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Laboratory Investigation of Outbreak of Hemorrhagic Colitis Caused by *Escherichia coli* O157:H7

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A severe outbreak of hemorrhagic colitis occurred in London, Ontario, during the month of September 1985. A total of 55 residents and 18 employees of a nursing home developed diarrhea, and 17 residents (age range, 78 to 99 years) died. Specimens from 38 patients, 37 employees and contacts, and 10 autopsies were investigated for all enteric pathogens. Specimens were also planted on MacConkey-sorbitol agar. Fecal extracts were tested on Vero cells for cytotoxin (FVT). *Escherichia coli* isolates were serotyped and tested for verotoxin and beta-glucuronidase production. Of the 38 symptomatic patients, 26 were positive for FVT, verotoxin-producing *E. coli* (VTEC), or both. Of the 105 specimens that were examined from these 38 patients, FVT and VTEC were both positive in 30 specimens, FVT only was positive in 13 specimens, and VTEC only was positive in 4 specimens. None of the 27 specimens from 10 autopsies was positive for FVT or VTEC. No other enteric pathogen was found in any of the cases. All asymptomatic individuals were negative for both FVT and VTEC. Of 19 VTEC strains that were isolated, 18 belonged to serotype O157:H7. These 18 strains and 2 more strains that were obtained from sporadic cases that had occurred within the 2 previous months were found to give similar biochemical reactions in a 36-test identification system. All isolates of serotype O157:H7 were beta-glucuronidase negative and susceptible to the antimicrobial agents that are used to treat *E. coli* infections. Testing for FVT and VTEC was found to be the most sensitive and specific technique for the laboratory diagnosis of this disease. Negative sorbitol, positive raffinose, and negative beta-glucuronidase tests appeared to be consistent markers for aiding in the detection of *E. coli* O157:H7.

Epidemic and sporadic cases of hemorrhagic colitis caused by *Escherichia coli* serotype O157:H7 have been reported since 1982 (12, 15–17). The outbreak that occurred in London, Ontario, in September 1985 has been the most severe so far (13). In a nursing home for the elderly, 55 residents and 18 employees developed diarrhea, and 17 residents died because of complications following hemorrhagic colitis.

The detection of a pathogenic strain of *E. coli* among a multitude of nonpathogenic *E. coli* strains and other lactose-fermenting coliforms is a difficult task unless differentiating markers are available. Laboratory diagnosis of hemorrhagic colitis is based on the demonstration of a cytotoxin produced by *E. coli* which is highly toxic for Vero cells (verotoxin) (10). The cytotoxin has been detected in *E. coli* culture broth filtrates and in stool extracts (6, 9). The duration of shedding of verotoxin-producing *E. coli* (VTEC) and the presence of verotoxin in feces (FVT) have been reported to be less than 8 days in adults after the development of symptoms (12, 15, 16, 21). Serotyping with specific O and H antisera is a valuable tool for the identification of VTEC (2, 6, 9, 16, 21). Useful markers that have been described for detection of *E. coli* O157:H7 are the absence of fermentation of sorbitol within 48 h (5, 11, 21) and a negative beta-glucuronidase reaction (1).

We report here the results of the laboratory investigation of the outbreak; this included the examination of pathologic specimens from 10 autopsies.

MATERIALS AND METHODS

Specimens. Stool specimens were referred to our laboratory from the nursing home and other hospitals in the city where the patients with hemorrhagic colitis were admitted. Specimens were also obtained from employees at the nursing home and other contacts. Specimens were received from 10 autopsies. These specimens included contents of the large intestine from one or more levels and pieces of intestinal tissue.

Bacterial strains. Additional VTEC strains included in the study were obtained from M. A. Karmali (Toronto, Ontario). These strains were O157:H7 (four strains), O1:K1:NM (one strain), O26:K60 (two strains), O91:H21 (one strain), O111:K58:H8 (one strain), O111:K58:NM (two strains), O113:K75:H21 (one strain), O121:H19 (two strains), and O145:NM (one strain).

Antisera. Neutralizing antiserum for *E. coli* O157:H7 verotoxin was also provided by M. A. Karmali. This serum sample was obtained from a patient who had recovered from hemolytic uremic syndrome associated with VTEC and had a high titer of neutralizing antibody to the verotoxin of *E. coli* O157:H7. Because of the limited availability of this antitoxin, it was used only in neutralization of cytotoxic fecal extracts in those cases in which cultures for VTEC were negative. Specific agglutinating antisera for O157 and H7 antigens were obtained from the *E. coli* Research Center, Pennsylvania State University, University Park. In case of doubtful and negative reactions for the O antigen, a suspension was heated at 100°C for 30 min, and the agglutination was repeated. Flagellar H7 agglutination was performed on overnight broth cultures or in semisolid agar containing H7 antiserum (2).

Culture. Specimens were examined for all enteric bacterial pathogens by standard methods. A primary plating medium of MacConkey agar that contained sorbitol instead of lactose was also included. A total of 5 to 10 sorbitol-nonfermenting colonies that appeared to be *E. coli* were picked for testing for verotoxin production and were also subcultured on

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TABLE 1. Results of testing for FVT and culture for VTEC

Samples obtained from:	No. of patients	No. of patients that were:		No. of specimens tested	No. of specimens that were:	
		FVT⁺	VTEC⁺		FVT⁺	VTEC⁺
Individuals with diarrhea	38	24	19	105	43	34
Asymptomatic individuals	37	0	0	40	0	0
Autopsies	10	0	0	27	0	0

4-methyl umbelliferyl glucuronide (MUG) medium (see below). In the absence of sorbitol nonfermenters, other colonies resembling *E. coli* were picked. In most cases sweeps of mixed growth were also tested for verotoxin production. The colonies were identified as *E. coli* by standard bacteriologic methods.

FVT. Approximately 1 volume of stool and 3 volumes of phosphate buffer (pH 7.2; 0.01 M) were thoroughly mixed, centrifuged, and filtered (pore size, 0.22 μm). The filtrate was tested on Vero cell monolayers grown in 96-well tissue culture trays (8). Neutralization of fecal verotoxin was done by using high-titer human antiserum (8).

Verotoxin production by *E. coli*. A filtrate of the overnight growth of the suspected organism in Penassay broth (antibiotic medium 3; Difco Laboratories, Detroit, Mich.) was tested as described above (8). Sweeps of mixed growth were also grown in Penassay broth and then subjected to polymyxin treatment before they were tested for toxin (7).

MUG medium. The test for beta-glucuronidase was performed by using the substrate MUG (Sigma Chemical Corp., St. Louis, Mo.) (1, 4, 19). MUG was dissolved in warm water, filter sterilized, and added to sterilized and cooled (50°C) MacConkey agar containing sorbitol or to Mueller-Hinton agar. The final concentration of MUG was 100 μg/ml (4). It was found to be useful to increase the concentration of agar to 3% because the enzyme and the fluorogenic product tended to diffuse rapidly through the medium. Use of petri dish dividers (Trives Precision, Scarborough, Ontario, Canada) was also an effective method for containing the fluorescence in the immediate vicinity of the colony. Plates were read under a long wave UV lamp after overnight incubation.

Biotypes of the isolates were based on the responses to a series of 36 tests that were used to identify enteric organisms. A replica-plate identification system was used that was based on overnight (18 to 20 h) test results (20). Acid production was determined in media containing Bacto-Peptone (10 g/liter; Difco), NaCl (5 g/liter), agar no. 1 (30 g/liter; Oxoid Ltd., Basingstoke, Hampshire, England), bromothymol blue (0.6%; 10 ml), and 10 g of the substrate being tested per liter (pH 7.6). The biotypes of the isolates in this study were compared with those on a computer file of enteric isolates that were identified at University Hospital, London, Ontario.

Antibiotic susceptibility tests. Antibiotic susceptibility tests were performed by the replica-plate method (20), with the inoculum standardized so that 10^3 to 10^4 organisms were deposited per spot of 1 to 2 μl.

TABLE 2. Positive results with FVT and VTEC in 26 cases[a]

Test result	No. of:	
	Cases	Specimens
FVT⁺, VTEC⁺	17	30
FVT⁺, VTEC⁻	7	13
FVT⁻, VTEC⁺	2	4

[a] A total of 47 specimens were examined.

RESULTS

A total of 172 specimens were examined for FVT, VTEC, and enteric bacterial pathogens. These were obtained from 38 diarrheic individuals, 37 employees and contacts (asymptomatic individuals), and 10 autopsies (Table 1). Of the 26 patients who were positive, 17 had both FVT and VTEC in their specimens, 7 had only FVT, and 2 were positive only for VTEC (Table 2). The last two were both mild cases that did not require hospitalization. One of them was an employee with watery diarrhea, and the VTEC isolated from this patient was untypable. This strain was sorbitol positive and beta-glucuronidase positive and was clearly unrelated to the strains involved in the outbreak (Table 3). The verotoxins in fecal extracts from 7 patients who were FVT⁺ but VTEC⁻ were all neutralized by specific antiserum to the verotoxin of *E. coli* O157:H7. Multiple samples were available from 19 of 26 laboratory-proven cases, and it was found that the duration of positive FVT, shedding of VTEC, or both was 4 to 8 days in 16 cases. In 3 cases FVT, VTEC, or both remained positive for 15, 17, and 25 days. Fatalities were 3 of 16 in the former group and 2 of 3 in the latter group. Specimens were received from 10 autopsies, but premortem samples were not sent to our laboratory in 5 of these cases. The other five cases had positive FVT, VTEC, or both during life but became negative 5 to 17 days before death. One patient had a positive blood culture for *E. coli* O157:H7 2 days before death. None of the asymptomatic individuals (contacts and employees) had a positive result for FVT or VTEC.

The use of MacConkey-sorbitol agar as a primary plating medium was highly successful in the early detection of *E. coli* O157:H7 during this outbreak. However, most other serotypes of VTEC, as well as the one untypable strain that was isolated in the outbreak, fermented sorbitol (Table 3).

The MUG reaction was negative with all strains of *E. coli* O157:H7. Other serotypes of VTEC, however, gave variable

TABLE 3. Sorbitol and raffinose fermentation and beta-glucuronidase reaction of VTEC strains

VTEC serotype	No. of strains	Sorbitol fermentation	Raffinose fermentation	Beta-glucuronidase reaction
O157:H7[a]	20	−	+	−
O157:H7[b]	4	−	+	−
O1:K1:NM[b]	1	+	−	−
O111:K58:H8[b]	1	+	+	−
O111:K58:NM[b]	2	+	+	−
O113:K75:H21[b]	1	+	+	+
O26:K60[b]	2	+	+	+
O91:H21[b]	1	+	+	+
O121:H19[b]	2	+	−	+
O145:NM[b]	1	+	−	+
Untypable	1	+	+	+

[a] A total of 18 strains were obtained from the outbreak; 2 strains were obtained from sporadic cases before the outbreak.
[b] Obtained from M. A. Karmali, Toronto.

TABLE 4. Biochemical test results of four biotypes of *E. coli* O157:H7

Test	Biotype				% of all *E. coli* Positive[a]
	A	B	C	D	
Glucose	+	+	+	+	100
Lactose	+	+	+	+	88
Sucrose	+	+	+	+	42
Maltose	+	+	+	+	98
Mannitol	+	+	+	+	99
Adonitol	−	−	−	−	3
Inositol	−	−	−	−	1
Salicin	−	−	−	−	0
Xylose	+	+	+	+	97
Arabinose	+	+	+	+	99
Cellobiose	−	−	−	−	0
Dulcitol	−	+	+	−	50
Sorbitol	−	−	−	−	95
Raffinose	+	+	+	+	20
Rhamnose	−	−	+	+	91
Trehalose	+	+	+	+	99
H_2S	−	−	−	−	0
Indole	+	+	+	+	99
Motility	+	+	+	+	67
Gluconate	−	−	−	−	0
Malonate	−	−	−	−	0
Citrate	−	−	−	−	0
Phenylalanine deaminase	−	−	−	−	0
Urease	−	−	−	−	0
Gelatin	−	−	−	−	0
DNase	−	−	−	−	0
Oxidase	−	−	−	−	0
Lysine decarboxylase	+	+	+	+	92
Ornithine decarboxylase	+	+	+	+	74
Beta-glucuronidase	−	−	−	−	96[b]

[a] A total of 9,714 strains were examined.
[b] See reference 4.

reactions, and the untypable strain gave a positive reaction (Table 3). It was noted that diffusion of fluorescence occurred rapidly through the medium, and plates had to be read within 12 to 16 h. The use of plate dividers delayed the spread and made it possible to test as many as 36 strains on one plate (diameter, 100 mm).

Serotyping with O157 antiserum on fresh isolates of sorbitol-nonfermenting *E. coli* gave clear agglutination with most isolates. VTEC from 18 of 19 cases agglutinated with both O and H antisera.

Biochemical results revealed four closely related biotypes for serotype O157:H7. The tests that were responsible for differentiating these biotypes were for dulcitol and rhamnose (Table 4). Subsequent retesting revealed that variable results were obtained with these tests; thus, the four biotypes that were observed initially during the outbreak probably represent alternate test scores for the same strain.

The biotypes of serotype O157:H7 differed from the majority of *E. coli* isolates by consistently producing negative test results for sorbitol and positive test results for sucrose and raffinose. These differences resulted in biotypes that are infrequently encountered. Biotypes A and B were not previously encountered in our file of 9,714 isolates of *E. coli*, whereas biotype C was encountered 13 times, and biotype D was encountered 52 times. The serotypes of these previous isolates were unknown. Two sporadic isolates of serotype O157:H7 that occurred before the outbreak both exhibited biotype D on initial isolation.

All O157:H7 strains were susceptible to the following antimicrobial agents: ampicillin, ticarcillin, cefazolin, tetracycline, gentamicin, tobramycin, polymyxin, sulfamethoxazole, trimethoprim-sulfamethoxazole, chloramphenicol, nalidixic acid, and nitrofurantoin.

DISCUSSION

The outbreak of hemorrhagic colitis that occurred in a nursing home in London, Ontario, in September 1985 has been the largest and most severe of all outbreaks reported to date (13, 17, 18). A case fatality rate of 31% among the nursing home residents was noted. Three outbreaks have occurred in nursing homes for the elderly, two in Ontario and one in the United States, in the past 3 years, which indicates that this is a population at high risk for acquiring this infection (13, 17, 18). No other bacterial, parasitic, or viral pathogen was found in the cases reported here. The association of *E. coli* O157:H7 with hemorrhagic colitis and hemolytic uremic syndrome is well established (8, 12, 16, 17).

Laboratory diagnosis of this condition has been difficult because of the fact that the definitive test for toxin is one that requires tissue culture facilities and is slow and cumbersome. The detection of verotoxin in stool filtrates is the most specific and sensitive one available (9, 12). In addition, the demonstration of toxin production by isolated colonies of *E. coli* or even from mixed cultures is highly specific, although it is somewhat less sensitive than testing for FVT (6, 7, 9). This requires the testing of 5 to 20 colonies that resemble *E. coli*. Tests for FVT and VTEC take between 2 and 5 days to complete, but they do detect all serotypes of *E. coli* that are capable of producing verotoxin. In agreement with results reported by others (9, 12), we found that testing for FVT was more sensitive than culturing for VTEC. Of 26 positive cases, 24 had FVT, whereas only 19 of 26 had VTEC (Table 2). It is noteworthy that two patients had positive VTEC but no detectable FVT. Both patients had only watery diarrhea and did not require hospitalization. One of these two VTEC strains was untypable serologically and was different from serotype O157:H7 on the basis of other reactions as well. Most of the patients had positive specimens for a period of 4 to 8 days after the onset of symptoms. This is within the range of duration reported by others (12, 15, 16, 21). However, three patients had persistence of FVT, VTEC, or both for 15 to 25 days. This is considerably longer than the mean of 4 to 7 days reported in adults (12, 21) and of 11 days reported in children (8, 9). Persistence of VTEC may be associated with a poor prognosis in elderly patients. Of these three patients, 2 died, whereas only 3 of 16 patients with shorter periods of positive tests (4 to 8 days) had a fatal outcome. The high fatality rate in this outbreak also provided an opportunity to examine postmortem specimens for verotoxin and VTEC. It is significant that all 27 specimens from 10 autopsies were negative for both FVT and VTEC. This may be because of the short period of shedding of the organism and the fact that most of these patients received large doses of antibiotics for several days before death. In those patients who died in whom premortem specimens were also available (5 of 10), it is significant that all 5 patients who were initially positive turned negative 5 to 17 days before death. One patient, although negative for verotoxin and VTEC in feces, had a positive blood culture for *E. coli* O157:H7 2 days before death. This organism is not considered invasive, but in an elderly patient who is terminally ill, it is difficult to attribute any significance to blood stream invasion.

The detection of a pathogenic strain of *E. coli* in feces is not easy unless distinguishing screening methods are available. In the case of enterotoxigenic *E. coli* and enteroinvasive *E. coli*, detection involves the testing of many colonies of *E. coli* from each specimen. For enteropathogenic *E. coli*, serotyping is the only tool as no pathogenecity tests have been described. However, Rappaport and Henig (14) described the use of a MacConkey-based medium that contained sorbitol instead of lactose. Two of the more common enteropathogenic *E. coli* serotypes (O111:B4 and O55:B5) failed to ferment sorbitol, whereas 93 to 95% of all *E. coli* are sorbitol fermenters. In 1982, in the two outbreaks in the United States, it was noted that *E. coli* O157:H7 failed to ferment sorbitol within 7 days (21). Other workers (2, 5, 11) noted that O157:H7 strains remained sorbitol negative for 48 h or more. Our *E. coli* O157:H7 strains reacted in a similar manner. This has led to the widespread use of MacConkey-sorbitol medium for screening stool specimens. A note of caution must be sounded here. We found all other serotypes of VTEC to be sorbitol fermenters (Table 3). Although it is true that most of the outbreaks of hemorrhagic colitis reported so far have been caused by serotype O157:H7, there is a likelihood that other serotypes will be missed, particularly in sporadic cases, if total reliance is placed in MacConkey-sorbitol medium. In infants serotype O26 appears to be as frequent as O157 (8, 9). Another differentiating biochemical reaction of *E. coli* O157:H7 is raffinose fermentation. Only 20% of all *E. coli* isolates are raffinose fermenters, as determined by our overnight, agar-based identification system; however, it should be noted that traditional tube tests that are incubated for 1 to 2 days produce a higher percentage (3). All strains of O157:H7 and four of the seven other serotypes of VTEC that we tested were raffinose fermenters (Table 3). Only 0.6% of *E. coli* isolates in our file are both sorbitol negative and raffinose positive.

The beta-glucuronidase reaction has been proposed as a rapid method for the detection of *E. coli* (4, 19). The substrate that we used, MUG, was particularly appropriate, as it could be incorporated with MacConkey agar or other media without changing the characteristics of the medium (1, 4, 19). About 96% of all *E. coli* isolates are positive (4), but we as well as others have found all strains of O157:H7 to be negative for beta-glucuronidase (1). In addition, we observed that two other serotypes (O111:K58 and O1) were beta-glucuronidase negative. A combination of sorbitol-negative, raffinose-positive, and MUG-negative results occurs only with O157:H7.

Serotyping was a valuable method in this outbreak. Nonfermenters of sorbitol were tested 24 h after the specimens were received and a presumptive identification with O157 antiserum was made. To our knowledge a report of nontoxigenic *E. coli* O157:H7 has not been published so far. However, we believe that confirmation of toxin production is essential for every strain that is isolated. It is clear from our results and those of others (5, 11, 12, 21) that isolation and identification of serotype O157:H7 is relatively easy because of the fortuitous occurrence of several unique markers. The same does not appear to be true regarding other serotypes of VTEC, and it may be only a matter of time before outbreaks caused by other serotypes of VTEC are reported. Although all outbreaks of hemorrhagic colitis reported to date have been caused by O157:H7, other serotypes of VTEC have been found that occur more sporadically (6, 9). We have found three strains of serotype O1 and one untypable strain in our laboratory in the past year. These VTEC isolates do not share the identifying markers that are used for detecting O157:H7. There is a need for rapid immunospecific methods or DNA probes for the early recognition of VTEC of all serotypes in feces.

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LITERATURE CITED

1. **Doyle, M. P., and J. L. Schoeni.** 1984. Survival and growth characteristics of *Escherichia coli* associated with hemorrhagic colitis. Appl. Environ. Microbiol. **48:**855–856.
2. **Farmer, J. J., III, and B. R. Davis.** 1985. H7 antiserum-sorbitol fermentation medium: a single tube screening medium for detecting *Escherichia coli* O157:H7 associated with hemorrhagic colitis. J. Clin. Microbiol. **22:**620–625.
3. **Farmer, J. J., III, B. R. Davis, F. W. Hickman-Brenner, A. McWhorter, G. P. Huntley-Carter, M. A. Asbury, C. Riddle, H. G. Wathen-Grady, C. Elias, G. R. Fanning, A. G. Steigerwalt, C. M. O'Hara, G. K. Morris, P. B. Smith, and D. J. Brenner.** 1985. Biochemical identification of new species and biogroups of *Enterobacteriaceae* isolated from clinical specimens. J. Clin. Microbiol. **21:**46–76.
4. **Feng, P. C. S., and P. A. Hartman.** 1982. Fluorogenic assays for immediate confirmation of *Escherichia coli*. Appl. Environ. Microbiol. **43:**1320–1329.
5. **Harris, A. A., R. L. Kaplan, L. J. Goodman, M. Doyle, W. Landau, J. Segreti, K. Mayer, and S. Levin.** 1985. Results of a screening method used in a 12-month stool survey for *Escherichia coli* O157:H7. J. Infect. Dis. **152:**775–777.
6. **Johnson, W. M., H. Lior, and G. S. Bezanson.** 1983. Cytotoxic *Escherichia coli* O157:H7 associated with hemorrhagic colitis in Canada. Lancet **i:**76.
7. **Karmali, M. A., M. Petric, C. Lim, R. Cheung, and G. S. Arbus.** 1985. Sensitive method for detecting low numbers of verotoxin-producing *Escherichia coli* in mixed cultures by use of colony sweeps and polymyxin extraction of verotoxin. J. Clin. Microbiol. **22:**614–619.
8. **Karmali, M. A., M. Petric, C. Lim, P. C. Fleming, G. S. Arbus, and H. Lior.** 1985. The association between idiopathic hemolytic uremic syndrome and infection by verotoxin-producing *Escherichia coli*. J. Infect. Dis. **151:**775–782.
9. **Karmali, M. A., B. T. Steele, M. Petric, and C. Lim.** 1983. Sporadic cases of haemolytic-uraemic syndrome associated with faecal cytotoxin and cytotoxin-producing *Escherichia coli* in stools. Lancet **i:**619–620.
10. **Konowalchuk, J., J. I. Speirs, and S. Stavric.** 1977. Vero response to a cytotoxin of *Escherichia coli*. Infect. Immun. **18:**775–779.
11. **March, S. B., and S. Ratnam.** 1986. Sorbitol-MacConkey medium for detection of *Escherichia coli* O157:H7 associated with hemorrhagic colitis. J. Clin. Microbiol. **23:**869–872.
12. **Pai, C. H., R. Gordon, H. V. Sims, and L. E. Bryan.** 1984. Sporadic cases of hemorrhagic colitis associated with *Escherichia coli* O157:H7. Ann. Intern. Med. **101:**738–742.
13. **Pudden, D., N. Tuttle, D. Korn, J. Carlson, A. Carter, and J. Hockin.** 1985. Hemorrhagic colitis in a nursing home—Ontario. Can. Dis. Weekly Rep. **11:**169–170.
14. **Rappaport, F., and E. Henig.** 1952. Media for the isolation and differentiation of pathogenic *Escherichia coli* (serotypes O111 and O55). J. Clin. Pathol. **5:**361–362.
15. **Remis, R. S., K. L. MacDonald, L. W. Riley, N. D. Puhr, J. G. Wells, B. R. Davis, P. A. Blake, and M. L. Cohen.** 1984. Sporadic cases of hemorrhagic colitis associated with *Escherichia coli* O157:H7. Ann. Intern. Med. **101:**738–742.
16. **Riley, L. W., R. S. Remis, S. D. Helgerson, H. B. McGee, J. G. Wells, B. R. Davis, R. J. Hebert, E. S. Olcott, L. M. Johnson, N. T. Hargrett, P. A. Blake, and M. L. Cohen.** 1983. Hemor-

rhagic colitis associated with a rare *Escherichia coli* serotype. N. Engl. J. Med. **308**:681–685.

17. **Ryan, C. A., R. V. Tauxe, G. W. Hosek, J. G. Wells, P. A. Stoesz, H. W. McFadden, Jr., P. W. Smith, G. F. Wright, and P. A. Blake.** 1986. *Escherichia coli* O157:H7 diarrhea in a nursing home: clinical, epidemiological, and pathological findings. J. Infect. Dis. **154**:631–638.
18. **Stewart, P. J., W. Desormeaux, and J. Chene.** 1983. Hemorrhagic colitis in a home for the aged—Ontario. Can. Dis. Weekly Rep. **9**:29–32.
19. **Trepeta, R. W., and S. C. Edberg.** 1984. Methylumbelliferyl-β-D-glucuronide based medium for rapid isolation and identification of *Escherichia coli*. J. Clin. Microbiol. **19**:172–174.
20. **Trust, T. J., and J. L. Whitby.** 1976. Antibiotic resistance of bacteria in water containing ornamental fishes. Antimicrob. Agents Chemother. **10**:598–603.
21. **Wells, J. G., B. R. Davis, I. K. Wachsmuth, L. W. Riley, R. S. Remis, R. Sokolow, and G. K. Morris.** 1983. Laboratory investigation of hemorrhagic colitis outbreaks associated with a rare *Escherichia coli* serotype. J. Clin. Microbiol. **18**:512–520.