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Molecular subtyping by genome and plasmid analysis of *Campylobacter jejuni* serogroups O1 and O2 (Penner) from sporadic and outbreak cases of human diarrhoea

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SUMMARY

Ribosomal RNA gene patterns, randomly amplified polymorphic genomic DNA (RAPD) profiles and plasmid profiles were used to discriminate between 28 strains of *Campylobacter jejuni* serogroups O1 and O2 (Penner). Most isolates were biotype I (Lior). The strains were representative isolates from a UK school outbreak of enteritis (7 cases) and from 21 sporadic human cases of enteritis in 4 countries. The molecular techniques discriminated to various degrees between strains in each of the serogroups. The outbreak strains were homogeneous in most molecular features but a variety of types was detected amongst the isolates from the sporadic cases. Five groups of two or more strains with identical ribopatterns were identified and within each, strains from different patients were homogenous with respect to serogroup. RAPD profile typing based on numerical analysis generally matched ribotyping. Plasmid profiling overall gave least discrimination but was useful in separating some strains similar in other features. We concluded that optimal discrimination of *C. jejuni* could best be achieved using a combination of phenotypic and genotypic properties. *Hae* III ribotyping was the single most discriminatory and reproducible technique investigated. Several strains of *C. jejuni* from sporadic infections had similar molecular profiles which have potential for general typing purposes.

INTRODUCTION

Despite the prevalence worldwide of campylobacter infections in man, only limited epidemiological data are available on the distribution of different strain types within populations, in animal hosts, in food and in the environment [1–4]. Epidemiological surveillance and monitoring of *Campylobacter jejuni*, the major bacterial causative agent of diarrhoeal disease in man [5–8], has been based mainly on biotyping according to the schemes of Skirrow [9] and of Lior [10] and

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on the internationally recognized serotyping systems of Penner and Hennessy [11] and of Lior and coworkers [12]. Phage typing has been developed but utilized in only a limited number of studies on *C. jejuni* [13–15]. Similarly, DNA (molecular) typing, based on total genomic DNA digest patterns from conventional [14, 16–19] and pulse field gel electrophoresis [20], ribosomal RNA gene restriction digest patterns [14, 17, 21–24], and plasmid profiling [25], has been applied to a limited extent. Recently, the polymerase chain reaction (PCR) was used to obtain randomly amplified polymorphic DNA (RAPD) profiles, which provide an additional and apparently discriminatory method of typing strains of *C. jejuni* [26, 27]. The bands in such profiles represent amplifications of genomic DNA between adjacent short (10-mer) random primer sites, and differences in band size are attributed to polymorphisms in distances between such sites in different genomes. The differences appear to provide specific strain markers in *C. jejuni*.

In this study, we investigated the rRNA gene restriction patterns (ribopatterns), genomic RAPD-profiles and plasmid profiles of strains of *C. jejuni* within Penner serotypes O1 and O2, which are two of the most prevalent serotypes in the UK [1] and South Africa [28]. The aim was to determine which method or combination of methods was most discriminatory and reproducible for general typing purposes in epidemiological studies.

MATERIALS AND METHODS

Bacterial strains

The strains of *C. jejuni* used in this study are listed in Table 1 with their sources, alternative strain numbers, and Penner serotype. Sixteen strains were isolated from blood or faeces submitted to the Department of Microbiology, Red Cross War Memorial Children's Hospital, Cape Town. All these isolates came from independent patients except 5 and 7, which were from faeces and blood of the same individual. Seven other clinical strains received from F. J. Bolton, Public Health Laboratory, Preston were examined because they were representatives from a school outbreak of enteritis in the north-west of England in 1988 associated with contamination of a private drinking water supply. Three reference cultures (NCTC 11322, NCTC 12500 and NCTC 12501) originated from the USA and Canada, respectively; the latter two strains were the reference cultures for Penner serotypes O1 and O2.

All bacteria were cultivated at 37 °C for 48 h on 5% (v/v) defibrinated sheep blood agar under microaerophilic conditions in an anaerobic jar (catalyst removed) that was evacuated to a pressure of 560 mm of Hg (*c.* 74·7 KPa) and filled with 10% CO_2, 5% O_2, and 85% N_2. Strains were preserved at −70 °C on glass beads on Nutrient Broth No. 2 (Oxoid CM67) containing 10% (v/v) glycerol, and were also lyophilized in 5% (w/v) inositol serum.

Biotyping and serotyping

The bacteria were examined using the following conventional bacteriological tests: Gram stain, growth at 42 °C, catalase production, hippurate hydrolysis, nitrate reduction, nalidixic acid and cephalothin susceptibility, H_2S production (TSI medium and rapid test) and DNA hydrolysis. The methods used were as

Table 1. *Details of strains of* Campylobacter jejuni *used and typing results*

Study no.	Strain number	Specimen	Country of origin	Serotype (PEN)	*Hae* III Ribotype (r-type)	Plasmid content (Md)	RAPD type (a-type)
1	A700/92	faeces	RSA	O2	1	60	4
2	A701/92	faeces	RSA	O2	11	nd*	12
3	A703/92	blood	RSA	O2	3	nd	8
4	A704/92	faeces	RSA	O2	5	nd	6
5	A705/92	faeces†	RSA	O2	5	30	6
6	A706/92	faeces	RSA	O2	12	3·2	12
7	A707/92	blood†	RSA	O2	5	30	6
8	A708/92	faeces	RSA	O1	10	nd	8
9	A709/92	faeces	RSA	O1	7	nd	5
10	A710/92	faeces	RSA	O1	7	nd	5
11	A711/92	faeces	RSA	O1	2	nd	13
12	A712/92	faeces	RSA	O1	6	35	nt
13	A713/92	faeces	RSA	O1	7	nd	5
14	A714/92	faeces	RSA	O1	7	nd	5
15	A715/92	faeces	RSA	O1	6	nd	nt
16	A716/92	faeces	RSA	O1	4	nd	7
17	NCTC 11168	faeces	UK	O2	8	nd	1
18	NCTC 10983	blood	UK	O2	8	nd	1
19	NCTC 11322	faeces	USA	O1	6	35	3
20	NCTC 12500‡	faeces	Canada	O1	6	nd	2
21	A622/89	faeces§	UK	O1	9	nd	9
22	A623/89	faeces§	UK	O1	9	nd	10
23	A624/89	faeces§	UK	O1	9	nd	11
24	A625/89	faeces§	UK	O1	9	nd	11
25	A626/89	faeces§	UK	O1	9	nd	11
26	A627/89	faeces§	UK	O1	9	nd	11
27	A628/89	faeces§	UK	O1	9	nd	11
28	NCTC 12501‖	faeces	Canada	O2	8	nd	nt

* Abbreviations used: nd, not detected; nt, non-typable.
† From the same individual.
‡ Reference strain for serogroup O1.
§ From different individuals but outbreak-associated.
‖ Reference strain for serogroup O2.

described previously [17, 29]. Isolates were biotyped according to the extended scheme of Lior [10]. Serotyping was performed according to the heat-stable (somatic) antigenic scheme of Penner and Hennessy [11].

DNA isolation, restriction digestion, vacublotting and RNA gene hybridization

Chromosomal DNA was isolated and purified using the guanidium thiocyanate reagent method [30]. All DNA samples (5 μg) were digested for 4 h at 37 °C with *Hae* III (2–3 U/μg of DNA) according to the manufacturer's instructions (Boehringer Mannheim). The digested DNA was electrophoresed at 30 V for 16 h in a horizontal 0·7% (w/v) agarose gel in a buffer containing 89 mM Tris hydrochloride, 89 mM boric acid, and 2 mM disodium ethylenediaminetetraacetic acid (EDTA) (pH 8·3). After electrophoresis the DNA fragments were transferred to nylon membranes (Hybond-N, Amersham International) by vacuum transfer blotting. The membranes were then hybridized by previously described procedures [19, 31] for 18 h at 42 °C. The biotinylated copy (c)DNA probe used was prepared

from a mixture of 16S and 23S rRNA from *Escherichia coli* (Sigma) using Moloney mouse leukaemia virus reverse transcriptase (Gibco-BRL). Biotinylation was achieved by the incorporation of biotin-16-dUTP [32]. The membranes were washed after hybridization and the hybridized probe detected colorimetrically using a BluGENE (Gibco-BRL) nonradioactive detection kit. The pattern of bands containing rRNA gene sequences was designated the ribopattern.

Band-size estimation

DNA band sizes in the Southern blot hybridization patterns were calculated from migration distances by an automated gel reader and analysis system (IBI, New Haven, Conn., USA). Biotinylated lambda phage (Gibco-BRL Ltd) digested with *Hin*d III was used to provide the size markers. Three lanes (outside left and right, and centre) containing the markers were included on each gel to correct for within-gel distortions.

Computation of strain similarities

To compare ribopatterns from different membranes, the bands were coded according to size to minimize errors when determining similarities by computer-assisted methods of analysis. The blot hybridization patterns were screened for bands within 21 different size ranges up to 10 kb and positive (presence) and negative (absence) results were recorded. Bands of faint intensity were excluded. Double bands falling within a given range were scored as a single band. Computed similarities among strains were estimated by means of the Dice coefficient (negative matches excluded) and clustering of strains was based on the unweighted pair group method to facilitate the generation and the plotting of a dendrogram [33]. All computations were performed using the DNAGE program, a modification of previously described software [34].

Plasmid DNA detection

Single colonies from overnight cultures were taken and plasmid DNA was prepared by a modification of the method of Kado and Liu [35]. Cells were suspended in 250 μl of 3% SDS at pH 12·5 and incubated at 55 °C for 90 min. An equal volume of phenol/chloroform (1:1) mixture was added with thorough mixing, followed by centrifugation at 12000 g for 15 min at 4 °C. The upper layer was removed, mixed with an equal volume of chloroform/2-pentanol (24:1), and centrifuged (12000 g for 15 min) at 4 °C. Twenty μl of the supernatant fluid were then mixed with 5 μl of dye solution and electrophoresed in a horizontal 0·7% agarose gel for 2 h at 100 V. The gels were stained with ethidium bromide, viewed in a u.v. transilluminator and photographed. Standard plasmids in *E. coli* K12 hosts were used to determine molecular weights.

RAPD analysis

A 200 μl suspension of bacterial cells was boiled for 10 min and centrifuged for 5 min. The OD_{260} of the supernatant was measured and dilutions were prepared to an $OD_{260} = 0{\cdot}15$ according to Mazurier and colleagues [26]. 10 μl of each dilution were used in the amplification reaction. The primer OPA-11 (Operon Technology Res. Inc., Alameda, CA) with sequence 5′-CAATCGCCGT-3′, was selected after preliminary testing of a number of different primers [27] as it gave a reproducible

and easy to read pattern of bands. A reaction volume of 100 μl was made up by addition of 2·5 units of *Taq* polymerase (BCL), 10 mM Tris-HCL pH 8·3 at 25 °C; 50 mM KCl; 2·0 mM-$MgCl_2$, 200 μM of each deoxynucleotide triphosphate, 0·3 μM of the primer, 10 μl of the cell suspension (OD_{260} = 0·15) and sterile distilled water. This solution was overlaid with 100 μl of paraffin oil and cycled through the following temperature profile: 1 cycle of 94 °C for 1 min, and 45 cycles of 94 °C for 1 min, 36 °C for 1 min, and 72 °C for 2 min. Incubation was in a thermocycler (PCR Heating Block, Hybaid Ltd, Teddington, Middlesex). Standard procedures were adopted to eliminate contamination [36]. The amplified DNA products were electrophoresed on 1·2% (w/v) agarose gels (ultra pure electrophoresis grade; Gibco-BRL) and stained in ethidium bromide solution. Bacteriophage λ DNA digested with *Bst*E II was used as a molecular weight marker (Appligene, Chester-le-Street, Co. Durham). Purified genomic DNA for RAPD analysis was obtained by the miniprep protocol of Wilson [37] using cetyltrimethylammonium bromide (CTAB).

RESULTS

Ribosomal RNA gene patterns

Chromosomal DNAs from the 28 strains of *C. jejuni* that comprised Penner serotype O1 (18 strains) and *C. jejuni* Penner serotype O2 (10 strains) were digested with *Hae* III to a high frequency to give multiple digest patterns. Characteristic ribosomal rRNA gene restriction patterns were produced when Southern blots were probed with a biotin-labelled cDNA from 16+23S rRNA from *E. coli* (Fig. 1). Bands in a region of the ribopattern, which comprised between 5 and 11 fragments ranging in size between 1 and 10 kb, were digitized and compared by numerical analysis. The resultant dendrogram (Fig. 2) shows diversity amongst strains of the same as well as different serotypes of *C. jejuni*. In the numerical analysis, 12 different ribopatterns (r-1 to r-12) were identified at the 95% similarity level. The ribotypes assigned to the various strains are listed in Table 1, and are also shown in Figure 2. Seven ribotypes (r-1, r-2, r-3, r-4, r-10, r-11 and r-12) were each represented by just a single strain. The other five ribotypes, however, were each represented by two or more strains as outlined below.

Ribotype 5: three serogroup O2 isolates were from two patients in South Africa, which included a blood and faecal isolate from one patient.

Ribotype 6: four serogroup O1 cultures from unrelated patients in South Africa, the United States and Canada. The reference strain (NCTC 12500) for serogroup O1 was in this ribogroup.

Ribotype 7: four serogroup O1 cultures from four unrelated patients in South Africa.

Ribotype 8: three serogroup O2 reference cultures (NCTC 11168 and NCTC 10983) from UK patients (original sources were faeces and blood respectively), and NCTC 12501 from Canada.

Ribotype 9: seven serogroup O1 cultures from different patients representing the school outbreak in the north-west of England in 1988.

In the dendrogram (Fig. 2) most strains were linked at $\geq$ 50%S indicating they were all members of *C. jejuni*. Strains 2 and 6 (both serogroup O2) were exceptions because they were linked at a low level (< 20%) to the other isolates, so were

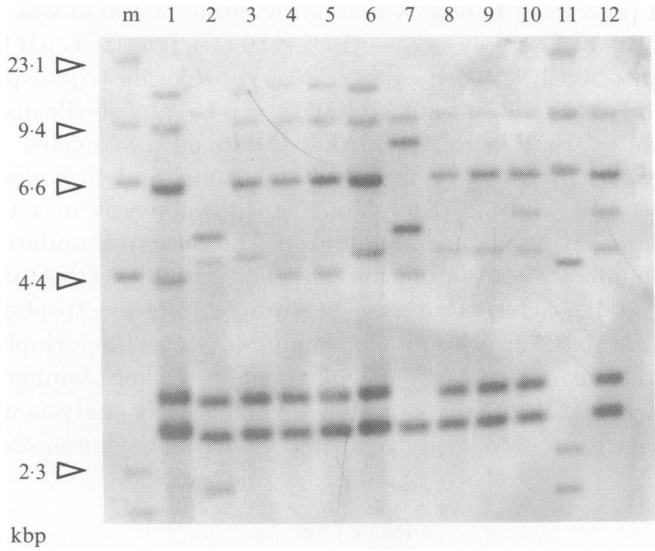

Fig. 1. Ribosomal RNA gene patterns for *Hae* III genomic DNA digests of representative strains of *C. jejuni* probed with biotinylated cDNA from 16+23S rRNA of *E. coli*; m, *Hind* III digests of bacteriophage λ.

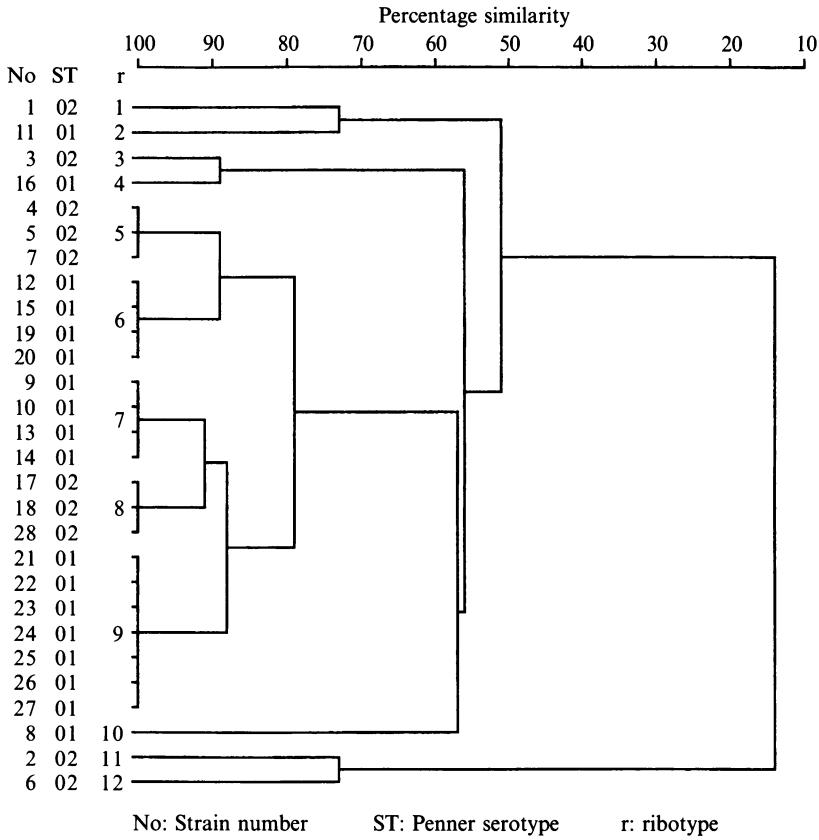

Fig. 2. UPGMA clustering dendogram of the *Hae* III ribopatterns of *C. jejuni* strains of serogroups O1 and O2. The simple matching coefficient was used. A ribotype was defined as comprising strains with profiles with $\geqslant 95\%$ similarity.

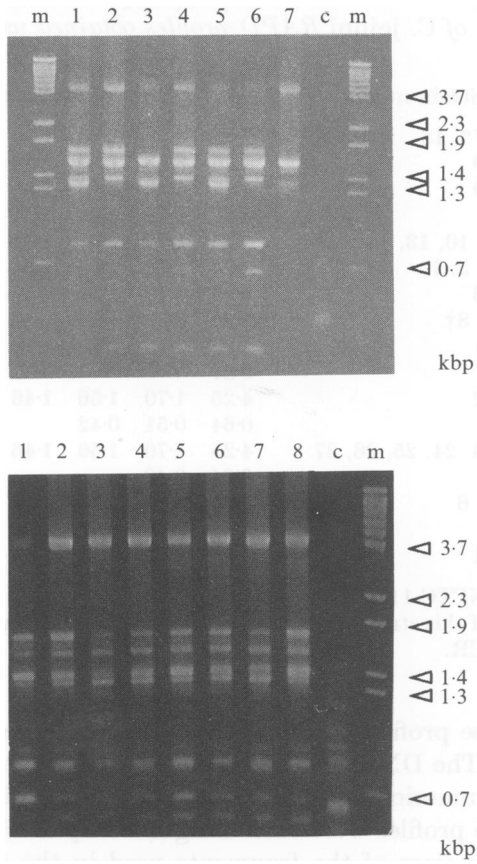

Fig. 3. RAPD profiles of representative strains of *C. jejuni* with primer OPA-11. Panel A, the results are for reproducibility tests on samples from NCTC 11168 (lanes 1,3,5 7) isolate 5 (lanes 2,4 6). M, marker DNA fragments are *Bst*E II digests of bacteriophage λ DNA. C, is the negative control sample. Panel B, the results are for outbreak-associated isolates. Lanes: 1, isolate 22; 2, isolate 23; 3, isolate 24; 4, isolate 25; 5, isolate 26; 6, isolate 27; 7, isolate 25; 8, isolate 27.

genomically distinct from typical *C. jejuni*. Most strains (21/28) were linked at 80% similarity to form a relatively homogeneous genomic grouping of the five ribotypes detailed above.

Plasmid profiles

The 28 strains of *C. jejuni* were screened and the plasmids were detected in six (22%). The plasmids had sizes of 3·2, 30, 35 and 60 Md and were present in the strains shown in Table 1. Frequency of plasmids in the serogroup O1 strains was 11% (2/18) and in the serogroup O2 strains was 40% (4/10).

RAPD analysis

The 28 strains of *C. jejuni* were subjected to RAPD analysis using the 10-mer oligonucleotide OPA-11. This primer was chosen for reproducibility and discrimination after preliminary investigation of ten other 10-mers [27]. The PCR products were electrophoretically separated on an agarose gel yielding multiple distinct DNA products with sizes in the range *c.* 420 bp to 4·2 kbp. The number of

Table 2. *Types of* C. jejuni *RAPD profiles obtained with primer OPA-11*

RAPD pattern (type)	Strain number	Sizes of fragments in profiles (kbp)							
a-1	17*, 18	4·25	3·53	1·70	1·56	1·34	0·96	0·42	
a-2	20	4·25	3·53	1·70	1·56	1·34	0·96		
a-3	19	4·25	3·53	1·75	1·56	1·34	0·96		
a-4	1	4·25	1·70	1·56	1·34	0·96	0·81	0·42	
a-5	9, 10, 13, 14	4·25	1·70	1·56	1·34	0·96	0·81		
a-6	4, 5, 7	4·25	1·70	1·56	1·34	0·81	0·44		
a-7	16	4·25	1·70	1·56	1·34	0·81			
a-8	3, 8†	4·25	3·53	1·70	1·56	1·34	0·81	0·68	
a-9	21	4·25	3·53	1·75	1·70	1·56	1·46	1·05	0·96
		0·81							
a-10	22	4·25	1·70	1·56	1·46	1·34	0·96	0·81	0·72
		0·64	0·51	0·42					
a-11	23, 24, 25, 26, 27	4·25	1·70	1·56	1·46	1·34	0·96	0·81	0·72
		0·64	0·42						
a-12	2, 6	4·25	3·53	1·70	1·34	1·05	0·81	0·72	0·68
		0·51	0·44	0·42					
a-13	11	4·25	3·53	1·70	1·56				

* Reference strain NCTC 11168.

† The RAPD type of this strain was equivocal because minor band differences were observed on repetition of the PCR.

DNA bands in these profiles ranged from 4 to 11. Representative examples are shown in Figure 3. The DNA profiles were compared by visual analysis and more rigorously by the numerical methods used for analysis of the ribopatterns (data not presented). The profiles were then designated by arbitrary numbers as listed in Table 2 with the sizes of the fragments used in the analysis. RAPD profile analysis allowed discrimination between isolates of *C. jejuni* sharing the same serotype. Thus, nine RAPD types (a-2, a-3, a-5, a-7 to a-11 and a-13) were found amongst the serogroup O1 strains, and five RAPD types (a-1, a-4, a-6, a-8, a-12) were found amongst the serogroup O2 strains. Also, minor band differences were revealed between the epidemiologically related strains in the UK school outbreak with three RAPD types identifiable (a-9, a-10, and a-11); however, in overall features these RAPD profiles were similar.

The analysis did not include strains (NCTC 12501, 12 and 15) for which no RAPD profiles could be obtained because DNA was degraded during heat lysis and PCR. This effect was attributed to DNAse activity by these particular isolates, and as an alternative, purified DNA was extracted by the CTAB method. However, no PCR products using the OPA-11 primer could be obtained with genomic DNA from these three strains which were recorded as non-typable. Types a-8 and a-12 were each represented by two strains with different serotypes. Repeated PCR confirmed similarities of strains 2 and 6 (type a-12) but the similarities between strains 3 and 8 (type a-8) were not completely reproducible, with minor fluctuations in the presence and the intensity of some bands. Reproducibility also was assessed by repeated PCR of NCTC 11168 DNA extracts, which generally gave constant profiles although occasionally bands were not present or were of faint intensity (Fig. 3). Standard precautions were taken to

eliminate contamination of the PCR with extraneous DNA [36]. Even so there was occasionally evidence of minor products in some 'negative' controls. The products were smaller than those in the campylobacter profiles and were not considered to invalidate the analyses performed.

DISCUSSION

Chromosomal and plasmid DNA diversity was detected both within and between clinical isolates of *C. jejuni* Penner serogroups O1 and O2. The investigation was based on 28 strains from 27 individuals in 4 countries although the strains were predominantly (57%) from patients with enteritis in South Africa. The genomic DNA heterogeneity of strains within a serogroup confirmed and extended previous molecular typing data on strains of *C. jejuni* serogroups O2 and 20 [21]. The results clearly demonstrated that a high degree of ribotype diversity exists between sporadic strains of the same serotype with six distinct ribotypes amongst the serogroup O1 strains, and six ribotypes amongst the serogroup O2 strains of *C. jejuni*. Previously, diversity was also reported in *C. coli* Lior serogroup 20 [38].

Biotypes of *C. jejuni* are relatively simple to determine and are a logical starting-point for typing strains but provide too little discrimination for use as an independent system for meaningful epidemiological investigations. However, knowledge of DNAse activity, a defining feature of Lior biotypes II and IV, is useful in choosing suitable methods for subsequent DNA analysis. A method such as the Wilson [37] miniprep is essential to avoid isolation of degraded DNA, which results in strains being untypable.

Ribopatterns provide a well validated and reproducible means of discriminating between strains in many bacterial species [39], and have proved to be particularly effective in identifying strains of *C. jejuni* [14, 17, 21–24]. We have shown in previous studies that overall ribopattern similarities broadly reflect the taxonomic affinities of strains within the thermophilic campylobacters. The identification in the present study of two serogroup O2 strains (isolates 2 and 6) with ribotypes r-11 and r-12 respectively, provide further support to our previous observations [21] that some isolates of *C. jejuni*, when defined just on conventional phenotypic criteria, are in fact genomically divergent. These serogroup O2 strains, nevertheless, have their major lipopolysaccharides, which are the antigenic determinants in the Penner scheme, in common with other more typical serogroup O2 members of the species. It appears from our findings that the serogroup O1 and serogroup O2 antigens are each expressed in a wide range of different *C. jejuni* genotype backgrounds.

In an evaluation of methods to distinguish epidemic-associated *C. jejuni* strains, Patton and co-workers [14] found that ribotyping was one of the most sensitive and stable methods that distinguished strains of the same serotype. Their ribotyping was based on the use of *Pvu* II- and *Pst* I-digested DNA with *E. coli* 16S and 23S rRNA, which in our experience agrees closely with ribotyping using *Hae* III digested DNA either from *E. coli* or *C. jejuni* (R. J. Owen and M. Desai, unpublished results).

As ribopattern analysis is a time consuming and technically complex activity

for routine clinical microbiological practice, there is a need for alternative methods that are simpler yet reproducible. Mazurier and colleagues [26] demonstrated that RAPD profiles had considerable potential in that respect for *C. jejuni* and, depending on the primers used for PCR, allowed discrimination of strains within Penner and Lior serotypes including serologically non-typable strains. Our results support the findings of Mazurier and colleagues [26] and show that RAPD profiling based on the OPA-11 primer, there were no sequence homologies between this 10-mer and HLWL 85, the most discriminating primer tested by Mazurier and colleagues [26], distinguished strains of the same serotype and gave a level of discrimination comparable to ribotyping. Furthermore, the molecular types of *C. jejuni* defined by analysis of ribopatterns and RAPD profiles were generally in close correspondence. Strain sets r-5/serogroup O2 (three strains), r-7/serogroup O1 (three strains) and r-8/serogroup O2 (three strains) were all homogenous with respect to RAPD type. Five other strains of *C. jejuni* had unique profiles in both methods. However, some anomalies were apparent in the RAPD data. Strains 2 and 6 were unusual in having unique ribotypes (r-11 and r-12) yet were identical in RAPD profiles. These strains were genomically atypical of *C. jejuni* according to overall ribopattern similarity so they possibly had a different genome organization which could explain why the OPA-11 primer profiles lacked the discrimination found with typical *C. jejuni*. In the epidemic-associated set (r-9/serogroup O1), most isolates had the same RAPD profile but two strains could be further discriminated on minor differences in RAPD profiles. Although phage typing was not evaluated in the present study, it is relevant to mention that our RAPD typing data did not exactly match the subtyping of these strains by the Preston phage typing scheme [15]. This strain set has been compared by total DNA restriction digest analysis and ribotyping with *Pvu* II, *Pst* I and *Hind* III and none of these investigations revealed any significant genetic diversity between the strains [R. J. Owen and M. Desai, unpublished results]. A disadvantage of RAPD profiling was that some strains were non-typable, possibly because the DNA was degraded and no PCR products could be obtained. This fact confirmed the value of using biotyping to establish which strains exhibited DNAse activity and potentially would be non-typable unless suitable DNA isolation procedures were used. The main problem we encountered with RAPD profiling was the difficulty of ensuring reproducibility from gel to gel and this is an aspect of the technique that needs more detailed investigation before the discriminatory power can be fully assessed.

Plasmids were detected with a low overall frequency in the strains examined and were of no discriminatory value in general typing of *C. jejuni*. Other studies differ considerably in the frequency of plasmid detection reported in this species and no consistent associations with serotyping were found [25, 40]. Although the numbers of strains examined were small, we found that the serogroup O2 strains had the highest frequency of plasmid occurrence (4/9). Ribotypes r-5 and r-6 each had strains bearing plasmids of a characteristic size; plasmids in type r-5/serogroup O2 were 30 Md and those in type r-6/serogroup O1 were 35 Md. In the latter two sets of strains, plasmid profiling was the only method of subtyping that provided evidence of additional differences between strains. In some cases therefore, plasmids are a useful adjunct to other typing methods.

We conclude that typing of *C. jejuni* by biotyping and serotyping does not provide sufficient discrimination for accurate epidemiological investigation. A polyphasic approach based on both phenotypic and genotypic data is desirable and has potential for larger scale studies with the availability of a widening range of different DNA fingerprinting methods. Novel techniques such as RAPD profiling provide a potentially significant advance, although further evaluations are needed on larger numbers of isolates of *C. jejuni* to assess fully the reproducibility and discrimination of different primers.

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REFERENCES

1. Fricker CR, Park RWA. A two-year study of the distribution of 'thermophilic' campylobacters in human, environmental and food samples from the Reading area with particular reference to toxin production and heat-stable serotype. J Appl Microbiol 1989; **66**: 477–90.
2. Griffiths PL, Park RWA. Campylobacters associated with human diarrhoeal disease. J Appl Bacteriol 1990; **69**: 281–301.
3. Park RWA, Griffiths PL, Moreno GS. Sources and survival of Campylobacters: relevance to enteritis and the food industry. J Appl Bacteriol Symp Suppl 1991; **70**: 978–1068.
4. Skirrow MB. A demographic survey of campylobacter, salmonella and shigella infections in England. Epidemiol Infect 1987; **99**: 647–57.
5. Butzler JP, Oosterom J. *Campylobacter*: pathogenicity and significance in foods. Int J Fd Microbiol 1991; **12**: 1–8.
6. Cover TL, Blaser MJ. The pathobiology of *Campylobacter* infections in humans. Ann Rev Med 1989; **40**: 269–85.
7. Healing TD, Greenwood MH, Pearson AD. Campylobacters and enteritis. Rev Med Microbiol 1992; **3**: 159–67.
8. Penner JL. The genus *Campylobacter*: a decade of progress. Clin Microbiol Rev 1988; **1**: 157–72.
9. Skirrow MB. *Campylobacter*, *Helicobacter* and other motile curved gram-negative rods. In: Parker MT, Duerden, BI, eds. Topley & Wilson's principles of bacteriology, virology, immunology, 8th ed, vol 2, Systematic bacteriology. London: Edward Arnold, 1992: 530–49.
10. Lior H. New extended biotyping scheme for *Campylobacter jejuni*, *Campylobacter coli*, and *Campylobacter laridis*. J Clin Microbiol 1984; **20**: 636–40.
11. Penner JL, Hennessy JN. Passive haemagglutination technique for serotyping *Campylobacter fetus* subsp. *jejuni* on the basis of soluble heat stable antigens. J Clin Microbiol 1980; **12**: 732–7.
12. Lior H, Woodward DL, Edgar JA, Laroche LJ, Gill P. Serotyping of *Campylobacter jejuni* by slide agglutination based on heat-labile antigenic factors. J Clin Microbiol 1982; **15**: 761–8.
13. Grajewski BA, Kusek JW, Gelfand HM. Development of a bacteriophage typing system for *Campylobacter jejuni* and *Campylobacter coli*. J Clin Microbiol 1985; **22**: 13–18.

14. Patton CM, Wachsmuth IK, Evins GM, et al. Evaluation of 10 methods to distinguish epidemic-associated *Campylobacter* strains. J Clin Microbiol 1991; **29**: 680–8.
15. Salama SM, Bolton FJ, Hutchinson DN. Application of a new phage typing scheme to campylobacters isolated during outbreaks. Epidemiol Infect 1990; **104**: 405–11.
16. Bruce D, Hookey JV, Waitkins SA. Numerical classification of campylobacters by DNA-restriction endonuclease analysis. Zbl Bacteriol Mikrobiol Hyg Ser A 1988; **269**: 284–97.
17. Hernandez J, Owen RJ, Costas M, Lastovica A. DNA-DNA hybridization and analysis of restriction endonuclease and rRNA gene patterns of atypical (catalase- weak/negative) *Campylobacter jejuni* from paediatric blood and faecal cultures. J Appl Bacteriol 1991; **70**: 71–80.
18. Owen RJ, Beck A. Evaluation of three procedures using a laser densitometer and microcomputer for estimating molecular sizes of restriction endonuclease digest fragments and application to *Campylobacter jejuni* chromosomal DNA. Lett Appl Microbiol 1987; **4**: 5–8.
19. Owen RJ, Costas M, Dawson C. Application of different chromosomal DNA restriction digest fingerprints to specific and subspecific identification of *Campylobacter* isolates. J Clin Microbiol 1989; **27**: 2338–43.
20. Yan W, Chang W, Taylor DE. Pulse field gel electrophoresis of *Campylobacter jejuni* and *Campylobacter coli* genomic DNA and its epidemiological application. J Infect Dis 1991; **163**: 1068–72.
21. Fayos A, Owen RJ, Desai M, Hernandez J. Ribosomal RNA gene restriction fragment diversity amongst Lior biotypes and Penner serotypes of *Campylobacter jejuni* and *Campylobacter coli*. FEMS Microbiol Lett 1992; **95**: 87–94.
22. Geilhausen B, Mauff G, Vlaes L, Goossens H, Butzler JP. Restriction fragment length polymorphism for identification of *Campylobacter jejuni* isolates. Zbl Bakteriol Mikrobiol Hyg Ser A 1990; **274**: 366–71.
23. Hernandez J, Fayos A, Owen RJ. Biotypes and DNA ribopatterns of thermophilic campylobacters from faeces and seawater in Eastern Spain. Lett Appl Microbiol 1991; **13**: 207–11.
24. Owen RJ, Hernandez J, Bolton F. DNA restriction digest ribosomal RNA gene patterns of *Campylobacter jejuni*: a comparison with bio-, sero- and bacteriophage-types of United Kingdom outbreak strains. Epidemiol Infect 1990; **105**: 265–75.
25. Tenover FC, Williams S, Gordon KP, Harris N, Norlan C, Plorde. Utility of plasmid fingerprinting for epidemiological studies of *Campylobacter jejuni* infections. J Infect Dis 1984; 149: 279.
26. Mazurier S, van de Giessen A, Heuvelman CJ, Wernan K. RAPD analysis of *Campylobacter* isolates: DNA fingerprinting without the need to purify DNA. Lett Appl Microbiol 1992; **14**: 260–2.
27. Owen RJ, Hernandez J. Ribotyping and arbitrary primer PCR-fingerprinting of campylobacters. In: Sussman M, ed. Application of new techniques in food beverage microbiology. SAB Technical Ser. 31. London: Academic Press, 1993. In press.
28. Lastovica AJ, Le Roux E, Congi RV, Penner JL. Distribution of sero-biotypes of *Campylobacter jejuni* and *C. coli* isolated from paediatric patients. J Med Microbiol 1986; **21**: 1–5.
29. Benjamin J, Leaper S, Owen RJ, Skirrow MB. Description of *Campylobacter laridis*, a new species comprising the nalidixic acid resistant thermophilic *Campylobacter* (NARTC) Group. Curr Microbiol 1983; **8**: 231–8.
30. Pitcher DG, Saunders NA, Owen RJ. Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. Lett Appl Microbiol 1989; **8**: 151–6.
31. Maniatis T, Fritsch EF, Sambrook J. Molecular cloning – a laboratory manual. Cold Spring Harbor: Cold Spring Harbor Laboratory, 1982.
32. Pitcher DG, Owen RJ, Dyal P, Beck A. Synthesis of a biotinylated DNA probe to detect ribosomal RNA cistrons in *Providencia stuartii*. FEMS Microbiol Lett 1987; **48**: 283–7.
33. Sneath PHA, Sokal RR. Numerical taxonomy. San Francisco: W. H. Freeman Co, 1973.
34. Costas M. Numerical analysis of sodium dodecylsulphate-polyacrylamide gel electrophoretic protein patterns for the classification, identification and typing of medically important bacteria. Electrophoresis 1990; **11**: 382–91.

35. Kado CI, Liu S-T. Rapid procedure for detection and isolation of large and small plasmids. J Bacteriol 1981; **145**: 1365–73.
36. Kwok S, Higuchi R. Avoiding false positives with PCR. Nature (London) 1989; **339**: 237–8.
37. Wilson K. Preparation of genomic DNA from bacteria. In: Ausubel FM, Brent R, Kingston RE, Moore DD, Smith JA, Seidman JG, Struhl K, eds. Current protocols in molecular biology. New York: Wiley, 1987.
38. Fraser ADE, Brooks BW, Garcia MM, Lior H. Molecular discrimination of *Campylobacter coli* serogroup 20 biotype I (Lior) strains. Vet Microbiol 1992; **30**: 267–80.
39. Owen RJ. Chromosomal DNA fingerprinting – a new method of species and strain identification applicable to microbial pathogens. J Med Microbiol 1989; **30**: 89–99.
40. Lind L, Sjogren E, Welinder-Olsson C, Kaijser B. Plasmids and serogroups in *Campylobacter jejuni*. APMIS 1989; **97**: 11097–102.