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Molecular Subtyping Scheme for Serotypes HS1 and HS4 of *Campylobacter jejuni*

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We describe a molecular subtyping scheme for two principal O (heat-stable [HS]) serotypes of *Campylobacter jejuni*, HS1 and the HS4 complex. A 16S rRNA gene-specific probe confirmed that almost all the *C. jejuni* strains had three copies of this gene, and strains could be assigned with complete typeability to 1 of 16 combined (*Pst*I and *Hae*III) 16S ribotypes. Macrorestriction profiles (mrps) consisting of up to 10 *Sma*I fragments from ~40 to ~480 kbp were resolved by pulsed-field gel electrophoresis (PFGE). There were 11 mrps among the HS1 strains and 9 mrps among HS4 strains which corresponded to valid types—they occurred in multiple isolates, hosts, places, and times. There were 14 additional single-strain mrp fingerprints in HS1 and 20 in HS4. PFGE exhibited complete typeability when formaldehyde fixation of cells was employed, and PFGE was generally more differential than ribotyping. The data presented elucidate a high-resolution genotypic subtyping scheme for these common subspecific phenotypes of *C. jejuni*, which is both coherent and efficient for epidemiological purposes.

Campylobacter jejuni is the most frequent causative agent of acute enterocolitis throughout the United Kingdom, the United States, and many other developed countries (15). Infections are predominantly sporadic, and four sources have been identified as responsible for most infections in industrialized countries: poultry, raw milk, untreated surface water, and pets (19). Avian, bovine, and porcine hosts constitute other animal reservoirs of significance (18).

Serotyping by the scheme of Penner and Hennessy (14) identifies 47 heat-stable (HS or O) serotypes of *C. jejuni*, while the scheme of Lior et al. (9) identifies 108 heat-labile (HL) serotypes of *C. jejuni* and *Campylobacter coli*. Two of the most commonly occurring O serotypes of *C. jejuni* in humans are HS1 and the HS4 complex; the HS4 complex is a group of strains which cross-react with the HS4, HS13, HS16, HS43, and HS50 antisera.

The serotyping of *C. jejuni* is problematical because both schemes require considerable time and labor investment to produce antisera and because a significant number of strains are nontypeable by either scheme (12). Since effective epidemiological surveillance and outbreak investigation require high-resolution identification of strains, it has been suggested that DNA-based (genotypic) typing might successfully address this need for *Campylobacter* spp. (1).

rDNA polymorphisms (ribotypes) have previously been examined in *C. jejuni* with general purpose probes consisting of 16 plus 23S rRNA and intergenic regions (4, 11–13). Ribotyping has also been done with specific PCR-generated 16S rRNA gene probes, giving data which can be related directly to gene copy number as well as epidemiological typing (16, 17). Pulsed-field gel electrophoresis (PFGE) has been applied to several strains of *C. jejuni* as a tool for chromosome mapping (3, 8), and it was further shown to have potential epidemiological application in a study of 12 strains (20).

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We have here described a genotypic subtyping scheme for large numbers of strains of the HS1 and the HS4 complex serotype of *C. jejuni*. These were the two most prevalent HS serotypes found among 2,500 isolates from 18 collaborating laboratories in the United Kingdom mainly over the period 1992 to 1994 (10a). Isolates from diseased humans and from diverse nondiseased animal hosts were examined. 16S rRNA gene polymorphisms and chromosome macrorestriction (PFGE) profiles were analyzed in order to resolve genotypic subtypes within these two major *C. jejuni* serotypes.

MATERIALS AND METHODS

Bacterial strains. Strains from human, cattle, sheep, dog, and poultry sources were received as part of a study funded by the United Kingdom Department of Health. They were isolated between 1989 and 1994 in 18 different laboratories; however, the study did not contain significant numbers of poultry isolates. Those typed to serotype HS1 comprised 37 sporadic and 7 outbreak-associated human isolates, plus 34 isolates from diverse animal hosts and 2 reference strains from the National Collection of Type Cultures catalog. Strains of the serotype HS4 complex comprised 59 sporadic human isolates, 26 isolates from diverse animal hosts, and 5 reference strains from the National Collection of Type Cultures catalog. They were cultivated at 37°C for 48 h on 5% (vol/vol) defibrinated sheep blood agar under microaerophilic conditions (5% CO_2, 5% O_2, 2% H_2, and 88% N_2) in a Variable Atmosphere Incubator (Don Whitley Scientific Ltd.). Neither the gas mixture nor the culture conditions had any effect on the antigenic profile of reference strains for serotyping (14). Bacteria were identified by standard methods (1) and serotyped by the method of Penner and Hennessy (14) using 45 O antisera provided by A. Lastovica (Red Cross War Memorial Children's Hospital, Cape Town, Republic of South Africa).

DNA preparation and hybridization, PCR, and PFGE. Preparation of genomic DNA from *C. jejuni* and hybridization were performed as previously described (17). Preparation of oligonucleotide forward and reverse primers (Pharmacia Biosystems) and PCR amplification of a 1,500-bp fragment from the 16S rRNA gene of NCTC 11168 were performed as previously described (16, 17). Preparation of *C. jejuni* strains for PFGE was done as described previously (5). Electrophoresis was carried out for 22 h at 200 V and 14°C constant temperature in a CHEF-DR II unit (Bio-Rad), with pulse times ramped from 10 to 35 s.

RESULTS

16S rRNA gene polymorphisms in serotypes HS1 and HS4. Restriction fragment length polymorphisms were examined around the 16S rRNA genes of the 80 HS1 strains and the 90 strains belonging to the HS4 complex. All strains could be

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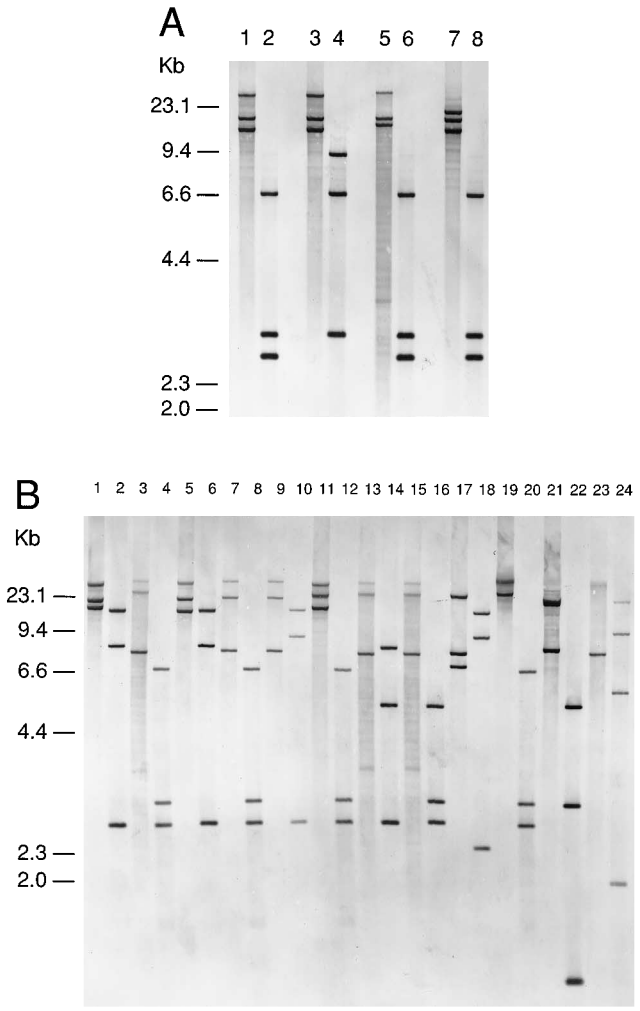

FIG. 1. 16S ribotypes of *C. jejuni*. Southern blots of *C. jejuni* genomic DNA hybridized with 16S rRNA gene-specific probe. (A) Four combined 16S ribotypes found in serotype HS1. Lanes 1 and 2, *Cj*-RI; lanes 3 and 4, *Cj*-RII; lanes 5 and 6, *Cj*-RIII; lanes 7 and 8, *Cj*-RIV. *Pst*I (component) types are shown in odd-numbered lanes, and *Hae*III (component) types are shown in even-numbered lanes. (B) Twelve combined 16S ribotypes found in the HS4 serotype complex. Lanes 1 and 2, *Cj*-RV; lanes 3 and 4, *Cj*-RVI; lanes 5 and 6, *Cj*-RVII; lanes 7 and 8, *Cj*-RVIII; lanes 9 and 10, *Cj*-RIX; lanes 11 and 12, *Cj*-RX; lanes 13 and 14, *Cj*-RXI; lanes 15 and 16, *Cj*-RXII; lanes 17 and 18, *Cj*-RXIII; lanes 19 and 20, *Cj*-RXIV; lanes 21 and 22, *Cj*-RXV; lanes 23 and 24, *Cj*-RXVI.

typed by this method. In the HS1 strains, the size of *Pst*I bands varied from ~12 to ~30 kbp, while in the HS4 complex strains, it varied from 6.5 to ~30 kbp. Of 80 HS1 strains, 78 belonged to the principal *Pst*I type, P1 (Fig. 1A, lanes 1 and 3), and 15 of 90 strains of the HS4 complex also belonged to type P1 (Fig. 1B, lane 5). The second *Pst*I ribotype, P2, occurred only in a single HS1 strain (A709/92 [Fig. 1A, lane 5]) but was the most common *Pst*I ribotype among HS4 complex strains (Fig. 1B, lane 1). The final HS1 ribotype, P3 (Fig. 1A, lane 7) was unique. Seven *Pst*I ribotypes (P4 to P10) were unique to the HS4 strains, and two of those (P4 and P5) contained 23% of the strains.

The *Hae*III band sizes varied from 2.5 to ~9 kbp in HS1 strains and from 1.0 to ~13.5 kbp in the HS4 complex strains. The principal *Hae*III type, H1, was found in 75 of 80 HS1 strains and was also found in 35 of 90 HS4 strains (compare Fig. 1A, lane 2, with Fig. 1B, lane 4). Ribotype H2 (Fig. 1A, lane 4) was found in only five HS1 strains. Seven other *Hae*III types (H3 to H9) were found among the HS4 strains, with one (H3) accounting for almost half of the HS4 strains.

The polymorphs were used to designate combined 16S ribotypes, as shown in Table 1. The 73 HS1 strains which were P1 H1, were termed *Cj*-RI, the five P1 H2 strains were termed *Cj*-RII, and the remaining two (P2 H1 and P3 H1) were termed *Cj*-RIII and *Cj*-RIV. Combined ribotype *Cj*-RI was found in 85% of all the human HS1 isolates, and in all of the HS1 isolates from animals, irrespective of host species (16 bovine, 13 ovine, 3 chicken, and 2 canine species). Among the HS4 serotype complex, there was greater diversity, and strains were also more evenly distributed among 12 combined ribotypes shown in Fig. 1B, whose composition and distribution is shown in Table 2. The four largest combined ribotypes in HS4 contained both human and animal (canine, bovine, and ovine) isolates. The eight minor combined ribotypes in HS4 all belonged to human isolates.

Chromosome macrorestriction profiles of serotype HS1 and HS4 complex strains. Endonuclease *Sma*I digests of genomic DNA of 80 HS1 strains and 90 strains belonging to the HS4 complex were resolved by PFGE as described in Materials and Methods. This technique was applicable to all strains as shown in Tables 1 and 2.

In the HS1 strains, 8 to 10 *Sma*I fragments were resolved and were sized from ~40 to ~480 kbp. Of the HS1 strains, 82.5% were assigned to 11 distinctive mrp types, which contained multiple strains (Fig. 2A, lanes 2 to 12). Fourteen mrps were found in only one strain (Fig. 2B, lanes 2 to 15). In Fig. 2, all the mrp types found within serotype HS1 are ordered according to their prevalence in this study. The commonest contained 25 strains including 7 strains epidemiologically traced to a common-source outbreak, the next commonest contained 12 strains, and so forth. Mrps and their relationship to 16S ribotype are shown in Table 1, which is also ordered according to their prevalence in this study.

In the HS4 complex strains, four to eight *Sma*I fragments were resolved and were sized from ~40 to ~480 kbp. Of the strains in the HS4 complex study, 70 were assigned to 9 mrp types, containing multiple strains (Fig. 3A, lanes 4 to 6 and 8 to 13), while the other 20 mrps were found only in a single strain (Fig. 3B, lanes 2 to 21). In Fig. 3 and Table 2, HS4 mrps are ordered according to prevalence in the study. The commonest contained 35 strains, the next commonest contained 9 strains, and so forth. Three HS4 mrp types, contained more than one combined ribotype, but the others corresponded to a single combined ribotype.

DISCUSSION

Three copies of the 16S rRNA gene were found in the individual strains of *C. jejuni* previously examined (3, 8). No sites for *Pst*I or *Hae*III were found in the published gene sequence from a type strain, CIP702 (GenBank no. L04315). Since the probe used was an almost full-length intragenic fragment of the gene amplified from NCTC 11168, the homologous *Pst*I or *Hae*III bands detected in our Southern blots were directly related to gene copy number. Three well-separated *Pst*I or *Hae*III bands were detected with this probe in all but three strains corresponding to three copies of the gene. While bacterial strains are believed to have consistent copy numbers of the rRNA genes, certain rare *C. jejuni* strains seem to have a variable 16S rRNA gene copy number as was found for some strains of *Campylobacter upsaliensis* (17).

16S ribotypes exhibited complete typeability for all the strains of *C. jejuni* in the study. Previously *Escherichia coli* 16

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TABLE 1. Macrorestriction profiles, origins, and 16S ribotypes of *C. jejuni* HS1 strains

HS1 mrp type and strain	Place of isolation[a]	Host	16S ribotype		
			*Pst*I	*Hae*III	Combined
Type A					
NCTC 12500	NK[b]	NK	P1	H1	*Cj*-RI
A622/89	Preston PHL	Human	P1	H1	*Cj*-RI
A623/89	Preston PHL	Human	P1	H1	*Cj*-RI
A624/89	Preston PHL	Human	P1	H1	*Cj*-RI
A625/89	Preston PHL	Human	P1	H1	*Cj*-RI
A626/89	Preston PHL	Human	P1	H1	*Cj*-RI
A627/89	Preston PHL	Human	P1	H1	*Cj*-RI
A628/89	Preston PHL	Human	P1	H1	*Cj*-RI
34/92	Weybridge CVL	Bovine	P1	H1	*Cj*-RI
48/92	Lancaster (Hosp)	Human	P1	H1	*Cj*-RI
72/92	Lancaster (Univ)	Ovine	P1	H1	*Cj*-RI
109/92	Lincoln PHL	Human	P1	H1	*Cj*-RI
151/92	Lincoln PHL	Human	P1	H1	*Cj*-RI
A709/92	South Africa (Hosp)	Human	P2	H1	*Cj*-RIII
34/93	Ashford PHL	Human	P1	H1	*Cj*-RI
35/93	Ashford PHL	Human	P1	H1	*Cj*-RI
84/93	Ashford PHL	Human	P1	H1	*Cj*-RI
85/93	Exeter PHL	Chicken	P1	H1	*Cj*-RI
109/93	Bristol (Univ)	Canine	P1	H1	*Cj*-RI
114/93	Bristol (Univ)	Canine	P1	H1	*Cj*-RI
379/93	Lancaster (Univ)	Bovine	P1	H1	*Cj*-RI
457/93	Lancaster (Hosp)	Human	P1	H1	*Cj*-RI
571/93	Guys Hosp, London	Human	P1	H1	*Cj*-RI
1132/93	Cardiff PHL	Human	P1	H1	*Cj*-RI
30/94	Cardiff PHL	Human	P1	H1	*Cj*-RI
Type B					
57/92	Lancaster (Hosp)	Human	P1	H1	*Cj*-RI
124/93	Ashford PHL	Human	P1	H1	*Cj*-RI
283/93	Lancaster (Univ)	Ovine	P1	H1	*Cj*-RI
284/93	Lancaster (Univ)	Ovine	P1	H1	*Cj*-RI
295/93	Lancaster (Univ)	Ovine	P1	H1	*Cj*-RI
404/93	Lancaster (Univ)	Bovine	P1	H1	*Cj*-RI
406/93	Lancaster (Univ)	Bovine	P1	H1	*Cj*-RI
631/93	Lancaster (Univ)	Ovine	P1	H1	*Cj*-RI
635/93	Lancaster (Univ)	Ovine	P1	H1	*Cj*-RI
638/93	Lancaster (Univ)	Ovine	P1	H1	*Cj*-RI
711/93	Exeter PHL	Chicken	P1	H1	*Cj*-RI
724/93	Exeter PHL	Chicken	P1	H1	*Cj*-RI
Type C					
301/93	Lancaster (Univ)	Bovine	P1	H1	*Cj*-RI
302/93	Lancaster (Univ)	Bovine	P1	H1	*Cj*-RI
303/93	Lancaster (Univ)	Bovine	P1	H1	*Cj*-RI
305/93	Lancaster (Univ)	Bovine	P1	H1	*Cj*-RI
384/93	Lancaster (Univ)	Bovine	P1	H1	*Cj*-RI
385/93	Lancaster (Univ)	Bovine	P1	H1	*Cj*-RI
606/93	Ashford PHL	Human	P1	H1	*Cj*-RI
1178/93	Lancaster (Hosp)	Human	P1	H1	*Cj*-RI
Type D					
NCTC 11168[a]	Worcester (Hosp)	NK	P1	H1	*Cj*-RI
16/92	Weybridge CVL	Bovine	P1	H1	*Cj*-RI
17/92	Weybridge CVL	Bovine	P1	H1	*Cj*-RI
527/93	Ashford PHL	Human	P1	H1	*Cj*-RI
Type E					
127/93	Lancaster (Hosp)	Human	P1	H1	*Cj*-RI
286/93	Lancaster (Univ)	Ovine	P1	H1	*Cj*-RI
287/93	Lancaster (Univ)	Ovine	P1	H1	*Cj*-RI
Type F					
45/92	Lancaster (Hosp)	Human	P1	H1	*Cj*-RI
117/92	Lancaster (Hosp)	Human	P1	H1	*Cj*-RI
206/92	Lancaster (Hosp)	Human	P1	H1	*Cj*-RI
Type G					
576/93	Guys Hosp, London	Human	P1	H1	*Cj*-RI
590/93	Guys Hosp, London	Human	P1	H1	*Cj*-RI
687/93	Guys Hosp, London	Human	P1	H1	*Cj*-RI
Type H					
620/93	Lancaster (Univ)	Ovine	P1	H1	*Cj*-RI
630/93	Lancaster (Univ)	Ovine	P1	H1	*Cj*-RI
Type I					
182/93	Institute of Animal Health, Compton	Bovine	P1	H1	*Cj*-RI
641/93	Lancaster (Univ)	Bovine	P1	H1	*Cj*-RI
Type J					
102/92	Lincoln PHL	Human	P1	H1	*Cj*-RI
432/93	Ashford PHL	Human	P1	H1	*Cj*-RI
Type K					
A1030/92	South Africa (Hosp)	Human	P1	H1	*Cj*-RII
281/93	Lancaster (Hosp)	Human	P1	H1	*Cj*-RII
Ungrouped					
116/92	Lancaster (Hosp)	Human	P1	H1	*Cj*-RI
157/92	Lancaster (Hosp)	Human	P1	H1	*Cj*-RI
A708/92	South Africa (Hosp)	Human	P1	H1	*Cj*-RII
A712/92	South Africa (Hosp)	Human	P1	H1	*Cj*-RI
A1033/92	South Africa (Hosp)	Human	P1	H1	*Cj*-RII
A1034/92	South Africa (Hosp)	Human	P1	H1	*Cj*-RII
228/93	Institute of Animal Health, Compton	Bovine	P1	H1	*Cj*-RI
292/93	Lancaster (Univ)	Ovine	P1	H1	*Cj*-RI
414/93	Lancaster (Hosp)	Human	P1	H1	*Cj*-RI
563/93	Ashford PHL	Human	P1	H1	*Cj*-RI
591/93	Guys Hosp, London	Human	P3	H1	*Cj*-RIV
607/93	Ashford PHL	Human	P1	H1	*Cj*-RI
628/93	Lancaster (Univ)	Ovine	P1	H1	*Cj*-RI
640/93	Lancaster (Univ)	Bovine	P1	H1	*Cj*-RI

[a] All isolates from the United Kingdom unless otherwise stated. Abbreviations: CVL, Central Veterinary Laboratory; PHL, Public Health Laboratory; Univ, university laboratory; Hosp, hospital laboratory.
[b] NK, not known.

plus 23S rRNA has been employed as a probe in a ribotyping study of five outbreaks caused by *C. jejuni* serotype HS2 or HL4 (12), and the best discrimination between strains was obtained with *Pst*I. For both serotypes in the present study, *Pst*I ribotypes comprised an evenly distributed number of strains, whereas a single *Hae*III ribotype comprised 94% of the HS1 strains, and two *Hae*III ribotypes composed about half the HS4 strains. A low numerical index of discrimination (*D* value [7]) was found for ribotyping the HS1 strains (0.05 for *Pst*I, 0.12 for *Hae*III, and 0.17 for combined ribotyping), but this increased substantially for ribotyping the HS4 complex (0.78 for *Pst*I, 0.61 for *Hae*III, and 0.81 for combined ribotyping).

None of the combined ribotypes found in the HS4 complex (*Cj*-RV to *Cj*-RXVI) occurred in serotype HS1, underlining its value for subtyping in this case. By contrast, the simple *Pst*I type P1 occurred in both serotypes. As an example of subtyping HS1, combined ribotype *Cj*-RII comprised five strains. Four of these strains were isolated from a single South African hospi-

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TABLE 2. Macrorestriction profiles, origins, and 16S ribotypes of *C. jejuni* HS4 strains

HS4 mrp type and strain	Place of isolation[a]	Host	16S ribotype		
			*Pst*I	*Hae*III	Combined
Type A					
5/92	Weybridge CVL	Bovine	P1	H3	*Cj*-RVII
7/92	Weybridge CVL	Bovine	P2	H3	*Cj*-RV
55/92	Lancaster (Hosp)	Human	P1	H3	*Cj*-RVII
94/92	Lancaster (Hosp)	Human	P1	H3	*Cj*-RVII
148/92	Lancaster (Hosp)	Human	P2	H3	*Cj*-RV
191/92	Manchester PHL	Human	P2	H3	*Cj*-RV
193/92	Manchester PHL	Human	P2	H3	*Cj*-RV
A1060/92			P2	H3	*Cj*-RV
10/93	Lancaster (Hosp)	Human	P1	H3	*Cj*-RVII
19/93	Ashford PHL	Human	P2	H3	*Cj*-RV
42/93	Lancaster (Hosp)	Human	P1	H3	*Cj*-RVII
46/93	Salford (Univ)	Human	P2	H3	*Cj*-RV
166/93	Ashford PHL	Human	P2	H3	*Cj*-RV
173/93	Institute of Animal Health, Compton	Bovine	P2	H3	*Cj*-RV
193/93	Institute of Animal Health, Compton	Bovine	P2	H3	*Cj*-RV
199/93	Institute of Animal Health, Compton	Bovine	P2	H3	*Cj*-RV
280/93	Lancaster (Hosp)	Human	P1	H3	*Cj*-RVII
285/93	Lancaster (Univ)	Bovine	P2	H3	*Cj*-RV
288/93	Lancaster (Univ)	Bovine	P2	H3	*Cj*-RV
296/93	Lancaster (Univ)	Bovine	P1	H3	*Cj*-RVII
298/93	Lancaster (Univ)	Bovine	P1	H3	*Cj*-RVII
306/93	Lancaster (Univ)	Bovine	P2	H3	*Cj*-RV
370/93	Ashford PHL	Human	P2	H3	*Cj*-RV
381/93	Lancaster (Univ)	Bovine	P1	H3	*Cj*-RVII
398/93	Lancaster (Univ)	Bovine	P1	H3	*Cj*-RVII
493/93	Lancaster (Hosp)	Human	P1	H3	*Cj*-RVII
504/93	Ashford PHL	Human	P2	H3	*Cj*-RV
551/93	Lancaster (Hosp)	Human	P1	H3	*Cj*-RVII
621/93	Lancaster (Univ)	Ovine	P2	H3	*Cj*-RV
626/93	Lancaster (Univ)	Ovine	P2	H3	*Cj*-RV
835/93	Lancaster (Hosp)	Human	P1	H3	*Cj*-RVII
911/93	Lancaster (Univ)	Bovine	P2	H3	*Cj*-RV
973/93	Ashford PHL	Human	P1	H3	*Cj*-RVII
1173/93	Lancaster (Hosp)	Human	P1	H3	*Cj*-RVII
NCTC 12559			P2	H3	*Cj*-RV
Type B					
55/93	Preston PHL	Human	P4	H1	*Cj*-RVI
56/93	Preston PHL	Human	P4	H1	*Cj*-RVI
58/93	Preston PHL	Human	P4	H1	*Cj*-RVI
148/93	Reading (Univ)	Canine	P4	H1	*Cj*-RVI
451/93	Lancaster (Hosp)	Human	P4	H1	*Cj*-RVI
479/93	Ashford PHL	Human	P4	H1	*Cj*-RVI
499/93	Guys Hosp, London	Human	P4	H1	*Cj*-RVI
592/93	Guys Hosp, London	Human	P4	H1	*Cj*-RVI
748/93	Lancaster (Hosp)	Human	P4	H1	*Cj*-RVI
Type C					
300/93	Lancaster (Univ)	Bovine	P2	H3	*Cj*-RV
423/93	Lancaster (Hosp)	Human	P2	H3	*Cj*-RVII
464/93	Lancaster (Hosp)	Human	P1	H3	*Cj*-RVII
397/93	Lancaster (Univ)	Ovine	P2	H3	*Cj*-RV
916/93	Lancaster (Univ)	Bovine	P2	H3	*Cj*-RV
Type D					
363/93	Lancaster (Hosp)	Human	P5	H4	*Cj*-RIX
520/93	Ashford PHL	Human	P5	H4	*Cj*-RIX
537/93	Harrogate (Hosp)	Human	P5	H4	*Cj*-RIX
568/93	Lancaster (Hosp)	Human	P5	H4	*Cj*-RIX
826/93	Harrogate (Hosp)	Human	P5	H4	*Cj*-RIX
Type E					
14/92	Weybridge CVL	Bovine	P2	H3	*Cj*-RV
199/92	Lancaster (Hosp)	Human	P2	H3	*Cj*-RV
12/92	Weybridge CVL	Bovine	P2	H3	*Cj*-RV
15/92	Weybridge CVL	Bovine	P2	H3	*Cj*-RV
Type F					
160/93	Bristol (Univ)	Canine	P5	H1	*Cj*-RVIII
307/93	Lancaster (Hosp)	Human	P5	H1	*Cj*-RVIII
757/93	Lancaster (Hosp)	Human	P5	H1	*Cj*-RVIII
1276/93	Weybridge CVL	Canine	P5	H1	*Cj*-RVIII
Type G					
289/93	Lancaster (Univ)	Bovine	P4	H1	*Cj*-RVI
310/93	Ashford PHL	Human	P4	H1	*Cj*-RVI
22/93	Barnstaple (Hosp)	Human	P4	H1	*Cj*-RVI
Type H					
144/93	Reading (Univ)	Canine	P4	H1	*Cj*-RVI
146/93	Reading (Univ)	Canine	P4	H1	*Cj*-RVI
147/93	Reading (Univ)	Canine	P4	H1	*Cj*-RVI
Type I					
573/93	Guys Hosp, London	Human	P5	H1	*Cj*-RVIII
589/93	Guys Hosp, London	Human	P8	H1	*Cj*-RXIV
Ungrouped					
122/93	Ashford PHL	Human	P4	H6	*Cj*-RXII
758/93	Lancaster (Hosp)	Human	P7	H7	*Cj*-RXIII
808/93	Lancaster (Hosp)	Human	P9	H8	*Cj*-RXV
357/93	Lancaster (Hosp)	Human	P4	H1	*Cj*-RVI
220/92	Lancaster (Hosp)	Human	P4	H5	*Cj*-RXI
37/93	Lancaster (Hosp)	Human	P4	H1	*Cj*-RVI
61/93	Preston PHL	Human	P5	H1	*Cj*-RVIII
793/93	Lancaster (Hosp)	Human	P6	H1	*Cj*-RX
A1054/92			P5	H1	*Cj*-RVIII
145/93	Reading (Univ)	Canine	P4	H1	*Cj*-RVI
601/93	Taunton	Human	P5	H1	*Cj*-RVIII
158/93	Bristol Univ	Canine	P4	H1	*Cj*-RVI
266/93	Lancaster (Hosp)	Human	P5	H1	*Cj*-RVIII
773/93	Ashford PHL	Human	P5	H1	*Cj*-RVIII
314/93	Ashford PHL	Human	P5	H1	*Cj*-RVIII
736/93	Lancaster (Hosp)	Human	P5	H4	*Cj*-RIX
NCTC 12561			P6	H1	*Cj*-RX
NCTC 12512			P6	H1	*Cj*-RX
NCTC 12514			P4	H1	*Cj*-RVI
NCTC 12548			P10	H9	*Cj*-RXVI

[a] All isolates from the United Kingdom unless otherwise stated. Abbreviations: CVL, Central Veterinary Laboratory; PHL, Public Health Laboratory; Univ, university laboratory; Hosp, hospital laboratory.

tal, and the fifth was isolated in the United Kingdom from a patient recently returned from South Africa. This group of strains probably therefore represents a geographically distinct (South African) clone of serotype HS1, and this supposition was confirmed by macrorestriction profiling (Table 1).

When the problem of DNase activity in certain strains was overcome by formaldehyde fixation (5), complete typeability by PFGE was obtained for all *C. jejuni* strains in the study. The use of *Sma*I for macrorestriction of the *C. jejuni* chromosome,

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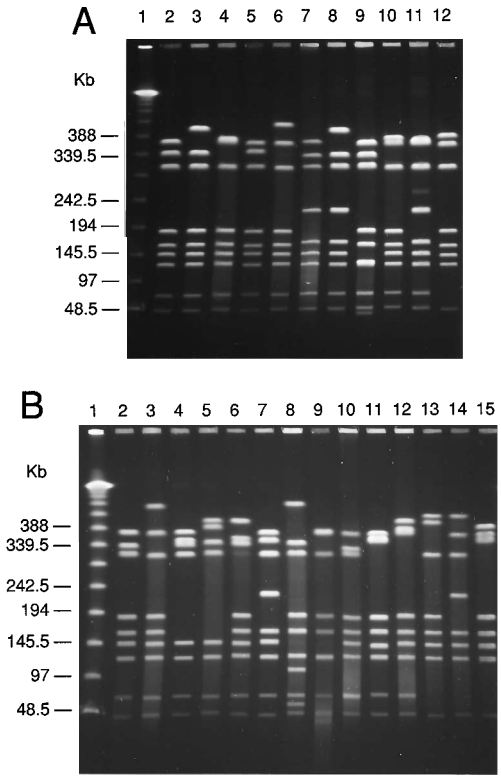

FIG. 2. *Sma*I mrps of HS1 serotype of *C. jejuni*. (A) mrp types. Lane 1, 48.5-kb ladder (New England Biolabs); lane 2, mrp type A; lane 3, mrp type B; lane 4, mrp type C; lane 5, mrp type D; lane 6, mrp type E; lane 7, mrp type F; lane 8, mrp type G; lane 9, mrp type H; lane 10, mrp type I; lane 11, mrp type J; lane 12, mrp type K. (B) mrps found only in single HS1 strains. Lane 1, ladder; lane 2, strain 640/93; lane 3, strain 414/93; lane 4, strain 116/92; lane 5, strain 157/92; lane 6, strain 563/93; lane 7, strain 607/93; lane 8, strain 292/93; lane 9, strain 628/93; lane 10, strain A712/92; lane 11, strain 228/93; lane 12, strain 591/93; lane 13, strain A1034/92; lane 14, strain A1033/92; lane 15, strain A708/92.

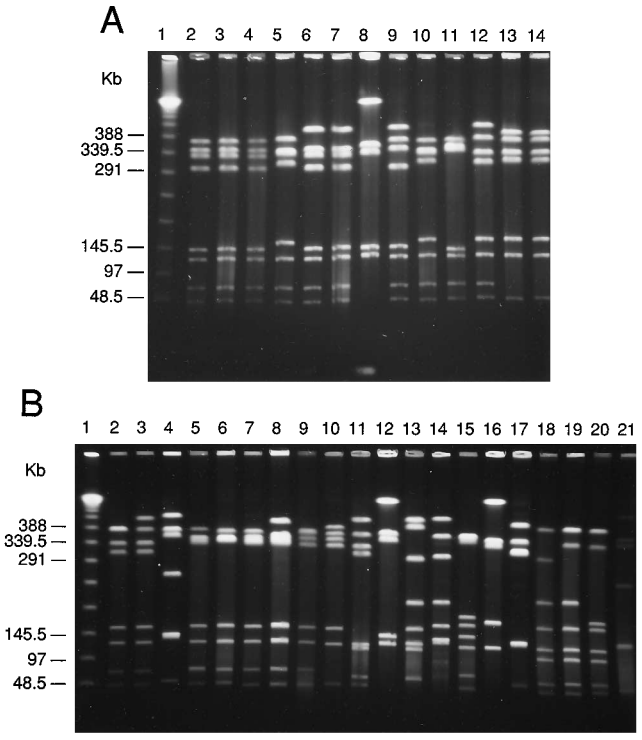

FIG. 3. *Sma*I mrps of HS4 serotype complex of *C. jejuni*. (A) mrp types. Lane 1, ladder; lanes 2 to 4, mrp type A; lane 5, mrp type B; lanes 6 and 7, mrp type C; lane 8, mrp type D; lane 9, mrp type E; lane 10, mrp type F; lane 11, mrp type G; lane 12, mrp type H; lanes 13 and 14, mrp type I. (B) mrps found only in single HS4 strains. Lane 1, 48.5-kb ladder; lane 2, strain 37/93; lane 3, strain 145/93; lane 4, strain 357/93; lane 5, strain 158/93; lane 6, strain 266/93; lane 7, strain 773/93; lane 8, strain 314/93; lane 9, strain 601/93; lane 10, strain 61/92; lane 11, strain A1054/93; lane 12, strain 736/92; lane 13, strain 220/93; lane 14, strain 122/93; lane 15, strain 793/93; lane 16, strain 758/93; lane 17, strain 808/93; lane 18, strain NCTC 12561; lane 19, strain NCTC 12512; lane 20, strain NCTC 12514; lane 21, strain NCTC 12548.

was first suggested by Yan et al. (20) in a study of 12 *C. jejuni* strains. In this study, we have validated it for the purposes of large-scale molecular epidemiology. The HS1 strains, largely belonging to a single combined ribotype, could be differentiated into 25 mrps by macrorestriction and PFGE. The corresponding *D* value (0.87) represented a considerable improvement over combined ribotyping for serotype HS1. The first mrp type (HS1 type A) included seven strains implicated epidemiologically in a common-source outbreak. Strains from diverse geographical locations in the United Kingdom and South Africa belonged to this type. They were also from a wide variety of hosts and were isolated over a 5-year period. The stability of this and the other mrp types is consistent with their being true genotypes.

The 11 mrp types within serotype HS1 (Fig. 2A) exhibited a high degree of relationship, and among all HS1 mrps (Fig. 2), a high proportion of *Sma*I fragments (six to eight) was conserved, with mrps being related by shifts only in the sizes of one, two, or three *Sma*I fragments (Fig. 1). Maslow et al. (10) noted that bacterial strains with identical mrps are obviously clonal, while strains exhibiting one or two band shifts, consistent with a single genetic event, are "clonally related." By these criteria, HS1 strains are indeed clonally related. Baquar et al. (2) noted that mrps of *Salmonella brandenberg* were uniform within a large-scale national outbreak, whereas epidemiologically unrelated strains of that serotype exhibited a single macrorestriction band shift, and that such mrp differences were maintained over a decade or more. In agreement with their findings for *Salmonella* species, another zoonotic enteropathogen, the key point emerging from this study is that macrorestriction indeed identifies stable subtypes of *C. jejuni* serotypes. These subtypes occur reproducibly in different host species and in humans at many different geographical locations and at different times.

While the HS1 strains mrps constituted a single phylogenetic complex with obviously common ancestry, those of the HS4 strains (Fig. 3) presented a much more heterogeneous picture. It should be noted that although the HS4 complex is defined by five cross-reacting antisera, the mrp types did not correlate with these primary antigenic profiles (data not shown). This result would seem to imply that the strains within the HS4 complex may be too broadly associated as presently organized. mrp types of HS4 were also stable genotypes found in different hosts and geographical locations. Their isolation from humans, cattle, sheep, and dogs was again consistent with a general zoonotic pattern of infection. *D* values for macrorestriction (0.84) and combined 16S ribotyping (0.81) were comparable in HS4.

Patton and Wachsmuth (12) have reviewed the relative merits and limitations of current typing methods for campylobacters. The main objective of subtyping is to support or reject hypotheses developed from epidemiological observa-

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tions. Furthermore, studies should extend beyond the investigation of outbreaks, ultimately to the population genetics of a given species. An issue which can be elucidated by the type of data reported here will be the incidence and distribution of clonal groups. We conclude that cost-effective epidemiological typing of *C. jejuni* can be achieved by a combination of (HS) serotyping with macrorestriction-PFGE, which constitutes a coherent scheme. Broad-spectrum phenotypic typing is thereby complemented by high-resolution genotypic subtyping. We propose the structure presented herein as a molecular subtyping scheme for serotypes HS1 and HS4 of *C. jejuni*.

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