Short Communication

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Received 19 August 2002

Accepted 5 December 2002

Prevalence of 11 pathogenic genes of *Campylobacter jejuni* by PCR in strains isolated from humans, poultry meat and broiler and bovine faeces

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Although many genes related to the pathogenicity of *Campylobacter jejuni* have been reported, the relationships between these genes and the sources of strains are not clear. In this study, the presence of 11 pathogenic genes responsible for the expression of adherence, invasion, colonization and cytotoxin production was examined in 111 *C. jejuni* isolated from human clinical samples, poultry meat, broiler faeces and bovine faeces. For most of the pathogenic genes, no difference in their presence in *C. jejuni* was found among the sources, but, for *racR*, *wlaN* and *virB11*, there were some variations among sources. The *racR* gene was present at rates of 98.2 (human clinical samples), 90.5 (poultry meat), 85.7 (broiler faeces) and 76.7 % (bovine faeces). Detection rates for the *wlaN* gene were 25.0, 23.8, 4.7 and 7.7 % and those for the *virB11* gene were 10.7, 9.5, 9.5 and 15.4 % in human clinical samples, poultry meat, broiler faeces and bovine faeces, respectively. One hundred and seven of 111 strains (96.4 %) carried from eight to 10 of the pathogenic genes. These data did not show remarkable differences in the presence of pathogenic genes carried by *C. jejuni* from various sources.

Introduction

Campylobacter jejuni is one of the most common causes of bacterial enteritis. Campylobacteriosis is mainly a food-borne infection, and poultry products may play an important role in transmission to humans (Park, 2002). *C. jejuni* contamination of poultry meat during processing has been reported (Kazwala *et al.*, 1990; Chuma *et al.*, 1994, 1997; Berrang *et al.*, 2001; Hartnett *et al.*, 2001). Adherence, invasion and cytotoxin production appear to be possible virulence factors. The clinical and epidemiological characteristics of the disease provide clues to the molecular mechanisms that play a role in *C. jejuni* infection. Recently, some genes have been recognized as responsible for the expression of pathogenicity. In this study, *flaA* (Nuijten *et al.*, 2000), *cadF* (Ziprin *et al.*, 2001), *racR* (Bras *et al.*, 1999) and *dnaJ* (Ziprin *et al.*, 2001) were selected as pathogenic genes responsible for the expression of adherence and colonization, *virB11* (Bacon *et al.*, 2000), *ciaB* (Konkel *et al.*, 1999a; Rivera-Amill *et al.*, 2001) and *pldA* (Ziprin *et al.*, 2001) as pathogenic genes responsible for the expression of invasion and *cdtA*, *cdtB* and *cdtC* as pathogenic genes responsible for the expression of cytotoxin production (Purdy *et al.*, 2000; Lara-Tejero & Galan, 2001), while *wlaN* (Linton *et al.*, 2000) was selected as a pathogenic gene responsible for the expression of Guillain-Barré syndrome. In this study, PCR was applied to analyse the relationship between pathogenic genes and sources of *C. jejuni*.

Methods

Bacterial strains and growth conditions. One hundred and eleven strains in total were tested in this study, including 56 isolates from human clinical samples, 21 from poultry meat, 21 from broiler faeces and 13 from bovine faeces. All strains were incubated on Müller-Hinton agar (Oxoid) at 42 °C under microaerobic conditions (85 % N₂, 5 % O₂, 10 % CO₂) for 24 h.

Preparation of DNA. Template DNAs for PCR were extracted by the conventional boiling method. Fresh cultures of *C. jejuni* were suspended in 300 µl TE buffer and the suspensions were boiled at 95 °C for 10 min. After centrifugation at 15 000 r.p.m. for 2 min, the supernatants were stored at –20 °C and used as template DNA.

PCR primer design and amplification. Three sets of primers were described by Konkel *et al.* (1999b) (*cadF*), Hickey *et al.* (2000) (*cdtA*) and Linton *et al.* (2000) (*wlaN*). Eight further sets of primers were designed based on sequences from GenBank accession numbers AF345999 (*flaA*), AF053960 (*racR*), AF052661 (*dnaJ*), AF226280 (*virB11*), AF114831 (*ciaB*), NC_002163 (*pldA*), AF289020 (*cdtB*) and AJ000859 (*cdtC*). Primer sequences, annealing temperatures and lengths of products are listed in Table 1.

All PCR amplifications were performed in a mixture (30 µl) consisting of 3 µl 10× ExTaq buffer, 0.6 U ExTaq (Takara), 2.4 µl dNTPs (dATP, dCTP, dGTP, TTP, each at 2.5 mM; Takara), 0.6 µl extracted template DNA and 0.3 µl of a 20 pM solution of each primer. Distilled water was added to make 30 µl. The reaction mixture was overlaid with mineral oil and subjected to 30 cycles of amplification in a DNA thermal cycler 480 (Takara). The cycling was as follows: denaturation at 94 °C for 1 min, annealing at a temperature specific to the primer pair for 1 min and

extension at 72 °C for 1 min. Part of this amplified sample was analysed by electrophoresis on 1.5 % NuSieve agarose gels (Rockland). DNA bands were stained with ethidium bromide, visualized with a UV transilluminator (ATTO) and photographed (Fig. 1).

Results and Discussion

Five of the 11 pathogenic genes, *flaA*, *cadF*, *cdtA*, *cdtB* and *cdtC*, were detected from all strains, and the detection rates of three genes, *racR*, *wlaN* and *virB11*, varied among sources of isolates. Rates of three other genes, *dnaJ*, *ciaB* and *pldA*, also varied among sources, but not so markedly (Table 2).

Only two strains from human clinical samples had all 11 pathogenic genes, and two strains from bovine faeces had six genes. The other 107 strains carried from eight to 10 genes related to pathogenicity, independent of source (Table 3).

C. jejuni strains from all sources tested in this study had most of the pathogenic genes examined. However, *racR*, *wlaN* and *virB11* showed some variation in prevalence according to source. The importance of these three genes in the pathogenicity of *C. jejuni* compared with other pathogenic genes is not clear. Interestingly, the detection rate of the *wlaN* gene from human clinical samples (25.0 %) was similar to that from poultry meat (23.8 %), but different from that from broiler and bovine faeces. We could not distinguish *C. jejuni* strains on the basis of source using data for the pathogenic

Fig. 1. Agarose gel electrophoresis of PCR products of 11 pathogenic genes of *C. jejuni*. Lanes: M, 100 bp marker; 1, *flaA*; 2, *cadF*; 3, *racR*; 4, *dnaJ*; 5, *virB11*; 6, *ciaB*; 7, *pldA*; 8, *cdtA*; 9, *cdtB*; 10, *cdtC*; 11, *wlaN*.

genes carried by *C. jejuni*. Further studies are needed to reveal the relationship between each pathogenic gene or combinations of these genes and the pathogenicity of *C. jejuni*.

Table 1. PCR primers and annealing temperatures

Target gene	Primers	Sequence (5'→3')	Annealing temperature (°C)	Product (bp)	Reference
<i>flaA</i>	flaA664 flaA1494	AATAAAATGCTGATAAAACAGGTG TACCGAACCAATGTCTGCTCTGATT	53	855	This study
<i>cadF</i>	cadF-F2B cadF-R1B	TTGAAGGTAATTTAGATATG CTAATACCTAAAGTTGAAAC	45	400	Konkel <i>et al.</i> (1999b)
<i>racR</i>	racR-25 racR-593	GATGATCCTGACTTTG TCTCCTATTTTTACCC	45	584	This study
<i>dnaJ</i>	dnaJ-299 dnaJ-1003	AAGGCTTTGGCTCATC CTTTTGTTCATCGTT	46	720	This study
<i>virB11</i>	virB-232 virB-701	TCTTGAGATTGCCTTACCCCTTTT CCTGCGTGTCTGTGTTATTTACCC	53	494	This study
<i>ciaB</i>	ciaB-403 ciaB-1373	TTTTTATCAGTCCTTA TTTCGGTATCATTAGC	42	986	This study
<i>pldA</i>	pldA-84 PlD-981	AAGCTTATGCGTTTTT TATAAGGCTTTCTCCA	45	913	This study
<i>cdtA</i>	DS-18 DS-15	CCTTGATGCAAGCAATC ACACTCCATTGCTTTCTG	49	370	Hickey <i>et al.</i> (2000)
<i>cdtB</i>	cdtB-113 cdtB-713	CAGAAAGCAAATGGAGTGTT AGCTAAAAGCGGTGGAGTAT	51	620	This study
<i>cdtC</i>	cdtC-192 cdtC-351	CGATGAGTTAAAAACAAAAGATA TTGGCATTATAGAAAATACAGTT	47	182	This study
<i>wlaN</i>	wlaN-DL 39 wlaN-DL 41	TTAAGAGCAAGATATGAAGGTG CCATTTGAATTGATATTTTTG	46	672	Linton <i>et al.</i> (2000)

Table 2. Presence of pathogenic genes in *C. jejuni* from various sources

Values are percentages of isolates testing positive.

Gene	Human clinical samples (n = 56)	Poultry meat (n = 21)	Broiler faeces (n = 21)	Bovine faeces (n = 13)
<i>flaA</i>	100	100	100	100
<i>cadF</i>	100	100	100	100
<i>racR</i>	98.2	90.5	85.7	76.7
<i>dnaJ</i>	98.2	90.5	100	100
<i>virB11</i>	10.7	9.5	9.5	15.4
<i>ciaB</i>	98.2	100	100	84.6
<i>pldA</i>	91.1	100	100	84.6
<i>cdtA</i>	100	100	100	100
<i>cdtB</i>	100	100	100	100
<i>cdtC</i>	100	100	100	100
<i>wlaN</i>	25.0	23.8	4.7	7.7

Table 3. Numbers of strains of *C. jejuni* carrying pathogenic genes

Values are numbers (percentages) of isolates carrying that number of pathogenic genes. No isolate carried fewer than six genes.

Number of pathogenic genes present	Human clinical samples (n = 56)	Poultry meat (n = 21)	Broiler faeces (n = 21)	Bovine faeces (n = 13)
11	2 (3.6)	0 (0)	0 (0)	0 (0)
10	16 (28.6)	5 (23.8)	2 (9.5)	3 (23.1)
9	30 (53.6)	14 (66.7)	16 (76.2)	7 (53.4)
8	8 (14.3)	2 (9.5)	3 (14.3)	1 (7.7)
7	0 (0)	0 (0)	0 (0)	0 (0)
6	0 (0)	0 (0)	0 (0)	2 (15.4)

Acknowledgements

We thank Dr D. G. Newell (Central Veterinary Laboratory, UK), Dr T. Itoh (Tokyo Metropolitan Research Laboratory of Public Health, Japan), Dr H. Lior (Laboratory Centre for Disease Control, Canada), Dr G. B. Lindblom (University of Göteborg, Sweden), Dr A. S. Greef (Medical University of Southern Africa, South Africa), Dr S. Yamai (Kanagawa Institute of Public Health, Japan) and Dr T. Chuma (Kagoshima University, Japan) for kindly providing *C. jejuni* strains used in this study.

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