

Paper

# Epidemiological study of *E. coli* O157:H7 isolated in Northern Ireland using pulsed-field gel electrophoresis (PFGE)

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## ABSTRACT

In Northern Ireland over the last 7 years, there is a mean of 41.9 laboratory reports per annum of human gastrointestinal infection (range 19-54) caused by *Escherichia coli* O157:H7. In the preceding years 1992-1996, reports were 5.4 per annum, whereas in 1997-2000, reports increased from 30 to 54 per annum. This high level has continued on an annual basis to date. The aim of this study was therefore to retrospectively examine this period of exponential increase in reports to help ascertain the genetic relatedness of strains employing pulsed-field gel electrophoresis (PFGE), as no data on the molecular epidemiology of *E. coli* O157:H7 in Northern Ireland has yet been published. Clinical isolates (n=84) were PFGE typed employing *Xba*I digestion and resulting band profiles demonstrated the presence of 13, 9 and 16 clonal types, for 1997, 1998 and 1999, respectively. In 1998, five clonal types remained from 1997 with the introduction of 4 new clonal types, whereas in 1999, 10 new clonal types were observed, accounting for over half (58%) of the *E. coli* O157 isolates for that year. These data suggest that, unlike gastrointestinal infections due to thermophilic campylobacters, there was considerable genetic evolution of PFGE clonal types of *E. coli* O157, through the displacement and emergence of genotypes. Further studies are now required to find the environmental reservoirs of these common clonal types of clinical *E. coli* O157:H7 in Northern Ireland to help define sources and routes of transmission of this infection locally.

## INTRODUCTION

Since verocytotoxin producing *Escherichia coli* (VTEC) was first recognised as an important gastrointestinal bacterial pathogen in humans, its microbiological characteristics, pathogenicity and epidemiology have been studied worldwide. The severity of symptoms with *E. coli* O157 infection may vary from mild diarrhoea to haemolytic uraemic syndrome (HUS). Following large outbreaks in Japan<sup>1</sup> and Scotland<sup>2</sup>, *E. coli* O157:H7, in particular, has been monitored closely. In Northern Ireland, reports on infection with *E. coli* O157 have risen from a few cases in the early 1990s (n=1; 1992: n=2; 1993) to 54 reports in 1999, which remains the highest yearly number of reports to date, where reports approximately doubled from 1997 to 1999.

Several methods have been used to subtype bacterial isolates and include biotyping<sup>3</sup>, phage typing<sup>4</sup> and molecular biological techniques, such as the random amplification of

polymorphic DNA polymerase chain reaction (RAPD-PCR). These systems have been invaluable for typing *E. coli* O157 isolates. In addition, pulsed-field gel electrophoresis (PFGE) techniques have been considered as a gold standard method for many epidemiological investigations due to the stability of the technique.

To date, no molecular genotyping data has been reported in the literature for clinical isolates of *E. coli* O157 in Northern Ireland. Therefore, PFGE analysis was applied retrospectively to a collection of archived *E. coli* O157 isolates obtained in Northern Ireland, during the period of predominant establishment of *E. coli* O157 disease, i.e. from 1997 to 1999. This was done in order to ascertain:- (i) the molecular relatedness among these strains examined, (ii) the clonal evolution of subtypes, i.e. whether different strain (PFGE) types were responsible for the doubling in incidence (>1997), or was this dramatic rise due to an increase in established clonal types already circulating in the community?

## MATERIALS AND METHODS

### Bacterial isolates used in this study.

*E. coli* O157:H7 isolates (n = 84) were obtained from fresh faecal specimens throughout Northern Ireland and archived at the Northern Ireland Public Health Laboratory, Belfast City Hospital, from 1997 to 1999. Twenty-nine isolates were recovered in 1997, 19 in 1998 and 36 in 1999. Isolates were stored in whole defibrinated horse blood (E&O laboratories, Scotland) at -80°C until required. *E. coli* O157:H7 reference strain NCTC 12079 was used throughout this study. Several *E. coli* non-O157 isolates (O26, O86, O111, O114, O126 and O128), were employed in the study as comparators.

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### DNA extraction

All DNA isolation procedures were carried out in accordance with the DNA contamination management guidelines of Millar *et al.*<sup>5</sup>.

**Method 1:** Bacterial suspension (1500 µl) was centrifuged at 8,000 x g for 10 minutes. The supernatant was decanted and the bacterial pellet was washed twice with Pett IV buffer (1M Tris-HCl [pH 7.6], 1M NaCl). The pellet was resuspended in 150µl of Pett IV buffer. The Pett IV-cell mixture was warmed to 30-40°C in a water bath. Lysozyme (4µl; 25mg/ml) was added to the Pett IV-cell mixture, mixed well and 150µl of 2% LMP-agarose (InCert®, FMC BioProducts, Rockland, USA) prepared in EC lysis buffer (polyoxyethylene (20) Cetyl Ether (Brij-58) [0.5%], NaCl [1M], 1M Tris-HCl (pH 7.6) [6mM], deoxycholic acid sodium salt solution [0.2% (w/v)], 0.5M EDTA [pH7.5] solution [100mM], sarkosyl NL30 [0.5%]) was added and pipetted gently. This mixture was pipetted carefully into an acrylic mould and allowed to solidify within the mould for a few minutes at room temperature, then for 10 minutes at 4°C. Plugs were gently removed from the mould and incubated overnight at 37°C in lysis buffer (1.5ml EC lysis buffer, 12µl lysozyme [100mg/ml], 3µl RNase [10mg/ml]). The lysis buffer was discarded and plugs were washed a few times with TE buffer (10mM Tris-HCl [pH7.4], 1mM EDTA [pH7.5]) and treated with 1.5ml of ESP buffer (0.5M EDTA [pH9-9.5], 1% N-lauroylsarcosine [Sigma L-5125], Proteinase K [1mg/ml]) at 50°C for 24-48 hours. Plugs were rinsed a few times with TE buffer and treated with 20µl of phenylmethylsulfonylfluoride (TE-PMSF)/isopropanol added to 1.5ml of TE buffer. After the plugs were incubated in TE-PMSF at room temperature for 30-60 minutes, the TE-PMSF solution was decanted carefully and the plugs were rinsed at least seven times with TE buffer. Finally, plugs were stored in TE buffer at 4°C until required.

**Method 2:** Bacteria suspension (1500 µl) was centrifuged at 8,000g for 10 minutes. The supernatant was decanted and the pellet was washed twice with TE buffer (pH 7.6) [10mM Tris-HCl, 5mM EDTA, 1M NaCl], and centrifuged at 4,000g for 20 minutes. The pellet was resuspended in 150µl of EC buffer (pH7.5) [6mM Tris-HCl, 1M NaCl, 0.1M EDTA, 0.5% (w/v) Brij 58, 0.2% (w/v) deoxycholate Na, 0.5% (w/v) Sarkosyl]. The cell suspension was mixed gently with 150µl of 2% (w/v) LMP-agarose prepared in EC buffer. The mixture was pipetted into the acrylic mould and left to solidify at 4°C for 20 minutes. LMP-agarose plugs were incubated in EC buffer at 37°C overnight, after which the EC buffer was decanted and 2ml of Proteinase K reagent (5mg/ml Proteinase K/EC buffer, Sigma P-0390) was added to each LMP-agarose plug and incubated at 50°C for 24-48 hours. The Proteinase K reagent was decanted and LMP-agarose plugs were washed at least seven times with TE buffer. Finally, the LMP-agarose plugs were stored in TE buffer at 4°C until required.

### PFGE analysis

Slices (1mm X 15mm) of LMP-agarose plugs were equilibrated in 100µl restriction enzyme buffer mixture (10X buffer, 100mM 2-mercaptoethanol, 0.1% (v/v) bovine serum albumin (Sigma A2153) and DNAase free distilled water). After 30 minutes, the buffer was removed and another 100µl of restriction enzyme mixture containing 10Units of

*Xba* I (Sigma R7260) or *Not* I (Sigma R8506) enzyme was added. The LMP-agarose plug slices were incubated at 37°C overnight. A 1% (w/v) agarose gel (Amresco, Agarose III pulsed-field application gel) was prepared in 0.5X TBE buffer (Tris-Borate-EDTA buffer, Sigma T-7527). The agarose gel was formed in an acrylic mould incorporating a 10 or 15 well comb. After complete solidification, the wells of the gel were loaded with the appropriate restricted LMP-agarose slices and DNA markers (DNA concatamers, ProMega-Markers® Lambda Ladders, G3011, USA). The wells were then sealed with molten (56°C) 1% (w/v) agarose gel. The electrophoresis tank, containing two litres of 0.5X TBE buffer, was allowed to cool from room temperature to 14°C using a mini chiller system. The prepared agarose gel was gently set into the electrophoresis tank and restriction enzyme generated DNA fragments were separated according to their molecular size by electrophoresis using a contour-clamped homogeneous electric field (CHEF) technique (CHEF-DRII system, BioRad Laboratories, Richmond, USA). Electrophoresis was carried out for 18 hours using the following parameters: linear ramp of 12.6-40.1 seconds (start ratio 1), at 200V (6 volts/cm) at 14°C. After electrophoresis, the agarose gel was stained in ethidium bromide buffer (1µg/ml ethidium bromide in distilled water) with gentle shaking for 30 minutes. The staining buffer was replaced with distilled water and left for 40 minutes with gentle shaking to remove residual background staining.

The agarose gel was visualised using a transilluminator (Vilber Lourmat, TFX-35M, France). Gels were photographed under UV exposure onto Polaroid black-and-white print film (type 667). Banding patterns were visually compared by eye and grouped according to interpretative PFGE criteria<sup>6</sup>.

## RESULTS

### Comparison of two agarose block methods

Two agarose block methods were compared in this study. Method 1 was shown to be more successful in extracting DNA from bacterial cells and gave clear and distinguishable banding patterns with *Xba* I restriction endonuclease. Using Method 2, it was found that extracted bacterial DNA was poor or not extracted from the bacterial cells and consequently complete endonuclease activity was hindered. In view of this, Method 1 was adopted as the method of choice for the extraction of bacterial DNA for further experimentation. The endonuclease enzymes, *Xba* I and *Not* I, were chosen for the initial analysis of *E. coli* O157 isolates obtained in Northern Ireland. Both enzymes have been previously used for *E. coli* study (Honda *et al.* 1995; Morooka *et al.* 1995) and provided some degree of discrimination between isolates in this study; however, as *Xba* I gave more distinctive patterns with well resolved bands and this endonuclease was selected for DNA analysis of further isolates.

### PFGE-CHEF<sup>®</sup> system with endonuclease *Xba* I

Fragments generated by *Xba* I digestion were separated by PFGE-CHEF<sup>®</sup>. On average, 15 DNA fragments were produced using this enzyme. Twenty-nine *E. coli* O157:H7 isolates obtained in 1997 were separated into 13 different groups (Group A to M) using PFGE-CHEF<sup>®</sup> the results of which are shown in Table I. Six of eight isolates in the predominant

TABLE I: PFGE genotype  
 PFGE-CHEF<sup>®</sup> results *E. coli* O157:H7 (1997-1999 isolates)

1997																					
A	B	C	D	E	F	G	H	I	J	K	L	M									
4N <sup>2*</sup>	12E	1N <sup>1</sup>	8E <sup>3</sup>	10N	21E	9E	11E	17S	18S	24E	29S	30E									
5N <sup>2</sup>	19E	2N <sup>1</sup>	15E	28S	21N																
6E <sup>2</sup>	25N <sup>5</sup>	3N <sup>1</sup>																			
7E <sup>3</sup>	26N <sup>5</sup>																				
13E	27N																				
16E <sup>4</sup>																					
20E <sup>4</sup>																					
22N																					
1998																					
A	B	C	D	E								N	O	P	Q						
9E <sup>7</sup>	6E	1W	3E	4N <sup>6</sup>								11N	14E <sup>8</sup>	23E <sup>9</sup>	2E						
10E <sup>7</sup>	8N			5N <sup>6</sup>								21E	16E <sup>8</sup>	24E <sup>9</sup>							
	19E			7W																	
				20E																	
				22N																	
1999																					
	C	E	G	L						O	P	R	S	T	U	V	W	X	Y	Z	α
	37N	6E <sup>11</sup>	19N	21E						26E	1N <sup>10</sup>	7E <sup>11</sup>	22N	29N <sup>13</sup>	32S	18N	20N	27N <sup>13</sup>	31E	34N	35N
		12E <sup>11</sup>								28E	2N <sup>10</sup>	8E <sup>11</sup>	23E <sup>12</sup>	30N <sup>13</sup>	33S						
		13E <sup>11</sup>									3N <sup>10</sup>	9E <sup>11</sup>	24E <sup>12</sup>								
		14E <sup>11</sup>									4N <sup>10</sup>	10E <sup>11</sup>	25E <sup>12</sup>								
		15E <sup>11</sup>									5N <sup>10</sup>	11E <sup>11</sup>	36N								
												16E <sup>11</sup>									

\*numbers on the isolate number's shoulder is outbreak number.

Group A consisted of isolates obtained from three different outbreak sources, viz. Outbreaks 2, 3 and 4. Isolates from Outbreak 1 were shown to be distinct from all other isolates and fell into Group C. Outbreak 5 strains fell into Group B and bore some relatedness to strains that were obtained from three sporadic cases (12E, 19E, 27N). All Group B strains were shown to possess VT1 and VT2. Two isolates (7E and 8E) in Outbreak 3 were categorised into Group A and D respectively showing more than seven banding differences according to the Tenover criteria. Comparisons between Groups A and C revealed three fragment differences while

comparisons between Group E and I, and Group E and L revealed them to differ by only two fragments, respectively.

PFGE-CHEF<sup>®</sup> analysis differentiated isolates (1997) belonging to phage type 2, 14, 32, and 49 into a number of smaller groups (Table 2). Two isolates belonging to phage type-2 could be divided into two different banding profiles (Groups H and J) while 10 phage type-14 strains could be divided into three different banding profiles (Groups A, B and G). Five phage type-32 isolates and five phage type-49 isolates, could each be divided into four groups (Groups D,

TABLE II: PFGE genotype  
Comparison of PFGE-CHEF<sup>®</sup> figures and phage types from 1997 to 1999

1997																		
A	B	C	D	E	F	G	H	I	J	K	L	M						
14 <sup>2*</sup>	<sub>1&amp;2</sub> 14	49 <sup>1</sup>	<sub>N</sub> 8 <sup>3</sup>	32	21	14	2	32	2	<sub>N</sub> RDNC	32	<sub>N</sub> RDNC						
14 <sup>2</sup>	<sub>1&amp;2</sub> 14	49 <sup>1</sup>	<sub>1&amp;2</sub> 32	32	21													
14 <sup>2</sup>	<sub>1&amp;2</sub> 14 <sup>5</sup>	49 <sup>1</sup>																
14	<sub>1&amp;2</sub> 14 <sup>5</sup>																	
49	<sub>N</sub> 14																	
49 <sup>4</sup>																		
32/14 <sup>3</sup>																		
RDNC <sup>4</sup>																		
1998																		
A	B	C	D	E						N	O	P	Q					
14 <sup>7</sup>	<sub>1&amp;2</sub> 8	49	32	32 <sup>6</sup>						32	49 <sup>8</sup>	21/28 <sup>9</sup>	<sub>N</sub> 34					
14 <sup>7</sup>	<sub>1&amp;2</sub> 14			32 <sup>6</sup>						32	49 <sup>8</sup>	21/28 <sup>9</sup>						
	32			32														
				32														
				32														
1999																		
	C	E	G		L		O	P	R	S	T	U	V	W	X	Y	Z	α
	49	32 <sup>11</sup>	21/28		RDNC		47	32 <sup>10</sup>	32 <sup>11</sup>	21/28	32 <sup>13</sup>	8	<sub>N</sub> RDNC	32	32 <sup>13</sup>	<sub>1&amp;2</sub> 32	<sub>1&amp;2</sub> 32	32
		32 <sup>11</sup>					47	32 <sup>10</sup>	32 <sup>11</sup>	21/28 <sup>12</sup>	32 <sup>13</sup>	RDNC						
		32 <sup>11</sup>						32 <sup>10</sup>	32 <sup>11</sup>	21/28 <sup>12</sup>								
		32 <sup>11</sup>						32 <sup>10</sup>	32 <sup>11</sup>	21/28 <sup>12</sup>								
		32 <sup>11</sup>						32 <sup>10</sup>	32 <sup>11</sup>	2								
									32 <sup>11</sup>									

\*numbers on the left of the phage types are outbreak number.

†Verocytotoxin type is VT2 unless otherwise stated on the right of the phage types.

G, I and L) and two different banding profiles (Groups A and C) respectively. Five isolates belonging to phage type-14 as well as being positive for VT1 and VT2, all had the same banding profile, Group B. *E. coli* O157 isolates obtained in 1998 were differentiated into nine groups A to Q. Twelve of 19 isolates were classified into five Groups, A, B, C, D and G. Seven other isolates could be classified into four new banding profiles, Groups N, O, P and Q. These results are shown in Table I. The Outbreaks 6, 7, 8 and 9 which occurred in 1998, were not connected with each other as all had distinct banding patterns. All outbreak isolates, other than Outbreak 6, did not share similar banding patterns with any other sporadic cases. Two Outbreak 6 isolates (4N and 5N) had a similar banding pattern with three sporadic cases (7W, 20E and 22N). Between Group C and O, and Group E and N, PFGE-CHEF banding patterns were different by three and two fragments, respectively. *E. coli* O157 isolates, phage types 14, 32 and 49 were differentiated into smaller groups by PFGE-CHEF

typing (Table II). Nine phage type-32 isolates could be divided into four groups (Groups B, D, E and N). Three *E. coli* O157 isolates belonging to phage types-14 and three *E. coli* O157 isolates belonging to phage types-49 were divided into two groups respectively.

Thirty-six *E. coli* O157 isolates obtained in 1999 were examined by PFGE-CHEF analysis. Fifteen isolates fell into the initial Groups A to Q (1997-1998). Groups C, G and L contained one isolate each while Groups E and P contained five isolates and Group O contained two isolates. Twenty-three other isolates were classified into 10 new Groups, R to Group α. Eleven isolates in Outbreak 11 were separated into Group E and R. Two fragment differences were observed between the two groups. Isolates in Outbreak 13 could be divided into Group T and X by four fragments difference. Outbreak 12 was related to two sporadic cases (22N and 36N). Every outbreak could be allocated into a different Group. Between Group E

and P, Group E and S, Group S and T, Group P and S, Group Y and Z, their fragment difference was four, two, three, three, and three, respectively.

PFGE-CHEF analysis separated isolates belonging to phage type-32, 21/28 and RDNC into smaller groups (Table 2). Twenty-three phage type-32 isolates fell into nine groups (Groups E, P, R, T, W, X, Y, Z and  $\alpha$ ). Five phage type 21/28 isolates fell into two groups (Group F and S).

Nine *E. coli* non-O157 isolates and one *E. coli* type strain NCTC 12079 were used in this study and of the nine *E. coli* non-O157 isolates, eight isolates showed unique banding patterns which do not resemble any patterns seen with other *E. coli* O157 isolates. The isolate which showed the closest similarity (four fragments difference) to E99/18N was serogroup O111 (C98.17). Type strain NCTC 12079 showed no similarity to *E. coli* O157 isolates obtained in Northern Ireland.

PFGE-CHEF groups obtained between 1997 and 1999, and related phage types were compared. Eight isolates in 1997 and two isolates in 1998 were placed into Group A. Among these, seven isolates were phage type-14 (70%). In Group B, six of eight isolates were phage type-14 (75%) and all were VT1 and VT2 positive. Of three isolates in Group D, two were phage type-32 (66%). In Group P, 71% of isolates were phage type-32. Eighty percent of isolates were phage type-21/28 in Group S. Each individual group (C, E, F, N, R and T) had isolates sharing the same phage type.

Six of eight *E. coli* O157 isolates were positive for VT1 and VT2 and fell into Group B. These isolates accounted for 75% of Group B isolates.

New PFGE-CHEF groups have emerged every year and more than 50% of the isolates obtained in 1998 had been categorised into the groups seen in 1997. However, most of the groups seen in 1997 were absent by 1999.

## DISCUSSION

In Northern Ireland, there has been a marked rise in laboratory reports of *E. coli* O157 from 1995 through to 2000, where infections approximately doubled from 1995 through to 2000, where they peaked at 54 laboratory reports per annum. The increase was particularly seen in two patient populations, namely the 1-4 year old age group and the 15-44 year old age group. Since 2000, laboratory have ranged from 19 to 53 reports per annum, where the most recent provisional data for 2006 recorded an annual total of 45 reports ([www.cdscni.org.uk/surveillance/Gastro/Escherichia\\_coli\\_O\\_157.htm](http://www.cdscni.org.uk/surveillance/Gastro/Escherichia_coli_O_157.htm))

In Northern Ireland, *E. coli* O157 was not routinely tested for in all faecal samples until 1997, accounting for the marked rise in laboratory reports. At this point, the Northern Ireland Public Health Laboratory based at Belfast City Hospital (NIPHL) tested over 5,000 human clinical faecal samples for verocytotoxin-producing *E. coli* (VTEC) including O157 using ELISA technique in conjunction with the Department of Agriculture veterinary laboratories at Stormont. Of the seven VTEC found in this surveillance study (unpublished data), three samples were *E. coli* O157, two were O26 and two were O128. Since 1997, the NIPHL has started to collect all *E. coli* O157 samples on a province-wide basis.

Since the introduction of pulsed-field gel electrophoresis using a contour-clamped homogeneous electric field technique, which provided the ability to analyse large DNA molecules (2Mb), the technique gained wide popularity very rapidly within biological sciences. It had the ability to work with and investigate large intact fragments of DNA, or non-damaged DNA molecules, which held the key to its success. The agarose gel block method used to gently embrace bacteria in a cocoon of LMP-agarose gel protects the bacterial DNA from physical damage during the extraction and subsequent manipulation or digestion of DNA. The relatively open pore size of the LMP-agarose also meant that reagents and enzymes could readily access the extracted DNA and when electrophoresis commences the DNA fragments rapidly leave the LMP-agarose and enter the electrophoretic agarose bed.

Two DNA preparation methods for PFGE-CHEF<sup>®</sup> were compared. Method 1 was designed especially for the analysis of *E. coli*. Method 2 was originally designed for the analysis of staphylococci and modified for Gram-negative bacterial strains. The basis of the two methods and the reagents used were very similar. It includes harvesting of the cultured bacteria, embedding of the washed bacteria in LMP-agarose, lysis of the cell membrane and purification of the DNA-contained agarose block. Although the components of the lysis buffer, TE buffer and pH values were almost identical, small differences were observed between the two methods. For example, method 1 was found to successfully extract DNA from bacterial cells, where this method employed RNase and PMSF. RNase was incorporated to denature any residual RNA present in the initial DNA preparation to help resolve DNA bands by reducing or preventing smearing of the lanes caused by excess RNA, which may be present in the DNA preparations. PMSF was incorporated in the method to inactivate proteinase K, thus preventing the destruction of the restriction enzymes that are used in the final digestion of bacterial DNA. In Method 2, proteinase K was also used, but removal of this agent was achieved by washing the agarose blocks several times with changes of buffer, however, because PMSF has been shown to be a neurotoxin it must be handled with extreme care. It was thought that changing the buffer several times may be inadequate to remove proteinase K or other reagents that might inhibit enzymatic activity. This final step is essential in order to deactivate all chemicals that might prevent enzyme activation.

PFGE-CHEF<sup>®</sup> analysis has been used successfully to describe the epidemiology of *E. coli* O157 between strains isolated from cattle, animal carcasses, food, environmental sources, human outbreaks and sporadic cases<sup>7,8</sup>. *Xba* I is the most frequently used restriction enzyme for investigations of *E. coli* O157 outbreaks and has been successfully applied here in the discrimination of isolates from Northern Ireland. The results obtained in this study using *Xba* I were compared with phage typing, and genotypes (verocytotoxin genes), and epidemiological data to assess the discriminatory power of PFGE-CHEF<sup>®</sup> analysis.

Throughout these experiments, some common bands generated by *Xba* I digestion were observed among all *E. coli* O157:H7 isolates examined. PFGE-CHEF<sup>®</sup> analysis also revealed the relationships within epidemiologically unrelated isolates. Arbeit *et al.*<sup>9</sup> illustrated that PFGE could

differentiate epidemiologically independent, but evolutionarily related isolates that were indistinguishable by multilocus enzyme electrophoresis and restriction polymorphisms associated with rRNA operons. PFGE-CHEF<sup>®</sup> analysis was able to differentiate phenotypically and genotypically indistinguishable strains. For example, phage type-32 and VT2 gene positive isolates were shown to be dominant between 1997 and 1999 (40.5%) but PFGE-CHEF<sup>®</sup> analysis revealed the great diversity among these isolates. Smith *et al.*<sup>10</sup> applied the PFGE technique and fluorescent amplified-fragment length polymorphism (FAFLP) for genotyping *E. coli* O157 strains of a single phage type and revealed that PFGE had more discriminatory power than FAFLP. This diversity might be explained as “clonal turnover” which resulted from mutations and rearrangements within the genome or the gain or loss of plasmids, but not because of genetic change in the plasmid as very little variation has been observed. In addition, Osawa *et al.*<sup>11</sup> reported that among *E. coli* O157:H7 strains, there was considerable variation in the Stx2-converting phage DNA found by using PFGE analysis on account of the alteration of phage genomes with those of host genomes and that PFGE-CHEF<sup>®</sup> analysis may have the potential to reveal minor changes in the bacterial genome.

To differentiate and interpret outbreak and sporadic isolates using PFGE-CHEF<sup>®</sup> analysis, standard criteria as recommended by Tenover *et al.*<sup>6</sup>, were employed in this study. Initially, outbreak and sporadic isolates that fell into the same PFGE-CHEF<sup>®</sup> group were compared. Three outbreaks (2,3 and 4) in 1997 were placed in Group A. Outbreak 2 and one of the Outbreak 3 isolates was obtained 10 days apart. According to Tenover's criterion, if their restriction patterns have the same numbers of bands and the corresponding bands are the same apparent size, isolates are indistinguishable. Indeed, using these criteria these two isolates may have been epidemiologically related. Outbreak 4 isolates and E97/22N were obtained eight days apart and their banding patterns were identical thus indicating that E97/22N strain could have been part of outbreak 4. This relationship was seen between other outbreaks and sporadic cases. Outbreak 5 and E97/27N were isolated 10 days apart and they were indistinguishable using *Xba* I digestion profiles. When isolates were collected from different Health Boards and/or the collection date differed by several days, isolates related to a possible outbreak might have been missed from the original outbreak. However, use of a single endonuclease with PFGE-CHEF<sup>®</sup> analysis compromises the ability to distinguish whether the isolate was part of an outbreak or not. Using other restriction enzymes and/or other fingerprinting methods such as RAPD-PCR heightens the accuracy of the epidemiological findings. In addition to this, when comparing epidemiologically unrelated sporadic isolates to outbreak isolates, Barrett *et al.*<sup>12</sup> suggested that isolates with PFGE patterns that differ from the PFGE pattern of the outbreak isolates by more than one band were probably not related.

Secondly, isolates in the same outbreak but with different PFGE-CHEF<sup>®</sup> patterns were closely examined. Two isolates from Outbreak 3 fell into two different groups (Groups A and D) with more than seven fragments differences between them. Applying Tenover's criteria, isolates in Outbreak 3 were different and not related to each other. Since two PFGE groups were obtained from one single outbreak, Outbreak

3 may have been initiated by two genetically unique *E. coli* O157 isolates.

Isolates from Outbreak 13 fell into Group T and X with four banding differences between them. These isolates were possibly related, though, on the bases of PFGE-CHEF<sup>®</sup> analysis alone it could not explain whether this outbreak was caused by two different *E. coli* O157:H7 or the mutation of a resident isolate into two new subtypes.

Eleven isolates from Outbreak 11 were divided into two groups (Groups E and R) with two banding differences. The minor variations in restriction DNA fragments produced may be explained by the different sites of integration of the phages into the *E. coli* O157 chromosome (Böhm and Karch, 1992). Since a single genetic event; i.e., a point mutation or an insertion or deletion of DNA into the recognition site or ‘motif’, can result in two to three fragment differences, isolates with PFGE patterns that differ by fewer than four bands should be considered subtypes of the same strain. This may indicate that Outbreak 11 might have been caused by a single *E. coli* O157 isolate.

*E. coli* non-O157 isolates were studied using PFGE-CHEF<sup>®</sup>. All but *E. coli* O111 (C98.17) showed unique banding patterns and bore no resemblance to *E. coli* O157. *E. coli* O111 (C98.17) may have originated from the same clone as E99/18N, however, PFGE-CHEF<sup>®</sup> analysis alone was insufficient to demonstrate this.

The results of phage typing and PFGE-CHEF<sup>®</sup> analysis obtained between 1997 and 1999 were examined. PFGE-CHEF<sup>®</sup> groupings showed a greater ability to discriminate *E. coli* O157 isolates and demonstrated some unique relations. More than 70% of isolates belonging to the same PFGE-CHEF<sup>®</sup> group were shown to have the same phage type over the study period. When banding patterns between the groups obtained in 1997 were compared, the majority of groups differed by two to three fragments, indicating that they were closely related. Some new PFGE-CHEF<sup>®</sup> groups which emerged in 1998 showed close relation to groups obtained in 1997. Most of the PFGE-CHEF<sup>®</sup> groups seen in 1997 had not re-emerged by 1999. However, the majority of new groups obtained in 1999 were also “closely related” or “possibly related” to the 1997 groups. These observations illustrated that most *E. coli* O157 causing human infection might have originated from a single clone which has remained in the environment within Northern Ireland.

Over the three years observation, a variety of PFGE-CHEF<sup>®</sup> groups were observed, illustrating the diversity of *E. coli* O157:H7 from which clonal subgroups could rapidly emerge during short intervals. This rapid genetic change of VTEC strains may explain their great ability to adapt to the environment with concomitant emergence of infection.

PFGE-CHEF<sup>®</sup> has been considered the most discriminating method for epidemiological typing of *E. coli* isolates. For most of the common bacterial pathogens, the validity of PFGE for molecular typing has been well established.<sup>6</sup> PFGE-CHEF<sup>®</sup> analysis has been useful for the study of *E. coli* O157:H7 isolates from Northern Ireland. This technique has proved to be a highly sensitive method for bacterial typing. As phage typing has been shown to be less

effective than PFGE-CHEF<sup>®</sup> typing, PFGE may provide the additional discriminatory power to support epidemiological investigations of *E. coli* O157 infections. Verocytotoxin PCR was found to be a useful adjunct to PFGE. The establishment of a good, reliable and reproducible platform for epidemiological analysis will be invaluable for further investigations of *E. coli* O157:H7 infections in Northern Ireland, particularly in the investigation of the stable PFGE types, from food, animals and the environment.

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