BACTERIAL CHARACTERISATION

Molecular characterisation of *Escherichia coli* O157:H7 isolates by pulsed-field gel electrophoresis and plasmid DNA analysis

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Summary. Foods of bovine origin have been identified as sources of *Escherichia coli* O157:H7. Genomic DNA of *E. coli* O157:H7 isolates from patients (six isolates), food samples (18 isolates from ground beef and six isolates from raw milk) and calf faecal samples (31 isolates) were characterised by pulsed-field gel electrophoresis (PFGE) and plasmid DNA analysis. These isolates originated from different locations in the USA during 1992 and 1993. Twenty-one distinct genomic profiles were generated from the 61 isolates by PFGE after digestion with the endonuclease *Xba*I. Four genomic profiles were identified among five patient isolates and the remaining patient isolate was not typable. Five different profiles were detected amongst the isolates from ground beef, one of which was associated with 13 ground beef isolates from an outbreak in the Pacific Northwest of the USA in 1993. The PFGE profile of five calf isolates from Washington and Wisconsin was identical to the profile of the ground beef isolates from the outbreak, suggesting that these isolates were related. Similarly, one PFGE profile accounted for three isolates from calf faeces and one from ground beef. Six raw milk isolates from Georgia were indistinguishable both from each other and from one isolate from calf faeces. Fourteen genomic profiles were identified among 31 calf faecal isolates from 18 different herds in 11 states. Only five plasmid profiles were identified among the 61 isolates. PFGE was shown to be a useful typing technique for *E. coli* O157:H7.

Introduction

Escherichia coli O157:H7 was first identified as a human pathogen in 1982. It causes several disease syndromes including diarrhoea, haemorrhagic colitis, haemolytic uraemic syndrome (HUS) and thrombotic thrombocytopenic purpura.^{1–3} The largest known outbreak of *E. coli* O157:H7 infection involved more than 500 cases and four deaths. This was associated with the consumption of undercooked ground beef sandwiches in the Pacific Northwest of the USA in 1993 and has brought both nationwide and international attention to this organism.⁴

Foods of bovine origin have been identified or suspected as the principal source implicated in outbreaks.^{1–3} Evidence suggests that in most instances the source was undercooked ground beef or, occasionally, unpasteurised milk.^{1–4} Cattle have been identified

as an important reservoir of *E. coli* O157:H7 and most bovine isolates have been obtained from apparently healthy cattle.^{1–3,5} Results of a recent study of dairy calves in 14 states in the USA revealed that 3·3 % of the animals excreted *E. coli* O157:H7 in their faeces at concentrations of 10²–10⁵ cfu/g.⁶

The epidemiology of *E. coli* O157:H7 infection at the molecular level is not well defined. Characteristics used to differentiate strains of *E. coli* O157:H7 include Shiga-like toxin genotypes,^{7–9} biochemical profiles,¹⁰ antimicrobial susceptibility,¹⁰ phage typing,^{10,11} plasmid profiles^{8–10} and bacteriophage λ -restriction fragment length polymorphism (λ -RFLP).^{9,12} Each of these techniques assigns *E. coli* O157:H7 strains to a limited number of subtypes. In the present study, plasmid analysis and pulsed-field gel electrophoresis (PFGE) were used to characterise and compare *E. coli* O157:H7 isolates from ground beef, calf faeces, raw milk and patients. The aim of the study was to determine the suitability of plasmid analysis and PFGE for epidemiological studies of *E. coli* O157:H7.

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Materials and methods

Bacterial strains

Sixty-one isolates of *E. coli* O157:H7 from different geographical locations in the USA isolated during 1992 and 1993 were tested. These included six isolates from HUS patients in Georgia, USA, 18 isolates from ground beef, six isolates from raw milk from six herds in Georgia and 31 isolates from calf faecal samples from 18 herds (table).

Verotoxin (VT) production

The type of verotoxin produced by the 61 *E. coli* O157:H7 isolates was determined by a sandwich enzyme-linked immunosorbent assay (ELISA), as described previously.⁶ Monoclonal antibodies 9C9 and BC5BB12, specific for VT-1 and VT-2 respectively, were used as the capture antibodies and rabbit polyclonal antibodies raised against each toxin individually were used as the detection antibodies.

Chromosomal analysis by PFGE

Genomic DNA of *E. coli* O157:H7 isolates was prepared by a modification of the method described by Böhm and Karch.¹³ Briefly, 0.5 ml of an overnight culture in Trypticase Soy Broth (TSB; Becton Dickinson Microbiology System, Cockeysville, MD, USA) at 37°C was transferred to 10 ml of TSB and grown at 37°C to an OD_{600nm} of 1.0. The cell suspension was then pelleted by centrifugation (14000 g, 5 min) and the pellets were washed three times with 75 mM NaCl, 25 mM EDTA, pH 7.4 (SE), and resuspended in 0.5 ml of SE. Finally, 150 µl of this suspension were mixed with 850 µl of SE and 1 ml of InCert agarose (FMC, BioProducts, Rockville, Maine, USA) 1.6% w/v in buffer consisting of 10 mM Tris, 10 mM MgCl₂, 0.1 mM EDTA, pH 7.5. The mixture was carefully dispensed into sample moulds (20 × 9 × 1.2 mm). After 10 min at 4°C, each solidified sample was cut into six plugs. Cells were lysed overnight at 56°C with vigorous agitation in 5 ml of 50 mM Tris, 50 mM EDTA, N-laurylsarcosine 1% and proteinase K 2 mg/ml, pH 8.0. On the following day, the agarose plugs were washed with 10 mM Tris, 5 mM EDTA, pH 7.5 (TE), and stored in 50 mM EDTA. Agarose-embedded DNA was digested with 50 U of *Xba*I (BRL, Gaithersburg, MD, USA) at 37°C overnight. The reaction was stopped by the addition of 20 µl of 0.5 M EDTA. Electrophoresis was performed with a contour-clamped homogeneous electric field device (CHEF DRII; BioRad Laboratories, Richmond, CA, USA) and agarose 1.2% gels in 0.5 × TBE buffer (10 × TBE is 0.89 M Tris, 0.025 M EDTA, and 0.89 M boric acid). Lambda concatamer ladder (Promega, Madison, WI, USA) was used as the size marker. After electrophoresis for 46 h at a constant voltage of 150 V, pulse times of 15–70 s with linear ramping and a temperature of 12°C, the gels

were stained with ethidium bromide and bands were visualised with UV transillumination. Each isolate was tested on at least two separate occasions.

Plasmid DNA analysis

A single bacterial colony was inoculated into 5 ml of TSB and incubated overnight at 37°C. Plasmid DNA was prepared by the alkaline lysis miniprep procedure of Lech and Brent,¹⁴ and electrophoresed in agarose 0.8% gels in 40 mM Tris-acetate, 2 mM EDTA at 100 V for 3 h. The gels were stained with ethidium bromide and bands were visualised with UV transillumination.

Results

VT production

Both VT1 and VT2 were detected in the majority of isolates (49 of 61). In the remaining 12 isolates, VT2 alone was detected (table).

Chromosomal DNA profiles by PFGE

Digestion of chromosomal DNA with *Xba*I yielded between 14 and 19 discernible bands, ranging from 30 to c. 1000 kb. Some bands (e.g., 440, 291, 150 and 125 kb) were common to all O157:H7 isolates examined. Twenty-one distinct genomic profiles were generated from the 61 isolates of *E. coli* O157:H7 and one isolate was not typable (table). The genomic fingerprints of 19 representative *E. coli* O157:H7 strains are shown in figs. 1 and 2. Comparison of genomic fingerprints revealed differences among strains of *E. coli* O157:H7 isolated from different sources and from the same source. Four different genomic profiles were identified among the six patient isolates from Georgia (fig. 1), one of which was shared by two isolates (fig. 1, lane D). A profile could not be determined for one patient isolate (isolate 58) which repeatedly appeared as a smear of degraded DNA (fig. 1, lane E). This may have been due to endogenous nuclease activity. Six raw milk isolates from six different herds in Georgia had an identical genomic profile (fig. 2, left, lane G); this profile was also given by a faecal isolate from Vermont (table). Four PFGE profiles were identified among five ground beef isolates from Oklahoma, Pennsylvania and Georgia (table), whereas only one profile was identified for the 13 ground beef isolates from the 1993 Pacific Northwest outbreak (fig. 2, left, lane F).

Restriction length polymorphisms were observed among the faecal isolates from different geographical locations. Fourteen genomic profiles were identified among 31 calf faecal isolates from 18 different dairy herds in 11 states. There was also considerable restriction length polymorphism among isolates from the same location; two calf faecal isolates from the same herd in Wisconsin had different profiles and

Table. Sources and characteristics of *E. coli* O157:H7 isolates

PFGE profile	Isolate no.	Source	Geographical location	Plasmid(s) (kb)	Verotoxin type
I	1	Raw milk	Georgia	97, 2.2	1 & 2
	2	Raw milk	Georgia	97, 2.2	1 & 2
	3	Raw milk	Georgia	97, 2.2	1 & 2
	4	Raw milk	Georgia	97, 2.2	1 & 2
	5	Raw milk	Georgia	97, 2.2	1 & 2
	6	Raw milk	Georgia	97, 2.2	1 & 2
II	44	Calf faeces	Vermont	97	1 & 2
	7	Ground beef	Washington	97	1 & 2
	8	Ground beef	Washington	97	1 & 2
	9	Ground beef	Washington	97	1 & 2
	10	Ground beef	Washington	97	1 & 2
	11	Ground beef	California	97	1 & 2
	12	Ground beef	California	97	1 & 2
	13	Ground beef	California	97	1 & 2
	14	Ground beef	California	97	1 & 2
	15	Ground beef	California	97	1 & 2
	16	Ground beef	California	97	1 & 2
	17	Ground beef	California	97	1 & 2
	18	Ground beef	California	97	1 & 2
	19	Ground beef	California	97	1 & 2
	40	Calf faeces	Washington ^a	97	1 & 2
	41	Calf faeces	Washington ^a	97	1 & 2
	42	Calf faeces	Washington ^a	97	1 & 2
	20	Calf faeces	Wisconsin ^b	97	1 & 2
	22	Calf faeces	Wisconsin	97	1 & 2
III	21	Calf faeces	Wisconsin ^b	97	2
IV	52	Ground beef	Oklahoma	97	1 & 2
V	53	Ground beef	Oklahoma	97	1 & 2
VI	54	Ground beef	Pennsylvania	97, 5	1 & 2
	55	Ground beef	Pennsylvania	97, 5	1 & 2
VII	45	Calf faeces	Ohio	97	1 & 2
VIII	23	Calf faeces	Ohio	97, 3	2
	24	Calf faeces	New York ^c	97, 3	2
	25	Calf faeces	New York ^c	97, 3	2
	26	Calf faeces	New York	97, 3	2
IX	46	Calf faeces	New York ^d	97	1 & 2
	47	Calf faeces	New York ^d	97	1 & 2
	48	Calf faeces	New York ^d	97	1 & 2
X	43	Calf faeces	New York	97	1 & 2
XI	27	Calf faeces	California ^e	97, 4	2
	28	Calf faeces	California ^e	97, 4	2
XII	30	Calf faeces	California ^f	97, 4	1 & 2
	31	Calf faeces	California ^f	97, 4	1 & 2
	32	Calf faeces	California ^f	97, 4	1 & 2
	33	Calf faeces	California ^f	97, 4	1 & 2
	34	Calf faeces	California ^f	97, 4	1 & 2
XIII	51	Calf faeces	California	97	1 & 2
XIV	36	Calf faeces	Nebraska ^g	97	2
	37	Calf faeces	Nebraska ^g	97	2
	38	Calf faeces	Maryland	97	2
	39	Ground beef	Georgia	97	2
	49	Calf faeces	Minnesota ^h	97	1 & 2
XV	50	Calf faeces	Minnesota ^h	97	1 & 2
	29	Calf faeces	Florida	97, 2.2	2
XVI	35	Calf faeces	Tennessee	97, 4	1 & 2
XVII	57	Human	Georgia	97, 2.2	1 & 2
XVIII	58	Human	Georgia	97, 2.2	1 & 2
XIX*	59	Human	Georgia	97	1 & 2
XX	60	Human	Georgia	97	1 & 2
XXI	61	Human	Georgia	97	1 & 2
XXII	62	Human	Georgia	97	1 & 2

a, b, c, d, e, f, g, h: the same letter indicates isolates from the same herd.

*Not typable.

produced different verotoxins (isolates 20 and 21) (table). However, some faecal isolates from the same location had indistinguishable restriction patterns and presumably represented a single strain that had spread between animals. Isolates with indistinguishable re-

striction endonuclease digestion patterns were found in Washington (one herd), New York (two herds), California (two herds), Nebraska (one herd) and Minnesota (one herd) (table). Five of six faecal isolates from Washington and Wisconsin had a PFGE profile

Fig. 1. Comparison of PFGE profiles of *E. coli* O157:H7 isolates from patients in Georgia (lanes A to E).

identical to the 13 ground beef isolates from the outbreak (table).

Different PFGE profiles were also obtained from isolates that produced the same verotoxins (fig. 2, left: lanes A–G, VT1 and VT2 producers; fig. 2, right: lanes B–E, VT2 only producers). However, isolates producing different verotoxins never had identical profiles.

Plasmid analysis

A total of five plasmid profiles was observed, although two profiles accounted for 77% of the isolates. A plasmid of 97 kb was present in all 61 isolates. No other plasmids were detected in 38 isolates. All six milk isolates, two human isolates and one

isolate from calf faeces had one additional plasmid of 2.2 kb. The remaining 14 isolates had one additional plasmid of 3 kb, 4 kb or 5 kb (table).

Discussion

Conventional epidemiological typing methods such as antimicrobial susceptibility profiles, biochemical profiles, serological typing, bacteriocin typing and bacteriophage typing have been useful in describing the epidemiology of many infections. However, for organisms such as *E. coli* O157:H7 that possess identical somatic and flagellar antigens and are of apparent clonal descent, simple serotype identification is insufficient to trace the source of the infecting organism.^{15,16} The ability to differentiate between bacterial isolates of the same serotype is essential, therefore, for epidemiological studies of this organism.

Plasmid profiles are useful when plasmids, preferably two or more, are present in most of the isolates examined. However, the loss, acquisition, or transfer of plasmids can create difficulties in interpretation. A particular problem is the possibility of conformational changes in plasmids. In one extract on a gel, the biologically active, closed circular supercoiled form of a plasmid can be present together with open circular and linear forms, produced from the same plasmid during isolation and purification procedures. Furthermore, plasmids of the same molecular size but with different DNA sequences are indistinguishable in a plasmid profile. When applied to the subtyping of *E. coli* O157:H7, this technique is less discriminatory than PFGE, with only five plasmid profiles compared to 22 PFGE profiles detected in the present study. Similar results have been reported by Ostroff *et al.* and Paros *et al.*^{8,9}

PFGE is a derivative of conventional DNA agarose gel electrophoresis in which the orientation of the

Fig. 2. Comparison of *Xba*I digests of genomic DNA of *E. coli* O157:H7 isolates from animals and foods. Left-hand side: lane A, PFGE profile XV; B, PFGE profile VII; C, PFGE profile IX; D, PFGE profile X; E, PFGE profile IV; F, PFGE profile II; G, PFGE profile I. Right-hand side: lane A, PFGE profile VIII; B, PFGE profile III; C, PFGE profile XI; D, PFGE profile XVI; E, PFGE profile XIV; F, PFGE profile XIII; G, PFGE profile XVII. Profiles V, VI and XII are not shown.

electric field is changed repeatedly. This enables the resolution of large DNA fragments generated by digesting the bacterial chromosome with restriction endonucleases that cleave the DNA infrequently.¹⁶ In contrast to patterns often obtained by conventional gel electrophoresis, which may be complex and difficult to compare, PFGE profiles can be easily compared. Genomic DNA fingerprinting by PFGE has been shown to be highly discriminatory for the intraspecies characterisation of a wide variety of organisms¹⁶ and has recently been applied to *E. coli* O157:H7.^{13,15,17} In the present study, there was considerable polymorphism among PFGE profiles of *E. coli* O157:H7 isolates from different geographical locations and different foods, and even among isolates within a single location. In addition, some isolates had identical PFGE profiles suggesting that these isolates were epidemiologically related. In particular, the present study showed that the profiles given by the ground beef isolates from the outbreak and the calf faecal isolates from Washington and Wisconsin were the same. An investigation by epidemiologists from the Centers for Disease Control and Prevention identified five slaughter plants in the USA and one in Canada as likely sources of carcasses used in the contaminated lots of meat in the outbreak. The animals slaughtered in USA plants were traced to farms and auction barns in six western states, including Washington.⁵ Nevertheless, with this or any other technique, the presence of what appears to be the same strain (identical profile) does not absolutely prove a direct epidemiological relationship. A more detailed epidemiological study is required to verify that a relationship exists. Interpretation of chromosomal patterns can also be difficult when isolates differ by only a few fragments. Such

differences could arise within a single strain from inversions, deletions, or other re-arrangements of the chromosome or from the acquisition or loss of a prophage, transposon, or insertion sequence. Alternatively, such differences could indicate that the isolates are distantly related. Although it is not known how to interpret small variations, an evaluation of the variation present in many different isolates from the same locality may be useful in epidemiological investigations. The possibility of in-vivo changes in phenotype or genotype is not limited to this technique and represents potential limitations of any typing scheme.

The results of this study suggest that PFGE with *Xba*I is unable to differentiate all epidemiologically independent isolates of *E. coli* O157:H7 and that some strains may not be typable by this technique. The use of other endonucleases or a combination of endonucleases may help overcome these limitations. However, the use of more than one method for characterising strains is preferable to any single technique and PFGE may be especially useful in conjunction with other molecular methods. PFGE generates a number of very diverse genomic DNA profiles and is useful for characterising *E. coli* O157:H7. A database consisting of genomic DNA profiles of *E. coli* O157:H7 strains obtained by PFGE would enhance the ability of epidemiologists to investigate outbreaks and to further characterise the role of *E. coli* O157:H7 as an inhabitant in cattle and pathogen in man, its mode of spread and the virulence of particular strains.

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References

- Doyle MP. *Escherichia coli* O157:H7 and its significance in foods. *Int J Food Microbiol* 1993; **12**: 289–301.
- Griffin PM, Tauxe RV. The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. *Epidemiol Rev* 1991; **13**: 60–98.
- Padhye NV, Doyle MP. *Escherichia coli* O157:H7: epidemiology, pathogenesis, and methods for detection in food. *J Food Prot* 1992; **55**: 555–565.
- Centers for Disease Control and Prevention. Update: multistate outbreak of *Escherichia coli* O157:H7 infections from hamburgers—western United States, 1992–1993. *Morbidity Mortal Weekly Rep* 1993; **42**: 258–263.
- Dorn CR. Review of foodborne outbreak of *Escherichia coli* O157:H7 infection in the western United States. *J Am Vet Med Assoc* 1994; **203**: 1583–1587.
- Zhao T, Doyle MP, Shere J *et al.* Prevalence of *Escherichia coli* O157:H7 in a survey of dairy herds, abstract P-79. Abstracts of the 94th Annual Meeting of the American Society for Microbiology, Las Vegas, Nevada, USA. 1994: 383.
- O'Brien AD, Melton AR, Schmitt CK *et al.* Profile of *Escherichia coli* O157:H7 pathogen responsible for hamburger-borne outbreak of hemorrhagic colitis and hemolytic uremic syndrome in Washington. *J Clin Microbiol* 1993; **31**: 2799–2801.
- Ostroff SM, Tarr PI, Neill MA, Lewis JH, Hargrett-Bean N, Kobayashi JM. Toxin genotypes and plasmid profiles as determinants of systemic sequelae in *Escherichia coli* O157:H7 infections. *J Infect Dis* 1989; **160**: 994–998.
- Paros M, Tarr PI, Kim H, Besser TE, Hancock DD. A comparison of human and bovine *Escherichia coli* O157:H7 isolates by toxin genotype, plasmid profile, and bacteriophage λ -restriction fragment length polymorphism profile. *J Infect Dis* 1993; **168**: 1300–1303.
- Ratnam S, March SB, Ahmed R, Bezanson GS, Kasatiya S. Characterization of *Escherichia coli* O157:H7. *J Clin Microbiol* 1988; **26**: 2006–2012.
- Khakhria R, Duck D, Lior H. Extended phage-typing scheme for *Escherichia coli* O157:H7. *Epidemiol Infect* 1990; **105**: 511–520.
- Samadpour M, Grimm LM, Desai B *et al.* Molecular epidemiology of *Escherichia coli* O157:H7 strains by bacteriophage λ -restriction fragment length polymorphism analysis: application to a multistate foodborne outbreak and a day-care center cluster. *J Clin Microbiol* 1993; **31**: 3179–3183.
- Böhm H, Karch H. DNA fingerprinting of *Escherichia coli* O157:H7 strains by pulsed-field gel electrophoresis. *J Clin Microbiol* 1992; **30**: 2169–2172.
- Lech K, Brent R. Minipreps of plasmid DNA. In: Ausubel FM, Brent R, Kingston RE *et al.* (eds) Short protocols in molecular biology—a compendium of methods from current protocols in molecular biology. New York, John Wiley and Sons. 1989: 19–20.
- Arbeit RD, Arthur M, Dunn R, Kim C, Selander AK, Goldstein

- R. Resolution of recent evolutionary divergence among *Escherichia coli* from related lineages: the application of pulsed field gel electrophoresis to molecular epidemiology. *J Infect Dis* 1990; **161**: 230–235.
16. Versalovic J, Woods CR, Georghiou PR, Hamill RJ, Lupski JR. DNA based identification and epidemiologic typing of bacterial pathogens. *Arch Pathol Lab Med* 1993; **117**: 1088–1098.
17. Harsono KD, Kaspar CW, Luchansky JB. Comparison and genomic sizing of *Escherichia coli* O157:H7 isolates by pulsed-field gel electrophoresis. *Appl Environ Microbiol* 1993; **59**: 3141–3144.
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