*Epidemiol. Infect.* (1990), **105**, 265–275
*Printed in Great Britain*



# DNA restriction digest and ribosomal RNA gene patterns of *Campylobacter jejuni*: a comparison with bio-, sero-, and bacteriophage-types of United Kingdom outbreak strains

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(*Accepted 4 May 1990*)

SUMMARY

DNA restriction endonuclease (*Hae* III and *Hin*d III) total digest and 16S and 23S ribosomal (r)RNA gene patterns (ribopatterns) were determined for 18 isolates of *Campylobacter jejuni* from three separate outbreaks of diarrhoea in the north of England. Strains were also characterized by biotyping, serotyping and phage typing. Comparisons of the DNA patterns by visual and numerical methods revealed five distinct strain groupings with clear differences between isolates from different outbreaks as well as some heterogeneity between strains within the community outbreak and one of the school outbreaks. An excellent correlation was observed between the genomic DNA fingerprints data and the Preston bacteriophage group, both of which gave better discrimination than biotyping and serotyping alone or in combination. Only one phage group (PG 37) was not confirmed by the DNA data. DNA fingerprints therefore provide additional information of value in studying the epidemiology of outbreaks of *C. jejuni*.

## INTRODUCTION

Campylobacter is one of the commonest causes of bacterial diarrhoea worldwide. In the United Kingdom over the past decade, infections with *Campylobacter jejuni* have increased five-fold to become the commonest single cause of food-poisoning associated with the consumption of undercooked poultry, but untreated water and raw milk may also act as vehicles of infection, sometimes with epidemic outbreaks [1–3]. Similar dramatic increases have been observed in other developed countries [4–6].

Epidemiological surveillance of *C. jejuni* is hampered by the lack of precise methods for strain identification, even though many different typing systems have been proposed. At present the most promising and widely used methods are biotyping according to the schemes of Lior [7] and Bolton [8], and serotyping using heat-stable (HS) [9] and heat-labile (HL) [10] surface antigens. Other schemes for typing *C. jejuni* have included fluorescent antibody tests [11],

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auxotyping [12], phage typing [13], and DNA restriction endonuclease analysis [14–16]. For epidemiological purposes, some investigators have proposed that improved discrimination can be achieved by the combination of several typing systems [17, 18].

The purpose of the present paper was to report our results on the DNA restriction endonuclease digest and ribosomal (r)RNA gene patterns (ribopatterns) of *C. jejuni* from three separate outbreaks in the United Kingdom. These novel genomic methods of strain characterization have been applied with promising results in epidemiological investigations of various other clinically important bacteria [19]. Ribosomal RNA genes, which are a small but highly conserved portion of the genome, provide particularly valuable markers for detecting and visualizing restriction fragment length polymorphisms. Differences that arise in these ribopatterns reflect minor nucleotide variations between strains occurring in the genes themselves or in flanking sequences. The DNA fingerprinting techniques offer high reproducibility and typability. Our aim was to investigate their discriminatory potential when applied to a well-defined set of outbreak strains of *C. jejuni*, by comparison with established methods of biotyping and serotyping (HL and HS systems), and with a new phage typing scheme [20].

## MATERIALS AND METHODS

### *Bacteria*

The present study includes 18 isolates of *Campylobacter jejuni* and their reference numbers are listed in Table 1. The strains were isolated from patients and a dog in three separate outbreaks of diarrhoea in the north of England in 1985 and 1988. The community outbreak in 1985 was associated with puppies and details have been documented previously [20, 21]. The outbreak at School 1 in 1988 was associated with contamination of a private drinking water supply. There was no epidemiological evidence for the source of the outbreak at School 2 in 1988.

All strains were grown on 5% (v/v) defibrinated horse blood agar. Cultures were incubated for 48 h at 37 °C under microaerobic conditions (5% $O_2$, 5% $CO_2$, 2% $H_2$, 88% $N_2$) in a Variable Atmosphere Incubator (Don Whitley Scientific Ltd, Shipley, Yorks). Strains were preserved at −70 °C on glass beads in Nutrient Broth No. 2 (Oxoid:CM 67) containing 10% (v/v) glycerol, and they were lyophilized in 5% (w/v) inositol serum.

### *Biotyping, bacteriophage typing and serotyping*

Biotyping and phage typing were performed at the Preston Public Health Laboratory using previously described methods [8, 20]. HL and HS serotyping were performed at the Manchester Public Health Laboratory according to standard methods [9, 10].

### *Chromosomal DNA extraction, digestion and separation of fragments*

Chromosomal DNA was extracted and rapidly purified after lysis of the bacteria with guanidium thiocyanate reagent [22]. The DNA (1–2 μg) was digested with the restriction endonuclease *Hae* III (5 units/μg DNA) for 4 h at 37 °C in the buffer recommended by the manufacturer (Northumbria). The digested DNA

Table 1. Campylobacter jejuni *strains studied and typing results*

| Name of outbreak | Source | Preston biotype | Serotype: Penner (HS) | Serotype: Lior (HL) | Preston phage group | DNA group* |
|---|---|---|---|---|---|---|
| Community (1985) | | | | | | |
| A615/89 | Human | 6100 | 4 | 1 | 86 | 1:1 |
| A616/89 | Human | 6100 | 4 | 1 | 86 | 1:ND |
| A617/89 | Human | 6100 | 4 | 1 | 86 | 1:ND |
| A618/89 | Human | 6100 | 13,50 | 1 | 86 | 1:1 |
| A619/89 | Human | 6100 | 4,50 | 1 | 86 | 1:1 |
| A620/89 | Human | 6154 | 15 | NT† | 59 | 2:ND |
| A621/89 | Dog | 6154 | 15 | NT | 59 | 2:2 |
| School 1 (1988) | | | | | | |
| A622/89 | Human | 6102 | 1 | 2 | 40 | 3:3 |
| A623/89 | Human | 6102 | 1 | 2 | 94 | 3:4 |
| A624/89 | Human | 6102 | 1 | 2 | 40 | 3:ND |
| A625/89 | Human | 6102 | 1 | 2 | 40 | 3:ND |
| A626/89 | Human | 6102 | 1 | 2 | 40 | 3:ND |
| A627/89 | Human | 6102 | 1 | 2 | 37 | 3:ND |
| A628/89 | Human | 6102 | 1 | 2 | 37 | 3:3 |
| School 2 (1988) | | | | | | |
| A629/89 | Human | 6100 | 4,13,16,50 | 7 | 44 | 4:5·2 |
| A630/89 | Human | 6100 | 14,13,16,50 | 7 | 44 | 4:5·1 |
| A631/89 | Human | 6100 | 14,13,16,50 | 7 | 44 | 4:ND |
| A632/89 | Human | 6100 | 14,13,16,50 | 7 | 44 | 4:ND |

* The LHS number is the total restriction digest group (*Hae* III and *Hind* III): the RHS number is the Southern blot hybridization (ribopattern) group; ND, not determined.

† NT, not typable.

(10–20 $\mu$l sample) was electrophoresed at 25 V for 18 h in an horizontal 0·7% (wt/vol) agarose (Gibco-BRL Ltd: ultrapure, electrophoretic grade) gel in a buffer containing 89 mM Tris-HCl, 89 mM boric acid, 2 mM disodium EDTA (pH 8·3). The DNA samples were also digested with *Hin*d III and electrophoresed as described above. The DNA fragments were then stained with ethidium bromide after electrophoresis and visualized using a u.v. transilluminator. A photograph of the DNA electrophoresis band pattern was taken as a permanent record. Patterns were compared visually.

*Probe synthesis*

A biotin-labelled cDNA probe, synthesized from a mixture of 16 and 23S rRNA from *Escherichia coli* (Sigma Chemical Co.), was prepared using methods described previously [23].

*Blotting and hybridization*

The DNA in the gel was nicked further after u.v. illumination by treatment with 0·25 N-HCl for 15–30 min, then denatured in 0·5 M-NaOH–1·5 M-NaCl for 1 h and neutralized in 0·5 M Tris hydrochloride (pH 7·2)–1·5 M-NaCl–1 mM disodium EDTA for 1 h. DNA transfer to Hybond-N membrane (0·45 $\mu$m pore size: Amersham International) was done overnight (18–20 h) in a Vacublot system (ABN, Emeryville, USA) as recommended by the manufacturer. The membranes were washed once in 2 × SSC (1 × SSC is 0·15 M-NaCl–0·015 M trisodium citrate), air dried and baked at 80 °C for 2 h.

The nylon membranes were prehybridized [24] at 42 °C for 3–4 h in a solution containing 50% (v/v) formamide, 5 × SSC, 5 × Denhardt solution (1 × Denhardt is 0·02% each polyvinylpyrolidone, Ficoll 400, and bovine serum albumin), 25 mM sodium phosphate (pH 6·5), 5% (w/v) SDS and 500 $\mu$g freshly denatured herring sperm DNA per ml. Nylon membranes were hybridized [24] at 42 °C for 18 h in a solution containing 45% (v/v) formamide, 5 × SSC, 1 × Denhardt solution, 20 mM sodium phosphate (pH 6·5), 5% (w/v) SDS, 5% (v/v) dextran sulphate 200 $\mu$g freshly denatured sheared herring sperm DNA per ml, and 1 $\mu$g heat-denatured biotinylated probe DNA. After hybridization, the nylon membranes were washed twice in 0·1% (w/v) SDS–2 × SSC for 5 min at room temperature, and twice in 0·1% (w/v) SDS–0·2 × SSC for 5 min at room temperature, and twice in 0·1% (w/v) SDS–0·16 × SSC for 15 min at 50 °C. The hybridization reactions were visualized colorimetrically with the BluGENE (Gibco-BRL Ltd) non-radioactive nucleic acid detection system, which contained streptavidin-alkaline phosphatase conjugate and dyes, as recommended by the manufacturer.

The hybridization patterns were screened for different bands, and positive (presence) and negative (absence) results were coded as 1 and 0 respectively. Similarity among strains was estimated by means of the simple matching coefficient (Ssm), which included negative matches and clustering was based on the UPGMA algorithm [25].

*Band size estimation*

Fragment sizes in the total digest and in the Southern blot hybridization patterns were calculated from migration distances by the DNA SIZE program as described previously [26].

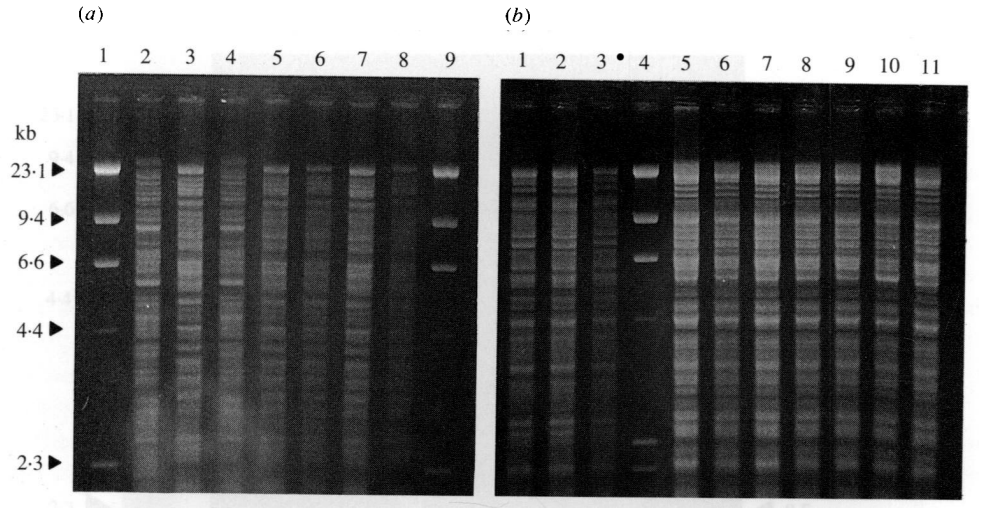


Fig. 1. Agarose gel electrophoresis of *Hae* III digest fragments of *Campylobacter jejuni* chromosomal DNA. Sizes indicated are for *Hin*d III digest fragments of bacteriophage λDNA. (*a*) Lanes 1 and 9; (*b*) lane 4. Other lanes from left to right in (*a*): lane 2, A620/89; lane 3, A619/89; lane 4, A621/89; lane 5, A618/89; lane 6, A617/89; lane 7, A616/89; lane 8, A615/89. These strains were all from the community outbreak. In (*b*): lane 1, A632/89; lane 2, A631/89; lane 3, A630/89; lane 5, A628/89; lane 6, A627/89; lane 7, A626/89; lane 8, A625/89; lane 9, A624/89; lane 10, A623/89; lane 11, A622/89. These strains were from the two school outbreaks.

## RESULTS

### *Biotyping, serotyping, and bacteriophage typing*

The results of biotyping, serotyping (HL and HS systems), and bacteriophage typing of the 18 strains of *C. jejuni* are listed in Table 1.

### *DNA restriction endonuclease digest patterns*

Chromosomal DNA from the 18 strains was cut with *Hae* III (recognition sequence GG.CC) at a high frequency to give multiple band patterns of 30 or more bands (Fig. 1). Visual inspection of the gels revealed four distinct patterns (Table 1). The DNA of representative strains of the above pattern types was then digested with *Hin*d III (recognition sequence A.AGCTT) to give further multiple band patterns (Fig. 2). The *Hae* III and *Hin*d III patterns were quite different as the *Hin*d III patterns generally were comprised of smaller sized bands but the grouping of strains was the same irrespective of the endonuclease used. The patterns obtained with both of these endonucleases were too complex for further detailed analysis.

### *Ribosomal RNA gene patterns*

Nine strains, representing each of the four *Hae* III and *Hin*d III pattern types identified above, were examined by Southern blot hybridization analysis using a 16S and 23S rRNA (*Escherichia coli*) gene probe. Only those strains with different biotypes, serotypes and phage types were studied. Fig. 3 illustrates the five and six band ribopatterns derived from the *Hae* III DNA digests, and Fig. 4 illustrates the four- and five-band ribopatterns derived from the *Hin*d III DNA digests. The

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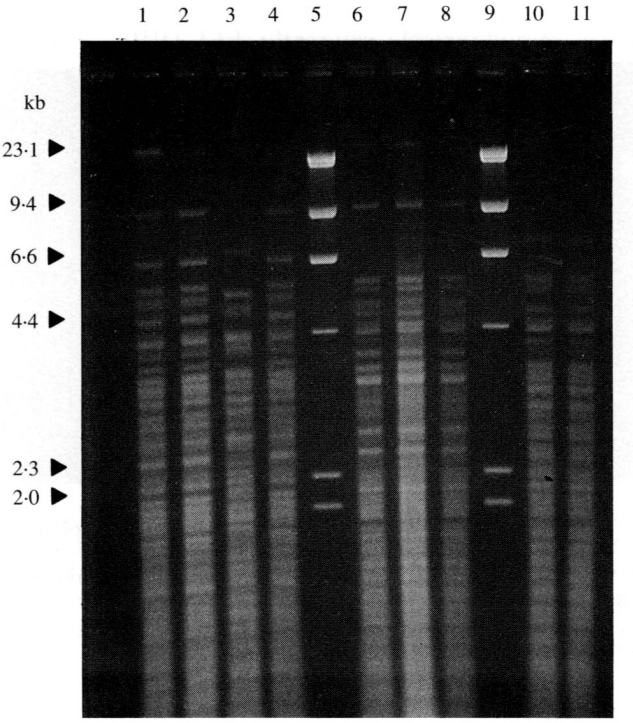


Fig. 2. Agarose gel electrophoresis of *Hin*d III digest fragments of *Campylobacter jejuni* chromosomal DNA. Sizes are indicated for *Hin*d III digest fragments or bacteriophage λDNA (lanes 5 and 9). Other lanes from left to right: lane 1, A615/89; lane 2, A618/89; lane 3, A621/89; lane 4, A619/89; lane 6, A622/89; lane 7, A623/89; lane 8, A628/89; lane 10, A629/89; lane 11, A630/89.

Table 2. C. jejuni *groups based on ribosomal RNA gene patterns* (*ribopatterns*)

| Ribopattern group* | Strain | band size (kb) | |
|---|---|---|---|
| | | *Hae* III digest | *Hin*d III digest |
| 1 | A615/89, A618/89, A619/89 | 11·1, 6·4, 5·1, 4·3, 2·7, 2·5 | 5·6, 5·1, 4·2, 1·9 |
| 2 | A621/89 | 14·3, 9·6, 6·1, 4·4, 2·3, 2·1 | 5·3, 4·7, 2·3, 1·9 |
| 3 | A622/89, A628/89 | 11·1, 6·4, 4·3, 2·7, 2·5 | 5·6, 4·2, 3·7, 1·9 |
| 4 | A623/89 | 11·1, 6·4, 4·3, 2·7, 2·5 | 5·6, 4·2, 3·7, (2·6)†, 2·3, (2·1), 1·9 |
| 5 | A629/89, A630/89 | (28.9), 15·4, 11·1, 8·5, 4·0, 2·5 | 5·4, 5·0, 4·2, 1·9 |

* Based on clusters in Fig. 5.
† Figures within parentheses represent weak intensity band.

relative molecular sizes (kb) of the various bands in each ribopattern are listed in Table 2. The combined results from the two Southern blot hybridizations, yielding 31 unique bands, were used as the basis of a numerical analysis to determine the similarities between the nine strains, and the dendrogram obtained is illustrated in Fig. 5. At the 90% similarity level, five clusters (ribopattern groups) were formed and they were comprised as follows:

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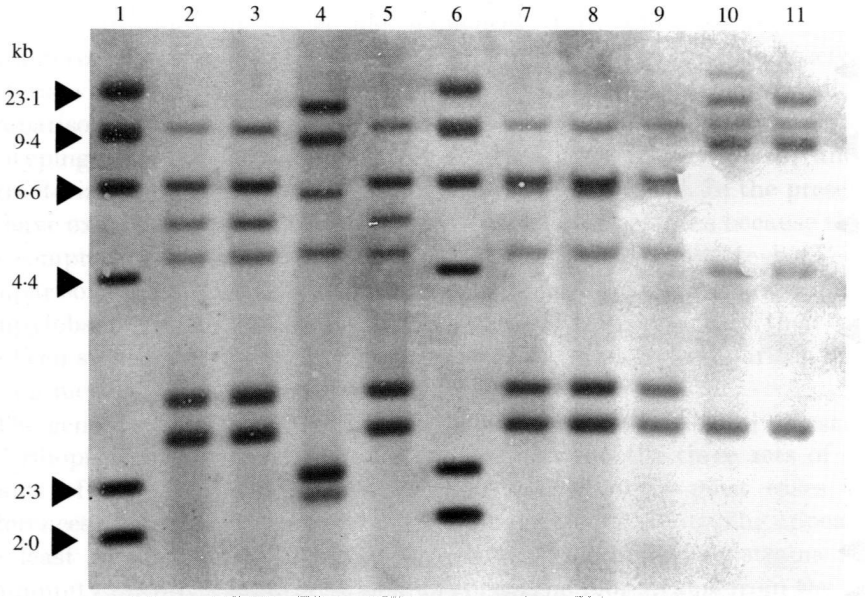


Fig. 3. Ribopatterns obtained by Southern blot analysis of an agarose gel (*Hae* III digest) hybridized with biotinylated cDNA probe transcribed from rRNA of *E. coli*. From left to right; lanes 1 and 6, bacteriophage λ*Hin*d III digest; lane 2, A615/89; lane 3, A618/89; lane 4, A621/89; lane 5, A619/89; lane 7, A622/89; lane 8, A623/89; lane 9, A628/89; lane 10, A629/89; lane 11, A630/89.

*Cluster 1* (ribopattern group 1) contained three strains (A615/89, A618/89, A619/89) from the community outbreak: they had identical biotypes, Lior serotype and phage group, but the Penner serotype antigens (4, 13 and 50) were expressed to different degrees.

*Cluster 2* (ribopattern group 2) contained one strain (A621/89), which was a dog isolate from the community outbreak.

*Cluster 3* (ribopattern group 3) contained two strains (A622/89, A628/89) from the outbreak at School 1, which had identical biotype and serotype but different phage types (PG40 and 37 respectively).

*Cluster 4* (ribopattern group 4) contained one strain (A623/89), also from the outbreak at School 1; it differed from the other strains in this outbreak in belonging to phage group 94.

*Cluster 5* (ribopattern group 5) contained two strains (A629/89, A630/89) from the outbreak at School 2: they had identical biotype, Lior serotype but differed in their Penner 4 and 14 cross-reactivity, phage group, and had a single-band difference in their ribopatterns.

*DNA group*

The DNA group designations of the various strains are shown in Table 1. The designation used is a combination of the total digest pattern type and where determined, the ribopattern group based on the numerical analysis.

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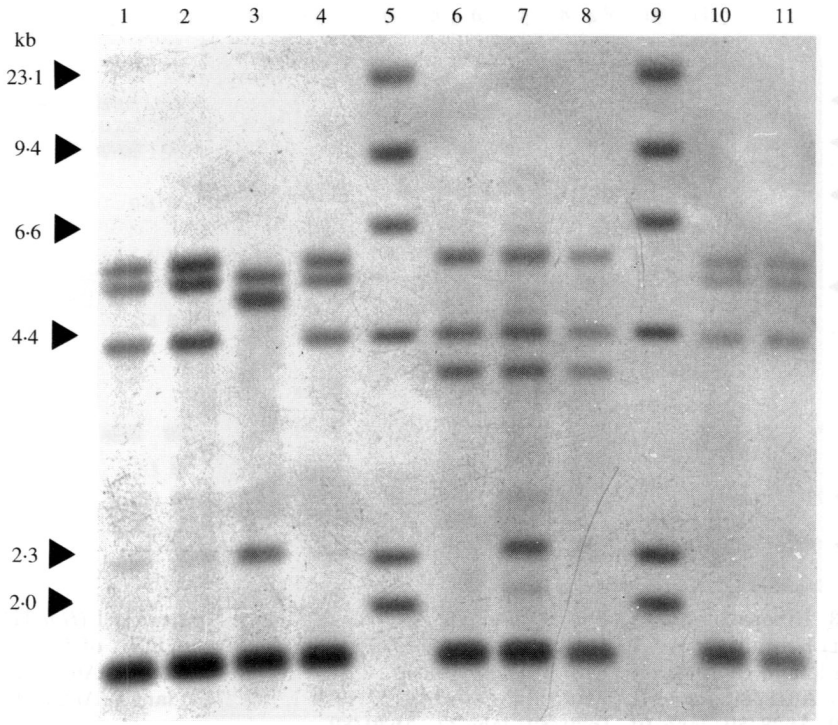


Fig. 4. Ribopatterns obtained by Southern blot analysis of *Hind* III digests. Lanes 5 and 9, bacteriophage λ*Hind* III digest; lane 1, A615/89; lane 2, A618/89; lane 3, A621/89; lane 4, A619/89; lane 6, A622/89; lane 7, A623/89; lane 8, A628/89; lane 10, A629/89; lane 11, A630/89.

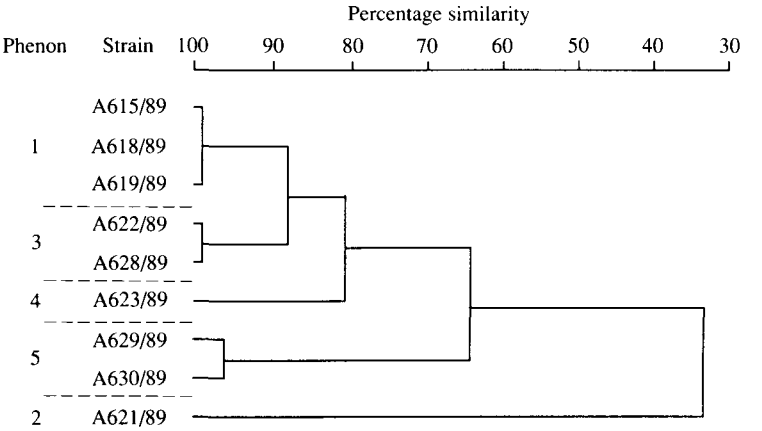


Fig. 5. Dendrogram of the cluster analysis of isolates of *C. jejuni* based on combined ribopatterns of *Hae* III and *Hind* III digests. Similarity amongst strains was estimated by means of the simple matching coefficient (Ssm).

## DISCUSSION

A development in the field of microbial identification of increasing interest to epidemiologists investigating and controlling hospital and community infection outbreaks is the use of DNA probes in providing molecular fingerprints for

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identification. Because these DNA fingerprints are highly sensitive to minor genomic variations in nucleotide sequences, they offer a precise means of characterizing species and of identifying individual strains of closely related bacteria when more traditional typing methods are unsuitable or unavailable [19]. Comparisons of DNA digest patterns have been used in conjunction with serotyping in several epidemiological studies of *C. jejuni* [15, 16] and in the characterization of cross-reacting serotypes of *C. jejuni* [27]. In the present study we have examined ribopatterns as well as total digest patterns because the former are comprised of less complex patterns of bands and facilitate between-strain comparisons. The potential of such DNA patterns as epidemiological markers for campylobacteria has been demonstrated [28, 29] but previously their value has not been systematically evaluated on outbreak strains by comparison with other typing methods.

The genomic data presented here, which comprises DNA total digest patterns and ribopatterns, revealed clear differences between the three sets of outbreak strains of *C. jejuni* and the results were consistent in most cases with the differences observed using serotyping and phage typing. Biotyping appeared to be the least discriminatory of the methods used because five strains from the community outbreak had the same biotype as the four strains from the outbreak at School 2. The limitations of biotyping for *C. jejuni* have been discussed previously [8, 21].

Two groups of strains within the community set were evident from the DNA data, and these were concordant with differences observed in biotype, serotype and phage groups. This outbreak was associated with puppies [21] but unfortunately none of the puppy isolates were available for testing by the DNA fingerprinting methods. It seems reasonable to assume, however, since all of these human and puppy strains were of the same serotype, biotype and phage group [20], that the puppy strains would have given the same DNA grouping results as the human isolates. The fact that two DNA group 1.1 human isolates (A618/89 and A619/89) had different Penner antigens was confusing, but the DNA data confirms that variations in expression of the 4, 13, 16 and 50 antigens do not necessarily indicate serotype specificity. This DNA grouping result supports the phage typing [20]. Two strains in DNA group 2.2 (A620/89 and A621.89) were different from the other community outbreak strains. One was isolated from a young child and the other from an adult dog; both isolates were epidemiologically linked. In this instance, the DNA grouping technique showed the same level of discrimination as the other typing methods, although the strains were non-typable in the Lior serotyping scheme.

The seven strains from the outbreak at School 1 were homogeneous with respect to serotype and biotype, but comprised three phage groups (PG 37, 40 and 94). The DNA data confirmed that all of the PG 40 strains were identical but did not substantiate PG 37. The two strains (A627/89 and A628/89) of this phage group were similar by biotyping, serotyping and DNA grouping to the PG 40 strains. This result suggests that these phage typing results need to be carefully evaluated. However, strain A623/89 of phage group 94, which was the same biotype and serotype as the other School 1 outbreak strains, was different according to the ribopatterns.

The isolates from the outbreak at School 2 were an homogeneous group by each

of the typing methods. However, they had affinities to the community outbreak strains in having a number of antigens of the Penner serogroup complex 4,13,50 in common. Both of these outbreak strain sets were different, however, by Lior serotyping, phage typing and DNA grouping. This finding highlights the excellent discriminatory power of the DNA grouping technique and confirms the value of using at least two different typing techniques when testing strains from different outbreaks [11–15]. Our results show clearly that both biotyping and DNA grouping have a major advantage over serotyping and phage typing in that strains of *C. jejuni*, which are untypable by the latter two methods, always give a result with biotyping and DNA grouping.

The DNA grouping method successfully discriminated between the causative strains in each of the three outbreaks and provided additional epidemiological information on some of these strains. The technique is therefore very useful for typing campylobacters isolated during such outbreaks. There is at present no generally agreed method of defining strain types based on total digest or ribopatterns because of the difficulties of comparing complex total digest patterns unless computer-assisted methods are used [29]. Furthermore, only limited ribopattern data is available on *C. jejuni* at present. The DNA groupings we have described are therefore provisional and were devised to combine both sets of DNA data. The ribopatterns were derived using *E. coli* ribosomal RNA as a template for probe synthesis and although available evidence indicates that it is useful broad spectrum probe [19], we have evidence from studies on *Helicobacter* and other genera, which are phylogenetically distinct from *E. coli*, that the probe has less specificity than probes derived using rRNA from the same species. In the case of *C. jejuni*, further ribopattern studies using a *C. jejuni* specific probe are needed to continue the development of the technique as a typing method.

ACKNOWLEDGEMENTS

J.H. was the recipient of a Senior Training Grant (BAP-0338-UK) provided within the framework of the Commission of the European Communities Research Action Programme for Biotechnology.

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