Use of Pulsed-Field Gel Electrophoresis for Epidemiological Study of Escherichia coli O157:H7 during a Food-Borne Outbreak

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Food and patient isolates from an Escherichia coli O157:H7 outbreak associated with undercooked ground beef were characterized by pulsed-field gel electrophoresis and Shiga-like toxin genotype. Pulsed-field gel electrophoresis confirmed the epidemiologically implicated source of the two-state outbreak and differentiated between outbreak and sporadic strains.

Escherichia coli O157:H7 is an important food-borne pathogen in North America and Europe. Pathogenicity is associated with the production of one or more Shiga-like toxins (SLT-I, SLT-II, or both) (8, 13). Most food-borne outbreaks, as well as sporadic cases of gastrointestinal illness, have been linked epidemiologically to undercooked ground beef (6). Other food and water sources, as well as person-to-person transmission, have also been documented (3, 5, 6, 9, 10).

In January 1994, the state health departments of Washington and Oregon began a joint epidemiological investigation of a two-state outbreak of E. coli O157:H7 infections. There were 33 culture-confirmed cases of E. coli O157:H7 infection, with onset from mid-January to mid-March 1994, in the two states. Using a recently improved method (15) for detection of E. coli O157:H7 in food, we analyzed 10 samples of raw or undercooked ground beef collected from the homes of six patients with culture-confirmed E. coli O157:H7 illness. Using SLT gene profiles and pulsed-field gel electrophoresis (PFGE) patterns, we then compared the E. coli O157:H7 strains isolated from the ground beef with isolates obtained from patients by the respective state health laboratories. The purposes of this study were to confirm the epidemiologically implicated source of the two-state E. coli O157:H7 outbreak and to differentiate unrelated sporadic strains from outbreak strains.

A total of 39 E. coli O157:H7 strains were analyzed: 26 patient isolates, 12 food-borne isolates from seven positive ground beef samples, and one control strain, EDL 933 (Table 1). The PCR method (15) was used to assess the presence of SLT-I and SLT-II genes in the E. coli O157:H7 isolates. PFGE was performed by modification of the method of Böhm and Karch (4). Briefly, isolates were grown in tryptic soy broth with 0.6% yeast extract at 35°C on a rotator to an optical density of 600 nm of 0.8 to 1.0. The cells were washed and resuspended in 1 M NaCl–10 mM Tris–50 mM EDTA, mixed with an equal volume of 1% low-melting-point preparative-grade agarose (Bio-Rad Laboratories, Hercules, Calif.), and dispensed into plug molds (Bio-Rad). After the plugs solidified, they were transferred to tubes that contained 1 mg of lysozyme per ml in 1 M NaCl–6 mM Tris–0.1 M EDTA–0.5% Sarkosyl–0.2% deoxycholate for 1 h at 37°C with gentle agitation. The solution was replaced with 1 mg of proteinase K per ml in 0.5 M EDTA–1% Sarkosyl and incubated overnight at 50°C. Plugs were then washed at room temperature with gentle agitation for two 1-h intervals in 10 mM Tris–1 mM EDTA containing 15 µl of 0.1 M phenylmethylsulfonyl fluoride per ml followed by three 1-h rinses in 10 mM Tris–50 mM EDTA. Plugs were stored in 10 mM Tris–50 mM EDTA at 4°C until used. Slices (1 to 2 mm thick) from the plugs were digested with XbaI (Sigma, St. Louis, Mo.) or SfiI (Sigma) in accordance with the manufacturer’s instructions and then transferred to a 1% agarose gel. PFGE was performed with a contour-clamped homogeneous electric field system (CHEF Mapper; Bio-Rad). For XbaI digests, a run time of 24 h with a voltage of 200 V and a linear ramped pulse time of 5 to 50 s was used. For SfiI digests, running conditions were 22 h with a voltage of 200 V and a linear ramped pulse time of 1 to 35 s. Lambda phage concatemers (New England BioLabs, Beverly, Mass.) were used as DNA size standards. Gels were photographed under UV transillumination after staining with ethidium bromide (1 µg/ml). DNA fragment patterns were examined visually. Isolates with identical restriction fragment patterns were assigned to the same group. Isolates that differed in band size or number were assigned to separate groups.

The presence of both SLT-I and SLT-II genes was confirmed by multiplex PCR in the 12 food isolates from the seven positive ground beef samples and in 24 of the 26 patient isolates, as well as in control strain EDL 933 (Table 1 and Fig. 1). Two patient isolates had the SLT-II genotype, strains SEA13A16 and SEA13A72 (Table 1 and Fig. 1).

After PFGE analysis with restriction enzyme XbaI, the 12 food isolates and 23 of the 26 patient isolates appeared to have the same banding pattern, PFGE pattern A (Table 1 and Fig. 2). PFGE pattern A contained 20 bands which ranged in molecular size from approximately 20 to 530 kb and was designated the characteristic PFGE pattern for this outbreak. All of the isolates with PFGE pattern A also had the same genotype, SLT-I and SLT-II.

The PFGE patterns of the three remaining patient isolates were distinct from the outbreak PFGE pattern and from each other. Five band differences were noted between the PFGE pattern of strain SEA 13A16 and PFGE pattern A: three missing bands at 500, 345, and 165 kb and two additional bands at 375 and 325 kb (Table 1 and Fig. 2). The PFGE pattern of strain SEA 13A55 differed from the outbreak pattern by three bands: one band missing at 250 kb and two additional bands at

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390 and 190 kb (Table 1 and Fig. 2). Strain SEA 13A72 could not be typed by PFGE because the DNA was consistently degraded and appeared as a smear on the gel (Table 1). The SLT-II genotype confirmed that this isolate was a sporadic case and was not associated with this outbreak. The control strain EDL 933 PFGE pattern varied from PFGE pattern A by four band differences: one band missing at 500 kb and three additional bands of 570, 225, and 95 kb (Table 1 and Fig. 2). Molecular weights of all PFGE bands were calculated with regression analysis against size standards run concurrently and are considered to be within 2.0% of the estimated value.

Analysis with a second restriction enzyme, SfiI, confirmed the XbaI PFGE pattern grouping of the isolates (data not shown). The PFGE patterns remained stable and reproducible when the analysis of some strains was repeated.

E. coli O157:H7 continues to be a major public health problem. The ability to differentiate between individual strains of the organism is essential for resolving epidemiologically related cases in outbreaks and for establishing a link to the causative food source. Several molecular subtyping techniques have been used to characterize strains of E. coli O157:H7 for epidemiological investigations. Phage typing (11), plasmid profile analysis (12), Shiga-like toxin genotype (12), multilocus enzyme electrophoresis (16), bacteriophage λ restriction fragment length polymorphism analysis (14), and PFGE (2, 4, 7) have been used. In our laboratory investigations, we used a new cultural isolation technique, as well as molecular subtyping by PCR and PFGE, to confirm the implicated source of an E. coli O157:H7 outbreak. PCR provided rapid identification of the Shiga-like toxin profile as a preliminary subgrouping of isolates. We used PFGE to further characterize the E. coli O157:H7 isolates by using DNA banding patterns to connect outbreak clinical isolates to the isolates from the incriminated food. These patterns were also used to distinguish patient strains involved in the outbreak from unrelated sporadic cases of E. coli O157:H7 disease.

One E. coli O157:H7 isolate in this study could not be typed by PFGE because persistent degradation of the DNA prevented resolution of the restriction fragments. We have noted the same problem with several other strains of E. coli O157:H7 (data not shown). To date, all of these isolates have been SLT-II genotype, but not all SLT-II genotype strains have been untypeable. Kristjansson et al. reported the same phenomenon with some strains of Clostridium difficile (11). This degradation of DNA during the PFGE procedure is likely due to the presence of endogenous nucleases. Modification of the method is necessary to solve this problem.

In conclusion, the results presented in this study further demonstrate the value of PFGE as an epidemiological tool for differentiating strains of E. coli O157:H7, identifying cases

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**TABLE 1. Sources, Shiga-like toxin genotypes, and PFGE patterns of E. coli O157:H7 strains**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Source</th>
<th>Genotype†</th>
<th>PFGE pattern</th>
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<tr>
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<td>B</td>
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<td>Patient 3</td>
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<td>A</td>
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<td>Patient 4</td>
<td>I</td>
<td>A</td>
</tr>
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<td>Raw ground beef</td>
<td>I + II</td>
<td>D</td>
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† I, SLT-I; II, SLT-II.

a NT, not typeable.
belonging to an outbreak, and linking isolates from incrimi-
nated food to outbreak strains. In this investigation, the tech-
nique was also of further value in that PFGE analysis was
conducted as soon as isolates became available during the
outbreak; thus, the results assisted in guiding the course of the
epidemiological study.

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