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Molecular epidemiological investigation of an outbreak of *Campylobacter jejuni* identifies a dominant clonal line within Scottish serotype HS55 populations

C. S. HARRINGTON[1]*, F. M. THOMSON-CARTER[2] AND P. E. CARTER[1]

Department of [1] Medical Microbiology, University of Aberdeen, and [2] Scottish Campylobacter Reference Laboratory, Aberdeen AB25 2ZD, Scotland, UK

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SUMMARY

Three molecular typing methods, pulsed-field gel electrophoresis (PFGE), ribotyping, and flagellin (*flaA*) gene typing, were used to discriminate within a group of 28 *Campylobacter jejuni*, heat-stable serotype 55 (HS55) isolates derived from cases of campylobacter enteritis occurring throughout Scotland, including 9 isolates associated with an outbreak. PFGE was found to be most discriminatory, identifying 6 distinct profiles, followed by ribotyping (5 profiles), and then flagellin gene typing (4 profiles). The coincidence of all three genotypic markers identified a dominant clonal line within the HS55 group, accounting for each of the outbreak strains, and for 9 of the 19 sporadic strains. A second, closely related, clonal line accounted for a further 5 of the sporadic strains, and also included the HS55 reference strain. Identification and monitoring of such clonal lines should facilitate more effective future epidemiological surveillance of *C. jejuni*.

INTRODUCTION

Campylobacter jejuni is the major cause of acute bacterial gastroenteritis throughout the United Kingdom [1], and in many other developed countries [2]. Campylobacteriosis infections are predominantly sporadic, but outbreaks have been described, associated mainly with the consumption of undercooked poultry or unpasteurized milk [1]. Routes of transmission in sporadic infections are thought to be predominantly foodborne, but are rarely established, since a wide range of zoonotic and environmental risk factors have been identified [1, 3, 4]. Effective and accurate subtyping methods are needed to help trace possible routes of transmission in sporadic infections.

Phenotypic methods such as biotyping and serotyping have been widely used for epidemiological monitoring of subtypes of *C. jejuni* [5]. Although such methods are useful for the primary characterization of isolates, they lack adequate discriminatory power for accurate subtyping of *C. jejuni*, and it is now recognized that additional methods are needed for high-resolution identification of strains. Several DNA-based (genotypic) methods have been developed to fulfil this need, notably pulsed field gel electrophoresis [6], ribotyping [7], and flagellin gene profiling [8, 9].

The Scottish *Campylobacter* Reference Laboratory routinely screens all submitted *Campylobacter jejuni* and *C. coli* isolates using the heat-stable (HS) serotyping method of Penner and colleagues [10]. In June 1996, nine isolates thought to be outbreak-associated were received from the Ayrshire and Arran (AA) Health Board area, and were identified as belonging to heat-stable serotype 55 (HS55). This is a relatively uncommon serotype in Scotland, accounting for just 0·5% (18/3337) of the annual sample load in the year preceding the outbreak [11]. Four additional HS55 isolates from outside the AA outbreak area were also received during the outbreak month. Further investigation was needed to determine whether the nine AA isolates did indeed constitute

* Author for correspondence: Danish Veterinary Laboratory, Bülowsvej 27, DK-1790 Copenhagen V, Denmark.

an outbreak, and if so, whether or not the four contemporaneous strains were also a part of the outbreak.

This study describes application of pulsed field gel electrophoresis (PFGE), ribotyping, and flagellin gene (*flaA*) typing to the genotypic characterization of the outbreak and contemporaneous HS55 sporadic isolates described above, and further describes characterization of 15 HS55 sporadic strains isolated throughout Scotland in the year preceding the outbreak. Each method was assessed for its ability to identify outbreak isolates, and to discriminate between outbreak and sporadic strains.

MATERIALS AND METHODS

Bacterial strains and growth conditions

Table 1 summarizes sources and isolation details of the three groups (A, outbreak-related; B, contemporaneous, sporadic; C, epidemiologically unrelated) of *Campylobacter jejuni* HS55 strains examined. The HS55 reference strain was also included in the study. Strains were received on charcoal transport swabs and were incubated microaerobically on *Campylobacter* selective agar [12] at 42 °C for 48 h, following which (or after resuscitation from storage) they were subcultured onto 5% (v/v) defibrinated sheep blood agar at 42 °C under microaerobic conditions for 48 h. Stocks of strains were preserved at −70 °C in storage medium containing 10% (v/v) glycerol.

Strain identification

All *Campylobacter jejuni* isolates were speciated by standard microbiological procedures [13]. Identification was based on growth at 42 °C, hippurate hydrolysis, catalase activity, and resistance to antibiotics.

Serotyping

Serotyping was performed according to the somatic O (formerly heat-stable) antigenic scheme of Penner and colleagues [10], using a panel of 42 O-antisera.

Pulsed-field gel electrophoresis

Preparation of genomic DNA agarose plugs and cleavage with restriction enzyme was as described previously [14], except that the initial lysis step was for 6–16 h at 37 °C in 1 ml EC lysis buffer (1 M NaCl, 100 mM EDTA, 6 mM Tris, 0·2% (wt/vol) deoxycholate, 0·5% (wt/vol) Brij-58 (Sigma, Poole, UK), 0·5% (wt/vol) *N*-Laurosyl sarcosine, pH 8·0), with lysozyme and RNase added to 1 mg/ml and 20 μg/ml respectively. *Sma*I (Promega, Southampton, UK) or *Sal*I (Boehringer–Mannheim, Poole, UK) digestion of 3 mm gel slices using the manufacturer's recommended buffer was for 6–16 h at 25 or 37 °C respectively. Restriction fragments were separated by electrophoresis through 1% (wt/vol) agarose, prepared and run in 0·5 × TBE (45 mM-Tris, 45 mM boric acid, 1 mM EDTA) at 14 °C in a CHEF DR-II apparatus (Bio-Rad), using Lambda concatemers (Bio-Rad) as molecular weight markers. Separation of *Sal*I and *Sma*I generated macrorestriction fragments was for 22 h and 27 h (respectively) at 200 V, with ramped pulse times from 20 to 25 s. Gels were stained in 0·5 × TBE containing 0·5 μg/ml ethidium bromide, transilluminated under UV light, and photographs taken.

DNA isolation and purification

Genomic DNA was isolated and purified by a modification of the procedure of Sambrook and colleagues [15], as described previously [16].

Ribotyping

Purified genomic DNA (~ 1 μg) was digested with 10 U *Pvu*II or *Hae*III (Boehringer–Mannheim) for 16 h at 37 °C under conditions recommended by the manufacturer. Digested DNA (20–30 μl final volume) was electrophoresed in 0·5 × TBE at 25 V for 18 h on a 0·8% (wt/vol) agarose gel. DNA transfer to Hybond-N membrane (Boehringer–Mannheim) was overnight using capillary transfer [15], with 20 × SSC as transfer buffer (1 × SSC is 0·15 M NaOH, 1·5 M NaCl, pH 7·0). Membranes were washed once in 2 × SSC, air-dried, and subjected to UV irradiation to fix the DNA. The ~ 1400 bp digoxygenin (DIG)-labelled 16 rRNA gene-specific probe was generated by polymerase chain reaction (PCR) amplification of purified genomic DNA from *C. jejuni* NCTC 11351^T using a commercially available DIG-labelling kit (Boehringer–Mannheim). Primers used were 27f (5′-AGAGTTTGATCA/CTGGCTCAG) and 1392r (5′-ACGGGCGGTGTGTA/GC), representing nucleotides 8–27 and 1392–1406 respectively of the *Escherichia coli* 16S rRNA gene [17]. Hybridization (68 °C) and detection of the DIG-labelled probe was according

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Table 1. *Sources, PFGE profile types, 16S ribotypes, flagellin-gene profile types, and combined genotypes of Penner 55 isolates characterized during this study*

Group*	Isolate†‡	Isolation date Mo/yr	Health Board area§	PFGE profile obtained with		Ribotyping profile obtained with		*Dde*I Flagellin type	Combined genotype
				*Sma*I	*Sal*I‖	*Hae*III	*Pvu*II		
A	1	6/96	AA	Sm1	Sa1	H1	P1	F1	G1
	2	6/96	AA	Sm1	Sa1	H1	P1	F1	G1
	3	6/96	AA	Sm1	Sa1	H1	P1	F1	G1
	4	6/96	AA	Sm1	Sa1	H1	P1	F1	G1
	5*	6/96	AA	Sm1	Sa1	H1	P1	F1	G1
	6	6/96	AA	Sm1	Sa1	H1	P1	F1	G1
	7	6/96	AA	Sm1	Sa1	H1	P1	F1	G1
	8*	6/96	AA	Sm1	Sa1	H1	P1	F1	G1
	9	6/96	AA	Sm1	Sa1	H1	P1	F1	G1
B	10	6/96	GR	Sm2	Sa2	H1	P1	F1	G2
	11	6/96	TY	Sm1	Sa1	H1	P1	F1	G1
	12	6/96	AC	Sm3	Sa3	H1	P2	F1	G3
	13	6/96	GR	Sm4	UT	H2	P3	F2	G4
C	14	4/96	TY	Sm2	Sa2	H1	P1	F1	G2
	15	3/96	AC	Sm5	Sa4	H3	P4	F3	G5
	16	2/96	GG	Sm2	Sa2	H1	P1	F1	G2
	17	2/96	AC	Sm2	Sa2	H1	P1	F1	G2
	18	2/96	GR	Sm2	Sa2	H1	P1	F1	G2
	19	2/96	GR	Sm1	Sa1	H1	P1	F1	G1
	20	2/96	GG	Sm1	Sa1	H1	P1	F1	G1
	21	12/95	GR	Sm6	Sa5	H4	P5	F4	G6
	22	9/95	GR	Sm6	Sa5	H4	P5	F4	G6
	23	7/95	LN	Sm1	Sa1	H1	P1	F1	G1
	24	6/95	LO	Sm1	Sa1	H1	P1	F1	G1
	25	6/95	GR	Sm1	Sa1	H1	P1	F1	G1
	26	6/95	LN	Sm1	Sa1	H1	P1	F1	G1
	27	5/95	GR	Sm1	Sa1	H1	P1	F1	G1
	28	5/95	GR	Sm1	Sa1	H1	P1	F1	G1
	NCTC 12546	Before 1983	North America	Sm2	Sa2	H1	P1	F1	G2

* Group A, outbreak-implicated isolates received from the AA Health Board area during first week of June 1996; Group B, sporadic isolates received during June 1996, but from outside the outbreak area; Group C, sporadic isolates received from all over Scotland during the year preceding the outbreak.

† Duplicate samples indicated by *.

‡ NCTC 12546 is the Penner serotype HS55 reference strain, isolated from human faeces, and first described in 1983 [10].

§ AA, Ayrshire and Arran; GR, Grampian; TY, Tayside; AC, Argyle and Clyde; GG, Greater Glasgow; LN, Lanarkshire; LO, Lothian (all Health Board areas in Scotland).

‖ UT, untypable.

to standard conditions detailed by the manufacturer of the DIG-labelling kit (Boehringer–Mannheim).

Flagellin gene typing

Flagellin (*flaA*) gene PCR using primers A1 and A2 was as described by Nachamkin and colleagues [9], but with modifications to the initial PCR reaction as described previously [16]. The concentration of *flaA* amplicons was estimated prior to digestion by visual comparison of band intensities against band intensities of molecular weight marker VI (Boehringer). Approximately 150 ng of product was digested (20 μl final volume) at 37 °C for 3 h using restriction enzyme *Dde*I (Boehringer–Mannheim) and the manufacturer's recommended buffer. Digests were analysed by electrophoresis through 2% (wt/vol) agarose gels using a 100 bp ladder (Gibco–BRL) as DNA size standard.

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Gels were run in 0·5 × TBE for 16 h at 30 V, then stained and photographed as above.

RESULTS

Epidemiological features

Outbreak-associated isolates (Group A) were obtained from persons living within the AA area, all of whom had eaten at the same canteen within this area. Sporadic isolates received during the same month as the outbreak (Group B) were obtained from persons living outside the AA area. Epidemiological investigations found that 3 of these 4 cases had not been exposed to the incriminated canteen, nor had they been in close contact with any of the primary cases. However, a possible epidemiological link to the outbreak was identified for the remaining case (isolate 11), having visited the outbreak area during the relevant time frame. Food histories however, proved inconclusive (W. J. Reilly, personal communication).

Strain identification and Penner serotyping

All nine outbreak-associated isolates (Group A), and the four contemporaneous sporadic strains from outside the outbreak area (Group B) were characterized as *C. jejuni*, Penner heat-stable serotype 55 (HS55).

Molecular epidemiological typing methods

Table 1 summarizes the results obtained for each of the 29 strains with respect to pulsed field gel electrophoresis (PFGE), ribotyping, and flagellin gene (*flaA*) typing. All three methods resulted in generation of indistinguishable profiles for each of the nine outbreak-associated isolates, but showed varying degrees of discrimination within the overall group, as detailed below.

PFGE

Analysis of *Sma*I-digested DNA yielded six distinct profiles (Sm1–Sm6), each with 5–6 fragments ranging in size from ~ 80 to > 440 kb (Fig. 1a). The outbreak profile (Sm1) was also shared by 9/19 of the sporadic isolates, including isolate 11, one of the contemporaneous group B strains (Table 1). Profile Sm2 differed from that of Sm1 only in the size of its third band (decreased from ~ 360 kb in Sm1 to ~ 330 kb in Sm2), and was also found to be prevalent among the

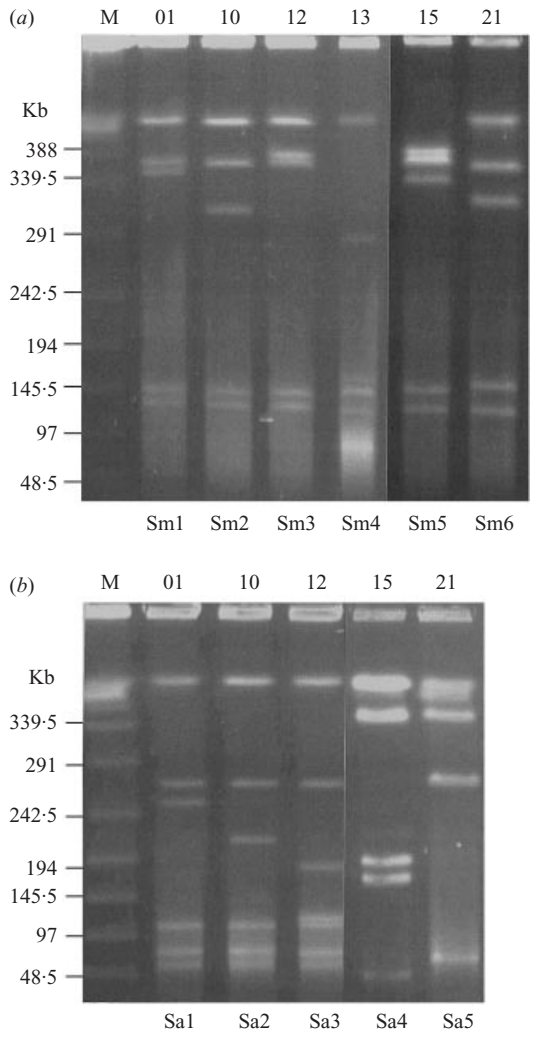

Fig. 1. PFGE profiles obtained from serotype HS55 isolates of *C. jejuni* using *Sma*I (*a*) or *Sal*I (*b*). Isolate numbers are given above the lanes, with corresponding *Sma*I (Sm) or *Sal*I (Sa) profiles identified below each lane. M, molecular size marker.

group, accounting for a further 5/19 of the sporadic strains, and also including the HS55 reference strain (Table 1). Aside from isolates 21 and 22 which shared an indistinguishable profile (Sm6), all remaining sporadic isolates were found to generate unique profiles. However, certain of these profiles differed only in minor band shifts compared to the predominant Sm1 and Sm2 profiles (Fig. 1a). Thus, Sm3 differed from Sm1 and Sm2 only in the size of a single band (increased from ~ 360 kb in Sm1 to ~ 410 kb in Sm3); the four remaining bands being shared between all three profiles. Profile Sm6 also appeared very similar to Sm2, differing only marginally in the spacing of its 2nd/3rd, and 4th/5th biggest bands. The latter two profiles were not differentiated using a

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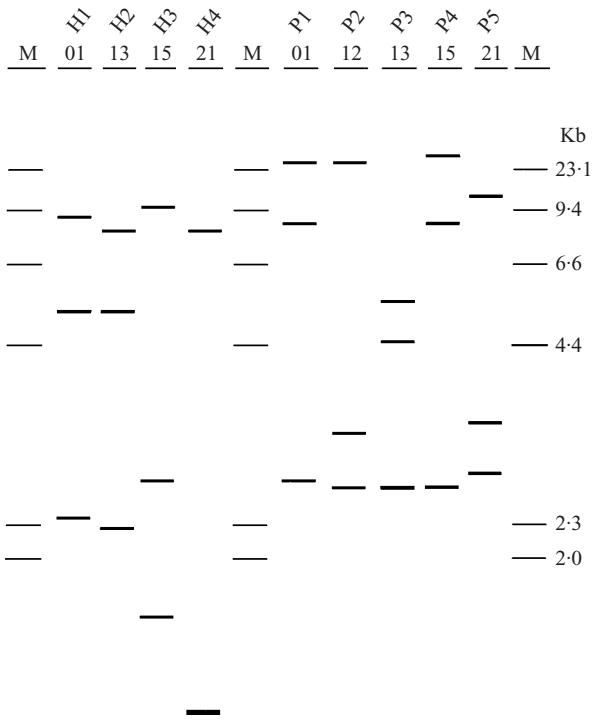

Fig. 2. Schematic diagram of 16S ribotypes obtained from serotype HS55 isolates of *C. jejuni*. Southern blots of *C. jejuni* genomic DNA digested with *Hae*III or *Pvu*II were hybridized with a 16S rRNA gene specific probe. Isolate numbers are given above the lanes, with corresponding *Hae*III (H) or *Pvu*II (P) ribotypes identified above each isolate. M, molecular size marker.

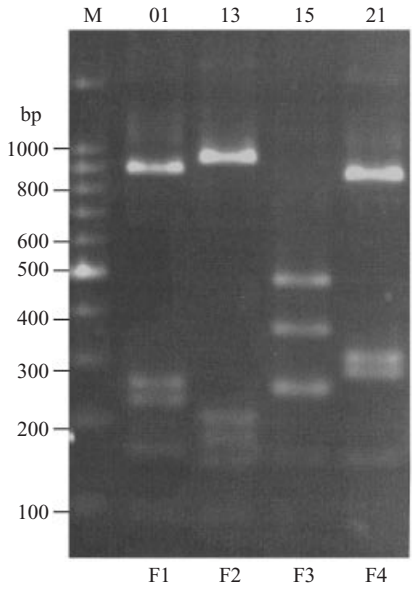

Fig. 3. Flagellin gene profiles obtained from serotype HS55 isolates of *C. jejuni* after restriction digestion of the amplified *fla*A gene with *Dde*I. Isolate numbers are given above the lanes, with corresponding flagellin (F) profiles identified below each lane. M, molecular size marker.

22 h PFGE run, nor was Sm3 differentiated from Sm1 (data not shown), but differences in profiles were found to be reproducible using the extended 27 h run, and were also validated by *Sal*I PFGE results (below).

*Sal*I digestion of DNA generated five profiles, resulting in the same grouping of strains as seen for *Sma*I digestion, with the HS55 reference strain again found to share the second most common (Sa2) profile (Table 1). Profiles typically consisted of 5–7 bands ranging in size from ~ 50 to > 440 kb (Fig. 1b), although isolate 13 was repeatedly found to be refractory to digestion (data not shown). Consistent with *Sma*I results, the Sa2 profile differed from that of the outbreak profile (Sa1) only in the size of its third largest band, which was again reduced in size by ~ 30 kb (from ~ 260 to 230 kb). Sa3 was again closely related to Sa1, sharing 4 of the 6 bands, but with 2 bands of ~ 190 and 120 kb, in place of the ~ 260 kb band in Sa1. This equates to an additional 50 kb of DNA in Sa3 compared to Sa1, as was found also for the corresponding *Sma*I profiles. All five *Sal*I profiles were clearly distinguishable using a 22 h PFGE run, including Sa1/Sa3 and Sa2/Sa5 profiles, whose equivalent pairs of *Sma*I profiles (Sm1/Sm3 and Sm2/Sm6 respectively) could only be distinguished by increasing the run time from 22 to 27 h.

Ribotyping

Digestion of genomic DNA with *Hae*III generated four distinct ribotypes (H1–H4), whereas five distinct ribotypes (P1–P5) were generated using *Pvu*II (Fig. 2). For both enzymes, profiles consisted of three well-separated bands, with the exception of profile H4 which consisted of two bands, although the smaller band of H4 had a higher intensity, suggestive of a co-migrating doublet (data not shown). *Hae*III bands ranged in size from $\sim 1{\cdot}25$ to $9{\cdot}5$ kb, whereas *Pvu*II bands ranged from $\sim 2{\cdot}7$ to > 23 kb. Combining the results for both enzymes led to identification of five combined ribotypes following exactly the distribution of *Pvu*II ribotypes. Outbreak strains shared the H1 P1 combined profile with the HS55 reference strain, and with 14/19 of the sporadic strains, including two (isolates 10 and 11) of the four contemporaneous sporadic strains. One further contemporaneous strain (isolate 12) shared the H1 profile, but was differentiated on the basis of its different (P2) *Pvu*II profile. The remaining four sporadic strains generated three distinct combined ribotypes, with isolates 21 and 22 sharing the same (H4 P5) combined ribotype.

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Flagellin typing

Flagellin gene (*flaA*) PCR amplicons of 1·7 kb were generated from every strain in the group. Four distinct profiles were obtained after digestion with *Dde*I (F1–F4; Table 1), each consisting of 4–5 bands ranging in size between ~ 100 and 1000 bp (Fig. 3). Distribution of profiles amongst the sporadic strains followed the same pattern as for *Hae*III ribotyping, i.e. the outbreak profile (F1) was shared by 15/19 of the sporadic strains, including 3/4 of the contemporaneous strains (isolates 10–12), and was also shared by the HS55 reference strain. The remaining four strains again generated an additional three profiles, with isolates 21 and 22 sharing the same (F4) profile.

DISCUSSION

The aim of the present study was to assess three molecular methods (PFGE, ribotyping, and flagellin gene typing) for their efficacy in positively identifying HS55 outbreak-associated strains of *C. jejuni*, and in differentiating these outbreak-associated strains from sporadic HS55 strains occurring throughout Scotland at the same time as the outbreak. All nine outbreak-associated (group A) strains shared identical profiles by each of the three methods (Table 1). Resolution of multiple indistinguishable genotypic markers in this way indicates these outbreak strains to be clonally derived, as indeed would be expected from a common-source outbreak. All three methods therefore fulfil the primary requirement of any bacterial typing system, i.e. that isolates clearly derived from a common source should generate the same subtype. However, outbreak profiles for all three methods were also shared by nine of the sporadic strains examined (Table 1), suggesting poor discrimination of outbreak from non-outbreak strains using these techniques. This lack of discrimination is in fact directly attributable to the limited genetic diversity represented by the Penner 55 group, within which there exists two closely related clonal lines (described later). Nevertheless, 3 of the 4 contemporaneous (group B) strains could be differentiated from the outbreak group by PFGE, 2 by combined ribotyping, and 1 by flagellin typing (Table 1). The fourth strain (isolate 11) was not differentiated using any of the methods. Retrospective investigations identified a possible epidemiological link between this strain and the outbreak area, but there remained the possibility that this strain was epidemiologically unrelated to the outbreak, and simply represented the dominant clonal line. Problems with accurate delineation of outbreak from non-outbreak strains are common to any bacterial subgroup representing a clonal population structure (e.g. methicillin-resistant *Staphylococcus aureus* strains [18]), and have been discussed previously [19].

Among the study group as a whole, PFGE was found to be most discriminatory (6 subtypes), followed by combined ribotyping (5 subtypes), and then flagellin subtyping (4 subtypes). This is in agreement with several previous studies finding PFGE to be more discriminatory than ribotyping and/or flagellin typing for subtyping within HS serotypes of *C. jejuni* [20–22] and of *C. coli* [23]. The greater discrimination achieved by PFGE is probably a consequence of subjecting the entire genome to restriction polymorphism analysis without subsequent selection of a subset of fragments by hybridization (ribotyping), or restricting the polymorphism analysis to a single genetic locus (as for flagellin typing). Combining the data to give overall genotypes resulted in identification of six genotypes (G1–G6), with exactly the same pattern of distribution as observed using *Sma*I PFGE alone (Table 1). Correlation between the three genotyping methods was good, and where differences in groupings were seen, these were confined to strains belonging to 1 of 3 closely related genotypes (G1–G3), each sharing an identical flagellin and/or ribotyping profile, and differentiated only by minor changes in their PFGE profiles.

The majority (9/19) of sporadic strains were found to share the same genotype (G1) as the outbreak strains (Table 1). A second genotypic group (G2) was also identified, accounting for a further 5/19 of the sporadic strains, and also including the HS55 reference strain. Existence of recurrent genotypes between strains distanced by time (12 months) and geographical location (throughout Scotland) demonstrates an evolutionary clonal relationship between such strains, and implies stability of the recurrent genotype to environmental pressures. In this respect, it is interesting to note that the HS55 reference strain, first isolated from human faeces in North America approximately 15 years ago [10], shares the G2 genotype with five of the Scottish strains isolated during 1996. Recurrent genotypic clones within serotypes have been demonstrated previously in both *C. coli* [23] and *C. jejuni* [21, 22]. For example, a study of HS1 and HS4 *C. jejuni* strains, identified one major recurrent genotype within each serotype, accounting for 29 and 33% respectively of the strains tested [22].

Such results suggest that expression of certain HS antigens can be linked with a conserved genotype. However, it is clear that genotypically-dissimilar strains can also express the same antigens, in this study represented by strains of genotypes G4–G6, none of which share any obvious genotypic character with either of the two dominant clones. We conclude that HS serotyping cannot be used alone as an indicator of strain identity, but that it does provide a useful reference point from which conserved genotypes may be identified.

Examination of the *Sma*I profiles representing each distinct genotype identified four apparently closely related profiles. Profiles Sm1, Sm2, and Sm3 (representing genotypes G1, G2 and G3, respectively) can all be seen to differ in the size of just 1 out of 5 bands, whereas Sm6 (representing G6) appears almost indistinguishable from Sm2 (Fig. 1a). In fact, the two strains representing G6 could clearly be distinguished from all the other strains using *Sal*I PFGE (Fig. 1b; Sa5 compared to Sa1/Sa2/Sa3), and were further distinguished by ribotyping, and flagellin typing (Table 1). G6 therefore represents a genotype quite distinct from the G1/G2/G3 group, despite its similar (and indistinguishable, under a 22 h run) *Sma*I profile. These results argue against the use of a single enzyme for inferring relatedness between strains when the PFGE profiles generated are similar or apparently identical. Such strains should be further examined using a second enzyme, as has been suggested previously [20, 24, 25].

Current interpretative criteria for PFGE profiles suggest that a 'close genetic' (i.e. clonal) relationship may be inferred between bacterial strains whose profiles differ by changes consistent with a single genetic event, involving up to three band differences between profiles [19, 26]. Strictly speaking, these criteria cannot be applied to the majority of *C. jejuni* strains examined by *Sma*I PFGE, since the profiles usually consist of < 10 distinct bands [20, 21, 24] and do not therefore satisfy the proposed guidelines. However, if the same single genetic event can explain profile differences between two strains using two different enzymes, then this provides strong evidence for the two strains being clonally related. This was found to be the case when examining PFGE profiles for strains of genotypes G1–G3. Thus, comparing *Sma*I and *Sal*I profiles of G1 and G2, a simple ~ 30 kb deletion from the genome of G1 accounts for the band differences seen using both enzymes (compare Sm1/Sm2 and Sa1/Sa2 in Figs 1a and 1b, respectively). Similarly, the decreased size of the ~ 410 kb band in Sm3 (G3) to ~ 360 kb in Sm1 (G1) could also be accounted for by a deletion of ~ 50 kb from the genome of G3. This would also account for the replacement of two bands of ~ 190 and 120 kb in Sa3 (G3) with a single band of ~ 260 kb in Sa1 (G1), provided that the deletion also contained a *Sal*I restriction site. A clonal relationship can therefore be inferred by PFGE between strains of genotypes G1–G3, and this relationship is further supported by conserved flagellin type and *Hae*III ribotypes across the three genotypes, whilst G1 and G2 also share the same *Pvu*II ribotype (Table 1).

The six *Sma*I profiles identified in this study were all broadly similar, each with 5–6 unevenly distributed bands. They formed a distinctive group compared to Scottish isolates representing serotypes other than HS55 (unpublished data), and compared to previously published PFGE profiles for *C. jejuni*, all of which consist typically of 7–9 more evenly distributed fragments [20, 21, 24]. It is therefore possible that serotype HS55 represents a distinct clonal line of *C. jejuni*, with genotypes G4–G6 representing isolates that have diverged further from the parental clone than have the G1–G3 genotypes. More work is needed to confirm this possibility.

Previous studies of HS1 and HS4 *C. jejuni* strains identified predominant genomic subgroups within each serotype, but these were identified against backgrounds of different genetic heterogeneity. Thus, HS1 strains appeared to consist predominantly of a single phylogenetic lineage, whereas HS4 strains were found to represent a far more heterogeneous population, likely to consist of several different clonal lines [21, 22]. The current study suggests that HS55 populations reflect more the HS1 than the HS4 population structure, and may in fact represent an even more conserved serotype than HS1, in that 14/19 sporadic strains (74%) share 1 of 2 very closely related genotypes (G1 and G2).

The degree of genetic diversity within a serotype will dictate the discriminatory potential of genotyping methods subsequently employed, and should, where known, be taken into account when selecting molecular methods for epidemiological surveillance of sporadic strains, and particularly for outbreak investigations, since such knowledge could significantly affect the interpretation of results. For example, although the rapidity and ease of flagellin typing has made it an increasingly popular epidemiological tool [8, 9, 27, 28] it will clearly have limited discriminatory

power in conserved serotypes such as HS55 in this study, or HS1 [22], where ~ 70% of sporadic strains are represented by one or two *Dde*I flagellin types respectively. Thus, in the current study, 2/3 contemporaneous HS55 sporadic strains (isolates 10 and 12) which were eliminated from the outbreak by PFGE, would not have been eliminated on the basis of flagellin typing alone, since they both shared the outbreak flagellin type (F1) common to the predominant HS55 clonal line.

HS serotyping combined with PFGE has been described previously as an effective epidemiological subtyping scheme for *C. jejuni* within serotypes HS1, HS2, and HS4 [20, 21]. In this study, HS-serotyping was effective in first identifying the outbreak group from among epidemiologically-unrelated sporadic strains present across Scotland at the same time, whilst PFGE was subsequently found to be more discriminatory than either ribotyping or flagellin typing for eliminating contemporaneous, sporadic HS55 strains from the outbreak. Our work therefore further supports the use of HS serotyping as the primary epidemiological tool for typing *C. jejuni*, followed by PFGE for high-resolution strain differentiation within serotypes.

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