ACTA VET. BRNO 2011, 80: 019–027; doi:10.2754/avb201180010019

Molecular characterization of *Campylobacter* spp. isolated from poultry faeces and carcasses in Poland

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Received January 20, 2010
Accepted September 21, 2010

Abstract

Campylobacter infection is one of the most common enteric human diseases world-wide but the mechanism of *Campylobacter* pathogenicity has not been exactly explained yet. One of the main reasons is genotypic, hence phenotypic diversity of the bacterial isolates. The aim of the present study was to perform a molecular characterization of randomly selected *C. jejuni* and *C. coli* strains isolated from poultry faeces and carcasses in Poland. Several virulence gene markers were identified by polymerase chain reaction (PCR). Furthermore, genetic typing has also been used by the macrorestriction profiling with pulsed-field gel electrophoresis (PFGE). The results of the present study showed that all analyzed isolates of *C. jejuni* (n = 24) and *C. coli* (n = 24) contained the *flaA* and *cadF* sequences. On the other hand, the *virB11* gene was present only in 6 of 48 (12.5%) of the analyzed isolates, whereas most of the strains contained the *cdt* genes. Other virulence gene *iam* linked to *Campylobacter* invasiveness was present in 34 of 48 (72.9%) strains. The restriction analysis of the whole genome digested with *Sma*I produced three main clonal groups designed as I, II (with two subgroups IIa and IIb), and III obtained by the comparison of macrorestriction profiling patterns. The results showed a poor correlation between *Campylobacter* profiles generated by a clonal molecular technique and the presence of virulence markers. Therefore, PCR detection of *Campylobacter* virulence markers can be utilized as a simple and rapid tool to discriminate stains recovered from different sources, especially when used in conjunction with the PFGE profile analysis as a complex strategy. These kinds of analyses had not been previously carried out in Poland and these results may generate more knowledge regarding the genetic diversity and molecular relationship of *Campylobacter.*

Campylobacter, virulence markers, molecular analysis, PCR, PFGE

According to the recent European Food Safety Authority (EFSA) report, the most frequently reported zoonotic disease in humans in the European Union in 2008 was *Campylobacter* infection, with incidences of 40.7 per 100,000 people (http://www.efsa.europa.eu). The infection with *Campylobacter* spp. (campylobacteriosis), especially with *C. jejuni,* and to a lesser extent with *C. coli*, is one of the leading causes of bacterial diarrhoea world-wide. During last years, laboratory confirmed incidence of campylobacteriosis in Poland. In 2008, according to the EFSA report, there were 257 cases of the disease (0.7/100,000 population), however, it seems that the number of cases is still underestimated due to a lack of proper identification of the infectious agent. Poultry and its meat is considered to be the main vector of *C. jejuni*; transmission occurs either as a result of cross contamination due to improper handling of raw meat or consumption of undercooked food of animal (mainly poultry) origin.

The mechanism of *Campylobacter* pathogenicity has not been exactly explained yet. One of the main reasons is genotypic, hence phenotypic diversity of the bacterial species belong to the genus of *Campylobacter*. For the consumers' safety it is essential to characterize pathogenicity markers in strains that are identified in food. It was found that some *C. jejuni* strains are not pathogenic at all or induce mild symptoms in humans, whereas other isolates cause a serious illness (Rivera-Amill et al. 2001). It is still not clear which factors of *Campylobacter* are essential to the disease development. However, it is known that mechanisms of movement, chemotaxy, adhesion, transcytosis and host cell penetration as

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well as toxin production are necessary to induce campylobacteriosis in humans (Snelling et al. 2005). Several putative or defined virulence markers of *Campylobacter* spp. have been described (Bang et al. 2003; Datta et al. 2003). One of the best characterized *Campylobacter* virulence markers is the *flaA* gene which determines the flagella formation, hence bacteria motility and enterocyte colonization (Nuijten et al. 2000). Molecular identification and differentiation of *Campylobacter* strains isolated from the same or different samples is very important in order to trace the sources of human infection. The methods used for genetic characterization differ in their taxonomic range, discriminatory power, reproducibility, easiness of interpretation, and standardization. Macrorestriction profiling (MRP) by pulsed-field gel electrophoresis (PFGE) has been proved to be useful for this purpose, and its discriminatory power can be enhanced by increasing the number of restriction enzymes used (On et al. 1998). This method is currently a golden standard for the typing of *Campylobacter* spp. (Schouls et al. 2003).

The aim of the present study was to perform a molecular characterization of randomly selected *C. jejuni* and *C. coli* strains isolated from poultry faeces and carcasses in Poland. For this purpose, 7 virulence genes important in pathogenesis of campylobacteriosis were chosen and identified by the PCR method. These molecular markers participate in adhesion and colonization (*flaA, cadF*), invasion (*virB11*) and toxin production (*cdtA*, *cdtB*, *cdtC*). Moreover, the *iam* sequence connected with diarrhoeal form of the disease was also identified. Furthermore, molecular characterization of the investigated *Campylobacter* strains were further performed by macrorestriction profiling with PFGE.

Materials and Methods

Bacterial strains

The following positive and negative reference strains were included: *C. jejuni* ATCC 33291, *C. coli* ATCC 43478, *Escherichia coli* EDL 933, and *Salmonella* Typhimurium ATCC 14028.

All *Campylobacter* isolates were isolated from randomly chosen poultry faeces and carcasses in Poland during the period of September 2004 and July 2005. The samples were obtained from different regions of the country (administrative division – voivodship), including the following areas: northern (Pomorskie – P; Warminsko-Mazurskie – WM, Zachodniopomorskie – ZP voivodships), western (Dolnoslaskie – D, Lubuskie – L voivodships), southern (Opolskie – O, Slaskie – S voivodships), and eastern (Lubelskie – LU, Ludzkie – LD, Mazowieckie – M voivodships). All faecal samples were taken using swab method at farm level. From each farm one pooled sample (taken from at least ten fresh droppings) were examined. The carcass samples were collected at slaughterhouse at the end of processing level, from final products stored chilled (< 4 °C). For isolation of thermophilic *Campylobacter* spp., swabs inoculated with faeces or carcass samples were plated onto *Campylobacter* blood-free selective medium such as mCCDA (Oxoid, UK) or Karmali Agar (Oxoid, UK) followed by incubation at 41.5 °C for 40-48 h in microaerofilic conditions generated by the Campy Gen gas-generating kit (Oxoid, UK). Bacteria from individual colonies were stored (at -80 °C in nutrient broth, with glycerol added to 15% for genotypic analyses. Suspected bacterial colonies were tested by multiplex PCR (m-PCR) for the simultaneous detection of the *C. jejuni* and *C. coli* in a single reaction based on *16S rRNA* (specific for theromphilic *Campylobacter*), *ceuE* (specific for *C. coli*), and *mapA* genes (typical for *C. jejuni*), respectively (Wieczorek and Osek 2005). Total of 48 isolates (24 from faeces and 24 from poultry carcasses) were used in this study.

Detection of putative virulence genes by PCR

Campylobacter strains were grown at 41.5 ºC in Karmali agar for 24 h under microaerophilic condition. A bacterial colony was suspended in 1 ml of sterile water and centrifuged at 13 000 *g* for 1 min. Afterwards, DNA was extracted using the Genomic – Mini kit (A&A Biotechnology, Poland) according to the manufacturer's instruction. The purity and concentration of the DNA preparations were estimated using spectrophotometry at 260 and 280 nm.

Characteristics of all primers used in the study are shown in Table 1. The PCR primers were commercially synthesised (Symbiosis, Poland).

All PCRs were carried out in a thermal cycler (PTC-100, MJ Research, USA) under the following conditions: initial DNA denaturation at 94 ºC for 5 min followed by 30 cycles of 94 ºC for 1 min, 55 ºC for 1 min (with the exception for the *flaA* gene – 48 ºC for 1 min) and 72 ºC for 1 min. The final extension step was run at 72 ºC for 5 min. The analysis of the amplified products was performed in 2% agarose (Sigma, USA) in Tris-Acetate–EDTA (TAE) buffer at 100 V. The DNA bands were visualised by staining with ethidium bromide, analysed under UV light (300 nm) and photographed using the Gel Doc 2000 documentation system (Bio-Rad, USA). The size of the PCR amplicons was compared to the 100 bp DNA marker (Fermentas, EU).

Table 1. Characteristics of PCR primers used in the study

Primer name	Sequence (5' → 3')	Target gene	Size of PCR amplicon (bp)	Annealing temperature (°C)	Reference
CDTAF	CCTTGTGATGCAAGCAATC	*cdtA*	370	55 °C	Bang et al. 2003
CDTAR	ACACTCCATTTGCTTTCTG				
CDTBF	CAGAAAGCAAATGGAGTGTT	*cdtB*	620	55 °C	Datta et al. 2003
CDTBR	AGCTAAAAGCGGTGGAGTAT				
CDTCF	CGATGAGTTAAAACAAAAAGATA	*cdtC*	182	55 °C	Datta et al. 2003
CDTCR	TTGGCATTATAGAAAATACAGTT				
F2B	TGGAGGGTAATTTAGATATG	*cadF*	400	45 °C	Konkel et al. 1999
R1B	CTAATACCTAAAGTTGAAAC				
IAMF	GCGCAAAATATTATCACCC	*iam*	518	55 °C	Korsak et al. 2005
IAMR	TTCACGACTACTATGCGG				
VirBF	GAACAGGAAGTGGAAAAACTAGC	*virB11*	708	55 °C	Bang et al. 2003
VirBR	TTCCGCATTGGGCTATATG				
flaAF	GGATTTCGTATTAACACAAATGGTGC	*flaA*	1700	48 °C	Datta et al. 2003
flaAR	CTGTAGTAATCTTAAACATTTTG				

Typing of *Campylobacter* strains by RFLP-PFGE

The DNA macrorestriction analysis was carried out using the One-Day (24-26 h) Standardized Laboratory Protocol for Molecular Subtyping of *C. jejuni* by pulsed field gel electrophoresis (PFGE) (Ribot et al. 2001). The plugs were digested with the restriction enzyme *Sma*I (Fermentas, EU) and the PFGE was run under the following conditions: pulse ramps from 5 to 40 s for 22 h at 4 °C at constant voltage of 6 V per 1 cm. Dendrograms were constructed on the basis of the obtained DNA fragment patterns using the GelCompar II software (BioNumerics, Belgium) by the Unweighted Pair Group with Mathematic Average Method (UPGMA). Similarities between the profiles based on band position were derived by using the Dice's coefficient. The discriminatory power of the typing methods was calculated by using Simpson's index (D). All the isolates within a similarity between 90% and 100% were subject to belong to the same cluster (Hunter and Gaston 1988).

Results

Identification of virulence-associated genes

The results of the present study have shown that all analyzed isolates of *C. jejuni* and *C. coli* derived both from poultry faeces and carcasses possessed the *flaA* sequence. Another gene responsible for the colonization properties of *Campylobacter* spp. – *cadF*, was identified in 100% *C. jejuni* and *C. coli* isolates. On the other hand, the next virulence marker determining invasiveness of *Campylobacter* isolates - the *virB11* gene, localized on pVir plasmid, was present only in 6 of 48 (12.5%) of the analyzed bacterial strains (Table 2).

Further PCR identification of *Campylobacter* virulence markers revealed that most of the strains tested possessed the *cdt* genes that are necessary for CDT toxin synthesis (Table 2). The majority of the isolates had the *cdtA* and *cdtC* markers (70.8% strains of each gene variant). It should be noted that 17 out of 24 *C. jejuni* isolates (70.8%) possessed all three toxin subunit genes. On the other hand, among *C. coli* only 5 out of 24 strains (20.8%) had all *cdt* toxin genes together. Altogether, many more *C. jejuni* (from 87.5 to 95.8%) than *C. coli* (45.8–70.8%) isolates were identified to possess the *cdt* genes (Table 2).

Identification of other virulence gene linked to *Campylobacter* invasiveness – *iam* has shown that this marker was present in 34 of 48 (72.9%) of the investigated strains. It was found that this gene was much more predominant in *C. coli* (22 positive isolates, 91.7%) than *C. jejuni* (13 positive isolates, 54.2%).

It was also shown that 34 out of 48 *Campylobacter* strains tested (70.8%) possessed five or more of the 7 virulence-associated genes (Table 2).

Table 2. Distribution of putative virulence PCR markers of *Campylobacter* strains according to the source and date of isolation

Strain No.	Species	Date of isolation	Place of isolation[1]	Virulence markers							Clonal group /clonal type
				cdtA	*cdtB*	*cdtC*	*cadF*	*virB11*	*iam*	*flaA*	
3C	*C. jejuni*	09. 2004	LU	-	+	+	+	-	+	+	I/5
6C	*C. jejuni*	09. 2004	LU	+	+	+	+	-	+	+	III/27
21C	*C. jejuni*	09. 2004	LU	+	+	+	+	-	-	+	I/1
7C	*C. jejuni*	04. 2005	LU	+	+	+	+	-	-	+	I/1
11C	*C. jejuni*	04. 2005	LU	+	+	+	+	-	-	+	I/1
18C	*C. jejuni*	04. 2005	LU	+	+	+	+	-	-	+	I/1
20C	*C. jejuni*	04. 2005	LU	+	+	-	+	+	-	+	I/1
25C	*C. jejuni*	04. 2005	LU	+	+	+	+	+	-	+	I/2
35C	*C. jejuni*	04. 2005	LU	+	+	-	+	-	-	+	I/2
40C	*C. jejuni*	06. 2005	LU	+	+	+	+	-	+	+	I/3
41C	*C. jejuni*	06. 2005	LU	+	+	+	+	-	-	+	I/3
47C	*C. jejuni*	06. 2005	LU	+	+	+	+	+	+	+	III/28
176F	*C. jejuni*	02. 2005	D	+	+	+	+	-	+	+	I/6
178F	*C. jejuni*	02. 2005	S	+	+	+	+	-	-	+	I/7
191F	*C. jejuni*	03. 2005	L	+	-	+	+	-	+	+	I/8
212F	*C. jejuni*	03. 2005	P	+	-	-	+	-	+	+	I/9
232F	*C. jejuni*	04. 2005	M	+	+	+	+	-	+	+	III/29
236F	*C. jejuni*	04. 2005	O	+	+	+	+	-	+	+	I/10
242F	*C. jejuni*	04. 2005	P	+	+	+	+	-	+	+	I/11
253F	*C. jejuni*	05. 2005	ZP	+	+	+	+	-	+	+	I/4
278F	*C. jejuni*	05. 2005	M	+	+	+	+	-	+	+	I/12
266F	*C. jejuni*	05. 2005	O	+	+	+	+	-	-	+	I/13
301F	*C. jejuni*	06. 2005	WM	+	+	+	+	-	-	+	III/30
318F	*C. jejuni*	07. 2005	O	+	-	+	+	-	+	+	I/4
2C	*C. coli*	09. 2004	LU	+	-	-	+	-	+	+	II/18
10C	*C. coli*	09. 2004	LU	-	+	-	+	-	+	+	II/18
15C	*C. coli*	09. 2004	LU	-	+	+	+	-	+	+	II/19
18C	*C. coli*	09. 2004	LU	-	+	+	+	-	-	+	II/19
20C	*C. coli*	04. 2005	LU	-	+	-	+	-	+	+	II/19
14C	*C. coli*	04. 2005	LU	-	-	+	+	-	+	+	II/19
17C	*C. coli*	06. 2005	LU	+	-	-	+	-	+	+	II/19
43C	*C. coli*	06. 2005	LU	+	+	+	+	-	+	+	II/17
46C	*C. coli*	06. 2005	LU	-	+	-	+	-	+	+	II/17

Table 2. Distribution of putative virulence PCR markers of *Campylobacter* strains according to the source and date of isolation

Strain No.	Species	Date of isolation	Place of isolation[1]	Virulence markers						Clonal group /clonal type
				cdtA	*cdtB*	*cdtC*	*cadF*	*virB11*	*iam*	*flaA*	
50C	*C. coli*	06. 2005	LU	-	+	+	+	+	+	+	II/17
54C	*C. coli*	06. 2005	LU	+	+	+	+	-	+	+	II/18
57C	*C. coli*	06. 2004	LU	-	+	+	+	+	+	+	II/18
168F	*C. coli*	02. 2005	M	-	-	-	+	-	+	+	II/16
176F	*C. coli*	02. 2005	D	+	+	-	+	-	+	+	II/16
179F	*C. coli*	02. 2005	LD	-	+	-	+	-	+	+	I/14
211F	*C. coli*	03. 2005	M	+	+	+	+	-	+	+	II/21
214F	*C. coli*	03. 2005	S	-	+	-	+	-	+	+	II/22
231F	*C. coli*	04. 2005	M	+	+	+	+	-	+	+	II/23
243F	*C. coli*	04. 2005	P	+	-	-	+	-	+	+	II/24
244F	*C. coli*	04. 2005	P	+	+	+	+	-	+	+	I/15
284F	*C. coli*	06. 2005	LD	+	+	+	+	-	-	+	II/20
287F	*C. coli*	06. 2005	S	+	+	+	+	-	+	+	II/20
320F	*C. coli*	07. 2005	ZP	-	-	+	+	+	+	+	II/25
325F	*C. coli*	07. 2005	S	-	-	-	+	-	+	+	II/26

[1]Place of isolation: LU – Lubelskie; D – Dolnoslaskie; S – Slaskie; L – Lubuskie; P – Pomorskie; M – Mazowieckie; O – Opolskie; WM – Warminsko-Mazurskie; LD – Ludzkie; ZP – Zachodniopomorskie voivodships

RFLP-PFGE typing

All MRPs were evaluated and assigned to arbitrarily defined profile groups (PGs). The results are summarized in Table 2 which also shows the information for each *Campylobacter* strain used in the study. The method was evaluated with respect to the discrimination index (D) and number of profiles obtained. All poultry faeces (n = 24) and carcass (n = 24) isolates were subjected to MRP using the restriction enzyme *Sma*I.

The mean differentiation index determined was 0.97. However, the PFGE D index was higher for the faeces strains (0.98) than for the carcass isolates (0.89).

In the dendrogram, pulsotypes were delineated with a 90% similarity of the cut-off level (Fig. 1). Thirty *Campylobacter* isolates displayed unique pulsotypes and six pulsotypes were shared by two or more isolates (Fig. 1). Two pulsotypes comprised 5 and another two pulsotypes contain 3 and 4 isolates, respectively. Three main clonal groups designed as I, II (with two subgroups – IIa and IIb), and III were formed by the comparison of MRP typing patterns (Fig. 1).

Clonal group I had 20 isolates belonging to *C. jejuni* whereas the 2 remaining isolates were identified as *C. coli*. This group comprised 15 unique *Sma*I pulsotypes, of which 2 clusters had 2 and 1 had 5 identical isolates.

Clonal group II consisted of 22 *C. coli* isolates. Restriction with *Sma*I split up them into two subgroups with 18 (subgroup IIa) and 4 (subgroup IIb) bacterial strains. Fourteen of the 18 isolates in the subgroup IIb were indistinguishable from each other after digestion with *Sma*I.

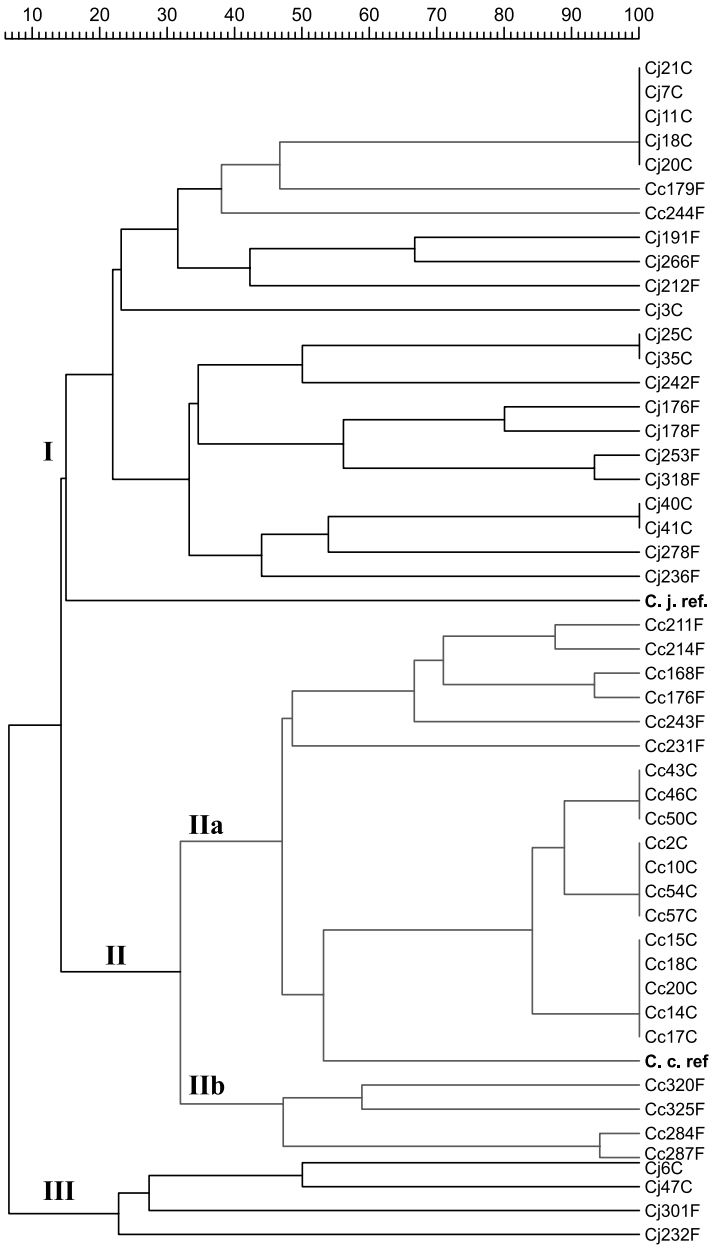

Fig. 1. Dendrogram based on RFLP-PFGE fragments patterns of 48 *C. jejuni* and *C. coli* isolates from poultry faeces and carcasses. The scale measures similarity values. Carcasse *Campylobacter* isolates marked with C letter and faeces *Campylobacter* isolates marked with F letter. Two reference *C. coli* ATCC43478 and *C. jejuni* ATCC 33291 strains are included and marked as C. c. ref. and C. j. ref., respectively.

The group III covered 4 *C. jejuni* isolates (2 carcass and 2 faeces origin) that displayed unique pulsotype (Fig. 1).

Discussion

Identification of putative virulence markers

One of the best characterized *Campylobacter* pathogenic marker is the *flaA* gene which determines flagella formation, hence bacteria motility and enterocyte colonization (Nuijten et al. 2000). Results of the present study showed that all analyzed isolates of *C. jejuni* and *C. coli* derived both from carcass and poultry faeces possessed the *flaA* sequence. Bang et al. (2003) examined the presence of this factor in *C. jejuni* and *C. coli* derived from swine and cattle (together 40 isolates), and obtained the same results, i.e. 100% positive *Campylobacter* strains. Similar results were found by Datta et al. (2003) who determined the *flaA* factor in the group of 111 *C. jejuni* derived from human clinical specimens, poultry carcass, faeces, and cattle. All these results may suggest that the *flaA* gene product is necessary for bacterial colonization of animal alimentary tract and determines the stability of bacteria on the surface of contaminated poultry carcass. However, other virulence markers, e.g. *cadF* gene product may also participate in the colonization and adherence process. The c*adF* gene is highly conservative among *C. jejuni* and *C. coli* which may also suggest its crucial role in campylobacteriosis development (Monteville et al. 2003). As detected in the present study, the c*adF* gene was presented in 100% of the examined *C. jejuni* and *C. coli* isolated both from poultry carcass and faeces. Other authors also identified this virulence marker in all or almost all isolates tested that were derived from poultry carcass and faeces and from human clinical specimens (Bang et al. 2003; Datta et al. 2003; Rozynek et al. 2005).

The next virulence factor examined in the present study was one of the markers determining the invasiveness of *Campylobacter* isolates - the *virB11* gene, localized on in pVir plasmid. The gene was present only in 6 from 48 (12.5%) analyzed bacterial isolates, equally in *C. jejuni* and *C. coli* strains (3 positive strains of each species). The low percentage of such *virB11*-positive *Campylobacter* isolates was also described by other authors (Bang et al. 2003; Datta et al. 20003; Louwen et al. 2006). However, the role of this gene marker in pathogenesis of campylobacteriosis in humans is still not clear. Tracz et al. (2005) suggested that products of the pVir plasmid genes may play a major role in the serious symptoms of the illness caused by *C. jejuni*. On the other hand, Louwen et al. (2006) claimed that this plasmid does not play any role in the induction of bloody diarrhoea in infected humans.

Toxins produced by *Campylobacter* might be another factor that potentially plays a role in the disease development. CDT toxin composed of three subunits: CdtA, CdtB, and CdtC is one of the best described toxin molecules produced by *C. coli* and *C. jejuni* isolates (Martinez et al. 2006). All the above mentioned subunits are necessary to induce a cytotoxic effect *in vitro*. In our present study, three *cdt* genes were examined. The majority of the isolates tested possessed the *cdtB* gene (79.2%). Several *Campylobacter* isolates had also the c*dtC* and *cdtA* markers (both 70.8% of isolates). Furthermore, 17 out of 24 *C. jejuni* isolates (70.8%) possessed all three toxin gene subunits. On the other hand, only 5 out of 24 *C. coli* (20.8%) isolates harbored all *cdt* gene markers. When the source of the isolates was included, a higher percentage of *C. jejuni* (87.5–95.8%) than *C. coli* (45.8–70.8 %) strains were positive for all *cdt* genes. The percentage of toxin-positive *Campylobacter* examined by other authors was from 90% to 100% (Bang et al. 2001, 2003; Datta et al. 2003; Martinez et al. 2006). However, little information concerning the prevalence of the *cdtA*, *cdtB* and *cdtC* genes had been published in Poland. Rozynek et al. (2005) identified these virulence markers in 77.5%, 93.8%, 77.5% of isolates derived from children with diarrhoea (n = 80) as well as in 94.6% and 98.9% of the isolates derived from poultry carcasses (n = 92).

The other putative virulence gene, i.e. *iam* connected with diarrhoea in patients infected with *Campylobacter*, was also detected during the present investigation. The *iam* PCR

product was found in 35 of 48 (72.9%) strains tested. The similar percentage of the positive isolates was identified by Korsak et al. (2005) who analyzed *Campylobacter* strains derived from chicken carcasses in Poland.

MRP by PFGE results

The discriminatory power of MRP obtained by the by PFGE method described in our study was rather high. Similar results have also been reported by other studies (Rivoal et al. 2005). The pulsotypes obtained after digestion with *Sma*I revealed that *C. jejuni* and *C. coli* are genetically diverse. Three main clusters comprising 30 out of the 48 *Sma*I-digested isolates were obtained. As shown in the present study, identical genotypes were commonly present in isolates from the same source and time of the isolation, e.g. 43C, 46C, and 50C that were recovered from poultry carcasses from the same region of Poland as well as on the same day. On the other hand, strains 3C and 6C were isolated from the same country region (Lubelskie voivodship) and at the same time but belong to different clonal groups - I and III, respectively. Previous studies have also shown that several *Campylobacter* genotypes can coexist among individual chickens (Schouls et al. 2003) and that individual farms can be contaminated with multiple clones (Stanley and Jones 2003; Rivoal et al. 2005).

As shown in the present study, *C. jejuni* and *C. coli* broiler isolates were fairly evenly distributed between the clonal clusters. Groups I and III comprised all *C. jejuni* isolates (plus 2 *C. coli* strains – 179F and 244F), whereas group II covered only *C. coli*. These results are in agreement with several other studies that have also demonstrated the ability of the PFGE method to discriminate between and within *Campylobacter* species (Rivoal et al. 2005). Furthermore, majority of the isolates of the same species, isolated from faeces and carcasses, were clustered together. Less diversity was found among *Campylobacter* strains derived from carcasses, which displayed eight different genotypes compared to 22 different PFGE profiles of the isolates of faecal origin (Fig. 1).

Relationship between present virulence genes and PFGE profile

The results obtained showed a poor correlation between profiles generated by a clonal molecular technique and the presence of virulence markers. However, this kind of comparative analysis sometimes allowed differentiating strains with identical patterns. For example, isolates W25 and W35 shared the same PFGE profiles but were differentiated by the presence of the different putative virulence genes because only strain W25 possessed the *cdtC* and *virB11* markers. The opposite observations were made when the strains were analyzed with respect to the presence of virulence markers. *Campylobacter* isolates with different PFGE profiles, such as 242D and 253C, were found to possess the same virulence genes. On the other hand, some strains e.g. *C. jejuni* 21C, 7C, 11C, 18C could not be distinguished even if both methods (PFGE and virulence gene determination) were used.

Our data show that most *C. jejuni* and *C. coli* strains isolated from poultry faeces and carcasses carrying genes linked to severe forms of human campylobacteriosis. Molecular profiles of *Campylobacter* spp. can contribute to microbial risk assessment by helping to assess the relative risks of cross-contamination of food of animal origin. The results also showed that MRP based on PFGE is a useful method for the determination of *Campylobacter* identification and eradication of the major reservoirs of the common bacterial clones. Furthermore, PCR detection of *Campylobacter* virulence markers can be utilized as a simple and rapid tool to discriminate stains recovered from different sources, especially when used in conjunction with the PFGE profile analysis as a complex strategy. These data, when considered with studies demonstrating that *Campylobacter* strains are significantly different in their ability to produce various toxins, may indicate that not all strains occurring in animals may be pathogenic for humans. Thus, the ability to identify

certain clones of known pathogenicity may be more relevant to public health protection than simply detecting the presence of *Campylobacter* spp.

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