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The application of genotyping techniques to the epidemiological analysis of *Campylobacter jejuni*

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SUMMARY

Campylobacter jejuni serogroup reference strains and collections of sporadic and outbreak-associated isolates were examined for restriction fragment length polymorphisms (RFLPs), using *C. jejuni* random chromosomal and 16S rRNA gene probes. A collection of 48 Penner (HS) and 14 Lior (HL) serogroup reference strains, plus 10 clinical isolates, generated 35 RFLP and 26 ribotype patterns. In combination the two loci generated 48 distinct genotypes. Both probes were able to differentiate between certain random isolates of the same HS/HL serogroups but greater discrimination was obtained with RFLP than with ribotyping. Genotyping distinguished accurately between related and unrelated strains when applied to several outbreaks. Genotypic analysis of *C. jejuni* by restriction fragment length polymorphisms is a valuable technique for epidemiological typing. Chromosomal variation detected by the two unlinked probe loci provides some information about the genetic relationship between isolates.

INTRODUCTION

Campylobacter jejuni is a major cause of human bacterial gastroenteritis. Most infections are sporadic, and of more than 44000 cases reported during 1994 to the Communicable Disease Surveillance Centre of the Public Health Laboratory Service (CDSC, Colindale, England), only 1% may have been outbreak associated [1]. Whilst current phenotyping methods provide adequate discrimination for outbreak investigations, the epidemiology of sporadic campylobacter infection remains ill-defined. Strain characterization by existing phenotypic techniques is sometimes ambiguous due to the unavailability of standardized reagents, cross-reactivity between certain antigens and the high proportion of non-typable strains. Recent studies comparing established techniques with molecular biological methods for epidemiological characterization of *C. jejuni* and *C. coli* indicate that the latter offer greater potential for differentiating between strains, even amongst isolates which share the same surface markers (e.g. serogroup or phagetype determinants) [2–5]. High resolution genotyping methods can additionally evaluate the phylogenetic as well as epidemiologic relationships between strains [4, 6].

Detection of restriction fragment length polymorphisms (RFLPs) with either generic ribosomal RNA (rRNA) or specific DNA probes is a relatively simple and reproducible technique, and has been useful in the epidemiological characterization of many pathogenic microorganisms [6, 7]. This paper describes the application of random chromosomal and 16S rRNA RFLPs for the epidemiological analysis of *C. jejuni*. Serogroup reference strains, and isolates obtained from environmental samples, cases of sporadic infection and defined *C. jejuni* outbreaks have been

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examined. We compared results obtained with RFLP and ribotyping with those obtained by Penner (heat-stable, HS) and Lior (heat-labile, HL) serogrouping, Preston phagetyping and biotyping. Conserved associations were seen between certain phenotypic and genotypic markers, which may represent stable cell lineages within the population structure of this important enteric pathogen.

MATERIALS AND METHODS

Bacterial strains

A selection of *C. jejuni* serogroup reference cultures, supplied by Drs J. Penner (48 strains) and H. Lior (14 strains), were examined in this study. A total of 42 epidemiologically unrelated clinical, environmental and animal isolates, and collections of outbreak-associated strains were also examined.

Phenotyping

Cultures were stored at −70 °C in Brain–Heart Infusion broth (Oxoid, Unipath, Basingstoke, UK) containing 15% glycerol. Bacteria were grown on Columbia agar (Oxoid) supplemented with 5% whole horse blood for 48 h at 37 °C, in a Variable Atmosphere Incubator (5% CO_2, 3% H_2, 85% N_2, 7% O_2; Don Whitley Scientific Ltd, Shipley, W. Yorks, UK). Identification to species level, biotyping and phagetyping were performed at the Preston Public Health Laboratory using previously described methods [8, 9]. Penner (HS) and Lior (HL) serogroups were determined at the Manchester Public Health Laboratory according to standard methods [10, 11].

Preparation of purified chromosomal DNA

Chromosomal DNA was extracted as follows: *C. jejuni* cells (50 mg wet weight) were harvested from 24 h plate cultures and suspended in DNA wash buffer (50 mM Tris pH 8·0, 5 mM EDTA, 50 mM NaCl). Washed cells were suspended in 400 μl of ice-cold 50 mM Tris (pH 8·0) containing 25% sucrose and 10 mg/ml lysozyme. After incubation on ice for 20 min, cells were lysed by the addition of 50 μl of 5% sterile sodium dodecyl sulphate (SDS) in 50 mM Tris (pH 8·0), followed by 200 μl Tris/EDTA buffer (10 mM Tris, 1 mM EDTA, pH 8·0), and 50 μl of 2 mg/ml proteinase K. Lysates were incubated at 56 °C for 1 h, and extracted once with an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1), and once with chloroform. Nucleic acids were recovered by ethanol precipitation.

Restriction endonuclease digestion

Approximately 5 μg of DNA was digested with 10 units of *Hae* III (Appligene, Chester-le-Street, Co. Durham, UK) at 37 °C for 3 h, in a total volume of 30 μl. Samples were electrophoresed for 3 h at 10 V/cm in 0·7% agarose gels prepared in 1 × TBE (90 mM Tris-borate, 2 mM EDTA), with 1 × TBE running buffer. Gels were photographed after staining in 1 × TBE containing 0·5 μg/ml ethidium bromide for 30 min.

Southern blotting

After gel depurination (0·25 N HCl, 20 min), denaturation (400 mM NaOH; 800 mM NaCl, 30 min), and neutralization (500 mM Tris, pH 6·0; 1·5 M NaCl, 30 min) DNA fragments were transferred to a nylon membrane (Hybond-N; Amersham International plc, Amersham, Bucks, UK) using a vacuum-assisted transfer device (Pharmacia Biotech Ltd., Knowlhill, Milton Keynes, UK) as instructed by the manufacturer. Membranes were then rinsed briefly in 2 × SSC (60 mM NaCl; 6 mM $C_6H_5Na_3O_7$), air-dried, and the DNA fixed by ultraviolet crosslinking (312 nm, 4 min).

Probe preparation

The probe E3CJC2 is a cloned *C. jejuni* DNA insert approximately 23 kb in size, obtained by screening a genomic library constructed from *Sau* 3AI-digested *C. jejuni* chromosomal fragments cloned into the lambda replacement vector, EMBL3 (Stratagene Ltd, Cambridge, UK). The screening probe, pBSKS33A, was a 2 kb *C. jejuni* random insert, cloned into the pBluescript II KS phagemid vector (Strategene), which demonstrated polymorphisms when hybridized to *Hae* III chromosomal DNA digests from a panel of phenotypically diverse strains of *C. jejuni*. Partial sequencing has identified a region of pBSKS33A with strong homology to an ATP-dependent helicase gene of *Haemophilus influenzae* (C. J. Jackson, unpublished). Extraction of purified bacteriophage DNA was accomplished using a commercial kit (Qiagen Lambda Midi-prep., Qiagen Ltd., Dorking, Surrey, UK). The 23 kb *C. jejuni* insert was not excised from the bacteriophage arms, and probe DNA was diluted to a working concentration of approximately 50 ng/μl.

16S ribosomal DNA gene probe

Primer sequences 5′-AGAGTTTGATC{C/A}TGGC-TCAG-3′ and 5′-AAGGAGGTG{A/T}TCCA{A/G}-CC-3′, representing nucleotides 8–27 and 1533–1550 respectively of the *Escherichia coli rrn*B sequence [12], were used to amplify a 1·5 kb fragment of the 16S rRNA gene from *C. jejuni* using the polymerase chain reaction (PCR). The product was purified by agarose gel electrophoresis and the DNA concentration adjusted to 50 ng/μl.

Probe labelling and hybridization

100 ng of E3CJC2 probe and 50 ng of lambda control DNA were labelled with horseradish peroxidase in a total volume of 20 μl, as instructed in the ECL Direct nucleic acid labelling protocol (Amersham ECL kit). The membrane was pre-hybridized for 1–2 h in ECL hybridization buffer containing 5% blocking agent and 0·5 M NaCl. After addition of probe, hybridization was continued at 38 °C for 18 h in a rotating oven (Appligene). Two stringent washes were done with primary wash buffer (4·5 M urea, 0·4% SDS, 0·5 × SSC) for 20 min each at 38 °C, followed by two 5 min non-stringent washes at room temperature in 2 × SSC. Signal was developed by addition of Detection Reagents 1 and 2 (Amersham ECL kit), and chemiluminescence was detected by exposure to Hyperfilm-ECL (Amersham) for 5–40 min. After probing with E3CJC2, blots were stripped in 0·1% SDS at 95 °C for 15 min, washed in 2 × SSC and rehybridized with the 16S rRNA probe.

Interpretation of patterns

Band matching and estimation of restriction fragment sizes was carried out with the 'Molmatch' software and digitization package (UV Products; Genetic Research Instrumentation Ltd., Dunmow, Essex, UK), with heated *Pst* I-digested lambda DNA as control.

RESULTS

RFLP and ribotyping

A number of *C. jejuni* random chromosomal probes and restriction enzyme combinations were examined for discrimination between selected Penner and Lior serogroup strains. The probe designated E3CJC2 in combination with the *Hae* III restriction digests gave the greatest discrimination amongst this collection, producing an average of seven bands ranging in size from 0·8–25 kb (Fig. 1*a*). Chromosomal DNA digests of *C. coli* and *C. jejuni* were clearly distinguished by the presence of species-specific hybridization profiles with this probe (data not shown). The PCR-generated 16S rRNA gene probe hybridized to an average of three fragments (0·5–8·5 kb) for *Hae* III-digested *C. jejuni* chromosomal DNA (Fig. 1*b*). RFLP typing for *C. jejuni* was highly stable and reproducible. The hybridization patterns were unaffected by the use of different growth conditions (media and atmosphere), repeated subculture, and freeze/thawing of isolates. Furthermore, patterns were reproducible through several probe preparations, and different batches of reagents, buffers and probe labelling kits.

Comparison of genotype versus phenotype markers for Lior and Penner reference strains

Table 1 lists the phenotype characteristics (HS and HL serogroups; biotype, and Preston phagegroup) and genotype profiles of 48 Penner and 14 Lior *C. jejuni* serogroup strains, and 10 clinical isolates. Strains with identical E3CJC2/16S ribotype profiles are grouped together, and placed in 48 unique RFLP/ribotype combinations. Genotype profiles of the 14 Lior serogroup strains are contained within those seen for the Penner and clinical strains, except for Lior 9, 11 and 18, which are unique (Table 1, combinations 4, 16 and 48). Individually, either probe shows reduced discrimination compared with serogrouping, identifying 35 RFLP types and 26 ribotypes respectively. The 48 combined genotypes provide the second highest discrimination (after serogrouping) amongst the techniques used here. For the strains listed, there are 22 biotypes and 13 phagegroups and generally, there is no correlation between biotype, phagegroup, serogroup or genotype. Of the 72 strains tested, 36 (50%) were non-typable using the panel of phages routinely employed in the Preston phagetyping scheme.

Serogroup strains from the Penner 4, 13, 16, 43, 50 complex, which are known cross-reactive serogroups, all share the same genotype (Table 1, combination 7), with the exception of Penner 50 (Table 1, combination 19). This finding supports the observation, based on a comparison of LPS profiles of this group [13], that the Penner 50 reference strain is unrelated to the other Penner 4 complex strains. The following serogroup strains, Penner 6, 7, 9, 12, 21, 27, 33, 38, 42, 45, 55, 57 and 60, were of identical genotype with the probe

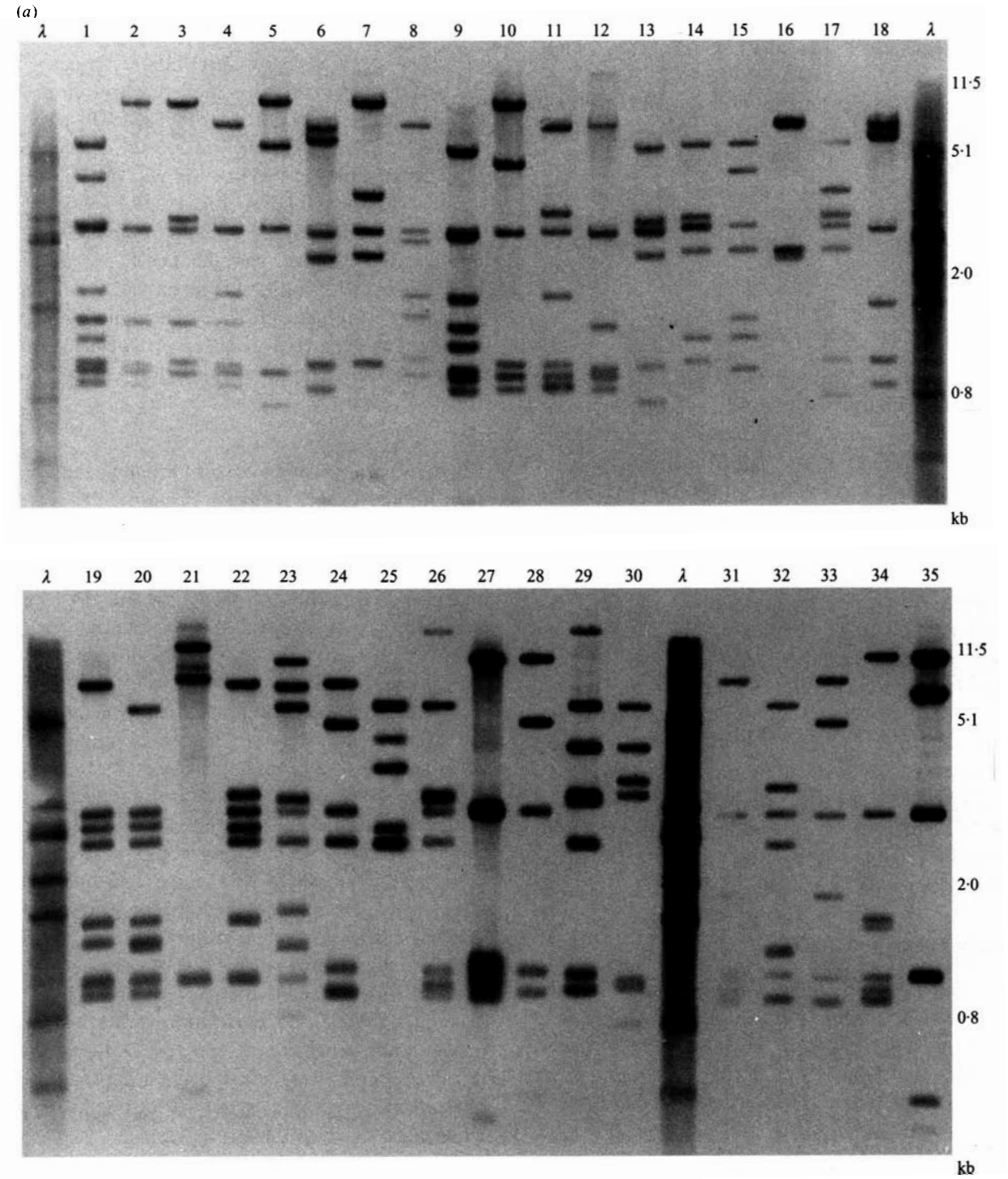

Fig. 1. (*a*) Unique RFLP types of Penner (HS) serogroup reference strains and laboratory isolates (LI) of *C. jejuni*, using the random chromosomal probe E3CJC2. Lane 1: HS 1, lane 2: HS 2, lane 3: HS 3, lane 4: HS 4, lane 5: HS 6, lane 6: HS 11, lane 7: LI C/13216, lane 8: HS 50, lane 9: HS 36, lane 10: HS 17, lane 11: HS 18, lane 12: LI C/13824, lane 13: HS 22, lane 14: HS 23, lane 15, HS 29, lane 16: HS 32, lane 17: HS 35, lane 18: HS 37, lane 19: HS 40, lane 20: HS 41, lane 21: HS 44, lane 22: HS 52, lane 23: HS 53, lane 24: HS 31, lane 25: HS 63, lane 26: HS 64, lane 27: LI C/19169, lane 28: LI B/23390, lane 29: LI B/4758, lane 30: LI B/2567, lane 31: LI C/8733, lane 32: LI B/2035, lane 33: LI C/20627, lane 34: LI B/11209, lane 35: Lior (HL) strain L 11. Lanes marked λ: *Pst* I-digested Lamba DNA marker.

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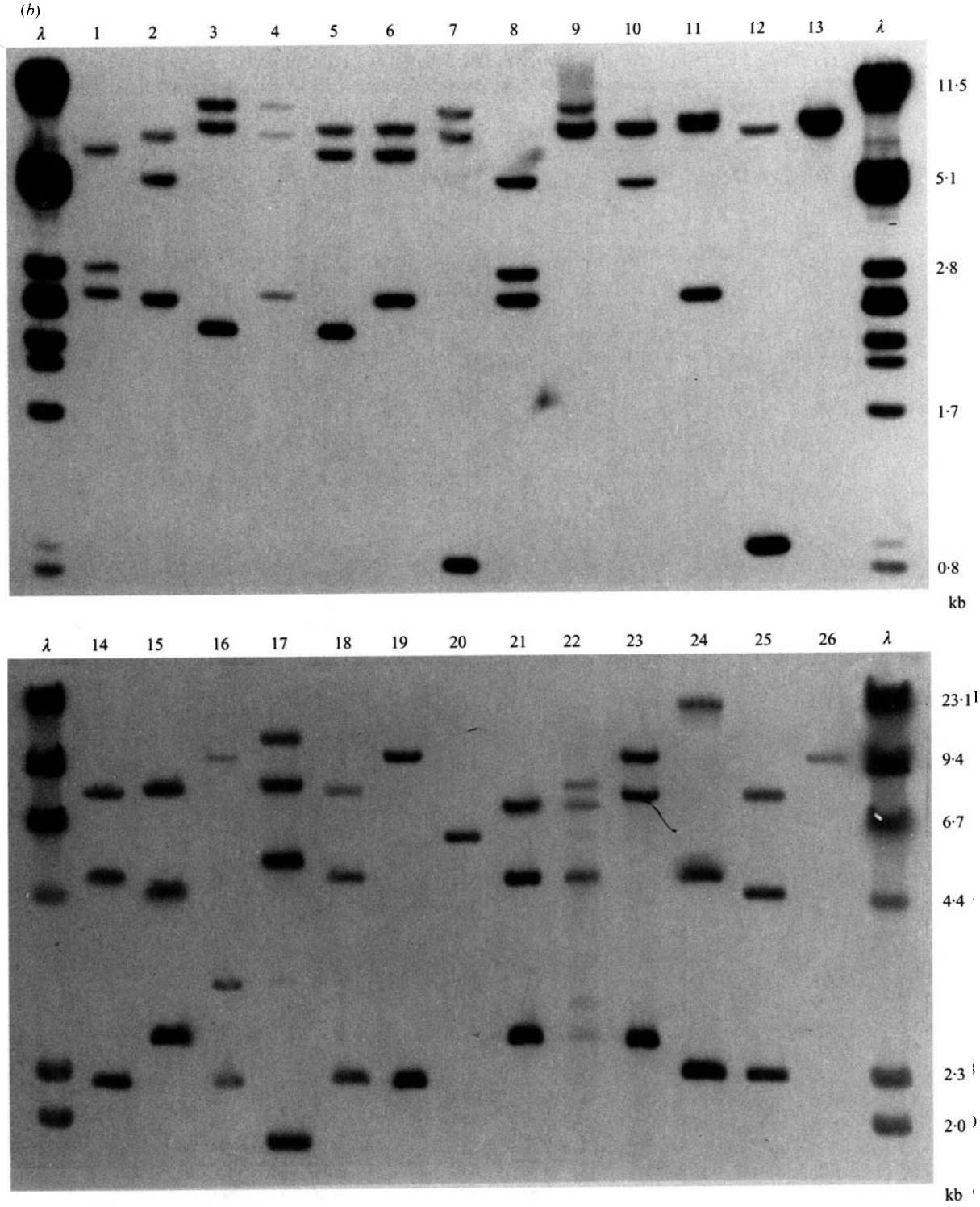

Fig. 1. (*b*) Unique 16S ribotypes of Penner (HS) serogroup reference strains and laboratory isolates (LI) of *C. jejuni*, using a PCR-amplified *C. jejuni* 16S rDNA probe. Lane 1: HS 1, lane 2: HS 3, lane 3: HS 6, lane 4: HS 5, lane 6: HS 10, lane 7: HS 12, lane 8: HS 17, lane 9: HS 21, lane 10: HS 22, lane 11: HS 29, lane 12: HS 32, lane 13: HS 36, lane 14: HS 52, lane 15: HS 40, lane 16: HS 42, lane 17: HS 43, lane 18: HS 53, lane 19: HS 57, lane 20, HS 58, lane 21: HS 41, lane 22: LI C/19701, lane 23: HS 07, lane 24: HS 66, lane 25: HS 64, lane 26: LI C/20627. Lanes marked λ: *Pst* I or *Hin*d III-digested Lamba DNA marker.

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Table 1. *Summary of phenotypic and genotypic characteristics for Penner (HS) and Lior (HL) serogroup reference strains and selected clinical isolates of* C. jejuni, *for 48 different RFLP/ribotype combinations*

RFLP/Rib combin.	RFLP type	Ribotype	Serogroup	Biotype	Phage group
1	1	1	HS 1	6004	52
	1	1	HS 8	6010	122
	1	1	HL 10	6010	146
2	2	1	HS 2	6004	52
	2	1	HS 15	6034	NT
	2	1	HL 4	6010	52
	2	1	HL 13	6074	NT
	2	1	HL 16	6014	56
	2	1	HL 17	6014	NT
3	2	2	HS 5	6130	NT
4	2	25	HL 9	6130	50/55
5	3	2	HS 3	6000	69
	3	2	HS 19	6000	69
6	3	21	HS 62	6004	NT
7	4	1	HS 4	6110	NT
	4	1	HS 13	6114	NT
	4	1	HS 16	6004	NT
	4	1	HS 43	6010	58
	4	1	HL 1	6102	NT
8	4	6	HS 10	6410	55
9	5	3	HS 6	6102	125
	5	3	HS 7	6130	NT
	5	3	HS 27	6106	69
	5	3	HS 33	6302	90
	5	3	HS 60	ND	ND
10	5	5	HS 9	6112	NT
	5	5	HS 38	6112	1
11	5	7	HS 12	6110	83
12	5	9	HS 21	6102	NT
13	5	14	HS 55	6102	14
14	5	16	HS 42	6102	69
	5	16	HS 45	6062	91
	5	16	HL 15	6002	NT
	5	16	HL 19	6112	NT
15	5	19	HS 57	6102	NT
16	5	25	HL 18	6102	NT
17	6	2	HS 11	6139	90
	6	2	HL 2	6010	NT
18	7	1	C/13217*	6130	52
19	8	4	HS 50	6020	NT
	8	4	HS 65	6000	055
	8	4	HL 7	6000	44
20	9	13	HS 36	6002	NT
	9	13	HL 5	6000	NT
21	10	8	HS 17	6124	146
22	10	20	HS 58	6010	NT
23	11	2	HS 18	6104	NT
24	12	1	C/13824*	6004	NT
25	13	10	HS 22	6004	NT
26	14	10	HS 23	4020	NT
27	15	11	HS 29	6002	146
28	16	12	HS 32	6002	125
29	17	10	HS 35	4004	NT
30	18	2	HS 37	6034	2

Table 1—*cont.*

RFLP/Rib combin.	RFLP type	Ribotype	Serogroup	Biotype	Phage group
31	19	15	HS 40	6012	69
32	20	6	HS 41	6012	97
33	21	17	HS 44	6006	NT
34	22	14	HS 52	6032	83
35	23	18	HS 53	6016	NT
36	24	8	HS 31	6120	NT
37	25	23	HS 63	4022	NT
38	25	24	HS 66	4030	NT
39	26	25	HS 64	6014	NT
40	27	21	C/19169*	6110	NT
41	28	8	B/22390*	6000	NT
42	29	25	B/4758*	6010	NT
43	30	3	B/2567*	6024	69
44	31	2	B/8733*	6110	NT
45	32	26	B/2035*	6002	90
46	33	27	B/20627*	6010	146
47	34	1	B/11209*	6010	1
48	35	8	HL 11	6010	90

* Penner and Lior (HS/HL) serogroups of clinical isolates are: *C/13217*: 1/2; *C/13824*: 2/NT; *C/19169*: 53/11; *B/22390*: NT/11; *B/4758*: 16/ND; *B/2567*: 4/NT; *B/8733*: 18/NT; *B/2035*: 19/17; *B/20627*: 41/09; *B/11209*: 10/ND.
NT = non-typable; ND = not done.

E3CJC2 (RFLP type 5) but were subdivided into seven 16S ribotypes (Table 1, combinations 9–16).

Genotypic similarity between common Penner and Lior serogroup associations

Previous analysis of the serogroup data from a large collection *C. jejuni* strains demonstrated that, for a high percentage of typable human isolates, stable associations exist between specific HS and HL antigens [14]. In several instances, reference strains from the respective serogrouping schemes showed identical genotype profiles, which reflect these expected antigenic associations; for example HS 2 and HL 4, and HS 4 and HL 1 (Table 1, combinations 2 and 7). The genotypes shared by these associated Penner and Lior serogroup strains also occurred amongst the collections of random isolates (Tables 2*a–c*) expressing the same HS/HL serogroup combinations.

Genotypes amongst random isolates of the same serogroup combination

Collections of epidemiologically unrelated isolates (clinical and environmental) expressing the three most common serogroup antigen combinations, and their corresponding typing results are listed in Tables 2*a–c*.

Three genotypes were identified using the E3CJC2 probe amongst the random isolates expressing the HS 1/HL 2 phenotype (Table 2*a*), but these strains were indistinguishable with the 16S rRNA gene probe, with the exception of the Lior 2 reference strain. The E3CJC2/16S ribotype combination identified for the Penner 1 reference strain was shared by five of the random strains expressing this serogroup; however the genotype of the Lior 2 reference strain was atypical. Amongst the HS 1/HL 2 isolates there was evidence of an association between genotype and phagegroup. The five HS 1/HL 2 isolates identified as E3CJC2 RFLP type 1 were of phagegroup 76 and the five isolates of RFLP type 7 were phagegroup 52. No such association was apparent for the biotyping results, which were heterogeneous, with 7 biotypes being detected amongst the 10 strains examined.

Table 2*b* shows the results obtained for random isolates expressing antigens of the HS 4, 13, 16, 50 complex. Isolates expressing antigens of one or more of these established cross-reacting serogroups are commonly associated with the HL 1 or HL 7 antigens. The results indicate that genotypically similar isolates may express either the HL 1 or the HL 7 antigens in association with HS antigens 4, 13, 16, 50. Amongst the 23 isolates examined, two genotypes were identified at both loci examined (RFLP/ribotype 4/1 and

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8/4). Although eight different phagegroups were detected amongst these strains, phagegroup 121 was only associated with strains of the combined genotype 4/1, whereas phagegroup 44 was predominantly associated with the 8/4 genotype. This second genotype (Table 1, combination 19) was identical to that obtained for the Penner 50 and Lior 7 reference strains. The genotype 4/1 (Table 1, combination 7) was shown by cross-reacting serogroup reference strains Penner 4, 13, 16, 43, as well as the associated Lior 1 strain. Of the seven biotypes observed, none appeared to be associated with other markers.

No genotypic variation was detected amongst eight random isolates with the HS 2/HL 4 serogroup combination or with the Penner 2 and Lior 4 reference strains (Table 2*c*).

Each group of random strains expressing different HS/HL antigen combinations (Tables 2*a–c*) were distinguished by unique E3CJC2 genotypes; however 16S ribotypes were shared by several strains between each of these separate groups.

Application of genotyping to outbreaks

Outbreak investigations are often used to assess the discriminatory ability of new typing methods. Isolates obtained during the course of a number of outbreaks of *C. jejuni* enteritis were examined by RFLP analysis, and the genotyping results compared with other available typing data are summarized in Tables 3*a–c*. RFLP data from a large milkborne outbreak in the Newcastle area is reported elsewhere [15].

The first outbreak was due to a pasteurization failure in a dairy, resulting in *C. jejuni* contamination of milk distributed to the local community (Table 3*a*). Amongst 32 isolates from cases thought to be associated with the outbreak, 26 were serogroup HS 17 and 6 were serogroup HS 23. All HS 17 strains were of identical genotype (4/1), phagegroup and biotype, but 16 were non-typable for HL antigen. Similarly, the 6 HS 23 strains had identical genotypes (9/13), biotypes and HL serogroups, but 3 were non-typable using Preston phagetyping. The different typing methods applied to this outbreak were therefore equally capable of recognising the two outbreak strains, but only HS serogrouping, biotyping and genotyping produced results for all isolates.

In the second outbreak (Table 3*b*), which occurred in a residential nursing home, the epidemic strain was again serogroup HS 17. All markers identified the epidemic strain, which could be distinguished from the HS 17 strain in Table 3*a* on the basis of biotype, HL serogroup and RFLP type. The 16S ribotype was not discriminatory, neither was the phagegroup. It is interesting to note that the E3CJC2 genotypes 1 and 4 identified for these two HS 17 outbreak strains are those found most frequently amongst the random strains of serogroups HS 1 and HS 4 (Tables 2*a*, *b*). This may imply some lability of HS serogroup antigens.

The third outbreak occurred at a children's boarding-school (Table 3*c*), in which a contaminated well supplying drinking water was implicated as the source. The relationship between the different case isolates associated with the outbreak could not be determined due to the ambiguity of the serogrouping results (HS 2/HL NT and HS NT/HL 7). However, phagegroup and RFLP results confirm that the HS NT/HL 7 human isolate is dissimilar from both the well and the other human isolates, and that the strains isolated from the well are epidemiologically unrelated to any of the human strains.

DISCUSSION

Campylobacter spp. are recognized as the commonest cause of bacterial gastroenteritis in many areas of the world. The vast majority of infections in developed countries are sporadic, and the sources of these infections are complex. Outbreaks from common sources are recognized infrequently, and when they do occur, almost any of the phenotyping methods, either alone or in combination will allow satisfactory discrimination [16]. The epidemiology of sporadic campylobacter infection must be clarified in order to design and implement effective intervention strategies, and this will require the provision of widely available, definitive and reproducible methods for strain characterization.

The two major campylobacter serogrouping schemes (Penner heat-stable [10] and Lior heat-labile [11]) require many reagents to cover the full spectrum of antigenic variants, and a high percentage of non-typable strains are seen in routine use [17]. The presence of weakly expressed, transient or cross-reacting serogroup determinants can cause further discrepancies, and additional phenotyping techniques, such as biotyping [8] and phagetyping [9] have been used to improve the quality of epidemiological analysis. Phagetyping is highly discriminatory and provides good resolution for epidemiological typing

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Table 2. *Summary of phenotype and genotype results*

Number	Source	Serogroup HS/HL	Biotype	Phage group	RFLP	Ribotype
(*a*) Random isolates (human and environmental) expressing the HS 1/HL 2 serogroup antigen combination						
C/13230	Environ	1/2	6410	76	1	1
C/13063	Human	1/2	6010	76	1	1
C/13405	Environ	1/2	6004	76	1	1
C/13715	Environ	1/2	6004	76	1	1
C/13940	Human	1/2	6044	76	1	1
C/14026	Human	1/2	6030	52	7	1
C/10828	Human	1/2	ND	ND	7	1
C/13088	Human	1/2	6020	52	7	1
C/13217	Human	1/2	6130	52	7	1
C/13790	Human	1/2	6030	52	7	1
Penner HS 1	Ref. strain	1/2	6004	52	1	1
Lior HL 2	Ref. strain	1/2	6010	84	6	2
(*b*) Random isolates (human and environmental) expressing the HS 4, 13, 16, 50 serogroup antigen complex						
C/12952	Chicken	16, 50/1	6000	97	4	1
C/13098	Human	4, 50/1	6114	121	4	1
C/13325	Human	4, 50/1	6114	44	4	1
C/13556	Human	4/1	6104	121	4	1
C/12699	Human	4, 50/1	6134	121	4	1
C/13751	Effluent	4, 50/1	6000	50/55	4	1
C/12930	Human	13, 16, 50/1	6000	121	4	1
C/12625	Human	13, 50/7	6004	69	4	1
C/13379	Human	13, 16, 50/7	6114	121	4	1
C/13548	Human	4, 13/7	6010	117	4	1
C/13604	Human	4, 13, 50/7	6114	121	4	1
C/13902	Human	4/7	6154	121	4	1
C/13947	Human	4, 13, 50/7	6154	121	4	1
C/12382	Environ	4, 50/NT	6034	44	4	1
C/13754	Human	4, 50/NT	6004	58	4	1
C/13781	Human	4, 13, 50/NT	6104	146	4	1
C/13794	Human	4/NT	6004	55	4	1
C/13833	Human	4/NT	6010	121	4	1
Penner HS 4	Ref. strain	4/NT	6110	NT	4	1
C/13383	Human	50/1	6000	44	8	4
C/13580	Human	4, 16, 50/7	6004	55	8	4
C/13796	Human	13, 50/7	6000	44	8	4
C/14000	Human	4, 13, 16, 50/7	6000	44	8	4
C/19607	Human	4, 13, 16, 50/20	6000	55	8	4
Lior HL 7	Ref. strain	ND/7	6000	44	8	4
(*c*) Random human case isolates expressing the HS 2/HL 4 serogroup antigen combination						
C/11783	Human	2/4	6004	52	2	1
C/12646	Human	2/4	6004	36	2	1
C/12898	Human	2/4	ND	75	2	1
C/12950	Human	2/4	6010	52	2	1
C/13298	Human	2/4	6020	52	2	1
C/13579	Human	2/4	6010	52	2	1
C/13844	Human	2/4	6104	69	2	ND
C/18204	Human	2/4	ND	ND	2	1
Penner HS 2	Ref. strain	2/4	6004	52	2	1
Lior HL 4	Ref. strain	ND/4	6010	52	2	1

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Table 3

Number of isolates	Serogroup HS/HL	Biotype	Phage group	RFLP	Ribotype
(*a*) Outbreak 1: Community outbreak associated with the consumption of contaminated milk					
10	17/2	6020	52	4	1
16	17/NT	6020	52	4	1
3	23/5	6006	69	9	13
3	23/5	6006	NT	9	13
(*b*) Outbreak 2: Residential nursing home, source unknown					
7	17/1	6014	52	1	1
(*c*) Outbreak 3: School outbreak, associated with a contaminated water supply					
Human					
5	2/NT	6004	52	2	1
1	NT/7	ND	55	8	4
Well					
2	4, 50/NT	ND	ND	4	1

but is compromized by the lability and lack of expression of phage receptors. The development of genotypic 'fingerprinting' methods for campylobacter epidemiology has increased discrimination as well as overcome problems (particularly non-typability) associated with established phenotyping methods.

The typing system described here uses a novel probe (E3CJC2) to detect RFLPs in an undefined chromosomal region encompassing an ATP-dependent helicase gene homologue of *C. jejuni*. Digestion of genomic DNA with *Hae* III restriction endonuclease provides good discriminatory power with this probe, along with ease of interpretation due to the relatively small number of restriction fragments produced (Fig. 1*a*).

The advantages of *Hae* III ribotyping as a molecular subtyping scheme for *Campylobacter* spp. have been described elsewhere [18], and our study confirms the potential for this technique. However, we observed only a single *Hae* III ribotype pattern for the majority of serogroup HS 1, 2 and 4 strains in this study (Tables 2*a–c*), a similar finding to that reported elsewhere [2, 5]. These three serogroups account for up to 80% of all serogroupable human isolates [17], and *Hae* III ribotyping appears inadequate for subtyping this group. An earlier paper [3] reported far greater diversity in the *Hae* III ribotypes of HS 1 and HS 2 serogroup strains, but many of these isolates came from geographical areas outside the UK. We found *Hae* III ribotyping most discriminatory for serogroups which are less frequently isolated from humans (Fig. 1*b*), and most of these reference strains are North American, rather than UK isolates. However, the description of identical genotypes amongst certain serogroup reference strains isolated in North America, and the collections of human and environmental strains from the UK (Tables 2*a–c*) suggests widespread geographical occurrence of specific genotypes. For example, the Penner 50 serogroup strain was originally isolated from a paediatric patient in Toronto, Canada, yet shares the same genotype and similar biotype to several UK isolates (Table 2*b*).

Other molecular techniques have been used for subtyping the three principal *C. jejuni* HS serogroups (HS 1, 2 and 4), including pulsed-field gel electrophoresis [2, 5] and RFLP analysis of the PCR-amplified campylobacter *fla* A gene [19, 20]. These studies confirm that within each HS or HL serogroup considerable genetic diversity exists at the loci studied. Our results with the E3CJC2 probe, using a limited panel of strains, also found variability within these groups, particularly amongst strains of HS 1 (Table 2*a*). No genotype variation was detected amongst the HS 2/HL 4 strains examined in this study, contrary to the variability reported elsewhere [2, 3, 21]. However, our analysis is restricted to strains showing the defined stable antigenic associations predominating in human infections (e.g. HS 1/HL 2, HS 2/HL 4), whereas other investigators have examined strains of a given HS serogroup irrespective of the HL characteristic. A larger genotyping study of random *C. jejuni* isolates has identified much greater polymorphism within the three main HS serogroups, particularly in strains with atypical HL associations (manuscript in preparation).

The outbreaks presented here demonstrate some of the ambiguities which may occur with the current

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phenotyping systems for *C. jejuni*. Strains from outbreaks 1 and 2 expressed an identical HS 17 antigen, but could be differentiated on the basis of RFLP fingerprint, biotype and HL serogroup. Serogroup and phagegroup markers were not expressed for a certain proportion of strains in each outbreak. Overall, the results confirm that maximum discrimination for outbreak-related isolates is achieved by using a number of phenotypic and genotypic characterization methods in combination [16].

Genomic polymorphism analysis, while useful for epidemiological subtyping, requires careful interpretation if used as a measure of the actual phylogenetic relationship between isolates [22, 23]. Similarly, phenotypic traits alone can mark stable cell lineages or clonality [24], but can also obscure the true genetic relationships between strains, particularly where surface determinants are labile, as with somatic and flagellar antigens, and phage adsorption sites. This may be one reason why multiple genotypes are found when single HS or HL serogroups are examined in isolation. In this study, we have identified a number of cell lines of *C. jejuni* which show strong associations between specific phenotypic and genotypic markers. Strains with the unique genotype profile 8/4 characteristically are biotype 6000, have the HS 50 and HL 7 antigens, and belong to either of the two closely related phagegroups 44 or 55. There is a strong correlation between HS 1/HL 2 strains of genotype 1/1 and phagegroup 76; similarly between HS 1/HL 2 strains of genotype 7/1 and phagegroup 52, although the biotypes of these isolates appear random.

These apparently similar strains might be differentiated by more sensitive subtyping procedures, but they may equally represent epidemiologically relevant clonal groupings or complexes. Whether such lineages are genetically definable clones in the evolutionary sense, or are simply the product of a recent 'epidemic' strain distribution is undetermined. A detailed examination of the phenotypic and genotypic associations within a large strain population of the three major HS serogroups of *C. jejuni* is currently in progress.

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REFERENCES

1. Cowden J. Campylobacter: Epidemiological paradoxes. BMJ 1992; **305**: 132–3.
2. Gibson JR, Fitzgerald C, Owen RJ. Comparison of PFGE, ribotyping and phage-typing in the epidemiological analysis of *Campylobacter jejuni* serotype HS2 infections. Epidemiol Infect 1995; **115**: 215–25.
3. Fayos A, Owen RJ, Hernandez J, Jones C, Lastovica A. Molecular subtyping by genome and plasmid analysis of *Campylobacter jejuni* serogroups 01 and 02 (Penner) from sporadic and outbreak cases of human diarrhoea. Epidemiol Infect 1993; **111**: 415–27.
4. Stanley J, Linton D, Sutherland K, Jones C, Owen RJ. High resolution genotyping of *Campylobacter coli* identifies clones of epidemiologic and evolutionary significance. J Infect Dis 1995; **172**: 1130–4.
5. Owen RJ, Sutherland K, Fitzgerald C, Gibson J, Borman P, Stanley J. Molecular subtyping scheme for serotypes HS1 and HS4 of *Campylobacter jejuni*. J Clin Microbiol 1995; **33**: 872–7.
6. Ni H, Knight AI, Cartwright KAV, McFadden JJ. Phylogenetic and epidemiological analysis of *Neisseria meningitidis* using DNA probes. Epidemiol Infect 1992; **109**: 227–39.
7. Frost EH, Deslandes S, Veilleux S, Bourgaux-Ramoisy D. Typing *Chlamydia trachomatis* by detection of restriction fragment length polymorphism in the gene encoding the major outer membrane protein. J Infect Dis 1991; **163**: 1103–7.
8. Bolton FJ, Holt AV, Hutchinson DN. Campylobacter biotyping scheme of epidemiological value. J Clin Pathol 1984; **37**: 677–81.
9. Salama SM, Bolton FJ, Hutchinson DN. Application of a new phagetyping scheme to campylobacters isolated during outbreaks. Epidemiol Infect 1990; **104**: 405–11.
10. Penner JL, Hennessy JN. Passive hemagglutination technique for serotyping *Campylobacter fetus* subspecies *jejuni* on the basis of soluble heat stable antigens. J Clin Microbiol 1980; **12**: 732–7.
11. 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.
12. Brosius J, Palmer ML, Kennedy PJ, Noller HF. Complete nucleotide sequence of 16S ribosomal RNA gene from *Escherichia coli*. Proc Natl Acad Sci USA 1978; **75**: 4801–5.
13. Preston MA, Penner JL. Characterization of cross-reacting serotypes of *Campylobacter jejuni*. Can J Microbiol 1989; **35**: 265–73.
14. Jackson CJ. Typing and environmental detection of *Campylobacter jejuni* [Ph.D. thesis]. Manchester: Manchester Metropolitan University, 1995.
15. Orr KE, Lightfoot NF, Sisson PR, *et al.* Direct milk excretion of *Campylobacter jejuni* in a dairy cow causing cases of human enteritis. Epidemiol Infect 1995; **114**: 15–24.
16. 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.
17. Jones DM, Abbott JD, Painter MJ, Sutcliffe EM. A comparison of biotypes and serotypes of *Campylobacter* sp. isolated from patients with enteritis and from animal and environmental sources. J Infect 1984; **9**: 51–8.
18. Kiehlbauch JA, Cameron DN, Wachsmuth IK. Evaluation of ribotyping techniques as applied to *Arcobacter*, *Campylobacter* and *Helicobacter*. Mol Cell Probes 1994; **8**: 109–16.
19. Owen RJ, Fitzgerald C, Sutherland K, Borman P. Flagellin gene polymorphism analysis of *Campylobacter jejuni* infecting man and other hosts and comparison with biotyping and somatic antigen serotyping. Epidemiol Infect 1994; **113**: 221–34.
20. Burnens AP, Wagner J, Lior H, Nicolet J, Frey J. Restriction fragment length polymorphisms among the flagellar genes of the Lior heat-labile serogroup reference strains and field strains of *Campylobacter jejuni* and *C. coli*. Epidemiol Infect 1995; **114**: 423–31.
21. Wachsmuth IK, Kiehlbauch JA, Bopp CA, *et al.* The use of plasmid profiles and nucleic acid probes in epidemiologic investigations of foodborne, diarrheal diseases. Int J Food Microbiol 1991; **12**: 77–90.
22. Forbes KJ, Fang Z, Pennington TH. Allelic variation in the *Helicobacter pylori* flagellin genes *fla* A and *fla* B: its consequences for strain typing schemes and population structure. Epidemiol Infect 1995; **114**: 257–66.
23. Tenover FC, Arbeit RD, Goering, RV, *et al.* Interpreting chromosal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 1995; **33**: 2233–9.
24. Achtman M, Mercer A, Kusecek B, *et al.* Six widespread bacterial clones among *Escherichia coli* K1 isolates. Infect Immun 1983; **39**: 315–35.