Molecular epidemiology of escherichia coli 0157:H7 strains by bacteriophage a restriction fragment length polymorphism analysis: Application to a multistate foodborne outbreak and a day-care center cluster

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Abstract
Genomic DNAs prepared from 168 isolates of Escherichia coli 0157:H7 were analyzed for restriction fragment length polymorphisms on Southern blots probed with bacteriophage A DNA. The isolates analyzed included strains from a recent large multistate outbreak of E. coli 0157:H7 infection associated with consumption of poorly cooked beef in restaurants, a day-care center cluster, and temporally and geographically unrelated isolates. E. coli 0157:H7 isolates recovered from the incriminated meat and from 61 (96.8%) of 63 patients from Washington and Nevada possessed identical A restriction fragment length patterns. The A restriction fragment length polymorphisms observed in 11 (91.7%) of 12 day-care center patients were identical, but they differed from that of the strain associated with the multistate outbreak. E. coli 0157:H7 from 42 patients temporally or geographically unrelated to either cluster of infection possessed unique and different A restriction fragment length patterns, except for paired isolates from three separate clusters of infection. These data demonstrate that the hybridization of DNA digests of E. coli 0157:H7 with radiolabelled bacteriophage K DNA can be a useful, stable, and discriminatory epidemiologic tool for analyzing the linkage between strains of E. coli 0157:H7.

Keywords
center, application, analysis, polymorphism, length, fragment, restriction, l, bacteriophage, strains, h7, 0157, coli, e, epidemiology, molecular, care, day, outbreak, foodborne, cluster, multistate

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Molecular Epidemiology of *Escherichia coli* O157:H7 Strains by Bacteriophage \( \lambda \) Restriction Fragment Length Polymorphism Analysis: Application to a Multistate Foodborne Outbreak and a Day-Care Center Cluster

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Genomic DNAs prepared from 168 isolates of *Escherichia coli* O157:H7 were analyzed for restriction fragment length polymorphisms on Southern blots probed with bacteriophage \( \lambda \) DNA. The isolates analyzed included strains from a recent large multistate outbreak of *E. coli* O157:H7 infection associated with consumption of poorly cooked beef in restaurants, a day-care center cluster, and temporally and geographically unrelated isolates. *E. coli* O157:H7 isolates recovered from the incriminated meat and from 61 (96.8%) of 63 patients from Washington and Nevada possessed identical \( \lambda \) restriction fragment length patterns. The \( \lambda \) restriction fragment length polymorphisms observed in 11 (91.7%) of 12 day-care center patients were identical, but they differed from that of the strain associated with the multistate outbreak. *E. coli* O157:H7 from 42 patients temporally or geographically unrelated to either cluster of infection possessed unique and different \( \lambda \) restriction fragment length patterns, except for paired isolates from three separate clusters of infection. These data demonstrate that the hybridization of DNA digests of *E. coli* O157:H7 with radiolabelled bacteriophage \( \lambda \) DNA can be a useful, stable, and discriminatory epidemiologic tool for analyzing the linkage between strains of *E. coli* O157:H7.

*Escherichia coli* O157:H7, an important and common human enteric pathogen (6, 7, 15), causes diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome. *E. coli* O157:H7 is among the most frequently isolated pathogens in North American microbiology laboratories which routinely seek its presence (5, 8, 10, 18).

Different isolates of *E. coli* O157:H7 possess relatively few characteristics with which one strain can be reliably distinguished from another. Characteristics which have been used to differentiate strains have included Shiga-like toxin (SLT) genotypes (11, 13, 19), plasmid profiles (13, 20), restriction digests of plasmids (20), electrophoretic types (21), restriction fragment length polymorphisms (RFLPs) generated by pulse-field gel electrophoresis (2), bacteriophage typing (1), and antibiotic susceptibilities (17). Each of these techniques assigns *E. coli* O157:H7 strains to a limited number of subtypes or is performed in only a few reference laboratories.

Recently, we studied the restriction fragment pattern generated by digesting *E. coli* O157:H7 genomic DNA and probing with radiolabelled \( \lambda \) DNA. The \( \lambda \) RFLP pattern identifies multiple subtypes of *E. coli* O157:H7 and discriminates among *E. coli* O157:H7 isolates better than does probing such digests with radiolabelled rRNA or toxin genes (16). Plasmid profiles and toxin genotypes are also less discriminatory than \( \lambda \) RFLP analysis of restriction fragments (14). However, the validity and utility of \( \lambda \) RFLP in an epidemic setting have not yet been established.

The largest known outbreak of *E. coli* O157:H7 infection to date occurred recently in Washington and other western states and was associated with the consumption of inadequately cooked ground beef at multiple outlets of a restaurant chain (3). The multistate outbreak strain contained genes encoding SLTs I and II, which is the most common SLT genotype (13, 19) in *E. coli* O157:H7 isolated from patients in the state of Washington, and was susceptible to a wide variety of antibiotics. *E. coli* O157:H7 was isolated from cultures of stool from multiple patients with primary and secondary infections and was also recovered from the incriminated ground beef served at the restaurants. The availability of multiple epidemiologically linked clinical isolates of *E. coli* O157:H7, including the organism isolated from the putative vehicle of transmission, provides an opportunity to study the genetic relatedness between strains by \( \lambda \) RFLP, to differentiate these isolates from epidemiologically nonrelated strains of *E. coli* O157:H7 from Washington State and elsewhere, and to assess the stability of the restriction fragment pattern as the same organism infects, and is recovered from, multiple people. Additionally, isolates from an unrelated day-care center cluster were subjected to \( \lambda \) RFLP analysis to determine the applicability of this technique to a second outbreak.

**MATERIALS AND METHODS**

**Bacterial cultures and culture conditions.** *E. coli* O157:H7 strains from patients involved in the Seattle outbreak were isolated from stools submitted for bacterial culture to the microbiology laboratory of the Children’s Hospital and Medical Center in Seattle or from strains provided to the Washington State Department of Health. The Washington State Department of Health also provided three strains of *E. coli* O157:H7 recovered from the incriminated ground beef.
E. coli O157:H7 strains from Nevada, linked to the same multistate outbreak as the one in Washington, and from an unrelated, earlier (August 1992) cluster in a Nevada day-care center were provided by the Bureau of Laboratory Services, Health Division, Nevada State Department of Human Resources. E. coli O157:H7 from previous years were initially isolated at the Children’s Hospital and Medical Center or were provided to the State of Washington and have been stored in LB-15% glycerol at -70°C (19). Additional strains were obtained from the Children’s Hospital of Philadelphia and the Province of British Columbia Center for Disease Control. All strains were initially identified by streaking stool cultures onto sorbitol-MacConkey agar and testing sorbitol-nonfermenting colonies for the presence of the O157 and H7 antigens by serologic techniques.

Isolation of bacterial DNA and restriction endonuclease digestion. DNA was isolated by a modification of a previously described method (16). Confluent bacterial cells on agar plates were scraped with several sweeps of a sterile flat-headed toothpick and were suspended in 0.8 ml of 50 mM Tris (pH 8.0) containing 50 mM EDTA. In sequence, 45 μl of 20% sodium dodecyl sulfate (SDS) and 10 μl of proteinase K (20 mg/ml; Pharmacia, Piscataway, N.J.) were added to this suspension, which was then incubated at 50°C for 15 min. The DNA was subjected to phenol-chloroform and chloroform-isooamyl alcohol extraction. DNA was precipitated by the addition of 2.5 volumes of absolute ethanol at room temperature, removed by pooling on a glass capillary pipette, and suspended in 50 μl of TE (10 mM Tris [pH 8.0], 1 mM EDTA). Four microliters of each DNA preparation was used for restriction enzyme digestions with EcoRI, HindIII, PstI, or PvuII (Bethesda Research Laboratories, Bethesda, Md.), which were performed according to instructions of the manufacturer.

Preparation of λ probe and Southern hybridization. Following digestion and electrophoretic separation in 0.8% agarose in Tris-borate-EDTA, DNA fragments were transferred to Nytran (Schleicher & Schuell, Keene, N.H.), baked, and probed with bacteriophage λ DNA (Bethesda Research Laboratories) which was labelled with [α-32P]dCTP by random priming (4). Hybridization was performed in 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) (9)-0.1% SDS–1 mM EDTA–5× Denhardt’s solution–25% formamide at room temperature overnight. Blots were washed twice in 2× SSC–0.1% SDS at 50°C for 20 min per wash. The blots were then air dried and exposed to X-ray film (Kodak, Rochester, N.Y.) overnight at -70°C in the presence of intensifying screens.

RESULTS

Comparison of restriction patterns demonstrated by λ RFLP probing. λ RFLP analyses were performed with EcoRI, HindIII, PstI, and PvuII on a limited number of strains in a pilot experiment. PstI and PvuII produced the most discriminating patterns. PvuII digestions were used in the present study.

λ RFLP of Washington outbreak strains. A total of 106 E. coli O157:H7 organisms were isolated from 61 patients evaluated for enteric infection during the Washington restaurant-associated outbreak at the Children’s Hospital and Medical Center in Seattle. An additional isolate was obtained from a patient from Thurston County, Wash., who was one of the earliest patients to be identified as an outbreak case. All but three strains possessed a common λ RFLP pattern. Identical λ RFLP patterns were displayed by three isolates of E. coli O157:H7 recovered from the incriminated ground beef. This common pattern among patient isolates is displayed in Fig. 1, lanes 1 to 5, 7 to 11, and 13 to 19, and Fig. 2, lanes 3 to 17. The λ RFLP pattern displayed by the E. coli O157:H7 strain isolated from the meat is shown in Fig. 2, lane 2.

Two of the three Washington State isolates with λ RFLP patterns which differed from the pattern of the outbreak strain were identical and from the same patient, and this patient’s family could not identify exposure to the incriminated vehicle or to primary cases (Fig. 2, lane 18). A third isolate from a patient in Washington State possessed a λ RFLP pattern which differed from the pattern of the multistate outbreak strain, but in this case, an exposure to the incriminated meat was reported. The λ RFLP pattern of this strain is shown in Fig. 1, lane 6.

λ RFLP of Nevada day-care center and multistate outbreak strains. Sixteen strains of E. coli O157:H7 were obtained from the Nevada State Health Department. Four strains were isolated from two patients who claimed exposure to ground beef served at Nevada outlets of the same restaurant chain implicated in the Washington State outbreak. The λ RFLP patterns of each of three strains isolated from one patient were identical, but this pattern was different from the λ RFLP pattern observed in the outbreak strains from Washington State. The single isolate studied from the second patient in Nevada with a claimed exposure to the incriminated ground beef had a λ RFLP pattern identical to the λ RFLP pattern of the multistate outbreak strain. The remaining 12 strains from Nevada were from 12 children in a day-care center cluster of infection which occurred in August 1992. Eleven of the 12 strains displayed the same λ RFLP patterns, while the λ RFLP pattern of 1 strain was quite different (data not shown). These λ RFLP patterns differed from the pattern displayed by the common multistate outbreak strain.

λ RFLP of strains recovered from unrelated cases. None of 42 E. coli O157:H7 strains from geographically or temporally unrelated sporadic (n = 36) or clustered (n = 6) cases in Philadelphia, Washington, British Columbia, or Oregon had
FIG. 2. λ RFLP analysis of DNA of E. coli O157:H7 isolated from patients involved in the Washington State outbreak and from incriminated meat. Lane 1, λ markers digested with HindIII; lanes 2 to 12, PvuII digestion of DNAs from E. coli O157:H7 isolated from the incriminated ground beef (lane 2), a Thurston County patient (lane 3), unrelated patients in Seattle with primary infection and symptoms that began on 6 January 1993 (lane 4), 8 January 1993 (lane 5), 12 January 1993 (lane 6), 13 January 1993 (lane 7), 16 January 1993 (lane 8), 17 January 1993 (lane 9), 20 January 1993 (lane 10), 26 January 1993 (lane 11), and 28 January 1993 (lane 12). Additionally, DNAs from E. coli O157:H7 isolated from five patients with secondary infections were analyzed. The dates of onset of symptoms for these patients were 20 January 1993 (lane 13), 22 January 1993 (lane 14), 23 January 1993 (lane 15), 26 January 1993 (lane 16), and 1 February 1993 (lane 17) (patients with secondary infections whose isolates were demonstrated in lanes 13 and 14 correspond to patients with primary infections whose isolates are shown in lanes 7 and 9, respectively). E. coli O157:H7 isolated from a Washington State resident with no exposure to the incriminated vehicle or to a patient with primary infection is shown in lane 18 (date of onset of symptoms was 17 January 1993). An E. coli O157:H7 strain isolated from an Oregon resident in an unrelated outbreak in February 1993 is shown in lane 19.

FIG. 3. Southern hybridization of PvuII digestion of E. coli O157:H7 DNA probed with λ DNA. Lanes 1 to 4, randomly selected nonoutbreak strains of E. coli O157:H7 isolated from Washington State patients with hemorrhagic colitis; lane 5, the outbreak strain.

λ RFLP probing for the identification of common strains depends on the stability of the λ RFLP pattern. The availability of multiple strains from a presumably identical source in the recent multistate outbreak of E. coli O157:H7 infection in four western states (3) provides a unique opportunity to assess this stability.

Our data demonstrate that λ RFLP analysis is quite specific for the identification of putatively common strains of E. coli O157:H7 in an outbreak and can differentiate these from synchronously isolated nonoutbreak strains. The E. coli O157:H7 strains recovered from stool cultures of 61 (96.8%) of 63 Washington and Nevada patients claiming ingestion of the incriminated vehicle or contact with a patient from the multistate outbreak displayed identical λ RFLP patterns, and this pattern was identical to the λ RFLP pattern observed in the strain isolated from the incriminated ground beef. The λ RFLP patterns of four E. coli O157:H7 strains isolated from two patients with a reported exposure to the incriminated vehicle differed considerably from the pattern of the multistate outbreak strain. In one case, the single strain tested contained many fewer fragments with homology to the λ probe, and the homologous fragments had mobilities quite different from those of fragments from the multistate outbreak strains (Fig. 1, lane 6). In the three other isolates, all from the same patient in Nevada, the major bands detected had mobilities different from those detected for the bands from the multistate outbreak strain, but were identical to each other. These findings suggest that these organisms were distinct from the multistate outbreak strain, because it is unlikely that such different patterns could have

the same λ RFLP pattern as the 1993 multistate outbreak strain or the Nevada day-care center common strain (examples provided in Fig. 3). The dissimilar λ RFLP pattern of one of these isolates, recovered from a patient in Oregon in February 1993 whose infection was epidemiologically linked to the consumption of mayonnaise, is shown in Fig. 2, lane 19.

Except for three pairs of strains obtained from six different patients in clusters of E. coli O157:H7 infection in Washington State, each λ RFLP pattern was unique. As expected, the paired cluster isolates had identical λ RFLP patterns.

DISCUSSION

The ability to differentiate between bacterial isolates of the same species is crucial for epidemiologic studies. However, this ability is limited when the organisms being examined possess identical somatic and flagellar antigens and are of apparent clonal descent, as is the case with E. coli O157:H7 (21). Because the utility of previously published methods (1, 2, 11, 13, 17, 19, 20, 21) is restricted by the inability of each of these techniques to distinguish among multiple strains of E. coli O157:H7 or by technical factors, we explored the use of λ RFLP in studying two outbreaks of E. coli O157:H7 infection. λ DNA was chosen as a probe because preliminary data demonstrated its superiority to toxin genes or rRNA probes (16) or plasmid profiles (14). However, the validity of

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arisen by bacteriophage induction or integration at a different site. A single additional distinct \( \lambda \) RFLP pattern was discerned in a patient from Washington State from whom \( E. \ coli \) O157:H7 was isolated and who claimed no known contact with the incriminated vehicle or an infected patient (Fig. 2, lane 18). Therefore, we conclude that the \( \lambda \) RFLP pattern is sufficiently stable for identifying epidemiologically linked cases.

The data presented above also confirm the ability of \( \lambda \) RFLP probing to distinguish among \( E. \ coli \) O157:H7 isolates of diverse origin. As negative controls in the present study, we studied 42 \( E. \ coli \) O157:H7 strains which were geographically or temporally unrelated to either the multistate outbreak or the Nevada day-care center cluster of infection. These 42 strains possessed 39 unique \( \lambda \) RFLP patterns. The three pairs of common \( \lambda \) RFLP patterns were epidemiologically linked as either residential or day-care contacts. These data demonstrate that the stability of the \( \lambda \) RFLP pattern is seen in additional strains of \( E. \ coli \) O157:H7.

Of the 12 strains of \( E. \ coli \) O157:H7 isolated from patients attending a day-care center in Nevada, 11 possessed identical \( \lambda \) RFLP patterns which did not resemble the \( \lambda \) RFLP pattern of the multistate outbreak strain. The single distinct \( \lambda \) RFLP pattern was quite different from the pattern displayed by the other strains isolated from day-care center attendees. The recovery of strains with discordant \( \lambda \) RFLP patterns from this day-care center patient and from single patients in Washington and Nevada suggests that some patients with epidemiologic associations with point source infections with \( E. \ coli \) O157:H7 may have acquired their infecting strains elsewhere.

The SLTs I and II produced by \( E. \ coli \) O157:H7 are encoded by genes on lambdoid bacteriophage \( \lambda \). The \( \lambda \) RFLP patterns that we presented in this report suggest that DNA sequences related to these lambdoid bacteriophage genes are quite variable. The origin of this variability remains speculative. It is unlikely that multiple different lambda bacteriophages separately infected different host strains of \( E. \ coli \) O157:H7. Rather, it is more plausible that an \( E. \ coli \) O157:H7 progenitor was infected with one or more SLT-encoding lambdoid bacteriophages and that the bacteriophage DNA has undergone subsequent evolution in the progeny of this initially infected strain. This evolution could be driven by recombination with other lambdoid bacteriophages that coinfect \( E. \ coli \) O157:H7 isolates during passage through animal and human hosts. However, our data suggest that the evolution and mutation of bacteriophage-related sequences in \( E. \ coli \) O157:H7 are not so rapid as to interfere with the ability of \( \lambda \) RFLP analysis to identify the outbreak-related strains of this pathogen.

\( \lambda \) RFLP probing of \( E. \ coli \) O157:H7 may have valuable epidemiologic applications. Most \( E. \ coli \) O157:H7 infections are sporadic, and the origin of infection is unknown. Cattle carry \( E. \ coli \) O157:H7, and it might be possible to use this technique to trace strains through the food supply. Additionally, apparently unrelated cases of \( E. \ coli \) O157:H7 infection might be investigated and identified as having originated from the same source. In this regard, synchronous infections with \( E. \ coli \) O157:H7 can occur in general populations (12), and this technique might be used to establish or refute the possibility of linkage between cases of infection. Finally, by using multiple restriction endonucleases, a sample of the base composition of the chromosome of \( E. \ coli \) O157:H7 can be obtained, albeit limited to integrated \( \lambda \) bacteriophage and the region surrounding the site(s) of integration. This sample can be used as a tool to discern the relatedness between strains of \( E. \ coli \) O157:H7, thereby providing an estimation of the genetic distances between strains of this serotype. Because \( E. \ coli \) O157:H7 isolates are so closely related to each other, it has not been possible to use phenotype analysis (multifocal enzyme patterns) to gauge these distances (21).

In summary, we demonstrated that the hybridization of restriction digests of multiple unrelated isolates of \( E. \ coli \) O157:H7 with radiolabelled bacteriophage \( \lambda \) DNA provides multiple differentiating patterns and that these patterns are stable between strains derived from multiple patients in a large multistate outbreak and a smaller day-care center cluster. This reproducible, stable, and discriminatory method is therefore useful for the epidemiologic analysis of strains of \( E. \ coli \) O157:H7 and can be extended to sporadic cases and possibly to phylogenetic studies of this important human pathogen.

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