JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2003, p. 174–180 Vol. 41, No. 1
0095-1137/03/$08.00+0 DOI: 10.1128/JCM.41.1.174–180.2003

Detection, Isolation, and Molecular Subtyping of *Escherichia coli* O157:H7 and *Campylobacter jejuni* Associated with a Large Waterborne Outbreak

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Received 14 June 2002/Returned for modification 8 August 2002/Accepted 27 September 2002

The largest reported outbreak of waterborne *Escherichia coli* O157:H7 in the United States occurred in upstate New York following a county fair in August 1999. Culture methods were used to isolate *E. coli* O157:H7 from specimens from 128 of 775 patients with suspected infections. *Campylobacter jejuni* was also isolated from stools of 44 persons who developed diarrheal illness after attending this fair. There was one case of a confirmed coinfection with *E. coli* O157:H7 and *C. jejuni*. Molecular detection of stx_1 and stx_2 Shiga toxin genes, immunomagnetic separation (IMS), and selective culture enrichment were utilized to detect and isolate *E. coli* O157:H7 from an unchlorinated well and its distribution points, a dry well, and a nearby septic tank. PCR for stx_1 and stx_2 was shown to provide a useful screen for toxin-producing *E. coli* O157:H7, and IMS subculture improved recovery. Pulsed-field gel electrophoresis (PFGE) was used to compare patient and environmental *E. coli* O157:H7 isolates. Among patient isolates, 117 of 128 (91.5%) were type 1 or 1a (three or fewer bands different). Among the water distribution system isolates, 13 of 19 (68%) were type 1 or 1a. Additionally, PFGE of *C. jejuni* isolates revealed that 29 of 35 (83%) had indistinguishable PFGE patterns. The PFGE results implicated the water distribution system as the main source of the *E. coli* O157:H7 outbreak. This investigation demonstrates the potential for outbreaks involving more than one pathogen and the importance of analyzing isolates from multiple patients and environmental samples to develop a better understanding of bacterial transmission during an outbreak.

Escherichia coli O157:H7 is a primary cause of severe and bloody diarrhea. Complications, particularly hemolytic-uremic syndrome (HUS) (39, 52), have made infections with this organism a public health priority. Ground beef and other bovine products have often been implicated as sources (19, 32), along with other food products (1, 6, 22, 30, 43, 51) and person-to-person transmission (5, 34). Occasional outbreaks have also been associated with public drinking water (42) and swimming in contaminated water (16).

Campylobacter spp. are the most commonly reported bacterial cause of gastrointestinal illness in the United States (25, 29). Although outbreaks have been reported, most cases occur sporadically (35). Cases have been associated most frequently with consumption of contaminated poultry (44), and less frequently with contaminated milk (31) and water (36). When pulsed-field gel electrophoresis (PFGE) is performed on outbreak isolates, multiple types are often identified, implying a polyclonal origin of the outbreak strains (20, 49). Only a few outbreaks involving both *E. coli* O157:H7 and *Campylobacter jejuni* have been reported (26; M. Cosgriff, www.promedmail.org).

The PCR is a rapid and reliable tool for the molecular-based diagnosis of a variety of infectious diseases (14). PCR analysis for screening drinking water and environmental samples has been reported (46–48) and has been utilized to identify *E. coli* in primary water specimens (15, 27), stool specimens (33, 56), and outbreaks (23, 24). In contrast, isolation of *E. coli* O157:H7 from water and other environmental samples is laborious. Culture is problematic due to large numbers of other flora that either overgrow or mimic the non-sorbitol-fermenting *E. coli* O157:H7 (12). Recently, immunomagnetic separation (IMS) has helped improve recovery by providing an antibody-based concentration procedure that uses magnetic beads coated with antibody against *E. coli* O157. Although many report the usefulness of IMS for testing artificially contaminated samples, few reports have documented the use of IMS with naturally occurring, epidemiologically linked specimens (11, 54). Furthermore, there are no reports documenting the use of IMS in support of a waterborne outbreak investigation.

PFGE is useful for subtyping *E. coli* O157:H7 isolates during outbreak investigations (1, 4, 5). PFGE is reproducible and has sufficient discriminatory power to allow detection of minor genetic variations among isolates (18, 53). Although the use of PFGE for the subtyping of *Campylobacter* spp. has not been as

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TABLE 1. Primers used for amplification of stx_1, stx_2, *eaeA,* and *hlyA* genes by PCR

Primer	Gene	Oligonucleotide sequence (5′→3′)	Amplicon product (bp)	Reference or source
VT1a	stx_1	GAAGAGTCCGTGGGATTACG	130	37
VT1b	stx_1	AGCGATGCAGCTATTAATAA		37
VT2aa	stx_2	CGACCCCTCTTGAACATATATCTC	397	Wadsworth Center (1996)
VT2b	stx_2	GCTCTGGATGCATCTCTGGT		37
AE19	*eaeA*	CAGGTCGTCGTGTCTGCTAAA	1,087	17
AE20	*eaeA*	TCAGCGTGGTTGGATCAACCT		17
MFS1F	*hlyA*	ACGATGTGGTTTATTCTGGA	166	13
MFS1R	*hlyA*	CTTCACGTCACCATACATAT		13

well described, it has been shown to be useful for strain discrimination under some circumstances (40, 55).

This study describes the laboratory analysis of patient and environmental isolates from the largest outbreak of waterborne *E. coli* O157:H7 and *C. jejuni* in the United States (8). This analysis illustrates the utility of screening diverse sample types using a PCR assay for stx_1 and stx_2. Moreover, it demonstrates the improved recovery of *E. coli* O157:H7 using IMS protocols which enabled a definitive match of PFGE patterns between isolates obtained from contaminated water and from ill patients.

MATERIALS AND METHODS

Primary specimens. The Washington County Fair (WCF) was held from 23 August through 29 August 1999 in New York State. There were approximately 111,000 admissions to this large agricultural fair, and exhibitors displayed hundreds of farm animals. The fairground that is the site of this fair does not have a public water supply and its 50 acres is situated on porous soil that is composed of sand and gravel. Additionally, drought conditions existed during this time period, causing a decrease in the water table, which necessitated the use of additional shallow wells for a water supply. Also, a heavy rainfall occurred on one day during the week of the fair.

Once a link between diarrheal illness and this fair was suspected, water was obtained from six shallow wells at the fairgrounds, four of which were unchlorinated in 1999. Additional samples were collected from distal points in the distribution system, a septic tank adjacent to the pump house for well 6, cow manure from a pile close to well 6, soil from a nearby dry well, and ice from a vendor at the fair. Stool samples were also received from several patients who attended the fair and were ill with HUS but whose routine cultures were negative for *E. coli* O157:H7. A comprehensive epidemiological analysis has been performed (J. Ackelsberg, B. Wallace, D. Schoonmaker-Bopp, D. Dziewulski, S. Sivapalasingam, R. Limberger, P. Smith, M. Burke, B. Sauders, S. Kondracki, S. Olson, D. Morse, D. L. Swerdlow, and the WCF Outbreak Team, unpublished data).

Primary isolation and IMS. Aliquots containing 100, 250, or 500 ml of liquid specimens (water, septic tank contents, or melted ice) were filtered through 0.45-μm-pore-size filters, and the filters were then placed in 225 ml of *E. coli* broth supplemented with novobiocin (ECN) (20 μg/ml). Twenty-five-gram aliquots of solid and semisolid specimens (cow manure, soil, or stool) were homogenized with 225 ml of ECN in a stomacher (model 400; Seward Ltd., London, United Kingdom) at normal speed for 2 min. Specimens were incubated in ECN with shaking for 12 to 18 h at 35 to 37°C (primary enrichment), and this was followed by plating onto sorbitol-MacConkey agar (SMAC) and onto SMAC supplemented with cefixime (0.05 mg/liter) and potassium tellurite (2.5 mg/liter) (CTSMAC). Colorless colonies suspected to be *E. coli* O157:H7 were subcultured and identified as described below. Primary enrichment cultures from most specimens were also subjected to IMS using beads coated with adsorbed and affinity-purified antibodies against all strains of *E. coli* O157 (Dynabeads anti-*E. coli* O157; Dynal, Wirral, United Kingdom) according to the manufacturer's directions, and this was followed by direct plating of beads onto SMAC and CTSMAC. Some cultures underwent a secondary overnight ECN enrichment of the beads at 42°C and plating of the broth and bead-bacteria complex onto SMAC and CTSMAC to further select for *E. coli* O157:H7 (7, 21). This method was adapted from the technique of Wells et al. (J. G. Wells, K. D. Greene, C. A. Bopp, M. E., Proctor, L. M. Slutsker, and P. Mead, Abstr. 99th Gen. Meet. Am. Soc. Microbiol., abstr. P-21, 1999). For selected specimens the IMS enrichment was repeated on the secondary enrichment overnight and an aliquot was transferred for a third enrichment at 42°C.

***stx*$_1$ and *stx*$_2$ PCR.** The primer sequences used for PCR are shown in Table 1. Primers VT1a, VT1b, and VT2b were identical to those described by Pollard et al. (37), whereas VT2aa was designed at the Wadsworth Center and is capable of identifying stx_2 as well as the additional antigenically distinct Shiga toxins stx_{2c} and stx_{2e}. Multiplex PCR amplification of stx_1 and stx_2 sequences was performed on primary and secondary enrichment broths from the samples. Crude template DNA was prepared as follows. A 200-μl aliquot of each broth sample was centrifuged at 13,000 × *g* for 2 min, and the pellets were resuspended in 200 μl of phosphate-buffered saline. The tubes were centrifuged and washed with phosphate-buffered saline again. After a third centrifugation, the pellets were resuspended in 1× GeneAmp PCR buffer II (100 mM Tris-HCl [pH 8.3], 500 mM KCl; Perkin-Elmer Cetus). The tubes were placed in a 95°C heat block for 15 min. DNA was amplified in a final reaction volume of 50 μl consisting of 1× PCR buffer, 2.5 mM $MgCl_2$, a 200 μM concentration of each deoxynucleoside triphosphate (dNTP), 1.25 U of Amplitaq Gold DNA polymerase (Perkin-Elmer Cetus), a 1 μM concentration (each) of the VT1-VT2 primer pair, and 10 μl of crude template DNA. Thermocycling conditions in a GeneAmp 9600 thermocycler (Perkin-Elmer Cetus) were as follows: initial cycle of 9 min at 95°C, followed by 30 s at 94°C, 30 s at 60°C, and 45 s at 72°C for 30 cycles, with a 7-min extension at 72°C. PCR products were visualized on a 2% agarose gel stained with ethidium bromide. Sample types such as cow manure, soil, and stools were tested for PCR inhibition by 1:10 dilution of the template DNA and/or by spiking of a duplicate PCR with a lysate of an *E. coli* O157:H7 strain known to produce Shiga toxin 1 and Shiga toxin 2.

***eaeA* gene PCR.** The primer sequences (AE19 and AE20) used for PCR are shown in Table 1. PCR assays were performed according to the methods in published studies (13) with slight modifications. Briefly, DNA was amplified in a final reaction volume of 100 μl consisting of 1× PCR buffer, 2.5 mM $MgCl_2$, a 200 μM concentration of each dNTP, 2.5 U of Amplitaq Gold DNA polymerase, a 1 μM concentration (each) of the AE19-AE20 primer pair, and 10 μl of crude template DNA. Thermocycling conditions were as follows: initial cycle of 9 min at 95°C, followed by 30 s at 94°C, 30 s at 55°C, and 60 s at 72°C for 35 cycles.

60-MDa plasmid (*hlyA* gene) PCR. The primer sequences (MFS1F and MFS1R) used for PCR are shown in Table 1. PCR assays were performed according to the methods in published studies (15) with slight modifications. Briefly, DNA was amplified in a final reaction volume of 100 μl consisting of 1× PCR buffer, 2.5 mM $MgCl_2$, a 200 μM concentration of each dNTP, 2.5 U of Amplitaq Gold DNA polymerase, a 1 μM concentration (each) of the MFS1F-MFS1R primer pair, and 10 μl of crude template DNA. Thermocycling conditions were as follows: initial cycle of 9 min at 95°C, followed by 30 s at 94°C, 30 s at 62°C, and 30 s at 72°C for 35 cycles.

Bacterial isolates. Patient isolates of *E. coli* O157:H7 were obtained from clinical laboratories, which processed stool samples by conventional methods. Briefly, stools were plated onto SMAC followed by identification of sorbitol-negative isolates as *E. coli* O157 using biochemical systems and O157 serological kits. At the Wadsworth Center, patient and environmental isolates of *E. coli* O157:H7 were confirmed using triple sugar iron, urea, citrate, indole, a latex agglutination kit for serogroup O157 (Oxoid, Hampshire, England), and tube agglutination for H7 (Difco, Detroit, Mich.).

Campylobacter isolates from clinical laboratories were plated on Mueller-Hinton II agar with 5% sheep blood (MHSBA) and incubated at 35 to 37°C microaerophilically in a tri-gas incubator with 85% N_2, 10% CO_2, and 5% O_2. Identification was confirmed by aerobic (37°C), anaerobic (37°C), and mi-

croaerophilic (25, 37, and 42°C) growth, as well as assays for oxidase; indoxyl acetate; hippurate hydrolysis; catalase; MacConkey agar growth; and susceptibility to nalidixic acid, cephalothin, and polymyxin B.

PFGE. DNA was prepared from *E. coli* O157:H7 cells grown for 24 h on brain heart infusion agar at 37°C according to established protocols (9). Briefly, bacteria were embedded in agarose, lysed, and treated with protease. DNA was digested with restriction endonuclease *Xba*I and in some cases *Avr*II (New England Biolabs, Beverly, Mass.), and the fragments were separated in 1.0% agarose gels on a clamped homogenous electric field apparatus (CHEF Mapper; Bio-Rad Laboratories, Richmond, Calif.). The initial pulse time of 2.2 s was increased linearly to 54.2 s over 22 h. Gels were stained with ethidium bromide, destained in water, and visualized with a Gel Doc 1000 gel analysis system (Bio-Rad Laboratories, Hercules, Calif.).

DNA was prepared from *Campylobacter* sp. cells that had been grown on MHSBA and incubated at 35 to 37°C for 24 h under microaerophilic conditions. The DNA was prepared as for the *E. coli* O157:H7 isolates with slight modifications (38). The DNA was digested with *Sma*I (New England Biolabs). The initial pulse time of 5 s was increased linearly to 35 s over 22 h.

We considered PFGE patterns to be identical if all bands greater than 50 kb in size were the same. Related subtypes were defined by three or fewer band differences, and unrelated types were defined by four or more band differences (45). Patterns were submitted to PulseNet (41) central for comparison to the national *E. coli* O157:H7 PFGE pattern database.

RESULTS

Epidemiological investigation. The outbreak was recognized when the Bureau of Communicable Disease Control of the New York State Department of Health became aware of an increased number of persons being evaluated for diarrheal illness at local hospitals. Some hospitalized children showed worsening kidney function, and *E. coli* O157:H7 was isolated from the stool of one patient. The next day, area hospitals were alerted to consider *E. coli* O157:H7 infection in patients who presented with diarrheal illness.

An epidemiological investigation revealed that many of these patients had attended the WCF, one of the largest agricultural fairs in New York State. The WCF 50-acre site does not utilize a public water supply, and in 1999 at the time of this outbreak, its water came from shallow wells. Water from the fairgrounds was obtained from six wells (four of which were unchlorinated). Analysis of samples from each of the wells showed that coliform bacteria were present in two of the wells and that one of these (well 6), an unchlorinated well that supplied drinking water, contained *E. coli*. Additionally, two potential environmental sources of *E. coli* contamination near well 6 were identified: a cow manure storage site (80 ft away) and a dormitory septic tank with a seepage pit only 36 ft from well 6. Environmental samples were collected from well 6, distal points in the distribution system of well 6, the septic tank for the dormitory, cow manure from the nearby storage site, soil from a dry well, and ice from a vendor. Details of the epidemiologic investigation will be published elsewhere (Ackelsberg et al., unpublished data).

Molecular screening for Shiga toxin genes stx_1 and stx_2. A summary of results obtained by screening specimens from this outbreak for Shiga toxin genes by PCR is shown in Table 2. Notably, stx_1 and stx_2 genes were detected in the well water from well 6 and distal points in the water distribution system (Fig. 1). Additional positive samples include primary specimens from the septic tank and cow manure sites as well as soil samples from the dry well and two submitted stool samples (Table 2). Ice specimens were negative by PCR upon initial screening and remained negative after the second and third enrichments (Table 2; Fig. 1). All PCR-positive enrichment broths were *E. coli* O157:H7 culture positive except for the cow manure specimens, but no PCR-negative enrichment broths were culture positive. The cow manure samples may have contained Shiga toxin genes from non-*E. coli* O157:H7 strains. The three water samples that were positive for stx_1 and stx_2 were also positive by PCR for the virulence factor genes *eaeA* and *hlyA* (data not shown). A reduction in the amount of amplified PCR product obtained from the spiked positive control indicated that a partial inhibition to the PCR occurred in some of the primary cow manure, stool, and soil samples. In previous validation tests, we noted that samples showing a partial inhibition did not detract from our ability to detect *E. coli* O157:H7 by PCR, since overnight growth amplification provided sufficient template for amplification. In addition, no inhibition occurred in the double-enriched and triple-enriched broths (not shown).

TABLE 2. Enriched specimens screened for stx_1 and stx_2 genes by PCR

Type of specimen tested	No. of specimens tested (no. positive) for stx_1 and stx_2		
	Primary enriched	Double enriched	Triple enriched
Water	34 (3)	0	0
Cow manure	6 (3)	3 (0)	3 (0)
Soil from dry well	2 (0)	2 (2)	2 (2)
Ice	10 (0)	6 (0)	2 (0)
Stool	6 (0)	5 (2)	0
Septic tank	5 (5)	0	0
Total (n = 86)	63 (11)	16 (4)	7 (2)

Selective enrichment and IMS treatment of environmental samples and clinical specimens. As shown in Fig. 2, no *E. coli* O157:H7 was recovered by direct plating on SMAC of primary or secondary enrichment broths from environmental samples. Direct plating of primary and secondary broths on CTSMAC, however, resulted in successful recovery of *E. coli* O157:H7 (Fig. 2). The implementation of IMS further increased the percentage of recovery from both the SMAC and CTSMAC

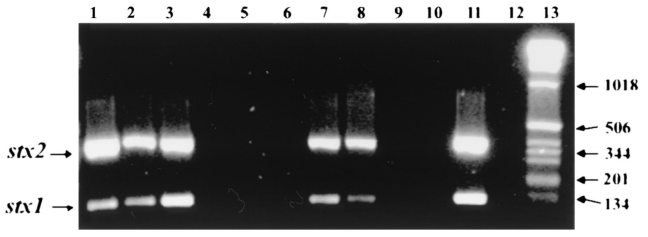

FIG. 1. Multiplex PCR amplification of Shiga toxin genes (stx_1 and stx_2) from selected environmental specimens. The expected amplified products were 397 bp for stx_2 and 138 bp for stx_1. Lanes 1 and 2, primary enriched water samples from well 6; lane 3, primary enriched sample from the distribution system of well 6; lanes 4 to 6, secondary enrichments of cow manure samples; lanes 7 and 8, secondary enrichments of samples from a dry well; lanes 9 and 10, secondary enrichments of ice samples from a vendor who utilized well 6; lane 11, positive control; lane 12, negative template control; lane 13, 1-kb DNA ladder (Gibco BRL) (base pairs indicated at right).

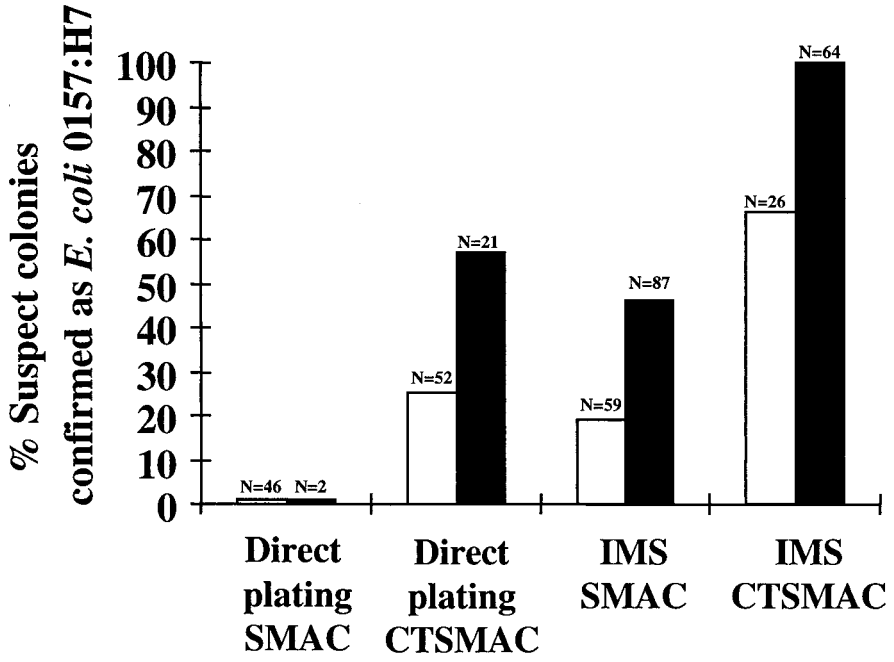

FIG. 2. Recovery of *E. coli* O157:H7 after primary enrichment (open bars) and secondary enrichment (solid bars), IMS treatment, and plating on various culture media. The *n* values indicate the total number of colonies isolated from these plates that were suspected to be *E. coli* O157:H7. The percentages were determined by dividing the number of *E. coli* O157:H7 confirmed identifications by the number of isolates examined. The first two columns, which encompass direct plating on SMAC, represent a value of 0%.

cultures (Fig. 2). Additionally, two stool samples that were previously culture negative, from patients with HUS, were received from a clinical laboratory. They were also selectively enriched and subjected to IMS, which resulted in the successful isolation of *E. coli* O157:H7 from these specimens (data not shown).

PFGE of *E. coli* O157:H7 isolates from patients and the environment. During this outbreak, 128 isolates of *E. coli* O157:H7 from 117 patients were examined by PFGE. From the group of patient isolates identified as *E. coli* O157:H7, it was observed that the majority had enzyme digestion restriction patterns designated as types 1 and 1a (91.5%). Related subtypes and types unrelated to the outbreak pattern comprised the remaining 8.5% (Table 3; Fig. 3). Multiple isolates from nine patients were analyzed (20 isolates), and each patient was found to harbor strains representing a single PFGE pattern (data not shown).

Among water distribution system isolates, 12 (63.1%) were type 1, 1 (5.3%) was subtype 1a, and 6 (31.6%) were subtype 1e

TABLE 3. PFGE patterns of *E. coli* O157:H7 isolates according to source

PFGE pattern	No. (%) of isolates			
	Patient	Water	Soil from dry well	Septic tank
1	76 (65.0)	12 (63.1)	0	1 (25)
1a	31 (26.5)	1 (5.3)	0	3 (75)
1b	2 (1.7)	0	0	0
1c	1 (0.9)	0	0	0
1e	0	6 (31.6)	0	0
1f	0	0	10 (100)	0
1g	2 (1.7)	0	0	0
1h	1 (0.9)	0	0	0
2	1 (0.9)	0	0	0
4	3 (2.6)	0	0	0
Total	117	19	10	4

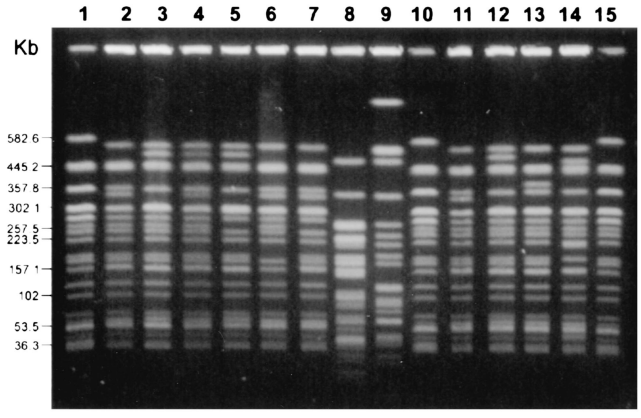

FIG. 3. PFGE analysis of selected *E. coli* O157:H7 isolates after digestion with *Xba*I. Lanes 1, 10, and 15 contain control *E. coli* strain G5244, with numbers at left indicating the band size in kilobase pairs. Lanes 1 to 9 contain clinical isolates from outbreak-associated patients, and lanes 11 to 14 contain environmental isolates associated with the outbreak. Lane 2, PFGE type 1; lanes 3 to 7, related subtypes (three or fewer band differences) 1a, 1b, 1c, 1g, and 1 h, respectively; lanes 8 and 9, PFGE types 2 and 4; lane 11, PFGE type 1; lanes 12 to 14, subtypes (three or fewer band differences) 1a, 1e, and 1f, respectively.

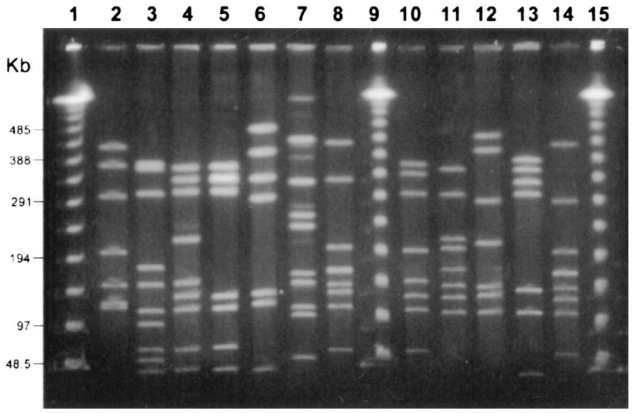

FIG. 4. PFGE analysis of selected *C. jejuni* isolates after digestion with *Sma*I. Numbers at left indicate band size in kilobase pairs. Lanes 1, 9, and 15 contain lambda ladder (48.5 kb) for size estimation. Lanes 2 to 8 contain clinical isolates from outbreak-associated patients. Lane 2, PFGE type 1, which comprised 83% of the patient isolates; lanes 3 to 8, unrelated types (more than four band differences) and subtypes (three or fewer band differences) 2, 3, 3a, 4, 5, and 6, respectively. Lanes 10 to 14 contain patient isolates not associated with the outbreak.

(which was not found in patient isolates). Among the dormitory septic tank isolates, one (25%) was type 1, and three (75%) were subtype 1a. All of the soil isolates were subtype 1f. Type 1 and type 1a, the predominant patient patterns in this outbreak, were not previously seen in the 165 *E. coli* O157:H7 isolates fingerprinted in our laboratory during the year prior to the outbreak. Additionally, since the outbreak, only 1 out of 300 isolates typed by our laboratory has matched the type 1 pattern, indicating that this pattern is rare. This predominant PFGE pattern, pattern 1, was also noted by four other states as part of the PulseNet database, and at least one of these cases was epidemiologically linked to this outbreak.

PFGE of *C. jejuni* isolates from patients. Clinical laboratories confirmed *C. jejuni* in specimens from 44 patients believed to be associated with the WCF outbreak, one of whom was coinfected with *E. coli* O157:H7 type 4. Thirty-five of the outbreak *C. jejuni* isolates were submitted to our laboratory for species confirmation and PFGE. Although multiple PFGE patterns were detected, the majority 29 (83%) were type 1 (Fig. 4), together with one isolate each of types 2, 3, 3a, 4, 5, and 6. *C. jejuni* was not isolated from well water or from the septic tank.

DISCUSSION

This outbreak, which included 775 patients with suspected cases of infection, was the largest outbreak of diarrhea associated with waterborne *E. coli* O157:H7 ever reported in the United States. Since water is a commonly consumed beverage and is used in food preparation and for hand washing, it was important to establish the presence of the pathogen in the water and to evaluate the genetic relatedness of the patient and water isolates from this investigation.

Given the long turnaround time associated with traditional culture methods, PCR was used as a primary screen for the detection of stx_1 and stx_2 toxin genes. PCR-positive results implicated well 6 and its distribution system as the source. This led to the laboratory's effort to isolate and fingerprint the organism, thus successfully confirming the suspected epidemiological link. We found no evidence that culturing of PCR-negative specimens would improve sensitivity. Also, positive results from the specific PCR tests for *eaeA* and *hlyA* genes, which are associated with virulence, helped to characterize the organisms isolated from water as pathogenic and probable *E. coli* O157:H7.

This investigation also demonstrated that IMS subculture improved the yield of recovery for *E. coli* O157:H7. Direct plating onto SMAC did not allow isolation of *E. coli* O157:H7, perhaps due to overgrowth of competing gram-negative organisms. Direct plating of primary and secondary broths to CTSMAC plates did, however, result in successful recovery of *E. coli* O157:H7. Use of CTSMAC increased selection of *E. coli* O157:H7 and decreased growth of non-O157 organisms. In addition, IMS further improved the success of isolation. When IMS was combined with secondary enrichment and plating on CTSMAC medium, a 100% confirmation rate was achieved for suspect colonies. The submitted stool samples that were previously culture-negative when processed by conventional methods and then found to be positive by our protocol demonstrated the usefulness of this protocol. We conclude from these experiments that the use of CTSMAC is necessary for successful recovery of *E. coli* O157:H7 from environmental samples. We also conclude, as have others (Wells et al., 99th Gen. Meet. Am. Soc. Microbiol.), that the implementation of the secondary IMS procedure combined with plating on CTSMAC medium is useful for both environmental and clinical samples in which the organism may be present in low numbers, and for which conventional isolation procedures are unsuccessful.

PFGE was used to genetically type patient isolates as well as multiple isolates from environmental samples. The two predominant PFGE types, 1 and 1a, comprised 91.5% of the *E. coli* isolates examined from patients with culture-confirmed infection. These two types were also present in isolates obtained from well 6, distal parts of the water distribution system, and the dormitory septic tank. The presence of isolates with this rare PFGE type in the vast majority of patients and in water from well 6 and parts of the distribution system provided evidence that the water was most likely the source of exposure for the illness. Analysis of the septic system from the nearby dormitory building using dye seepage tests demonstrated a hydraulic connection between the septic system and the water in well 6. Patient interviews and multivariate analysis also suggested that water could be a potential source of exposure (Ackelsberg et al., unpublished data). Thus, the laboratory and epidemiological data convincingly implicated water as the primary source of *E. coli* O157:H7.

The vast majority of the *E. coli* O157:H7 isolates from patients were of one related type, suggesting primary contamination of water and subsequent patient infection from a single unknown source. It is unclear whether the source was of bovine or human origin, since both cattle and humans can excrete multiple subtypes (2, 3, 10, 28). One hypothesis is that cow manure contaminated with *E. coli* O157:H7 was carried into the dormitory on muddy boots, was washed into the septic system, and subsequently seeped into well 6 (Ackelsberg et al., unpublished data). Unfortunately, we were unable to detect *E. coli* O157:H7 in a limited sample of six cow manure specimens

and are unable to confirm this hypothesis. Laboratory data also cannot rule out the possibility of a primary contamination from a human source in the septic tank. Moreover the presence of multiple PFGE types and subtypes, although few, further complicates the analysis. Only PFGE patterns 1, 1a, and 1e were detected in the water, although patient samples contained additional types and subtypes. There are several explanations that can account for these results. One possibility is that not all of the types and subtypes were able to be cultured from the water. Alternatively, an exogenous source of *E. coli* O157:H7 contamination could have contributed to the number of other types noted in isolates from ill patients. For example, some patients could have become infected through direct contact with the farm animals or even outside of the fair, which could explain the few patient isolates for which types different from the predominant pattern found in the water were found. In addition, the public announcement of an outbreak likely led to an increase in laboratory testing by concerned patients and physicians, which may have resulted in isolation of additional diverse *E. coli* O157:H7 strains from patients not involved in the outbreak. Conceivably, a combination of the above factors could explain the presence of the few other PFGE types found in the patient isolates.

There are some caveats to the genetic analysis of these isolates. Isolates from patients were obtained from a local laboratory (the isolates were presumably from a single colony) and sent to the Wadsworth Center on agar slants. Unlike the environmental samples, from which independently isolated colonies were typed by PFGE, the patient isolates were only typed from one original colony. Thus, there may have been genetic variation in additional patient isolates tested that we were unable to detect because only single colonies had been sent for analysis. Of the 117 *E. coli* O157:H7 isolates cultured from patient specimens, 96.6% were related to the major type 1 pattern (45). Testing with another enzyme confirmed that various subtypes of pattern 1 were genetically related (data not shown). Furthermore, the predominant PFGE patterns, 1 and 1a, have rarely been detected by our laboratory, suggesting that this outbreak originated primarily from a single genetically related strain. Studies have demonstrated that multiple subtypes can be shed from both bovine and human infections (2, 3, 10, 28). Although this is often explained by a loss of either the 60-MDa plasmid or the bacteriophage harboring the Shiga toxin genes, the exact mechanisms involved are unclear. Unfortunately, outbreak analysis often focuses on isolation of *E. coli* O157:H7 from a patient sample, followed by PFGE of a single colony. This complex outbreak may have benefited from PFGE typing of additional patient isolates much in the same way the environmental isolate typing resulted in demonstration of multiple subtypes from the same source.

The occurrence of 29 concurrent *Campylobacter* cases with isolates having a single PFGE type also suggests primary contamination from one source, with some secondary contamination from other sources. The presence of a predominant PFGE type in 83% of the *C. jejuni* isolates that were available for PFGE testing places this outbreak as one of the few outbreaks in which a predominant *C. jejuni* clone has been demonstrated. Our laboratory and others have demonstrated broad heterogeneity among PFGE types from *Campylobacter* isolates, which is believed to be related to genetic instability of the organism (20, 49, 50). Therefore, the finding of essentially one PFGE type suggests a single source of contamination, with the other types resulting from rare exogenous sources or genetic instability. Because we were unable to culture *C. jejuni* from the water samples, we were unable to definitively link the *C. jejuni* infections to the water. However, the predominance of a single type suggests a massive contamination from a single source.

In summary, since Shiga toxins are important virulence factors of *E. coli* O157:H7, the initial PCR screening for stx_1 and stx_2 was an effective method for identifying samples to be processed for culture isolation and fingerprinting. Successful isolation was accomplished using IMS subculture and selective culture medium. Furthermore, we demonstrated the relatedness of isolates from patients and water by PFGE fingerprinting, thereby implicating the water distribution system as the main source of exposure in this large outbreak of *E. coli* O157:H7 infection. The presence of multiple PFGE types among patient and environment isolates demonstrates the importance of testing multiple isolates and of using a discriminating fingerprinting technique during an outbreak investigation.

ACKNOWLEDGMENTS

We gratefully acknowledge the New York State Department of Health, the regional laboratory partners, and the Centers for Disease Control and Prevention and the contributions of numerous personnel from these institutions who were involved in the activities associated with this outbreak.

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