EPIDEMIOLOGICAL TYPING

Subtyping of *Campylobacter jejuni* Penner heat-stable (HS) serotype 11 isolates from human infections

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High resolution molecular subtyping was applied to *Campylobacter jejuni* Penner heat-stable (HS) 11 isolates from human infections and other sources. Strains were genotyped by restriction fragment length polymorphism analysis involving PCR-based flagellin gene (*flaA*) profiling with *Hinf*I and *Dde*I, and pulsed-field gel electrophoretic (PFGE) profiling with *Sma*I and *Kpn*I. *Fla*-genes of the strains were highly conserved as most (95%) had the same *fla*-profile. PFGE analysis of *Sma*I digests was more discriminatory with 15 profile subtypes identified, although 36% of isolates had a common profile. The study showed that strains of *C. jejuni* HS11, unlike those of HS1 and the HS4 complex, were relatively homogeneous at the genomic level and that high resolution molecular techniques were essential for detailed epidemiological subtyping.

Introduction

The importance of *Campylobacter jejuni* as a major enteric pathogen has become established since 1977 following the development of effective selective isolation media [1]. An estimated 40 000 cases of diarrhoeal disease in England and Wales are now attributed annually to infection with *Campylobacter* spp. [2]. The majority of speciated strains are *C. jejuni* and most are from sporadic cases [3].

The transmission routes of campylobacters remain unclear and are probably complex because of the involvement of various potential animal hosts and environmental reservoirs [2]. The use of phenotypic and molecular typing schemes in the laboratory provides the means for investigating the origins of such infections. For example, the Penner heat-stable (HS) serotyping scheme identifies 45 serotypes [4] within *C. jejuni*, and strains can be further subtyped by phage typing, resistotyping and various molecular techniques to provide more detailed epidemiological information [5, 6].

A recent 12-month study of clinical isolates of *C. jejuni* in England showed that the HS serotypes were diverse, but four (HS1, HS2, HS4 complex and HS11) currently predominated [7]. Strains of *C. jejuni* HS1,

HS2 and HS4 complex were genomically diverse, although some distinct clonal lines comprising isolates from mammals, chickens and cattle were identified [5, 6]. The aim of the present study was to determine diversity amongst strains of *C. jejuni* HS11, because such strains represented *c.* 10% of the isolates in the above study, and to assess methods for their epidemiological subtyping.

Materials and methods

Bacterial strains and culture conditions

The 78 strains of *C. jejuni* studied were from human infections (62 isolates), cattle (three isolates) and poultry (five isolates). Seven strains of unknown origin were also included. The HS11 sero-reference strain (NCTC 12510) was obtained from the National Collection of Type Cultures. All strains were cultured under micro-aerobic conditions as described previously [5]. Pure cultures were stored over liquid nitrogen (–196°C) in the Cryobead system (Prolab-Diagnostics Neston, Wirral).

Phenotyping

Bacteria were identified by standard methods [8] and serotyped by the method of Penner and Hennessy [4] with a panel of 45 antisera for the Penner heat stable (HS) serotyping scheme. The antisera were provided by Dr A. Lastovica (Red Cross War Memorial Children's Hospital, Cape Town, South Africa).

Flagellin gene polymorphism

The *flaA* gene was amplified by PCR with the primers and conditions described previously [9]. A single Cryobead was used as the DNA source for each sample. DNAase activity of the Lior biotype II strains was eliminated by boiling cells for 10 min in 30 ml of sterile distilled water. The final PCR mix contained 5 ml of lysate prepared from beads in a total volume of 45 ml of PCR reagents. For DNAase negative strains, a 5-ml cell suspension was used directly without boiling.

Pulse-field gel electrophoresis (PFGE)

Preparation of *C. jejuni* DNA for PFGE after digestion with *Sma*I was as described previously [10]. To digest with *Kpn*I (Gibco BRL, Life Technologies), DNA samples in blocks were incubated in React 4 buffer for 3 h at 37°C and then re-treated with fresh enzyme and buffer for a further 3-h incubation. The PFGE conditions described previously were used for *Sma*I digests [10]. Modified conditions were used for *Kpn*I digests, in which pulsed-field times were ramped from 2 to 20 s with electrophoresis for 40 h at 200 V. The *Kpn*I analysis was used only for strains that had identical *Sma*I profiles.

Results

Fla-profiles

The fla-profiling results are given in Table 1. The majority of strains had profile H2D5 (74 of 78; 95%), but three other profiles were observed. Profile H2D5*, which was a feature of NCTC 12510 and one field strain, differed from profile H2D5 by a *Dde*I digest band deletion at the 350-bp position (Fig. 1). H1D4

Table 1. Summary of molecular typing results for <i>C. jejuni</i> HS11 strains			
Fla-profile	PFGE (<i>Sma</i> I) profile	Number of isolates	PFGE (<i>Kpn</i> I) profile
H2D5	L1U1	28 (36%)	13 strains (type 1: 11 strains) (type 2: 1 strain) (type 3: 1 strain)
	L1U2	15 (19%)	
	L1U3	8 (10%)	
	L2U4	3	
	L1U5	1	
	L1U8	2	
	L1U9	4	
	L1U10	2	
	L1U11	1	
	L4U7	1	
	L6U2	2	
	L7U8	6	
	L8U9	1	
	L1U4	1†	
H2D5*	L1U5	1	
H1D4	L5U3	1	
H8D6	L3U6	1	

†NCTC 12510, sero-reference strain for HS11.

Fig. 1. Fla-type profiles digested with *Hinf*I and *Dde*I for *C. jejuni* HS11 strains. Lane 1, 123-bp ladder, *Hinf*I digests: 2, C670/93; 3, C236/93; 4, C339/93. *Dde*I digests: lane 5, C670/93; 6, C236/93; 7, C339/93; 8, NCTC 12510.

and H8D6 patterns were each a feature of single HS11 strains. The fla-types of isolates C670/93 (H1D4) and C627/94 (H2D5) gave total digest sizes of 200 and 650 bp in excess of that expected for the flagellin A gene (1.448 bp), although the PCR products were not detectably larger. Single colony picks were taken from each strain and on repeat fla-typing, both strains had the predicted total digest size.

PFGE profiles

The pulsed-field gel patterns generated with *Sma*I (typically six band profiles) were designated according to the upper (≥ 290 bp) and lower (≤ 235 bp) band profiles. The different types observed are shown in Fig. 2. Separation of the profiles on this basis allowed discrimination in the more variable upper band regions whilst highlighting the conserved lower band patterns. Lower band pattern 1 (L1) was seen in 67 (86%) of 78 strains.

The PFGE profiles with *Kpn*I contained more bands (typically 16) and potentially provided more discrimination than *Sma*I. The *Kpn*I patterns are shown in Fig. 3. Running conditions for *Kpn*I were optimised as several bands (five-to-nine) were present in the ≥ 48.5 –97-bp region. A total run time of 40 h was necessary to give adequate separation. Complete digestion to avoid partials was enhanced by adding fresh *Kpn*I and buffer after 3 h at 37°C followed by a further incubation for 3 h (J. Gibson, personal communication).

Fig. 2. PFGE profiles with *Sma*I for *C. jejuni* HS11 strains. Lane 1, lambda ladder; 2, C236/93; 3, C25/93; 4, C1160/93; 5, C1128/93; 6, C424/93; 7, C339/93; 8, C264/94; 9, C372/94; 10, C777/93; 11, C358/94; 12, C633/94; 13, C641/94; 14, C670/93; 15, C467/94; 16, lambda ladder.

Thirteen distinct PFGE profiles with *Sma*I were detected within the fla-type H2D5 strains (Table 1). PFGE pattern types L1U1, L1U2 and L1U3 accounted for 65% of these strains. The upper band and lower band sizes are given in Tables 2 and 3. Total genome sizes were calculated to be between 1659 and 1931 bp with *Sma*I digests and between 1877 and 2096 bp with *Kpn*I digests.

Three different profiles were observed with *Kpn*I among 13 strains with the same *Sma*I profile (Fig. 3). The seven largest bands were conserved within these strains with the differences observed in the 48.5–97-bp region.

Isolates from two human cases, in whom the infection had possibly been acquired abroad, had the uncommon *Sma*I L1U9 and L3U7 patterns. Two other human isolates from cases within the same household had a common *Sma*I profile (L1U1) and the same profile with *Kpn*I (type 1). Bovine isolates included in the study also had the common *Sma*I profile (L1U1). Chicken isolates had the common H2D5 fla-type but had more diverse PFGE profiles – L1U2, L1U3, L1U4, L1U10 and L5U2.

The discrimination indices for PFGE and fla-typing given in Table 4 show that the combination of fla-typing with PFGE profiling did not markedly improve discrimination over PFGE profiling alone.

Discussion

Penner heat-stable serotype HS11 represents one of the commoner serotypes currently isolated from patients infected with *C. jejuni* [7]. The study of this serotype in relation to current typing methods is, therefore, important and relevant to developing an understanding of the contribution of individual serogroups to the epidemiology of *C. jejuni* infections. Fla-typing and PFGE profiling were assessed as molecular typing methods for this set of pathogenic strains.

The majority of strains (79%; 62 of 78) were isolated from sporadic cases of human illness at widely separated locations (Ashford, Belfast, Cardiff, Harrogate, Lancaster, London and Preston). It is of interest that sporadic infections with *C. jejuni* HS11 arise among a diverse human population yet strains appear to be remarkably homogeneous with respect to fla-type with 95% (74 of 78) strains sharing a common profile.

Strain homogeneity in serological properties and fla-types was also reflected in the similarities of PFGE profiles. Detailed analysis of the PFGE profiles showed that the largest number of band differences in *Sma*I digests between any two strains was five polymorphisms. Most differences (69%; 54 of 78) were only one-to-two bands. Previous studies found that strains of HS1 and the HS4 complex were highly heterogeneous in their PFGE profiles, a fact consistent

Fig. 3. PFGE profiles with *Kpn*I for *C. jejuni* HS11 strains. Lane 1, lambda ladder; 2, C362/94; 3, C567/93; 4, C987/94; 5, C1215/94; 6, C694/93; 7, C237/93; 8, C447/93; 9, C1122/93; 10, C1124/93; 11, C1125/93; 12, C752/93; 13, C28/94; 14, C363/94; 15, lambda ladder. *, indicates from the same household.

Table 2. Sizes of lower (L) region bands in PFGE profiles (*Sma*I) of *C. jejuni* HS11 strains

Band no.	Lower region bands present in profile								Size (bp)
	L1	L2	L3	L4	L5	L6	L7	L8	
1				+			+		261
2			+						220
3	+	+			+	+		+	216
4				+				+	190
5				+				+	181
6						+			177
7	+	+	+	+	+	+	+	+	169
8					+				159
9			+						150
10	+	+		+			+	+	143
11		+	+			+		+	132
12					+				128
13				+					97
14					+				80
15			+	+					52

*Doublets present at these positions.

Table 3. Sizes of upper (U) region bands in PFGE profiles (*Sma*I) of *C. jejuni* HS11 strains

Band no.	Upper region bands present in profile												Size (bp)
	U1	U2	U3	U4	U5	U6	U7	U8	U9	U10	U11	U12	
1			+										498
2					+					+	+	+	469
3	+	+		+	+			+	+				446
4				+				+			+		427
5						+							404
6	+	+	+			+	+		+	+			385
7							+						365
8		+						+		+	+		363
9	+		+	+	+								317
10						+							312

Table 4. Discrimination indices for *C. jejuni* typing methods applied to HS11 strains

Method	Number of types	Strains in main type (%)	Discrimination index [11]
Fla-type	4	95	0.100
<i>Sma</i> I PFGE	15	36	0.820
Combined	17	36	0.821

with the greater number of *Sma*I [5] sites. The latter serotypes typically gave eight- or nine-band *Sma*I profiles whereas the typical HS11 *Sma*I profiles had only six bands. It is possible that *Sma*I was not the best enzyme for demonstrating diversity within the HS11 serogroup, and when *Kpn*I was used, three different profiles were detected among 13 strains that were identical with *Sma*I. The running conditions were increased to 40 h to separate effectively the bands in the 48.5–97-bp region. Even under these conditions, 11 of the 13 strains still had the same *Kpn*I profile (type 1).

Tenover *et al.* [13] emphasised the need to include epidemiologically unrelated isolates when investigating outbreaks by PFGE to ensure that endemic strains were differentiated from outbreak strains. The results of the present study show that more precise molecular typing such as PFGE with *Kpn*I would be needed for *C. jejuni* HS11 because of the inherent homogeneity of strains with that particular serotype. The extent that other HS serotypes of *C. jejuni* are conserved is not established, although data on strains representing common types in the UK, such as HS1, HS2 and HS4 complex, indicated a high level of molecular diversity [5, 6].

The present study found that fla-typing of *C. jejuni* HS11 with *Dde*I and *Hinf*I provided low discrimination and even when combined with PFGE, discrimination was not significantly improved. Furthermore, the commonest fla-profile, H2D5, was diverse as it was subdivided by *Sma*I PFGE analysis into 13 different profile types. The results showed that the use of fla-typing as a supplementary typing method to Penner serotyping was of limited use in the case of HS11 strains as it separated only 5% of isolates. *Sma*I and

*Kpn*I PFGE analysis would be the preferred subtyping method.

In conclusion, these results indicate that strains within serotypes of *C. jejuni* can vary significantly in genotypic homogeneity and it is necessary to optimise the choice of molecular subtyping method according to the particular serotype.

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References

1. Goossens H, Wachsmuth IK. Isolation and identification of *Campylobacter* spp. In: Nachamkin I, Blaser MJ, Tompkins LS (eds) *Campylobacter jejuni*: current status and future trends. Washington, DC, ASM Press. 1992: 93–109.

2. Linton D. Old and new campylobacters. *PHLS Microbiol Dig* 1996; **13**: 10–15.

3. Pearson AD, Healing TD. The surveillance and control of campylobacter infection. *Commun Dis Rep CDR Rev* 1992; **2**: 133–139.

4. Penner JL, Hennessy JN. Passive hemagglutination technique for serotyping *Campylobacter fetus* subsp *jejuni* on the basis of soluble heat-stable antigens. *J Clin Microbiol* 1980; **12**: 732–737.

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**: 872–877.

6. 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**: 215–225.

7. Owen RJ, Slater E, Telford D, Donovan T, Barnham M. Subtypes of *Campylobacter jejuni* from sporadic cases of diarrhoeal disease at different locations of England are highly diverse. *Eur J Epidemiol* 1997; **13**: 837–840.

8. Committee on the Microbiological Safety of Food. Interim report on *Campylobacter*. London, HMSO. 1993.

9. Santesteban E, Gibson J, Owen RJ. Flagellin gene profiling of *Campylobacter jejuni* heat stable serotype 1 and 4 complex. *Res Microbiol* 1996; **147**: 641–649.

10. Gibson JR, Sutherland K, Owen RJ. Inhibition of DNase activity in PFGE analysis of DNA from *Campylobacter jejuni*. *Lett Appl Microbiol* 1994; **19**: 357–358.

11. Hunter PR, Gaston MA. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. *J Clin Microbiol* 1988; **26**: 2465–2466.

12. Owen RJ, Gibson JR. Update on epidemiological typing of *Campylobacter*. *PHLS Microbiol Dig* 1995; **12**: 2–6.

13. Tenover FC, Arbeit RD, Goering RV *et al.* Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 1995; **33**: 2233–2239.