Toronto is comparable to the isolation rate of 0.7% reported by regional public health laboratories in Timmins and Peterborough, Ontario (19). In the United States, MacDonald et al (16) recovered *E coli* 0157:H7 from only 25 (0.4%) of 6485 diarrheal stool specimens obtained from members of a health maintenance organization in the state of Washington. The organism was isolated less frequently than campylobacter or salmonella and about as often as shigella. *E coli* 0157:H7 was recovered from only two (0.08%) of 2552 stools submitted to a hospital clinical laboratory in Chicago (17). In contrast, *E coli* 0157:H7 was recovered from 2.5% of 5414 patients with diarrhea seen at three Calgary hospitals in two years and was, after salmonella, the second most common bacterial enteric pathogen identified (7). In British Columbia, *E coli* 0157:H7 was second only to campylobacter as a bacterial cause of infectious diarrhea in a tertiary care pediatric hospital (20); it was isolated from 1.9% of 1425 stools processed over 14 months. The relatively low isolation rate of *E coli* 0157:H7 found in the Metropolitan Toronto region may be attributable to geographic variability in prevalence of the organism. Alternatively, the low rate may reflect culture results from a patient population with a milder spectrum of diarrheal illness. The patients studied were generally outpatients who sought medical attention and had stool cultures requested by their physicians. However, these patients did not come to hospital emergency departments because of their symptoms and were, perhaps, less likely to have had grossly bloody diarrhea or hemorrhagic colitis.

The results of this study provide further evidence that there is variation in the proportion of *E coli* 0157:H7 infections causing nonbloody diarrhea (6). In a survey of 92 patients with enterocolitis in Newfoundland, *E coli* 0157:H7 was recovered from seven of 47 patients (15%) with grossly bloody diarrhea but from none of 45 patients with nonbloody diarrhea (8). In a prospective study conducted at three Calgary hospitals, almost all patients with *E coli* 0157:H7 infection had bloody diarrhea, and the organism was isolated from 40% of the 137 patients who presented with bloody diarrhea (7). In the present study, nearly half (48%) of patients with verotoxin-producing *E coli* infection did not give a history of bloody diarrhea, and only two provided stool specimens that were grossly bloody at the time of culture. Moreover, both salmonella and campylobacter were recovered more frequently than *E coli* 0157:H7 from patients whose stools were grossly bloody on receipt in the laboratory.

The present finding that 14.5% of specimens yielded non-sorbitol-fermenting organisms on MacConkey-Sorbitol agar is similar to the 15% reported by March and Ratnam (13) and the 16% found by Walker *et al* (18). Organisms that grow on this medium and that may not ferment sorbitol include non-0157 *E coli*, *Proteus* species, *Morganella* species, and occasionally other coliforms or *Pseudomonas* species (13). Although MacConkey-Sorbitol agar is useful in identifying the majority of stool specimens that are negative for *E coli* 0157:H7, a more selective screening test or medium is desirable. Other potentially useful markers for *E coli* 0157:H7 include lysine and ornithine decarboxylase reactions (21) and raffinose fermentation (22). However, it is important to note that these tests, as well as the use of

MacConkey-Sorbitol agar, fail to detect non-0157 serotypes of verocytotoxin-producing *E coli*. The detection of verotoxin in fecal filtrates remains the most sensitive and specific method of establishing the diagnosis (23), but is slow and labour intensive, and requires tissue culture facilities. A simple, rapid and accurate assay for the detection of fecal verotoxin would therefore be desirable.

The present data indicate that many patients with this infection in the community do not have typical symptoms of hemorrhagic colitis, and that the isolation rate of *E coli* 0157:H7 in southern Ontario is much lower than that reported elsewhere in Canada (7,8,20). These results also confirm that sorbitol-containing MacConkey agar is useful for screening stool specimens for *E coli* 0157:H7, although even with this medium there was considerable work and expense incurred in identifying nonsorbitol-fermenting isolates. It has been estimated that even with a prevalence of *E coli* 0157:H7 in diarrheal stools of 0.9%, the cost of identifying one patient with this infection would be approximately US\$465.00 (24).

The authors' estimate of \$350.00 per isolate substantiates the high cost of identifying infected patients. Whether routine use of MacConkey-Sorbitol agar for processing stool specimens is justified when isolation rates of *E coli* 0157:H7 are well under 1% is not certain. Further studies are required to determine whether establishing a specific etiologic diagnosis or early intervention would ameliorate the risk of subsequent complications such as hemolytic uremic syndrome. However, it seems reasonable to use this medium at least for the investigation of outbreaks of diarrheal illness, for culturing bloody stools, and for specimens obtained in summer months or from children.

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