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Genetic diversity of the *Campylobacter* genes coding immunodominant proteins

Dariusz P. Pawelec [a], Dorota Korsak [b], Agnieszka K. Wyszyńska [a], Elżbieta Rożynek [c], Janusz Popowski [b], Elżbieta K. Jagusztyn-Krynicka [a,*]

[a] *Institute of Microbiology, Warsaw University, Nowy Œwiat 67, 00-046 Warsaw, Poland*
[b] *National Food and Nutrition Institute, Powsińska 61/63, 02-903 Warsaw, Poland*
[c] *Child Health Center, Al. Dzieci Polskich 20, 04-736 Warsaw, Poland*

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Abstract

Three *Campylobacter jejuni* 72Dz/92 genes (*cjaA* (*ompH1*), *cjaC* (*hisJ*) and *cjaD* (*omp18*)) encoding immunodominant proteins are considered to be potential chicken vaccine candidates. The presence and conservation of *cjaA*, *cjaC* and *cjaD* genes among different *Campylobacter* clinical isolates were determined. The genes were detected in thirty *Campylobacter* strains using hybridization as well as Western blot analysis. However, PCR products of the predicted size were amplified only from ten out of thirty examined strains regardless of the employed primer pair. The nucleotide sequence of the *C. jejuni* 72Dz/92 genes was compared with the nucleotide sequences of their homologs cloned from other *Campylobacter* strains as well as with the whole genome sequence of *C. jejuni* NCTC 11168. The examined sequences revealed 0 to 16% divergence. Strain-dependent levels of divergence were observed. The polymorphism detected in *cjaC* was mainly within the 5′ region of the gene, while the nucleotide substitutions in *cjaA* and *cjaD* are distributed uniformly along the whole genes. Most of the observed nucleotide substitutions occurred at the third base of the codons. This observation is consistent with the results of Western blot experiments.

Keywords: Immunodominant protein; Genetic diversity; Conservation of the gene sequences; MLST; *Campylobacter* spp.

1. Introduction

Campylobacter strains are recognized as a major agent of bacterial diarrhea in both developing and developed regions of the world. Additionally, *Campylobacter jejuni* as well as other members of the *Campylobacter* genus are increasingly identified as causes of bacteremia and a variety of systemic and localized infections in immunocompromised hosts [1]. Moreover, an association between *Campylobacter* infection and the acute inflammatory polyneuropathy, known as Guillain-Barre syndrome has been established [1].

The differentiation between two related species *C. jejuni* and *Campylobacter coli* is considered to be of particular concern as they are both major human enteropathogenic *Campylobacters*. The enteritis caused by *C. coli* requires a different medical treatment than disease due to *C. jejuni*. It has been proved that erythromycin-based therapy is not effective when *enteritis* is caused by *C. coli* infection. The most frequently used method to discriminate between the two *Campylobacter* species is based mainly on the hippurate hydrolysis test. The distinction, however, is estimated to be accurate in only 90% of all cases [2]. Several recently developed methods for identification of *Campylobacter* species relay on molecular genetic approaches. Genes for 16S rRNA, 23S rRNA, and the CeuE protein have been evaluated as target sequences for PCR assays to discriminate between two thermophilic *Campylobacter* species [3–5].

Moreover, the possibility to distinguish between individual *Campylobacter* strains is considered to be fundamental for epidemiological as well as vaccine studies. The various *Campylobacter*-biotyping methods at the subspecies level have demonstrated an enormous diversity within *Campylobacter* spp. [6]. There are two major serotyping schemes for *Campylobacter* spp. [6]. The first one, based on differ-

* Corresponding author. Tel./Fax: +48 (22) 826 89 82; E-mail: ejk@plearn.edu.pl

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ences in the bacterial lipopolysaccharide antigens (Penner HS scheme) includes more than 60 serogroups. The second one is the heat-labile antigen scheme devised by Lior (HL scheme) which recognizes more than 100 serogroups. Originally the later antigen was thought to be the flagellin, but recently presented genetic evidence has indicated that the flagella are not Lior serodeterminants [7]. Molecular techniques such as PFGE, RFLP or AFLP have been used as alternatives to phenotyping methods. All of them supported, as evidenced previously by phenotyping, diversity among the two major *Campylobacter* species implicated in human disease [8,9].

Polymorphism of the *Campylobacter* induced symptoms of illness, the lack of univocal correlation between molecular traits of the strains and clinical symptoms caused and the identification of the vast amount of *Campylobacter* serotypes provoked us to conduct the studies presented here. We undertook to determine the presence and conservation of the *Campylobacter* genes encoding immunodominant proteins among different clinical isolates of *Campylobacter*. The genes are potential anti-*Campylobacter* vaccine candidates.

A number of investigators have used different kinds of antisera, mainly raised against whole cells, glycine-extracted proteins or OMPs, to identify *C. jejuni* proteins involved in pathogenesis [10–16]. Immunological screening of the *C. jejuni* 72Dz/92 genomic libraries performed in our laboratory revealed several reactive clones. Three genes (*cjaA* (accession number Y10872), *cjaC* (accession number Y10873) and *cjaD* (accession number AJ132802)) encoding proteins which react with rabbit antiserum raised against whole formalin-inactivated bacterial cells were cloned, sequenced and characterized [10,11]. The CjaA and CjaC proteins exhibit relevant homology to several prokaryotic solute-binding protein (family 3) components of the ABC transport system, whereas the *cjaD* gene product, an 18 kDa protein, is similar to peptidoglycan-associated lipoproteins from other Gram-negative bacteria (Pawelec, D. and Jagusztyn-Krynicka, E.K., unpublished).

2. Materials and methods

2.1. Bacterial strains: isolation and growth conditions

The *C. jejuni* strain 81-176 isolated from an outbreak of *Campylobacter* diarrhea and widely used in pathogenesis studies was a gift of M. Blaser. *C. jejuni* 129108, a gift of B.A.M. van der Zeijst was originally obtained from a patient with recurrent *C. jejuni* infection. *C. jejuni* 72Dz/92 (Lior 71) was obtained from a Warsaw Child Health Centre patient. Lior 71 is one of the most common serotypes isolated in Poland. *C. jejuni* 72Dz/92 chromosomal DNA used for genomic library preparation was the source of the tested genes. The remainder of the *Campylobacter* strains were clinical isolates. They were obtained from Warsaw Child Health Centre patients, who showed different kinds of symptoms of illness, during the last ten years. The fecal swabs from child patients were spread over a 0.65 μm cellulose nitrate filter and placed on the surface of Brucella agar plates supplemented with 7% horse blood and *Campylobacter*-selective supplement (consisting of five antimicrobial agents: vancomycin, polymyxin B, trimethoprim, amphotericin B and cephalotin). After 4 h incubation under microaerophilic conditions at 42°C, the filters were removed and the plates were incubated for another 48 h under the same conditions. Single colonies were taken for further analysis. The strains were kept frozen at −80°C. Routinely *C. jejuni* strains were grown at 37°C on Müeller-Hinton agar supplemented with 7.5% sheep blood under microaerophilic conditions using the recommended gas generating kit (Linegal Chemicals Gmbh, Warsaw, Poland). The speciation and Lior serotype identification were performed at the moment of strain isolation by employing the API test (BioMerieux) and specific absorbed antisera raised against heat-labile antigen (a gift of H. Lior), respectively.

All *Escherichia coli* strains were routinely cultured in Luria Broth (LB) or on 1.5% agar LB plates.

2.2. DNA manipulation

Plasmid DNA from *E. coli* was isolated by the rapid alkaline lysis procedure [17] and *C. jejuni* chromosomal DNA was prepared as described previously [9].

Dot-blot hybridizations of *Campylobacter* chromosomal DNA were performed under high stringency conditions. Three recombinant plasmids were used as probes to determine the presence of the *C. jejuni* 72Dz/92 *cjaA*, *cjaC* and *cjaD* genes in other strains of *Campylobacter*. The probes were: pUWM207 containing a 0.8 kb fragment of *cjaA*, pUWM200 carrying a 0.8 kb fragment of *cjaC* and pUWM228 carrying a 0.46 kb fragment of *cjaD*. All constructs comprised of pBluescript SK and the fragment of appropriate gene coding for mature protein. Plasmid DNA was labeled with DIG-11-dUTP by random priming using a kit from Boehringer Mannheim under the conditions described by the supplier.

Oligonucleotide primers, based on known sequences of *cjaA*, *cjaC* and *cjaD* genes of *C. jejuni* 72Dz/92, were synthesized by Universal DNA Inc., USA. Two pairs of primers were designed for each gene; one corresponding to the start and end of the sequences encoding the mature proteins and the second set flanking the sequence of the most conserved motifs of the studied proteins. The only exception was the reverse *cjaC* primer, which is complementary to the sequence located downstream of the translation stop codon. Oligonucleotide sequences of the employed primers are given in Table 1B. Polymerase chain reaction (PCR) was performed with bacterial chromosomal DNA extracted according to Nachampkin et al. [18].

2.3. Protein immunoblot analysis

Preparation of bacterial protein extracts, SDS–PAGE and blotting procedures with *E. coli* absorbed rabbit anti-CjaA and anti-CjaC antibodies were done by standard methodologies [16]. The antigen–antibody complexes were visualized with goat anti-rabbit immunoglobulin G-alkaline phosphatase conjugate (Promega). Prestained markers supplied by Gibco BRL were used as molecular mass standards. CjaAx6His and CjaCx6His proteins for rabbit immunization were purified by affinity chromatography (Qiagen) under non-denaturing conditions following cloning of the mature proteins coding nucleotide sequences amplified by PCR into pQE plasmids.

3. Results

3.1. Species identification

The discrimination between two *Campylobacter* species, *C. jejuni* and *C. coli* by biotyping method is not with great accuracy. Thus, in our initial series of experiments it was of interest to confirm previously done species identification (biotyping) by the method based on molecular genetic techniques (PCR). Eyers et al. described specific primer sets complementary to variable regions of *Campylobacter* 23S rRNA [4]. According to the authors PCR with their specific primers is able to discriminate between four *Campylobacter* species: *C. jejuni*, *C. coli*, *Campylobacter upsaliensis* and *Campylobacter lari*. The strains, which we used, were classified as *C. jejuni* or *C. coli*. Thus, three sets of primers were selected: TERM1-COLI, TERM3-JEJ1, TERM3-JEJ2. The two different techniques used for *Campylobacter* species identification showed some discrepancies. Only about 80% of the clinical isolates were classified as the same *Campylobacter* species. Table 1A summarizes the data concerning species identification and the examined gene conservation (see further in the text). It should be noted that *C. jejuni* 72Dz/92 strain which was originally used to prepare genomic DNA libraries was classified as *C. coli* by the 23S rRNA assay. However, its genomic DNA contains the gene encoding the HipO enzyme catalyzing the reaction of hippurate hydrolysis [10].

3.2. Presence and conservation of the cjaA, cjaC and cjaD genes

Three kind of experiments were carried out in order to investigate the presence and conservation of the *C. jejuni* 72Dz/92 *cjaA*, *cjaC* and *cjaD* genes among different *Campylobacter* clinical isolates. Initial analysis was performed by dot-blot hybridization. Under high stringency conditions the probes hybridized to the genomic DNA of all strains examined (Table 1A). For every probe the most intensive reaction was observed for *C. jejuni* 129108. This strain was the only one that showed positive reaction when cloning vector was used as a probe (data not shown).

In order to determine the gene conservation genomic DNA from 30 *Campylobacter* strains was subjected to PCR amplification with several primer pairs specific to the genes tested. PCR products of the predicted sizes were amplified only from 10 out of 30 *Campylobacter* strains tested. The rest of them failed to generate a detectable PCR product. For every examined *Campylobacter* strain the result of the PCR reaction was the same regardless of the employed pair of primers. All strains, which were positive with one pair of primers, were also positive if the other five pairs of primers were used for PCR assay. It is worthwhile to point out that all but one *Campylobacter* strains which were examined by PCR and found positive were classified as *C. coli* by PCR test based on the 23S rRNA sequence. On the other hand when biotyping Lior method was employed three PCR-positive *Campylobacter* isolates were classified as *C. jejuni* and seven as *C. coli* (Table 1A). The majority (28) strains used in this study were clinical isolates from human infant taken at the central part of Poland. The remaining two originated from USA (Minnesota) and from The Netherlands. Both of them were found among the PCR-negative strains. Moreover *C. jejuni* strain 129108 appeared to be classified as *C. coli* using 23S rRNA sequence-based assay.

We wondered whether PCR-negative *Campylobacter* strains express the examined gene products. To determine this Western blot experiments with specific anti-CjaA and anti-CjaC serum were performed. The cell lysates prepared from all *Campylobacter* clinical isolates revealed the positive reactions with both specific antisera. All isolates showed identical banding pattern regardless of the used antiserum (Fig. 1). CjaA and CjaC proteins which display very similar, generated in silica, three-dimensional structure are immunologically related (Jagusztyn-Krynicka, E.K. and Pawelec, D., unpublished results). Further studies employing *cjaA* or *cjaC* isogenic mutants will allow us to identify band corresponding to each analyzed protein. Nevertheless, the obtained data convinced us that both proteins were present in every examined cell lysate.

4. Discussion

Campylobacteriosis is a food-borne disease, especially in developed countries. Epidemiological investigations indicate that eating incorrectly cooked poultry is responsible for more than 50% of all campylobacteriosis cases [19]. Studies involving combinations of sero- and biotyping methods demonstrated that the sero- and biotypes isolated from live and processed chicken generally correspond to those responsible for human infection [6]. CjaA, CjaC and CjaD proteins, which are highly immunogenic and prob-

Table 1A
Presence and conservation of the *C. jejuni* genes *cjaA*, *cjaC* and *cjaD* among 30 *Campylobacter* strains of different species and serotypes

Strain	Species identification		*cjaA*				*cjaC*				*cjaD*		
	API test	23S rRNA	PCR, pair of primers		Dot-blot	Western	PCR, pair of primers		Dot-blot	Western	PCR, pair of primers		Dot-blot
			1+2	1+3			1+2	1+3			1+4	2+3	
72Dz/92 Lior 71	jejuni	coli	+	+	+	+	+	+	+	+	+	+	+
81-176 Lior 5	jejuni	jejuni	−	−	+	+	−	−	+	+	−	−	+
129108a	jejuni	coli	−	−	+	+	−	−	+	+	−	−	+
11Dz/94 Lior 71	jejuni	jejuni	−	−	+	+	−	−	+	+	−	−	+
4Dz/96 Lior 28	coli	coli	+	+	+	+	+	+	+	+	+	+	+
48Dz/92[a]	coli	coli	+	+	+	+	+	+	+	+	+	+	+
498/91 Lior 7	jejuni	jejuni	−	−	+	+	−	−	+	+	−	−	+
52N/90 Lior 71	jejuni	jejuni	−	−	+	+	−	−	+	+	−	−	+
313/92 Lior 71	jejuni	jejuni	−	−	+	+	−	−	+	+	−	−	+
357/92 Lior 4	jejuni	jejuni	−	−	+	+	−	−	+	+	−	−	+
103/94 Lior 44	coli	jejuni/coli	+	+	+	+	+	+	+	+	+	+	+
256/93[a]	jejuni	jejuni	−	−	+	+	−	−	+	+	−	−	+
632/91 Lior 72	coli	jejuni	−	−	+	+	−	−	+	+	−	−	+
138/94 Lior 71	jejuni	jejuni	−	−	+	+	−	−	+	+	−	−	+
202/95[a]	jejuni	jejuni	−	−	+	+	−	−	+	+	−	−	+
68/94 Lior 72	coli	coli	+	+	+	+	+	+	+	+	+	+	+
45Dz/92[a]	jejuni	jejuni	−	−	+	+	−	−	+	+	−	−	+
322/90 Lior 2	jejuni	jejuni	−	−	+	+	−	−	+	+	−	−	+
332/93 Lior 28	coli	jejuni	−	−	+	+	−	−	+	+	−	−	+
79/93[a]	coli	coli	+	+	+	+	+	+	+	+	+	+	+
569Ba Lior 28	coli	coli	+	+	+	+	+	+	+	+	+	+	+
173/90 Lior 2	jejuni	jejuni	−	−	+	+	−	−	+	+	−	−	+
72Dz/92 Lior 4	jejuni	coli	+	+	+	+	+	+	+	+	+	+	+
4Dz/88[a]	jejuni	coli	+	+	+	+	+	+	+	+	+	+	+
302/91[a]	jejuni	jejuni	−	−	+	+	−	−	+	+	−	−	+
38Dz/89 Lior 72	coli	jejuni	−	−	+	+	−	−	+	+	−	−	+
404/96[a]	jejuni	jejuni	−	−	+	+	−	−	+	+	−	−	+
229/93 Lior 72	coli	coli	+	+	+	+	+	+	+	+	+	+	+
199/89[a]	jejuni	jejuni/coli	−	−	+	+	−	−	+	+	−	−	+

[a]Not determined.

ably surface-exposed, seem likely to be promising chicken vaccine candidates.

The species *C. jejuni* shows considerable variation in molecular typing and biotyping techniques. The most variable, so far studied, *C. jejuni* genes are *flaA* and *flaB* coding for essential virulence factor, flagellin. The *flaA* sequences from 18 strains of *C. jejuni* have been recently examined for their mosaic structure. In this case the sequence divergence ranged from 0.3% to 24%. The majority of the changes occurred within the middle part of the gene [20]. It has been proven that intragenomic as well as intergenomic recombination is responsible for the observed *flaA* gene diversity [20,21]. Hybridization and Western blot experiments presented in this paper proved the existence of *cjaA*, *cjaC* and *cjaD* genes within the genomes of all *Campylobacter* strains examined. However, the results of PCR assays suggested variability of the gene sequences. Also the sequence encoding 23S rRNA seems to be variable. The genetic diversity was studied in detail for *Helicobacter pylori*, a closely related species, which is considered to be genetically more diverse than most bacterial species [22]. To understand better *Campylobacter* spp. gene variability we conducted MLST (multilocus sequence typing) small-scale analysis taking advantage of the databases. The homologs of the presented above genes have been recently described: *cjaC* (*hisJ*) by Garvis et al. [13];

Table 1B
Primers used for *cjaA*, *cjaC* and *cjaD* gene amplifications

cjaA			*cjaC*			*cjaD*		
Primer	Priming site	Orientation	Primer	Priming site	Orientation	Primer	Priming site	Orientation
1	67–86	forward	1	24–41	forward	1	58–76	forward
2	836–814	reverse	2	863–846	reverse	2	495–475	reverse
3	410–395	reverse	3	311–292	reverse	3	253–269	forward
						4	405–386	reverse

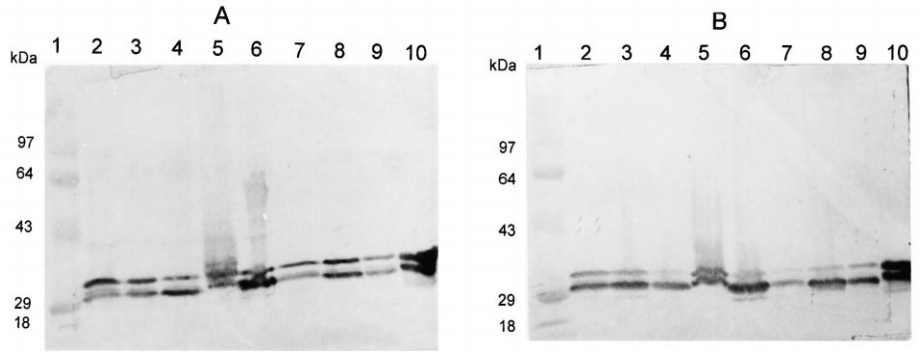

Fig. 1. Immunoblot analysis of various *Campylobacter* spp. clinical isolates with rabbit antisera against recombinant CjaA-6xHis (A) and CjaC-6xHis (B). *Campylobacter* protein extracts were resolved by SDS–PAGE, transferred to nitrocellulose and reacted with specific antisera. Lanes: 1, molecular mass standards; 2, *C. coli* 632/91; 3, *C. jejuni* 173/90; 4, *C. jejuni* 45Dz/92; 5, *C. coli* 68/94; 6, *C. jejuni* 13/92; 7, *C. jejuni* 322/90; 8, *C. jejuni* 165/933; 9, *C. jejuni* 81-176; 10, *C. jejuni* 72Dz/92. The molecular mass standards are given on the left (in kilodaltons).

cjaD, named *omp18*, by Burnens et al. [16] and Konkel et al. [12]; *cjaA*, termed *ompH1*, by Meisnersmann et al. [15]. CjaC (HisJ) is required for histidine transport as was demonstrated by complementation experiment [13]. The functions of two other studied genes were only deduced from translation of their nucleotide sequences into amino acid sequences and searching databases. It was demonstrated that the *C. jejuni* 81-176 Peb1 protein, which displays much the same homology as CjaA to the *Bacillus subtilis glnH* gene product (responsible for glutamine transport) is involved in adhesion and invasion of bacteria to HeLa cells [23]. The presence of the *peb1* gene, unlike *cjaA*, seems to be species-restricted [14]. Preliminary experiments conducted in our laboratory did not confirm the role of the CjaA in glutamine transport; thus the function of the gene product still remains undefined. Martin and Mulks have recently cloned and characterized the *Actinobacillus pleuropneumoniae apaA* gene which product displays high degree of homology to CjaA and is probably involved in the pathogenesis [24]. All three analyzed proteins are most probably outer membrane-located, thus might be the target for the host immune system. Burnens et al. showed that the Omp18 protein is really immunogenic during human infection [16]. Results of immunoblot experiments conducted in our laboratory also confirmed this observation.

The nucleotide sequences of the analyzed genes were compared with the nucleotide sequences of their homologs cloned from other *C. jejuni* strains (M275, A74/0 and ATCC 29428). Corresponding sequences from *C. jejuni* NTCC 11168 whose entire genome has been sequenced were also included in the analysis. Pairwise comparison showed the sequence divergence range from 0% to a maximum 16%. Strain-dependent level of divergence was observed. *CjaD* (*omp18*) and *cjaC* (*hisJ*) genes have been cloned and sequenced from *C. jejuni* M275 strain (human isolate). Both genes revealed 100% sequence identity to those ones obtained from *C. jejuni* 72Dz/92. On the other hand, the comparison of the *C. jejuni* 72Dz/92 *cjaC* gene sequence to its homolog from *C. jejuni* NTCC 11168 (*cj0734c*) showed 90% of identity. The polymorphism detected in *cjaC* was mostly within 5′ region of the gene. There are 63 polymorphic sites within this sequence, with the majority of the substitutions (36) occurring at the gene fragment coding for the N-terminal of the protein (codons 1–40). Two gaps were also identified within this region of the *cjaC*.

Among four complete *cjaD* sequences available two variants were identified which showed 83% identity. *cjaD* gene sequence from *C. jejuni* M275 is identical to that obtained from *C. jejuni* 72Dz/92. The comparison of the *C. jejuni* 72Dz/92 *cjaD* sequence to its homolog from *C. jejuni* NTCC 11168 (*cj0113*) revealed 83% identity. The Omp18 coding gene was also sequenced from *C. jejuni* ATCC 29428 strain (human infant isolate). Its nucleotide sequence is identical to that of *C. jejuni* NTCC 11168. Burnens et al. also studied the conservation of the Omp18 coding gene among 41 clinical isolates from different sources [16]. The primers were designed by them based on nucleotide sequence of the gene from *C. jejuni* ATCC 29428. In contradistinction to our observation they were able to amplify the PCR product of expected size from every tested strain. The limited data available to date on population genetics of this organism render the interpretations of the incompatible results difficult. One can suggest that the strains used by Burneus et al. were closely related.

As far, there are three *cjaA* (*ompH1*) nucleotide sequences available. Similarly, as for *cjaD* gene, in comparing *cjaA* gene sequences coming from different sources several divergences were noticed. Sequence from *C. jejuni* 72Dz/92 differs to those of *C. jejuni* A74/0 (chicken isolate) and *C. jejuni* NTCC 11168 (*cj0982*) by several positions (about 16% of divergence) while the other two are almost identical (0.4% of divergence). The observed nucleotide substitutions are distributed rather uniformly along the gene sequence. The same was noticed when different *cjaD* alleles were analyzed.

Analysis of the corresponding sequences derived from four *C. jejuni* strains originated from different geographi-

cal regions manifested the high level of genetic variability what is consistent with the results of PCR experiments performed by us. Among 30 Polish originated strains 10 appeared to be clonally related, the sequence of their variable regions of 23S rRNA seems to be also conserved.

The observed genotypic variation does not result in phenotypic changes. All hitherto examined strains expressed CjaA and CjaC proteins, which react with specific antibodies. Most of the observed nucleotide substitutions which occur at the third base of the codon do not influence the function of the protein. Similar gene variability seen in PCR analysis (microdiversity) was reported for many *H. pylori* genes such as urease (*ureA*, *ureB*, *ureC*) or flagellin (*flaA*, *flaB*) genes [22]. The observation has been recently extended by Achtman et al. who compared sequences of fragments from seven house-keeping and two virulence-associated genes and demonstrated the high level of synonymous sequence variation among 20 *H. pylori* strains from different geographical regions [25]. Such variation may arise by at least two mechanisms: either by accumulation of spontaneous point mutations or by horizontal gene transfer. As far, there is no information concerning *C. jejuni* mutator genes. One can speculate that as among other bacterial species there are in the population mutator strains bearing some defect mainly in a DNA repair enzyme/s.

In summary, this work presented evidence that *C. jejuni cjaA*, *cjaC* and *cjaD* genes coding immunopositive proteins undergo genetic diversity. MLST technique seems to be useful to study clonal relationship among *Campylobacter* isolates from different parts of the world. Sequence variability shows very high level of silent mutations, which do not influence the immunological properties of the proteins. The last information is essential if the genes would be used for vaccine construction.

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References

[1] Scott, D., Baqar, S., Pazzaglia, G., Guerry, P. and Burr, D.H. (1997) Vaccines against *Campylobacter jejuni*. In: New Generation Vaccines, 2nd Edn., revised and expanded (Levin, M.M., Woodrow, G.C., Kaper, J.B. and Cobon, G.S., Eds.). Marcel Dekker, Inc., New York.

[2] Totten, P.A., Patton, C.M., Tenover, F.C., Barett, T.J., Stamm, W.E., Stegerwalt, A.G., Lin, J.Y., Holmes, K.K. and Brenner, D.J. (1987) Prelevance and characterization of hippurate-negative *Campylobacter jejuni* in King County, Washington. J. Clin. Microbiol. 27, 147–151.

[3] Van Camp, G., Flerens, H., Vandamme, P., Goossens, H., Huyghebaert, A. and De Wachter, R. (1993) Identification of enteropathogenic *Campylobacter* species by oligonucleotide probes and polymerase chain reaction based on 16S rRNA genes. Syst. Appl. Microbiol. 16, 30–36.

[4] Eyers, M., Chapelle, S., Van Camp, G., Goossens, H. and De Wachter, R. (1993) Discrimination among thermophilic *Campylobacter* species by polymerase chain reaction amplification of 23S rRNA gene fragments. J. Clin. Microbiol. 31, 3340–3343.

[5] Gonzalez, I., Grant, K.A., Richardson, P.T., Park, S.F. and Collins, M.D. (1997) Specific identification of the enteropathogens *Campylobacter jejuni* and *Campylobacter coli* by using a PCR test based on the *ceuE* gene encoding a putative virulence determinant. J. Clin. Microbiol. 35, 759–763.

[6] Patton, Ch.M. and Wachsmuth, I.K. (1992) Typing schemes: are current methods useful? In: *Campylobacter jejuni*, Current Status and Future Trends (Nachampkins, I., Blaser, M.J. and Tompkins, L.S., Eds.), pp. 110–128. ASM, Washington, DC.

[7] Alm, R.A., Guerry, P., Power, M.E., Lior, H. and Trust, T.J. (1991) Analysis of the role of flagella in the heat-labile Lior serotyping scheme of thermophilic Campylobacters by mutants allele exchange. J. Clin. Microbiol. 29, 2438–2445.

[8] Nishimura, M., Nukina, M., Yuan, J.M., Shen, B.Q., Ma, J.J., Ohta, M., Saida, T. and Uchiyama, T. (1996) PCR-based restriction fragment length polymorphism (RFLP) analysis and serotyping of *Campylobacter jejuni* isolated from diarrheic patients in China and Japan. FEMS Microbiol. Lett. 142, 133–138.

[9] Kokovic, B. and On, S.L.W. (1999) High-resolution genomic fingerprinting of *Campylobacter jejuni* and *Campylobacter coli* by analysis of amplified fragment length polymorphisms. FEMS Microbiol. Lett. 173, 77–84.

[10] Pawelec, D., Rożynek, E., Popowski, J. and Jagusztyn-Krynicka, E.K. (1997) Cloning and characterization of a *Campylobacter jejuni* 72Dz/92 gene encoding a 30 kDa protein, component of ABC transport system; expression of the gene in avirulent *Salmonella typhimurium*. FEMS Immun. Med. Microbiol. 19, 137–150.

[11] Pawelec, D., Jakubowska-Mróz, J. and Jagusztyn-Krynicka, E.K. (1998) *Campylobacter jejuni* 72Dz/92 *cjaC* gene coding 28 kDa immunopositive protein, a homologue of the solute-binding components of the ABC transport system. Lett. Appl. Microbiol. 26, 69–76.

[12] Konkel, M.E., Mead, D.J. and Cieplak Jr., W. (1996) Cloning, sequencing and expression of a gene from *Campylobacter jejuni* encoding a protein (Omp18) with similarity to peptidoglycan-associated lipoproteins. Infect. Immun. 64, 1850–1853.

[13] Garvis, S.G., Puzon, G.J. and Konkel, M.E. (1996) Molecular characterization of a *Campylobacter jejuni* 29-kilodalton periplasmic binding protein. Infect. Immun. 64, 3537–3543.

[14] Pei, Z. and Blaser, M. (1993) PEB1, the major cell-binding factor of *Campylobacter jejuni*, is a homolog of the binding component in Gram-negative nutrient transport system. J. Biol. Chem. 286, 18717–18725.

[15] Meinersmann, R.J., Hiett, K.L. and Tarplay, A. (1997) Cloning of an outer membrane protein gene from *Campylobacter jejuni*. Curr. Microbiol. 34, 360–366.

[16] Burnens, A., Sticki, U., Nicolet, J. and Frey, J. (1995) Identification and characterization of an immunogenic outer membrane protein of *Campylobacter jejuni*. J. Clin. Microbiol. 33, 2826–2832.

[17] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

[18] Nachampkin, I., Bohachick, K. and Patton, C. (1993) Flagellin genotyping of Campylobacter jejuni by restriction fragments length polymorphism RFLP analysis. J. Clin. Microbiol. 31, 1531–1536.

[19] Stern, N.J. (1992) Reservoirs for *Campylobacter jejuni* and approaches for intervention in poultry. In: *Campylobacter jejuni*, Current Status and Future Trends (Nachampkins, I., Blaser, M.J. and Tompkins, L.S., Eds.), pp. 49–57. ASM, Washington, DC.

[20] Harrington, C.S., Thomas-Carter, F.M. and Carter, P.E. (1997) Evidence for recombination in the flagellin locus of *Campylobacter jeju-*

ni: implication for the flagellin gene typing scheme. J. Clin. Microbiol. 35, 2386–2392.

[21] Wassernaar, T.M., Fry, B.N. and van der Zeijst, B.A.M. (1995) Variation of the flagellin gene locus of *Campylobacter jejuni* by recombination and the horizontal gene transfer. Microbiology 41, 95–101.

[22] Marshall, D.G., Dundon, W.G., Beesley, S.M. and Smyth, C.J. (1998) *Helicobacter pylori* - a conundum of genetic diversity. Microbiology 144, 2925–2939.

[23] Pei, Z., Burucoa, Ch., Girgnon, B., Baquar, S., Huang, X., Kopecko, D.J., Bourgeois, A.L., Fauchere, J.L. and Blaser, M.J. (1998) Mutation in the *peb1* locus of *Campylobacter jejuni* reduces interactions with epithelial cells and intestinal colonization of mice. Infect. Immun. 66, 938–943.

[24] Martin, P.R. and Mulks, M.H. (1999) Cloning and characterization of a gene encoding an antigenic membrane protein from *Actinobacillus pleuropneumoniae* with homology to ABC transporters. FEMS Immunol. Med. Microbiol. 15, 245–254.

[25] Achtman, M., Azuma, T., Berg, D.E., Ito, Y., Morelli, G., Pan, Z.J., Suerbam, S., Thompson, S.A., van der Ende, A. and van Doorn, L.J. (1999) Recombination and clonal grouping within *Helicobacter pylori* from different geographical regions. Mol. Microbiol. 32, 459–470.