Original article

Occurrence of virulence genes among *Campylobacter jejuni* and *Campylobacter coli* isolates from domestic animals and children

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Abstract

The presence of the *flaA*, *cadF*, *cdtB* and *iam* genes of *Campylobacter* spp. was determined with the PCR method. The materials to investigate were 56 *C. jejuni* and 23 *C. coli* strains isolated from clinical samples (children and domestic animals).

It was found that all of the *Campylobacter* spp. isolates from children with diarrhoea and domestic animals had *cadF* gene, responsible for adherence.

The *flaA* gene was present in all *Campylobacter* spp. isolates derived from children and cats. Occurrence of *flaA* gene was confirmed in 100% of *C. jejuni* strains obtained from dogs.

The high prevalence of the *cdtB* gene associated with toxin production was observed in this study (100%-*Campylobacter* spp. isolates obtained from dogs and cats, 97.9%-*Campylobacter* spp. isolates from children).

The isolates showed a wide variation for the presence of *iam* gene. The lowest prevalence (23.5%) was detected in *Campylobacter* spp. obtained from dogs. The highest rates of *iam* detection (91.6%) were revealed in *C. coli* isolates from children.

Key words: *Campylobacter jejuni*, *Campylobacter coli*, virulence genes, PCR, domestic animals, children

Introduction

Microorganisms of the genus *Campylobacter* predominate as a zoonotic factor worldwide. Importance of these organisms has increased during last 30 years since it was recognized as an emerging pathogen. Gram-negative *Campylobacter jejuni* and *Campylobacter coli* are now the most common bacterial cause of human gastroenteritis in industrialized countries (Bereswill et al. 2003, Moore et al. 2005).

A natural reservoir of the bacteria is the gastrointestinal tract of birds, dogs, cats, and other animals that can carry the microorganism. The bacteria are therefore common in natural environment, e.g. surface waters (Wysok et al. 2009).

Campylobacter spp. infects humans and animals. *C. jejuni* is commonly isolated from birds. Particularly chickens are considered a major source of human campylobacteriosis. Transmission to humans occurs by ingestion of contaminated food or water, including

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undercooked poultry and unpasteurized milk (Lin 2009).

Another important way of transmission is direct contact with animals (Steinhauserova et al. 2000, Moser et al. 2001). The role of *Campylobacter* spp. as an enteric pathogen among dogs and cats is much less evident. Bacteria is frequently isolated both from animals with symptoms of *enteritis* and from healthy animals (Koane et al. 2004). Dogs and cats often live in close proximity to humans, especially children, what brings probability of direct transmission of these pathogens (Hald et al. 1997, Hald et al. 2004).

Infection caused by *Campylobacter* spp. can be severely debilitating, but is rarely life-threatening. It has been linked with subsequent development of Guillain-Barré syndrome (GBS), which usually develops 2 up to 3 weeks after the initial illness (van Doorn 2009). This wide range of clinical symptoms cannot be explained as pertaining only to the host's response; characteristics of the bacterial pathogen may contribute (Rożynek et al. 2005, Al-Mahmeed et al. 2006). Adherence, invasion and cytotoxin production appear to be essential virulence factors (Wardak et al. 2006). Recently, some genes have been recognised as responsible for the expression of pathogenicity: *flaA* gene-motility, *cadF*-adhesion, *cdtB*-toxin production, *iam*-invasion (Rożynek et al. 2005, Wardak et al. 2006, Rizal et al. 2010).

The aim of this report was to estimate occurrence of pathogenic genes: *cadF*, *flaA*, *cdtB* and *iam* and examine differences in the presence of these genes between *Campylobacter* spp. isolates obtained from children and domestic animals, which are one of the most important sources of human campylobacteriosis.

Materials and Methods

Strains identification

A total of 79 *Campylobacter* spp. strains isolated from humans and from animals were used in this study. The 49 strains were isolated from the faeces of children with campylobacteriosis, hospitalized in the Provincial Observational-Infectious Hospital in Bydgoszcz (Poland). The 30 strains were isolated from rectal swabs from cats and dogs with *enetritis* obtained in veterinary clinics.

Campylobacter strains were grown at 42°C in Preston agar (Oxoid) for 48 hrs under microaerophilic condition. *Campylobacter* spp. identification was done using multiplex PCR for the simultaneous detection of the *C. jejuni* and *C. coli* as described previously (On et al. 2003). The following positive strains: *C. jejuni* ATCC 33291, *C. coli* ATCC 33559 were also included. All strains were preserved in 20% glycerol at -70°C.

DNA preparation

Extraction of DNA was done using "Chelex-100" chelating resin (Bio-Rad). A bacterial colonies were suspended in 100 μl TRIS and 45 μl 20% Chelex and boiled for 10 min. Samples were then immediately placed on ice for 1 min and centrifuged at 13.000 g for 10 min at room temperature. Supernatant (2 μl) was used in PCR reaction. The purity and concentration of the DNA was estimated using spectrophotometry at 260 and 280 nm.

Amplification of virulence genes

The presence of the *cadF*, *flaA*, *cdtB* and *iam* genes was determined with the primers listed in Table 1. PCR primers were synthesized by Oligo (Poland). All PCR amplifications were performed in a mixture (25 μl) containing: 2.5 μl of the PCR buffer (10 – times concentrated), 2.5 μl of $MgCl_2$ (25 mM), 0.5 μl of dNTPs (10 mM), 1 μl of each primer (100 μM), 0.5 μl (1U) of the Taq thermostable DNA polymerase (Fermentas), 2 μl of the bacterial template DNA and 15 μl nuclease free water. The PCR products were analyzed by electrophoresis in 1.5% agarose gel. The DNA bands were visualized by staining with ethidium bromide and photographed using the IG/L-E In-Genius L documentation system (TK Biotech). The size of the PCR amplicons was compared to the 100 bp DNA marker (Fermentas).

Results

The detection of the virulence genes in *C. jejuni* and *C. coli* isolates is shown in Table 2. The distribution of virulence genes in all *Campylobacter* spp. from different sources is summarised in Fig. 1.

All *Campylobacter* spp. isolates from children and domestic animals had *cadF* gene, responsible for adherence.

The *flaA* gene was present in all *Campylobacter* spp. isolates from children and in all isolates from cats. The results showed that all analysed *C. jejuni* strains from dogs possessed *flaA* gene. The presence of this gene was not confirmed in only one *C. coli* strain obtained from dogs (85.7%).

Another virulence gene-*cdtB* was detected in all *Campylobacter* spp. isolates from domestic animals with *enteritis* and in all *C. jejuni* strains from children with diarrhoea. *CdtB* gene was present in 11 out of 12 *C. coli* strains from children (91.6%).

The occurrence of *iam* marker differs between both species and source. *Iam*-gene linked with invasiveness of *Campylobacter* spp., was indicated in 91.6% of *C. coli* strains and in 32.4% of *C. jejuni* derived

Table 1. PCR primers used in the study.

Primers	Sequence (5' → 3')	Product (bp)	References
cadF-F *cadF*-R	TGGAGGGTAATTTAGATATG CTAATACCTAAAGTTGAAAC	400	Konkel et al. 1999
flaA-F *flaA*-R	GGATTTCGTATTAACACAAATGGTGC CTGTAGTAATCTTAAAACATTTTG	1728	Nachamkin et al. 1993
cdtB-F *cdtB*-R	GTTAAAATCCCCTGCTATCAACCA GTTGGCACTTGGAATTTGCAAGGC	495	Bacon et al. 2001
iam-F *iam*-R	GCGCAAAATATTATCACCC TTCACGACTACTATGCGG	518	Carvalho et al. 2004

(F) – forward primers, (R) – reverse primers

Table 2. Detection of virulence genes in *C. jejuni* and *C. coli* by PCR.

| Isolate group (source/species)	Number of positive isolates (%)			
	cadF	*flaA*	*cdtB*	*iam*
Children/*C. jejuni* (n=37)	37(100)	37(100)	37(100)	12(32.4)
Children/*C. coli* (n=12)	12(100)	12(100)	11(91.6)	11(91.6)
Children/ Both (n=49)	49(100)	49(100)	48(97.9)	23(46.9)
Dogs /*C. jejuni* (n=10)	10(100)	10(100)	10(100)	3(30)
Dogs/ *C. coli* (n=7)	7(100)	6(85.7)	7(100)	1(14.2)
Dogs/ Both (n=17)	17(100)	16(94.1)	17(100)	4(23.5)
Cats/*C. jejuni* (n=9)	9(100)	9(100)	9(100)	8(88.8)
Cats/*C. coli* (n=4)	4(100)	4(100)	4(100)	2(50)
Cats/ Both (n=13)	13(100)	13(100)	13(100)	10(76.9)
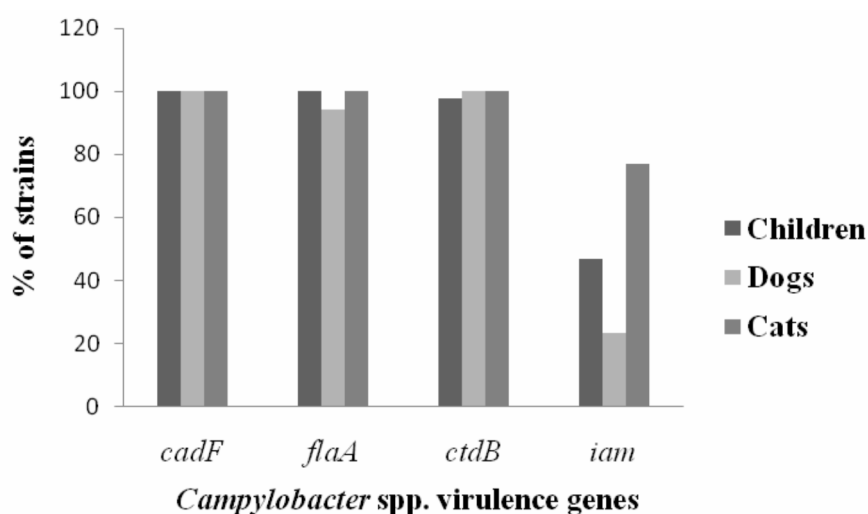
Fig. 1. Distribution of virulence genes in *Campylobacter* spp. from different sources.

from children. The prevalence rates for *iam* detection in *C. jejuni* and *C. coli* isolates from dogs were 30% and 14.2% respectively. Investigations on the occurrence of virulence genes among *Campylobacter* spp. strains from cats revealed that *iam* was predominant in *C. jejuni* strains (88.8%) and it was present in 50% of *C. coli* isolates obtained from cats.

Discussion

Identification of *Campylobacter* spp. virulence genes in domestic animals has not been examined in Poland yet, although the contact with an animal is a significant element of campylobacteriosis risk. The complete understanding of the epidemiology of *Campylobacter* spp. infection requires further studies. Analyses of *Campylobacter* strains similarity are needed to define the way of infection. The high prevalence of the same virulence genes among *Campylobacter* spp. isolates from children and domestic animals indicates that the transmission of these bacteria to humans from animals should be taken into consideration.

The first stage in pathogenesis process for bacteria that enter the host organism via the alimentary tract is to overcome the mucous membrane covering the intestine epithelium. The virulence factor used at this stage is motility. *In vitro* studies established a role for flagella in invasion and adherence (Wassenaar et al. 1999).

The study confirmed high frequency of the occurrence of the flagellin gene *flaA* in *Campylobacter* spp. strains obtained from hospitalized children, cats and dogs. There is lack of reports of *flaA* occurrence in *Campylobacter* spp. amongst domestic animals.

The results obtained in the group of strains from children with campylobacteriosis are similar to those of previous studies (Datta et al. 2003, Talukder et al. 2008).

The next virulence factor examined in the study was *cadF* gene. The product of the gene is an adhesin involved in the process of invasion and adhesion (Ketley 1997). Many authors underline that *cadF* gene is conservative among *C. jejuni* and *C. coli* strains and is necessary in the development of human campylobacteriosis (Wardak et al. 2006, Talukder et al. 2008).

In the present study *cadF* was found in all *Campylobacter* spp. isolates from children. The findings of *cadF* gene in clinical samples from children confirm observations of other studies (Datta et al. 2003, Rożynek et al. 2005, Talukder et al. 2008).

There are no reports about detection of *cadF* gene in *Campylobacter* spp. isolates from cats and dogs. Our study has revealed the high occurrence rate of *cadF* gene in *Campylobacter* spp. animal isolates which may indicate an important role of *cadF* gene in pathogenesis process.

Another virulence gene of *Campylobacter* spp. is the invasion associated marker (*iam*). *In vitro* studies (Carvalho et al. 2004) have shown that this chromosomal genetic marker, associated preferentially with both adherence and invasion, was detected in 85% of *Campylobacter* spp. isolates from children with diarrhoea, compared to only 20% of isolates from asymptomatic patients.

In our study we observed the *iam* gene in 46% of *Campyloabcter* spp. strains from children. Similar results were obtained by Al-Mahmeed et al. (2006). However, Rizal et al. (2010) estimated *iam* gene occurrence in human *C. jejuni* samples to be 77.7%.

We also found that the presence of *iam* marker varies between species. High occurrence of this gene in *C. coli* strains obtained from symptomatic patients (91.6%) was also shown in studies of other authors (Rożynek et al. 2005). This may confirm the idea that the marker is associated stronger with *C. coli* than *C. jejuni* species.

There is no data on the prevalence of *iam* marker among *Campylobacter* spp. isolates derived from domestic animals. In the present study the occurrence of *iam* gene was higher in *Campylobacter* spp. isolated from cats (76%) in contrary to isolates obtained from dogs where the marker was confirmed in only 23% of strains. The varying *iam* detection rates from different sources need more researches to be carried out to understand the specific role of this gene.

One of well characterized *Campylobacter* spp. virulence factor is the cytolethal distending toxin (CDT), a toxin that causes cell cycle arrest and eventual death in sensitive eukaryotic cells (Hickey et al. 2000, Heywood et al. 2005). It has also been shown that CDT is involved in inducing the release of pro-inflammatory cytokine IL-8 from intestinal epithelial cells (Smith et al. 2006).

The highest frequency of the occurrence of *cdtB* gene was detected in *Campylobacter* spp. strains coming from cats and dogs. In the available literature there is no information about the prevalence of *cdtB* gene in *Campylobacter* spp. isolates from these animals. The participation of *cdtB* gene in the development of infection in animals seems to be significant considering its high detection (100%) in both *C. jejuni* and *C. coli* strains.

Similar results were received in a group of clinical strains obtained from children. The study confirmed the presence of *cdtB* gene in all *C. jejuni* strains. The high prevalence of *cdtB* gene in human *C. jejuni* isolates had been detected in previous studies (Datta et al. 2003, Wardak et al. 2006).The presence of the gene responsible for toxin production in clinical *C. coli* samples was estimated in this work to be 91.6%. A similar percentage of detection of *cdtB* gene in *C. coli* strains obtained from symptomatic patients was found by Rożynek et al. (2005).

In conclusion, the high prevalence of *cadF*, *flaA* and *cdtB* genes among *C. jejuni* and *C. coli* isolates obtained from children with diarrhoea and domestic animals suggests their crucial role in pathogenesis of campylobacteriosis infection among humans and animals.

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