Differentiation of *Campylobacter coli* and *C. jejuni* by length and DNA sequence of the 16S–23S rRNA internal spacer region

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The internal spacer region (ISR) between the 16S and 23S rRNA genes of *Campylobacter* was investigated by PCR fragment length typing and DNA sequencing of clinical and chicken wild-type isolates. PCR fragment length typing showed one fragment of 859 nt in length for the 12 strains of *Campylobacter coli* investigated. Thirty-six of the *Campylobacter jejuni* subsp. *jejuni* strains possessed one fragment, which varied in size between 727 and 802 nt. Three strains showed two fragments between 501 and 923 nt. Strains of *C. jejuni* subsp. *doylei*, *Campylobacter lari* and *Campylobacter upsaliensis* possessed one or two fragments with lengths different from those of *C. coli* and *C. jejuni* subsp. *jejuni*. DNA sequences were obtained from 54 nt downstream of *rrs* up to *rri* of four strains of *C. coli*, eight strains of *C. jejuni* subsp. *jejuni*, and one strain each of *C. jejuni* subsp. *doylei* and *C. lari*, selected to represent the different biotypes of *Campylobacter*. ISR lengths determined by PCR fragment length typing and DNA sequencing corresponded for 12 strains. For two strains of *C. coli*, PCR fragment length typing underestimated ISR lengths by 159 and 193 nt, probably related to incomplete resolution of the distal helical structures, which were not fully denatured during PAGE. For the 14 strains and the published *C. jejuni* subsp. *jejuni* sequence, the first 206–211 nt were conserved and included the two tRNA genes in the characteristic tRNA_{Ala} to tRNA_{Ile} order separated by a short 8–9 nt spacer region. Within the region downstream of tRNA_{Ile}, conserved regions were identified which allowed a separation of *C. lari* from *C. coli* and *C. jejuni* but not separation of *C. coli* from *C. jejuni*. The 69–282 nt longer variable regions in *C. coli* strains allowed separation of this species from *C. jejuni*, confirming results obtained by PCR typing. Certain nucleic acid positions in variable regions were related to the Lior biotypes. Sequence information from ISRs of more strains is needed to ascertain if separation of species and biotypes will be possible for diagnostic purposes.

Keywords: *Campylobacter coli*, *Campylobacter jejuni*, rRNA, internal spacer region, ISR

INTRODUCTION

The identification of *Campylobacter coli* and *Campylobacter jejuni* is limited by low discrimination of pheno-

typic tests (On, 1996). Genotyping has the potential to improve separation, including typing below the species level (Owen *et al.*, 1990; Steele *et al.*, 1998); however further evaluation and standardization of genotyping methods are needed (Wassenaar & On, 1998). It is possible to standardize PCR-based methods for identification and typing but improvements are needed in relation to specificity (On, 1996). Direct sequencing of DNA for identification and typing of campylobacters is

Abbreviation: ISR, internal spacer region.

The GenBank accession numbers for the sequences reported in this paper are AF074828–AF074841.

another alternative that might become favourable with the increasing automatization of this technique.

The internal spacer region (ISR) between 16S rRNA and 23S rRNA genes has been used as a target for PCR-based identification and typing of many bacteria (Gürtler & Stanisich, 1996). In *Escherichia coli* the sequences which form the strong base-paired stems at each end of the mature rRNA genes are conserved (Pace & Burgin, 1990; Srivastava & Schlessinger, 1990). Other regions of 16S–23S rRNA gene spacers are highly variable and have been used for genotyping of bacteria by fragment length profiling, restriction fragment length profiling or DNA sequencing (Gürtler, 1993; Gürtler & Stanisich, 1996; LeBlond-Bourget *et al.*, 1996; Zavaleta *et al.*, 1996).

C. jejuni possesses three copies of *rrn* genes (Kim *et al.*, 1993). Within the *rrnA* operon, rRNA genes are separated by a 805 bp long spacer region which includes the tRNA_{Ala} and tRNA_{Ile} genes (Kim *et al.*, 1993, 1995). Not all ribosomal genes may be contained in all *rrn* operons. In *C. jejuni* strain NCTC 11168, *rrs* (16S rRNA) genes were separated from *rrl* (23S rRNA) genes and only one operon remained intact with linkage between genes (Taylor *et al.*, 1992; Newnham *et al.*, 1996).

The aim of the present investigation was to analyse ISRs at the fine structural level to obtain basal knowledge of the genetic diversity of wild-type and reference strains representing the phenotypic spectrum of *C. coli* and *C. jejuni*, and to evaluate this information for differentiating the two species.

METHODS

Bacterial strains and media. *C. jejuni* subsp. *jejuni* ($n=39$), *C. jejuni* subsp. *doylei* ($n=2$) and *C. coli* ($n=12$) were selected to cover six biotypes and 28 serotypes. Two strains of the related *C. lari* and one strain of *C. upsaliensis* were included for comparison (Table 1, Fig. 2). Bacteria were cultured from the freeze-dried state on Blood Agar (Base no. 2, Oxoid) in a micro-aerophilic atmosphere of 85% H₂/10% CO₂/5% O₂ for 2 d at 37 °C.

DNA extraction. Colonies were scraped from plates and resuspended in 0.5 ml TE buffer (50 mM Tris, 50 mM EDTA, pH 8). Cells were washed in TE buffer by centrifugation and lysis was initiated by addition of 50 µl of a solution containing 10 mg lysozyme ml⁻¹. After incubation at 37 °C for 30 min, 50 µl of a solution containing 10 mg Proteinase K ml⁻¹ in TE buffer and 20 µl 10% SDS pH 7.2 was added, gently mixed and further incubated for 2 h at 56 °C. Cell debris and protein were precipitated with 297 µl 3 M potassium acetate. After centrifugation at 16000 *g* for 10 min, the supernatant was added to 0.54 vol. 2-propanol, gently mixed and centrifuged again at 16000 *g* for 10 min. The pellet was washed twice in ice-cold ethanol by centrifugation, vacuum-dried and resuspended in sterile distilled and filtered ultrapure water.

PCR amplification. Three nested sets of oligonucleotide primers were used to amplify the spacer between 16S and 23S rRNA genes. The criterion for the identification of fragments was the presence of the same number of fragments with all three PCR primer-sets in order to eliminate PCR products

generated by misannealing of primers. Oligonucleotide primers were designed with identity to the *C. jejuni rrnA* sequence (Kim *et al.*, 1995). The primer for forward directional amplification was 16S-1498f (5' GGTGGATCACCTCCTT) in all three sets and was located on the 16S gene. The three reverse sets of primers, located on the 23S rRNA gene, were 23S-30r (5' CAGGGCATCCACCGT), 23S-123r (5' GGTT-GCCCCATTCGG) and 23S-494r (5' CTTTTCGCCTTTC-CCTC) (Fig. 1). Fragments generated with primer-sets 1, 2 and 3 have overhangs with 16S and 23S gene sequences of 45, 138 and 504 nt, respectively. The primer sequences were searched for similarity to *Campylobacter* DNA sequences deposited in the GenBank/EMBL database. At least five mismatches to genes other than *rrn* were found. The PCR primers therefore were predicted to amplify *rrn* regions only. For analysis on the ALF DNA sequencer (Pharmacia Biotech), one of the primers in each primer-set was labelled with FITC. PCR amplification was performed as described by Vogel *et al.* (1997) with the modifications of 0.1 µM concentration of each primer and 1 min annealing time.

Analysis of fragments. Fragments amplified by PCR were separated by denaturing PAGE on the ALF DNA sequencer. The size-marker Sizer 50–500 (Pharmacia Biotech) and a size-marker with 367, 583, 641, 740, 905 and 1161 nt double-stranded DNA were included on the gel. The size-marker of 367–1161 nt long fragments was made by PCR amplification of 23S rRNA gene from *Salmonella bongori* strain BR1859 by the primer-sets 114f (5' CCGAATGGGGAAACCC)/484r (5' GAGGGAAAGGCGAAAAG), 1939f (5' GTAGCGAAATTCCTTGTC)/2498r (5' CCTCGATGTCCGCTC), 481f (5' GAGGGAAAGGCGAAAAG)/1104r (5' AGAAAGCGTAATAGCTCAC), 1939f/2654r (5' AGTACGAGAGGACCGG), 1608f (5' AAACCGACACAGGTGG)/2498r, and 481f/1608r (5' AAACCGACACAGGTGG), respectively, with both primers labelled by FITC. The lengths of fragments were calculated by comparison with the published 23S rRNA gene sequence with GenBank accession number U77927.

Polyacrylamide gels (ReadyMix gel, Pharmacia Biotech) composed of 6% acrylamide, 7.0 M urea and 100 mM Tris/borate (pH 8.3) were cast with 0.5 mm spacers and loaded with PCR product which had been mixed with an equal volume of Stop-mix (AutoRead sequencing kit, Pharmacia Biotech), denatured at 94 °C for 2 min, spun down in a microcentrifuge and immediately cooled on ice. Electrophoresis was performed at 1500 V and 45 °C for 700 min. Peak positions and intensity were analysed by the program Fragment Manager (Pharmacia Biotech). Fragment lengths were reduced to allow for the overhangs with the 16S and 23S rRNA genes created during PCR amplification.

Partial sequencing of spacer fragments. PCR-amplified fragments were purified on Microspin columns (Pharmacia Biotech) and cycle-sequenced (Thermo Sequenase Fluorescent Labelled Primer Cycle Sequencing kit, Amersham) on an ALF sequencer (Pharmacia Biotech) using the primers 16S-1498f, 23S-123r (see above) and 289f (5' CTTTAAAATCTAAAG-CAAG), labelled by FITC. Comparison was performed with the published sequence for the *rrnA* operon of *C. jejuni* strain TGH 9011 (GenBank accession no. Z29326). Alignment and analysis of sequence structure were performed by use of the Wisconsin Sequence Analysis Package (Genetics Computer Group, Madison, USA) including the use of Mfold (Zuker, 1989) and GeneCompar (Applied Maths, Kortrijk, Belgium).

rrnA operon region of *C. jejuni* TGH 9011

Fig. 1. Structure of *C. jejuni* strain TGH 9011 *rrnA* operon (GenBank accession number Z29326; Kim *et al.*, 1995) and alignment of the region between *tRNA*_{Ile} and *rrl* of *C. coli*, *C. jejuni* and *C. lari* for positions 2042–2581 of strain TGH 9011.

RESULTS AND DISCUSSION

Typing by PCR fragment length profiling of ISRs

PCR amplification of ISRs allowed separation of *C. coli* from *C. jejuni* subsp. *jejuni* by the length and number of fragments (Fig. 2). The 12 strains of *C. coli* showed one

ISR with mean length of 859 nt. Thirty-six out of the 39 *C. jejuni* subsp. *jejuni* strains showed only one fragment, but this varied in size between 727 and 806 nt. The remaining three *C. jejuni* subsp. *jejuni* strains showed two fragments of which the shorter was between 501 and 735 nt and the longer between 763 and 923 nt,

Fig. 2. Typing of *Campylobacter* based on PCR amplification of ISRs. The cross hatching represents fragment length variation between strains of each species. ND, Not determined.

clearly separated from the length of the one *C. coli* fragment. One *C. jejuni* subsp. *doylei* strain showed one fragment of 828 nt, whilst the other strain showed two fragments of 794 and 986 nt. *C. lari* possessed one fragment of 678 nt or two fragments of 678 and 831 nt. *C. upsaliensis* showed two fragments with lengths of 633 and 653 nt (only one strain).

The number of fragments was confirmed by use of the three nested primer-sets. The data reported in Fig. 2 are means of fragment lengths obtained with primer-sets 1 and 2 because fragment lengths obtained with these primer-sets were comparable. Up to 91 nt underestimation was obtained by use of primer-set 3 as the longest fragments were greater than the length of the 1161 nt size marker.

Correspondence between PCR fragment length and DNA sequence analysis of ISRs

Selected strains from the PCR fragment length profiling of ISR were further investigated by sequencing (Table 1). DNA sequences of four *C. coli*, nine *C. jejuni* and one *C. lari* strain were obtained. These strains possessed only one spacer fragment (Table 1). Sequences were determined from position 1830 of the *C. jejuni* TGH 9011 *rrnA* sequence (GenBank accession no. Z29326), 54 nt downstream of *rrs* up to the *rml* gene (see Fig. 1). The lengths of ISRs determined by PCR typing and measured as DNA sequence corresponded within the experimental precision of 10 nt except for *C. coli* strains JEO 2772 and JEO 2777. Low accuracy was also found with *C. jejuni* subsp. *jejuni* strain NCTC 11392, where the spacer length was overestimated by 60 nt by PCR typing compared to sequencing. When *C. coli* strains JEO 2772 and JEO 2777 were sequenced, the lengths were determined as 999 and 967 nt, respectively (Table 1). The underestimation found by PCR typing might be related to incomplete resolution of the distal helical structures which were not fully denatured during PAGE. The mobility of such structures is known to be faster than that of single-stranded DNA and thus they will be underestimated in length (Sambrook *et al.*, 1989).

Sequences of *C. coli* and *C. jejuni* were conserved in the first 51 nt upstream from the tRNA_{Ala} gene, through the tRNA_{Ala} and tRNA_{Ile} genes including the 8–9 nt long tRNA spacer (data not shown). Base number 42 (G) of tRNA_{Ile} in the *C. jejuni* TGH 9011 *rrnA* sequence was absent from all of the campylobacter strains sequenced in the present study.

Downstream from tRNA_{Ile} four conserved regions of 15, 120, 40 and 84 nt were identified (Fig. 1). Within these regions and the conserved region upstream of tRNA_{Ala} variation between the nine strains of *C. jejuni* subsp. *jejuni* was 0–1.3%. Variation in the same range was found between *C. coli* strain JEO 2772 and *C. jejuni* subsp. *jejuni* strains and between strains CIP 70.80 and NCTC 11353 of *C. coli*. The variation was 1.6–2.9% between *C. jejuni* subsp. *doylei* strain NCTC 11951 and the *C. coli* strains and 2.9–3.6% between strain NCTC

11951 and the *C. jejuni* subsp. *jejuni* strains. Variations of 0.3–3.6% were found between *C. coli* strains and up to 4.6% variation between *C. coli* and *C. jejuni* subsp. *jejuni*. The variation between *C. lari* and *C. coli* and *C. jejuni* was 11.5–13.8%. The sequence variation between conserved parts of ISRs was more than twice the variation of 1.9–2.2% found between 16S rRNA sequences of *C. coli* and *C. jejuni* (Paster & DeWhirst, 1988; Thompson *et al.*, 1988). The variation of up to 14% between *C. lari*, *C. coli* and *C. jejuni* was much higher than the 2.2–3.7% variation determined by 16S rRNA sequence comparison (Paster & DeWhirst, 1988; Thompson *et al.*, 1988). The variability between *C. coli*, *C. jejuni* and *C. lari* within conserved regions of ISRs (tRNA genes excluded) therefore was more than twice that of 16S rRNA sequences.

One of the most likely reasons for conservation of parts of ISRs is the preservation of processing stems for rRNAs. Based on the published sequence of *C. jejuni* strain TGH 9011, a processing stem could form between the region between *rrs* and tRNA_{Ala} genes with the first 110 bases downstream of tRNA_{Ile} (79% complementarity), or between the latter region and the region upstream of *rrs* (80% complementarity) (see Fig. 1). Complementarities of 75–83% were determined in all strains for the first possibility.

Divergence in sequence length of variable regions was responsible for the variable ISR lengths observed by PCR typing. These sequence mosaics crossed the species border of *C. coli* and *C. jejuni* as the first 25 nt insert downstream from tRNA_{Ile} was found in CIP 70.80, NCTC 11353 and NCTC 11392 and the region from nt 75 to 210 (consensus numbering according to Fig. 1) was deleted in strains NCTC 11353 and NCTC 11392. The region from nt 566 to 755 was only found in *C. coli* strains, explaining the longer fragment length identified by PCR typing. For strains with only one ISR fragment, *C. coli* was separated from *C. jejuni* on the basis of the 152–193 nt insertion upstream of *rml*.

Signature nucleic acid positions

On the basis of variable regions it was possible to identify 19 signature nucleic acid positions where strains of *C. jejuni* subsp. *jejuni* could be separated according to the four Lior biotypes (Lior, 1984) (Fig. 3). For the first 11 positions up to consensus position 303, variations occurred at random between biotypes. However, for the last eight signatures biotype I differed from II, III and IV.

The correlation between DNA sequence and biotype is in accordance with the high correlations observed between PFGE groups and biotypes (Steele *et al.*, 1998) and between ribotyping, biotyping and serotyping of *C. jejuni* and *C. coli* (Owen *et al.*, 1990), which point to a clonal population structure. The clonal population structure of *C. coli* and *C. jejuni* has not been investigated in detail; however 88% of *C. coli* strains were in one sero-, ribo- and flagellin-type specific clone (Stanley *et al.*, 1995), and 62% of Danish *C. jejuni*

Table 1. Origin, and bio- and serotype characteristics of strains analysed, and nucleotide lengths of 16S–23S rRNA ISRs obtained by PCR typing and DNA sequencing

Strain*	Origin	Lior biotype	Serotype†		PCR typing				DNA sequence‡	
			HS	HL	Fragment length	No. of strains	n	SD	Length	Accession no.
<i>C. jejuni</i> subsp. <i>jejuni</i>										
JEO 2677 (3-1 a)	Chicken	I	ND	2	796	1	2	2	803	AF074831
NCTC 11168 (KJ6, JEO 4128)	Human faeces	I	2	4	781	1	2	1	804	AF074828
JEO 2693 (12583/87)	Human faeces	I	ND	9	801	1	2	2	804	AF074833
JEO 2762 (22-5 b)	Chicken	I	ND	11	793	2	2	6	803	AF074835
NCTC 11392 (JEO 4130)	Human faeces	II	6	ND	777	1	2	2	717	AF074829
KJ7	Human	III	ND	6	780	1	2	2	806	AF074830
JEO 2759 (16-1 b)	Chicken	III	ND	40	796	1	2	5	805	AF074834
JEO 2688 (775)	Chicken	IV	ND	19	799	1	2	2	804	AF074832
<i>C. jejuni</i> subsp. <i>doylei</i>										
NCTC 11951 ^T (JEO 4156)	Human faeces	ND	ND	ND	828	1	2	4	831	AF074836
<i>C. coli</i>										
CIP 70.80 ^T (NCTC 11366)	Porcine faeces	ND	4	ND	867	1	2	4	875	AF074837
NCTC 11353 (KJ4)	ND	ND	ND	ND	863	1	2	2	865	AF074838
JEO 2772 (11-8)	Chicken	I	ND	29, 55	862	1	4	7	999	AF074839
JEO 2777 (783)	Chicken	II	ND	28	856	1	4	7	967	AF074840
<i>C. lari</i>										
CCUG 29406 (JEO 4161)	ND	I	ND	1	678	1	2	5	685	AF074841

* The strain designation given for the culture collection from which the strain was obtained. Alternative strain designations are given in parentheses. CCUG, Culture Collection of the University of Göteborg, Sweden; NCTC, National Collection of Type Cultures, Central Public Health Laboratory, London, UK; JEO and KJ, Royal Veterinary and Agricultural University, Copenhagen, Denmark.

† HS, heat-sensitive (Penner); HL, heat-labile (Lior) serotypes. ND, Not determined.

‡ DNA sequence 54 nt downstream of tRNA_{Ala} from position 1830 to 2581 of *C. jejuni* *rrnA* gene (GenBank accession no. Z29326). Sequence lengths were increased by 53 nt for comparison with the full length of the 16S–23S rRNA spacer region.

isolates were distributed in three serotypes (Nielsen *et al.*, 1997). If the mosaic pattern of DNA sequences found in ISRs had been the result of recent recombination events between *Campylobacter* species, the observed correlations with biotypes of *C. coli* and *C. jejuni* would have been very unlikely.

Structure of *rrn* operons

Fifty out of the 56 strains analysed contained only one PCR-amplified spacer fragment. These strains were assumed to contain only one intact *rrn* operon because this was found for strain NCTC 11168 of *C. jejuni* by chromosome mapping (Taylor *et al.*, 1992; Newnham *et al.*, 1996) and DNA sequences determined for *C. coli* and *C. jejuni* strains were structurally similar. The presence of two or three operons with equal spacer

lengths cannot be excluded. However variations would have been expected in the light of the high degree of chromosomal instability observed with *Campylobacter* (On, 1997; Wassenaar *et al.*, 1998). Recently, all three paired copies of *rrs* and *rml* genes were mapped pairwise within the same restriction fragments of strain NCTC 11168 (Karlyshev *et al.*, 1998). The present study shows that the distance between two of the pairs of *rrs* and *rml* were above PCR-amplifiable lengths. It remains to be determined which part of the ISRs there are accompanying *rrs* and *rml* genes of split-up operons.

The ISR sequence was characterized by a low GC content of 9–38 mol% for variable regions and 22–31 mol% for conserved regions, in sharp contrast to *E. coli* where a value of 57–61 mol% was found for both these regions (Brosius *et al.*, 1981). Such low GC contents

Species/biotype/strain	Nucleotide number																			
	1	1	1	1	1	2	2	3			3	3	3	3	3	3	4	4		
	4	4	9	0	0	6	7	7	3	5	0	5	5	5	6	6	6	0	0	
	1	2	5	3	4	0	3	9	9	1	3	2	3	4	3	4	6	5	6	
<i>C. jejuni</i>																				
I NCTC 11168 JEO 2677 JEO 2693	T	A	T	-	-	G	A	C	T	T	A	-	-	A	G	C	G	G	A	
JEO 2762 TGH 9011																				
II NCTC 11392	-	-	.	-	-	-	C	-	.	A	.	T	A	T	A	T	A	A	T	
III JEO 2759	.	.	C	A	A	A	.	T	C	.	G	T	A	T	A	T	A	A	T	
IV JEO 2688 KJ7	.	.	.	-	-	A	.	T	.	.	.	T	A	T	A	T	A	A	T	
<i>C. coli</i>																				
I JEO 2772	.	.	C	-	-	A	.	T	.	.	A	T	A	T	A	T	A	T	T	
II JEO 2777	.	.	.	-	-	.	.	T	.	.	A	T	A	T	A	T	A	A	T	
<i>C. lari</i>																				
I	.	.	A	A	A	A	-	A	-	A	A	-	-	-	-	-	-	-	-	

Fig. 3. DNA sequence signatures of the 165–235 rRNA ISRs supporting differences between Lior biotypes of *C. coli*, *C. jejuni* and *C. lari* strains. Numbering of nucleotides starts downstream of tRNA_{Ile} according to the consensus sequence in Fig. 1 based on the alignment of the 14 sequences and the published sequence of *C. jejuni* strain TGH 9011. Deleted nucleotides are shown by dashes and nucleotides identical to those in the first row are shown by dots.

provide low possibilities for stable helix formation (Srivastava & Schlessinger, 1990). The less stable AT-rich secondary structures would be important during transcription of *rrn* genes (Richardson & Greenblatt, 1996) and the GC content of ISRs is at the same level as for the whole *C. coli* and *C. jejuni* genome of 30–33% (Van Damme & Goossens, 1992). The predicted helices mostly ended in loops of five nucleotides, which is in contrast to 16S and 23S rRNA where tetraloops dominate (Gutell *et al.*, 1994).

Secondary helical formations were predicted in the regions 76–134 and 151–190 of the consensus sequence for all strains except CIP 70.80, NCTC 11353 and NCTC 11392, where this region was deleted (Fig. 1). In these strains a helix was predicted instead from nt 52 to 232 of the consensus sequence (68 nt). Helix formation was further predicted in the conserved regions from nt 277 to 312 and from nt 367 to 388. The last helix predicted in all strains was from nt 439 to 478. In *C. coli*, additional helices were predicted in the inserted region from positions 567 to 619 of JEO 2777, CIP 70.80 and NCTC 11353 and from 642 to 658 of strain JEO 2772 (Fig. 1). The GC content of 38 mol% for these regions was somewhat higher than the value of 12–28 mol% of the other helices. It was characteristic that the predicted helices were located within variable regions, indicating that the conserved regions without these structures

could be involved in formation of processing stems for rRNAs.

A FASTA search showed low homology to the spacer regions of other bacteria. Only a sequence (GenBank accession no. I23675) listed from an unknown organism was 91–99% similar to the spacer of *C. coli* and *C. jejuni*. The paired order of tRNA_{Ala} and tRNA_{Ile} with the 8–9 nt short spacer and the ISR region between tRNA_{Ile} and *rrs* are therefore characteristic of *C. coli*, *C. jejuni* and *C. lari*.

Conclusion

The insertion of between 152 and 193 nt found in *C. coli* but not in *C. jejuni* could provide a target for rapid separation of the two species based on PCR or DNA hybridization. The study showed diverging DNA sequences between all the strains; however investigation of more strains is needed to ascertain if certain biotypes can be recognized based on ISR signature nucleic acid positions. The association between other variable regions and well-recognized genotypic and phenotypic characters is poorly understood. For example, there were deletions within similar regions of the reference strains NCTC 11353 and NCTC 11392, representing both *C. coli* and *C. jejuni*, but not in the other predominant wild-type clinical and chicken isolates.

These deletions might represent recent genomic rearrangements related to frequent laboratory cultivation as reported by On (1997) and Wassenaar *et al.* (1998).

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