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Molecular epidemiology of a fast-food restaurant-associated outbreak of Escherichia coli O157:H7 in Washington State

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Abstract

We studied the molecular epidemiology of the recent fast-food restaurant chain-associated Escherichia coli O157:H7 outbreak in Washington State. Genomic DNAs prepared from strains isolated from 433 patients were probed with radiolabelled Shiga-like toxin (SLT) I and SLT II genes and bacteriophage l DNA and were subsequently analyzed for their restriction fragment length polymorphism (RFLP) patterns. The SLT RFLP and l RFLP profiles of an E. coli O157:H7 strain isolated from the incriminated beef and prototype patient were compared with those of the patient isolates for determination of the concordance between patterns. Of the 377 patients with primary and secondary cases of infection epidemiologically linked to the outbreak, isolates from 367 (97.3%) of the patients displayed SLT RFLP and l RFLP profiles identical to those of the outbreak strains. Isolates from 10 of the 377 (2.6%) patients possessed SLT RFLP and l RFLP profiles different from those of the outbreak strains, and the patients from whom those isolates were obtained were subsequently characterized as having non-outbreak-related infections. The E. coli O157:H7 strains isolated from 31 of 44 (70.4%) patients who were epidemiologically excluded from the outbreak were linked to the outbreak by RFLP typing. Our results indicate that SLT RFLP and l RFLP analyses are stable and sensitive methods, and when they are used in conjunction with an epidemiological investigation they could result in an earlier recognition of outbreaks and their sources, hence prompting measures to prevent the continued transmission of E. coli O157:H7.

Keywords

h7, molecular, fast, food, washington, restaurant, state, associated, outbreak, coli, o157, escherichia, epidemiology

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Molecular Epidemiology of a Fast-Food Restaurant-Associated Outbreak of *Escherichia coli* O157:H7 in Washington State

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We studied the molecular epidemiology of the recent fast-food restaurant chain-associated *Escherichia coli* O157:H7 outbreak in Washington State. Genomic DNAs prepared from strains isolated from 433 patients were probed with radiolabelled Shiga-like toxin (SLT) I and SLT II genes and bacteriophage λ DNA and were subsequently analyzed for their restriction fragment length polymorphism (RFLP) patterns. The SLT RFLP and λ RFLP profiles of an *E. coli* O157:H7 strain isolated from the incriminated beef and prototype patient were compared with those of the patient isolates for determination of the concordance between patterns. Of the 377 patients with primary and secondary cases of infection epidemiologically linked to the outbreak, isolates from 367 (97.3%) of the patients displayed SLT RFLP and λ RFLP profiles identical to those of the outbreak strains. Isolates from 10 of the 377 (2.6%) patients possessed SLT RFLP and λ RFLP profiles different from those of the outbreak strains, and the patients from whom those isolates were obtained were subsequently characterized as having non-outbreak-related infections. The *E. coli* O157:H7 strains isolated from 31 of 44 (70.4%) patients who were epidemiologically excluded from the outbreak were linked to the outbreak by RFLP typing. Our results indicate that SLT RFLP and λ RFLP analyses are stable and sensitive methods, and when they are used in conjunction with an epidemiological investigation they could result in an earlier recognition of outbreaks and their sources, hence prompting measures to prevent the continued transmission of *E. coli* O157:H7.

Escherichia coli O157:H7 has recently emerged as an important food-borne pathogen (4). *E. coli* O157:H7 produces cytotoxins known as Shiga-like toxin I (SLT I) and SLT II (also called verocytotoxin I and II) and a number of SLT variants (9). SLT I is nearly identical in its structure and function to Shiga toxin, the cytotoxin produced by *Shigella dysenteriae* type I. SLT II is approximately 60% homologous to SLT I (3).

E. coli O157:H7 was first associated with human illness in 1982, when *E. coli* of this serotype was isolated from the stools of patients in two food-borne outbreaks associated with the consumption of poorly cooked hamburgers (11). This *E. coli* clone has also been found in the stools of children with hemolytic-uremic syndrome (5). Infection with *E. coli* O157:H7 is associated with a spectrum of symptoms which include diarrhea, hemorrhagic colitis, and hemolytic-uremic syndrome (5).

Recently, a massive food-borne outbreak caused by *E. coli* O157:H7 was recognized in the western United States (1). The outbreak, which was linked to the consumption of undercooked hamburger in multiple outlets of a fast-food chain restaurant (chain A), included more than 500 documented cases of infection and three deaths (1).

We examined the molecular epidemiology of the recent outbreak in Washington State by analyzing a collection of 583 *E. coli* O157:H7 strains isolated from 433 patients in whom the beginning of the onset of symptoms ranged from November 1992 to March 1993. Such a large collection of strains provided the opportunity to perform a detailed analysis of the molecular epidemiology of the outbreak strains, to compare the SLT restriction fragment length polymorphism (RFLP) and bacteriophage λ DNA RFLP data with the results of the classical epidemiological investigation, and to assess the concordance between the patterns generated by probing with radiolabelled SLT I and SLT II toxin genes (SLT RFLP) and λ DNA (λ RFLP). We previously used bacteriophage λ RFLP to study a subset of the cases from the outbreak (13).

MATERIALS AND METHODS

Bacterial cultures and culture conditions. *E. coli* O157:H7 strains isolated from patients and from incriminated beef were obtained from the Washington State Department of Health.

DNA isolation and restriction endonuclease digestion. Bacterial cultures were streaked onto MacConkey sorbitol agar plates, and the plates were incubated overnight at 37°C. Bacterial cells were harvested from the agar plates and were suspended in 800 μl of 50 mM Tris HCl (pH 8.0)–50 mM EDTA. Forty-five microliters of 20% sodium dodecyl sulfate (SDS) and 10 μl of proteinase K (20 mg/ml; Pharmacia, Piscataway, N.J.) were added, and the suspension was incubated at 50°C for 60 min. DNA obtained by sequential phenol-chloroform and chloroform-isoamyl alcohol extractions was precipitated by adding 2.5 volumes of absolute ethanol, and the DNA was suspended in 50 μl of TE buffer (10 mM Tris HCl [pH 8.0], 1 mM EDTA). Four microliters (1 to 2 μg) of DNA was digested with *Pvu*II restriction endonuclease overnight at 37°C.

Agarose gel electrophoresis, λ DNA probe, SLT probes, and Southern hybridization. Following digestion, the DNA fragments were electrophoretically separated in 0.8% agarose in Tris-borate-EDTA. The DNA fragments were transferred to Nytran (Schleicher & Schuell, Keene, N.H.), baked, and probed with α-^{32}P-labeled SLT I and SLT II gene fragments (1, 142-bp *Taq*I-*Hin*cII fragments of parts of the SLT IA and SLT IB genes and 842-bp *Sma*I-*Pst*I fragments of the SLT IIA gene, respectively) (8) or bacteriophage λ DNA (12, 13). The blots were hybridized at room temperature in 5× SSC (1× SSC is 150 mM NaCl plus 15 mM sodium citrate)–0.1% SDS–1 mM EDTA–50% (SLT probes) or 25%

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TABLE 1. Epidemiology for Groups 1 to 7

Group no.	Primary exposure[a]	Secondary exposure[b]	Onset[c]	Incubation period[d]
1	+	−	+	+
2	−	+	+	+
3	+	−	+	−
4	−	−	+	NA[e]
5	+	No info[f]	−	+
6	−	No info	−	NA
7	No info	No info	No info	No info

[a] +, patients who ate at a chain A restaurant.
[b] +, patients who did not eat at a chain A restaurant but who had contact with symptomatic individuals who ate at a chain A restaurant.
[c] +, an onset of symptoms from 1 January 1993 to 28 February 1993.
[d] +, onset of symptoms within 10 days of eating at a chain A restaurant or having had contact with a symptomatic individual who ate at a chain A restaurant or a symptomatic individual who did not eat at a chain A restaurant but who had a close contact with a symptomatic individual who ate at a chain A restaurant within 10 days of developing symptoms.
[e] NA, not applicable.
[f] No info, no information.

(λ probe) formamide overnight. The blots were washed twice in 2× SSC–0.1% SDS at 65°C (SLT I and SLT II probes) or 50°C (λ probe) for 30 min per wash, air dried, and exposed to X-ray film (Kodak, Rochester, N.Y.) overnight at −70°C with an intensifying screen.

Epidemiological classification. The patients were divided into groups on the basis of their epidemiological history, which was derived from a standardized questionnaire (1). Although a subsequent epidemiological investigation showed that the outbreak began before 1 January 1993, for the purposes of the present study we used the working definition of the outbreak at the time that the study was conducted. A patient with diarrhea and positive stool culture for *E. coli* O157:H7 or postdiarrheal hemolytic-uremic syndrome with the onset of symptoms from 1 January 1993 to 28 February 1993 was defined as having a case of infection. Cases of infection were considered to be primary if the onset of symptoms occurred up to 10 days after eating at a chain A restaurant between 1 January 1993 and 28 February 1993. Patients who did not eat at a chain A restaurant but who had household or close contact with a patient with a primary case of infection, with the onset of symptoms occurring within 10 days of the contact, were defined as having secondary cases of infection. Patients who did not fit the definitions for primary or secondary cases of infection were arranged into five other groups on the basis of the available epidemiological information, including eating at a chain A restaurant, contact with a patient with an identified case of infection, time of symptom onset, and incubation period (Table 1).

RESULTS AND DISCUSSION

Four hundred thirty-three patients were identified as having had a culture-confirmed *E. coli* O157:H7 infection between 1 January 1993 and 28 February 1993, and the isolates from these patients were submitted to the Washington State Department of Health. Five hundred eighty-three *E. coli* O157:H7 strains isolated from these 433 patients were evaluated for their SLT RFLP and λ RFLP patterns. The SLT RFLP and λ RFLP patterns of the patient isolates were compared with the SLT RFLP and λ RFLP patterns of an *E. coli* O157:H7 strain isolated from the incriminated beef and from a patient whose isolate was designated the prototype strain. The SLT RFLP profiles of the patient prototype isolate and incriminated beef isolate are displayed in Fig. 1, lanes 10 and 11, respectively. The SLT RFLP patterns of the clinical isolates which were different from the outbreak strains are displayed in Fig. 1, lanes 1 to 9. Results of SLT RFLP and λ RFLP typing are presented in Table 2.

On the basis of the data from the epidemiological investigation, patients with *E. coli* O157:H7 infections were divided into seven groups. Groups 1 and 2 included all patients who met the criteria of the original outbreak case definition (2). Groups 3 through 7 consisted of patients who were excluded from the outbreak case definition because of (i) an incubation period longer than 10 days after known exposure, (ii) lack of known exposure to the outbreak strain, (iii) an onset of symptoms outside the appropriate outbreak period, or (iv) inadequate epidemiological information.

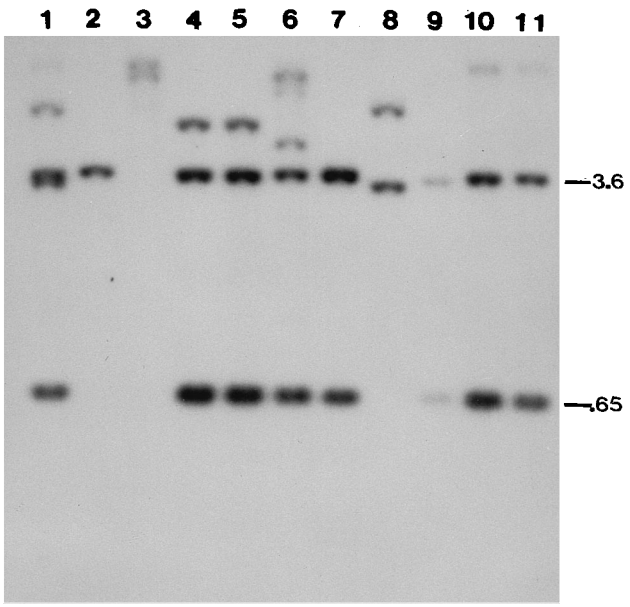

FIG. 1. SLT I RFLP and SLT II RFLP profiles of genomic DNAs isolated from patients with *E. coli* O157:H7 infection during the outbreak period, from the incriminated beef (lane 10), and from the prototype patient (lane 11). Eight SLT RFLP patterns were found among patient isolates (lanes 1 to 9: in lanes 1, 2, 3, 4 and 5, 6, 7, 8, and 9, respectively). The strains whose patterns are shown in lanes 4 and 5 were shown to be different from each other by λ RFLP.

Group 1 consisted of 338 patients with primary cases of infection. Of the 470 strains isolated from the 338 patients in this group, 460 strains from 330 (97.6%) patients showed SLT RFLP and λ RFLP patterns identical to those of the outbreak strains (patient prototype strain and the beef isolate) (Table 2). Of these 330 patients, from two to six independent *E. coli* O157:H7 isolates were recovered from the stool samples of 83 of them. The SLT RFLP and λ RFLP patterns of all of the multiple isolates from these patients matched.

From each of two patients in group 1, two different clonal types of *E. coli* O157:H7 strains were isolated from their stool

TABLE 2. Number of patients and isolates recovered from patients which displayed and did not display patterns of prototype outbreak strains

Group no.	No. of patients (% of total)			
	Patients infected with prototype outbreak strain	Isolates with pattern of prototype outbreak strain	Patients infected with non-prototype outbreak strain	Isolates without pattern of prototype outbreak strain
1	330 (97.6)[a]	460 (97.9)	8 (2.4)	10 (2.1)
2	37 (94.8)	51 (96.2)	2 (5.1)	2 (3.8)
3	5 (100)	6 (100)	0 (0)	0 (0)
4	20 (76.9)	20 (74.1)	6 (23.1)	7 (25.9)
5	3 (75.0)	3 (75.0)	1 (25.0)	1 (25.0)
6	3 (33.3)	3 (33.3)	6 (66.7)	6 (66.7)
7	12 (100)	14 (100)	0 (0)	0 (0)

[a] Two or more isolates were recovered from two patients displayed and did not display the patterns of the prototype outbreak strain.

samples; one of these strains was identical to the outbreak strains and the other one was different from the outbreak strains. Since the RFLP patterns of the non-outbreak strains were different from the outbreak strains in all three parameters tested (SLT I, SLT II and λ), it is unlikely that the change in patterns can be attributed to recombination, mutation, or the loss of a phage. It is also unlikely that the contaminated beef carried more than one clone of *E. coli* O157:H7. Had there been more than one clone, we would have expected to isolate these clones from other patients associated with the outbreak. Three possible explanations could account for the recovery of two different *E. coli* O157:H7 strains from these two patients: (i) the patients ingested *E. coli* O157:H7 strains from two different sources carrying two different strains of *E. coli* O157:H7, (ii) the second *E. coli* O157:H7 isolate from these patients is part of their normal or transient flora, or (iii) there was a laboratory error in the processing of these isolates.

Of the 338 patients in group 1, single *E. coli* O157:H7 strains were recovered from 8 (2.4%) patients; these strains displayed SLT RFLP and λ RFLP patterns different from those of outbreak strains (Table 2), suggesting that these patients contracted *E. coli* O157:H7 from a source other than the implicated beef. Of these non-outbreak strains, two appeared to be identical, although no epidemiological data were available to link the strains.

Group 2 included the 39 patients with secondary cases of infection. Fifty-three strains of *E. coli* O157:H7 were recovered from these 39 patients. Fifty-one strains isolated from 37 (94.8%) patients were identical to the outbreak strains, while each of two strains isolated from 2 (5.1%) patients were different from the outbreak strains and from each other (Table 2). The fact that these two isolates were not identical to the outbreak strain suggests that these patients were infected from a source unrelated to the outbreak.

Group 3 consisted of five patients who ate at a chain A restaurant and who developed illness during the outbreak period, but more than 10 days after eating at chain A (Table 1). There was no known exposure to a patient with an identified outbreak-related case of infection to explain the prolonged incubation times ranging from 11 to 14 days, a period greater than the 10-day criterion set for the definition of an outbreak-related case of infection. While the patients in this group were thus not linked to a patient with primary exposure to the outbreak or a patient with a secondary case of infection, SLT RFLP and λ RFLP typing profiles indicated that the six strains isolated from these five patients were identical to the outbreak strains (Table 2). Three possibilities could account for these results: (i) these patients have unrecognized secondary cases of infection, (ii) they have primary cases of infection with longer incubation periods, or (iii) their reported dates of exposure or onset are not correct. Incubation periods of longer than 10 days have been reported previously (6, 7). Differences in dose, condition of the pathogen, and host factors may account for different incubation periods among individuals.

Group 4 consisted of 26 patients who neither ate at a chain A restaurant nor had a known contact with a patient with an identified case of infection, but the onset of their symptoms occurred during the outbreak period (Table 1). Twenty-seven isolates were obtained from these 26 patients. Twenty strains isolated from 20 (76.9%) patients displayed SLT RFLP and λ RFLP patterns identical to those of the outbreak strains, and 7 strains from 6 (23.1%) patients showed patterns different from those of the outbreak strains (Table 2). It is likely that the 20 patients whose isolates were linked to the outbreak by RFLP typing had contact with a person with outbreak-related asymptomatic infection or failed to recall having eaten at a chain A restaurant.

Group 5 consists of patients who were not initially considered to be part of the outbreak because their illness began before 1 January 1993 (MMWR, 1993) (Table 1). Four strains of *E. coli* O157:H7 were isolated from four patients in this group. Three patients were infected with *E. coli* O157:H7 with SLT RFLP and λ RFLP profiles identical to those of the outbreak strains. One strain isolated from one of these four patients displayed SLT RFLP and λ RFLP profiles different from those of the outbreak strains (Table 2).

Of the three patients infected with outbreak strains in group 5, two ate at a chain A restaurant in mid-December and late November 1992, respectively. While the exposure history of the third patient is unknown, the onset of illness occurred in November 1992. Chain A representatives estimated that 4 weeks elapsed between the 19 November 1992 production date and the sale of the contaminated hamburger at retail stores in Washington State (1). It is possible that the chain A restaurants received the contaminated beef earlier than previously assumed, which would account for the recovery of the outbreak strain from the patients who ate at chain A restaurants in late 1992. However, it is unlikely that the patient with a November 1992 onset consumed a contaminated patty from the 19 November 1992 production date. Although testing of previous batches of beef patties supplied to chain A from the same distributor did not indicate contamination with *E. coli* O157:H7 (1), the possibility that previous batches of beef sent to the chain A restaurant from the same supplier were contaminated with this particular strain of *E. coli* O157:H7 cannot be excluded.

Group 6 consisted of nine patients who did not eat at a chain A restaurant and whose symptoms began before 1 January 1993 or after 28 February 1993 (Table 1). Nine strains of *E. coli* O157:H7 were recovered from these nine patients. Three strains were identical to the outbreak strains and six strains were different from the outbreak strains and from each other (Table 2). The three patients from whom outbreak strains were recovered became ill in March 1993. While there is no epidemiological information to link these patients to a primary or secondary source of infection, it is possible that they represent tertiary cases of infection. Although we have not yet found a match between the outbreak strain and isolates from subsequently tested patients in the region with sporadic cases of *E. coli* O157:H7 infection (300 isolates tested), we cannot exclude the possibility that this particular *E. coli* O157:H7 clone was introduced through other sources of contaminated beef or from a different vehicle altogether.

Group 7 consisted of 12 patients for which no epidemiological information regarding exposure was available (Table 1). The 14 strains isolated from these 12 patients were identical to the outbreak strains (Table 2).

The 1992 and 1993 *E. coli* O157:H7 outbreak in Washington State was linked to the consumption of undercooked beef associated with a fast-food restaurant chain. The primary and secondary cases of infection were epidemiologically linked to the outbreak, and SLT RFLP and λ RFLP typing results confirmed this for 367 of 377 (97.3%) patients. The isolates from 10 of 377 (2.6%) patients with primary and secondary cases of infection were different from the outbreak strains by SLT RFLP and λ RFLP typing, suggesting that they acquired *E. coli* O157:H7 from another source.

Patients in groups 3 through 6 did not meet the case definition because of the lack of one or more appropriate outbreak-related criteria. However, SLT RFLP and λ RFLP analyses suggest that 31 of the 44 (70.4%) patients in these groups were in fact part of the outbreak. The incubation period in 5 of

the 31 (16.1%) patients was longer than the 10 days specified in the case definition. Since these patients fit all other case definition criteria and their isolates were linked to the outbreak by SLT RFLP and λ RFLP typing, it is suggested that future studies reevaluate the incubation period for *E. coli* O157:H7 infection. Twenty-six of 39 (66.7%) patients who were epidemiologically excluded from the outbreak because of the lack of primary or secondary exposure and/or onset times longer than the 10 days specified in the case definition were linked to the outbreak by SLT RFLP and λ RFLP typing.

Our results demonstrate the stability of the target sequences (SLT genes and SLT-converting phages). Multiple strains (two to six) were isolated from 85 patients and were then typed after three to six passages in culture and 1 year in storage. Of the 85 patients from whom multiple strains were isolated, the pairs of strains from 83 (97.6%) of the patients had the same SLT RFLP and λ RFLP profiles. Our results also indicate that even after the large-scale introduction of a new clone of *E. coli* O157:H7 into the area, the clone does not persist in the human population after the incriminated vehicle is removed. Since the 1993 outbreak, we have not identified this particular clone of *E. coli* O157:H7 in this region among more than 300 isolates tested.

SLT RFLP and λ RFLP analyses appear to be very sensitive and specific methods for the interstrain differentiation of *E. coli* O157:H7 and could assist in the epidemiological investigation of outbreaks and sporadic cases of infection caused by this organism. The methods use standard techniques of molecular genetic analysis which are rapid (we can currently provide results in 2 to 3 days) and reasonably easy to perform. We believe that molecular linkage analysis by SLT RFLP or λ RFLP analysis in conjunction with active surveillance and reporting systems (10) could be useful for the detection of clusters of cases of *E. coli* O157:H7 infection. We propose that the routine usage of these typing methods for *E. coli* O157:H7 be implemented in a national reference center for the rapid detection of evolving outbreaks. Early detection of outbreaks will avert continued transmission of *E. coli* O157:H7. Had a national reference center been functioning at the time of this particular outbreak, the infections in November and December 1992 in California, Idaho, Nevada, and Washington could have been linked to the source earlier. This knowledge might have resulted in measures which could have halted subsequent cases of disease.

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