Journal of Animal and Veterinary Advances 11 (6): 704-711, 2012
ISSN: 1680-5593

Characteristics of *in vitro* Virulence Properties in *Campylobacter* sp. Isolated from Chicken Carcasses

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Abstract: Campylobacter is one of the leading cause of food poisoning in several countries. The major goal of this study was to provide the characteristics of virulence factors related with pathogenesis in *Campylobacter* sp. isolated from chicken carcasses in Korea. In the comparison of prevalence of *C. jejuni* and *C. coli* carrying virulence-associated genes, *C. jejuni* carried from 23-25 of virulence genes but *C. coli* carried from 15-25 of virulence genes. Campylobacter isolates contained different virulence-associated genes were performed for adhesion assay. Campylobacter isolates possessed all virulence genes had a significantly greater capacity ($p<0.05$) to adhere than isolates with partial virulence genes. But a clear association between presence of virulence genes and adhesion ability were not reflected in this study. In CDT titer of isolates with different virulence genes, isolates showed a variable titer from 0-128 in *C. jejuni* and 8- 64 in *C. coli*. Although, isolates possessed all virulence genes tested had higher CDT activity titer than isolates with partial virulence genes but cytotoxic acitivities of isolates were not in accord with presence of virulence genes.

Key words: Food poisoning, pathogenesis, chicken, isolates, virulence genes, Korea

INTRODUCTION

Campylobacter species have emerged as a major cause of human bacterial gastrointestinal disorders characterized by profuse diarrhea, acute abdominal pain and fever occurring worldwide. *Campylobacter jejuni* has also been involved in a variety of other human systemic diseases such as Guillian-Barre Syndrome (GBS) and arthritis (Smith, 1995). Therefore, a number of reports are described recently regarding the virulence factor and mechanisms of Campylobacter infections.

Specific properties are considered to be necessary in the process of infection such as flagella-mediated motility, chemotaxis, bacterial adherence to intestinal mucosa, invasion of epithelial cells and the ability to produce toxins (Ketley, 1997; Wassenaar and Blaser, 1999; Konkel *et al*., 2001). Especially, the motility by the flagella contributes to the ability of *C. jejuni* to colonize the intestinal tract of animals (Nachamkin *et al*., 1993; Wassenaar *et al*., 1993; Van Vliet and Ketley, 2001). To produce functional flagella, bacteria must coordinate both the temporal expression of >40 *flagella* genes and secretion of the encoded protein (Caldwell *et al*., 1985; Fernando *et al*., 2007). The prevalence of flagella-associated genes suggests their potential role as important virulence factor involved in *Campylobacter* sp. infection. Also, adhesins are surface-exposed molecules that facilitate a pathogen's attachment to host cell receptor molecules. The ability of *Campylobacter* sp. to enter, survive and replicate in intestinal cells also suggests their important factors in infection.

Bacterial toxin has been considered important factors for the pathogenesis of Campylobacter infection. The only verified Campylobacter toxin is Cytolethal Distending Toxin (CDT) (Dasti *et al*., 2010). The CDT is composed of three subunits encoded by three adjacent genes, *cdtA*, *cdtB* and *cdtC*. This toxin was found to induce progressive cell distension in different mammalian cell lines such as HeLa cells, Caco-2 cells and Chinese Hamster Ovary (CHO) cells which is characterized by elongation, swelling and eventually cell death (Whitehouse *et al*., 1998).

Lipooligosaccharide (LOS) and chemotaxis are thought to be critical factors for virulence mechanism. LOS is a major component of the outer membrane in gram-negative bacteria. The *wlaN* gene is LOS-associated gene

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and presumably involved in the expression of ganglioside mimics in Guillian-Barre syndrome. Chemotaxis is the movement of an organism towards or away from a chemical stimulus and an important virulence determinant. Several studies have examined *Campylobacter* sp. in poultry and the findings have indicated prevalence ranges of the bacteria from 3-98% (Newell and Wagenaar, 2000; Newell and Fearnley, 2003). Poultry and their products are commonly consumed in modern Korean diets and the prevalence of *Campylobacter* sp. in poultry meat is also described (Han *et al*., 2007; Kim *et al*., 2010). However, virulence properties and pathogenesis of Campylobacter are rarely reported in Korea. Therefore, the aims of this study were to investigate the characteristics of virulence properties in *Campylobacter* sp. isolated from chicken carcasses in Korea.

MATERIALS AND METHODS

Bacterial isolates and growth conditions: A total of 54 Campylobacter isolates including 43 *C. jejuni* and 11 *C. coli* isolated from domestic or imported chicken carcasses were used for the study (Table 1). *Campylobacter* sp. isolates were subcultured onto Campylobacter blood free selective agar (Oxoid CM0739) with CCDA selective supplement (Oxoid SR0155E) and Campylobacter growth supplement (Oxoid SR0232E) and incubated at 42°C in microaerobic conditions of 5% O_2, 10% CO_2 and 85% N_2. The isolates were confirmed by PCR assay to distinguish *C. jejuni* and *C. coli* (Nayak *et al*., 2005).

Preparation of DNA: Template DNAs for PCR were extracted using a QIAamp DNA Mini kit (Qiagen, France) according to manufacturer's directions. The DNA concentration was measured spectrophotometrically at A260.

PCR primer design and amplification: Primers and PCR condition for detection of virulence and toxin genes from Campylobacter isolates are shown in Table 2. PCR was performed in a DNA Thermal Cycler (Biometra) using standardized cycling parameters; 94°C for 5 min for initial denaturation followed by 30 cycles of denaturation at 94°C for 45 sec, annealing for 45 sec with variable temperature and extension at 72°C for 1 min and final extension step at 72°C for 5 min. The results were obtained by electrophoresis on 1.2% agarose gels stained with ethidium bromide.

Table 1: Campylobacter isolates from chicken carcasses tested in this study

Countries	No. of sample tested	No. of *C. jejuni*	No. of *C. coli*	Years isolated (No. of isolates)
Korea	17	13	4	2007 (1), 2008 (16)
Brazil	30	24	6	2008 (17), 2009 (13)
Denmark	2	1	1	2007 (1), 2008 (1)
USA	5	5	0	2008 (5)
Total	54	43	11	-

Preparation of HeLa cells: Human cervical adenocarcinoma cell lines, HeLa cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS) and 1% antibiotics (Anti-Anti, GibcoBRL 15240) at 37°C in a humidified atmosphere containing 5% CO_2. Medium was replenished every 2 days and confluent monolayers were passaged with 0.25% trypsin-EDTA (GibcoBRL, 25200).

Adherence assay: The adhesion assays were performed as described by Hanel *et al*. (2007). Trypsinized cells were seeded in 24 well cell culture plates at a density of 4.5×10^4 cells per well. The plates were incubated overnight to allow the cells to attach to the bottom of the plates. Prior the assay, the cells were once washed with Phosphate-Buffered Saline (PBS). Campylobacter isolates were grown microaerobically on blood agar plates for 48 h at 37°C. Bacteria were harvested from plates with PBS containing 1% FBS and inoculated into duplicated wells of a 24 well tissue culture plate after adjustment spectrophotometrically to approximately 1×10^8 cfu mL^{-1}. The actual numbers of bacteria in the inoculum added to monolayers were determined retrospectively by serial dilution and plate counting. The infected monolayers were incubated for 3 h at 37°C in 5% CO_2 to allow bacterial adherence and internalization. To assess adherence, the cells were rinsed three times with PBS and lysed with 100 μL Trypsin-EDTA and 900 μL 1% Triton X-100 in PBS for 15 min at room temperature. Total intracellular and extracellular bacteria associated with the cells were enumerated by plating serial dilutions of the lysates on blood agars and counting the resultant colonies. The results were the mean of at least three separate determinations and expressed as percentage bacteria adhered relative to inoculums.

Preparation of cell-free bacterial culture supernates for toxin assays: Cell free bacterial culture supernates were prepared according to the method described by Jain *et al*. (2008) with modification. Bacteria from frozen stocks were inoculated on blood agar plates and incubated under micro-aerobic conditions at 42°C for 48 h. Bacteria were suspended in PBS and the bacterial suspensions were adjusted to a defined optical density at 600 nm of 0.125 (approximately 2×10^8 cfu μL^{-1}) to enable a comparison of the ability of different strains to produce CDT. The bacteria were lysed by sonication (4×30 sec bursts with 30 sec intervals between each burst). The lysates were

Table 2: List of primers and PCR conditions used in this study

Target genes	Sequences	Annealing temperature (°C)	Product size (bp)	References
Flagella				
flaA	5'-ggatttcgtattaacacaaatggtgc-3' 5'-ctgtagtaatcttaaaacattttg-3'	50	1,728	Bang *et al.* (2003)
flaB	5'-ataaacaccaacatcggtgca-3' 5'-gttacgttgactcatagcata-3'	50	1,670	Muller *et al.* (2006)
flhA	5'-atggtgcaatggatggttct-3' 5'-ttgtacctcttggcgtgtga-3'	50	1,010	This study
flhB	5'-gcaggcgaagatcaagaaaa-3' 5'-ccaaaaccccagcaatcata-3'	55	310	This study
flgB	5'-ctggtgctttagcgggtaga-3' 5'-tgatgcccttcttcagttacg-3'	50	187	This study
flgE2	5'-catctcaccacgacctcctgttc-3' 5'-gcaaaaatcgcaatggcttca-3'	52	132	This study
fliA	5'-tgctaaaagagccgcctaaa-3' 5'-gcgctctttaagatcatctagca-3'	50	554	This study
fliM	5'-gccaagcccaacaagtttta-3' 5'-cctcctcttcaggctcatca-3'	50	770	This study
fliY	5'-aaatgaatgcatcagcacca-3' 5'-gcaaacgcacatccatgata-3'	55	590	This study
Adherence				
cadF	5'-gcaccagggattagacttgg-3' 5'-tcattgccttgagcgaggat-3'	52	770	This study
peblA	5'-agcaatgctaatgcagcaga-3' 5'-tggttcaaaactatctggcaaa-3'	52	600	This study
porA	5'-caatttgactataatgctgctgatg-3' 5'-atgctgagaagttaagttttggaga-3'	50	932	This study
jlpA	5'-tgcaatgcagatggtgattt-3' 5'-gctccgcccattaacataga-3'	52	931	This study
CJE1415	5'-agagcttgccaaaggttgaa-3' 5'-ctccccaccatagcgtctta-3'	52	974	This study
CJE1538	5'-tatttttgatcttactcgtgcaatg-3' 5'-ttaaggtataatcgacccaatacga-3'	52	1,115	This study
Invasion				
ciaB	5'-gctagccatacttaggcgtttg-3' 5'-catcaacccttttgccaagaa-3'	50	1,340	This study
iamA	5'-gcacaaaatatatcattacaa-3' 5'-ttcacgactactatgagg-3'	55	518	Muller *et al.* (2006)
LOS*				
wlaN	5'-tgctgggtatacaaaggttgtg-3' 5'-aattttggatatgggtgggg-3'	60	330	Muller *et al.* (2006)
Chemotaxis				
docA	5'-ataaggtgcggttttggc-3' 5'-gtctttgcagcagtagatatg-3'	55	725	Muller *et al.* (2006)
docB	5'-cggagagtttagaggcacc-3' 5'-ccgcaaattccatagcag-3'	50	1,418	Muller *et al.* (2006)
docC	5'-tgagctacgctatcattg-3' 5'-gcttacgctatgggttgg-3'	62	1,835	Muller *et al.* (2006)
CDT**				
cdtA	5'-gagcagctttaacggtttgg-3' 5'-tcatcgtacctctccttggc-3'	62	440	This study
cdtB	5'-gttggcacttggaatttgc-3' 5'-tcaggccttgaaagagttcc-3'	65	220	This study
cdtC	5'-gcctttgcaactcctactgg-3' 5'-cagctgaagttgttgttggc-3'	62	340	This study
Cdt-cluster	5'-atgtaaatcctttggggcgt-3' 5'-cagctgttaaaggtggggtt-3'	62	2,088	This study

*LOS: Lipooligosaccharide; **CDT: Cytolethal Distending Toxin

sterilized by filtration using a millipore filter membrane with pore size of 0.22 um and stored at -80°C until needed. About 10 μL of the filtrates were inoculated on blood agar plates and incubated at 37°C under both aerobic and micro-aerobic conditions to test for sterility (Bang *et al.*, 2001).

Detection of CDT activity: HeLa cell adjusted at a concentration of 1×10^4 cells mL^{-1} were seeded to each of wells in a 24 well cell culture plates and incubated overnight at 37°C in a 5% CO_2 incubator. The medium were replaced with fresh DMEM containing 1% FBS. Two-fold dilutions of culture filtrates and bacterial lysates

were prepared in DMEM and 0.5 mL of each dilution was added to each well and incubated for 72 h at 37°C in an atmosphere of 5% CO_2. All experiments were done in duplicate at the desired dilutions. Toxin production by each isolate was tested in at least three independent assays. Morphological changes in the cells were examined by giemsa staining and under phase-contrast microscope at every 24 h. The toxin titre of a given sample was expressed as the reciprocal of the highest dilution that caused at least 30% of the cells in a well to be rounded or distended.

RESULTS

Results of PCR detection of 25 virulence-associated genes among 43 *C. jejuni* and 11 *C. coli* isolates are shown in Table 3. All *C. jejuni* tested in this study showed fragments of *flaA*, *flaB*, *flhA*, *flhB*, *flgB*, *flgE2*, *fliA*, *fliM* and *fliY* genes. *C. coli* also showed the presence of all flagella-associated genes tested expect for *flhB* (63.6%). In the prevalence of adherence-associated genes, *C. jejuni* showed 100% genes including *cadF*, *jlpA*, *CJE1415* and *CJE1538* except for *peblA* (97.7%) and *porA* (93.0%) genes and *C. coli* showed a lower prevalence than *C. jejuni* in *peblA* (36.4%), *jlpA* (72.7%), *CJE1415* (72.7%) and *CJE1538* (90.0%), except for *cadF* (100%) and *porA* (100%) genes. Invasion-associated genes, *ciaB* and *iamA* were detected in the all *C. jejuni* however, showed a lower prevalence in *C. coli* (54.5% and 72.7%, respectively). In the prevalence of chemotaxis-associated genes, all *C. coli* showed the presence of *docA* (100%) and *docB* (100%) except for *docC* gene (54.5%) while *C. jejuni* showed a lower prevalence for *docA* (79.1%), *docB* (79.1%) and *docC* (72.1%). The prevalence of CDT encoding genes was evaluated using cdtA, cdtB, cdtC and cdt clutster specific primers for each subunit. All genes were detected in the *C. jejuni* and *C. coli* isolates tested in this study.

Table 4 shows the comparison of prevalence of *C. jejuni* and *C. coli* carrying virulence-associated genes. *C. jejuni* isolates with different country origin tested in this study carried from 23-25 of virulence genes. Twenty six (60.5%) of 43 *C. jejuni* had all 25 virulence-associated genes tested. Only 2 *C. jejuni* had 23 virulence genes and 15 isolates (34.9%) had 24 virulence genes which is devoid of only one gene. Whereas there was large difference between the *C. jejuni* and *C. coli* isolates. *C. coli* isolates carried insufficient virulence-associated genes from 15-25 genes. Only one *C. coli* isolate had all

Table 3: Prevalence of virulence-associated genes in 54 *Campylobacter* isolates

	Flagella								
Species	*flaA*	*flaB*	*flhA*	*flhB*	*flgB*	*flgE2*	*fliA*	*fliM*	*fliY*
C. jejuni	43***	43	43	43	43	43	43	43	43
(43)	(100)	(100)	(100)	(100)	(1000)	(100)	(100)	(100)	(100)
C. coli	11	11	11	7	11	11	11	11	11
(11)	(100)	(100)	(100)	(63.6)	(100)	(100)	(100)	(100)	(100)
Total	54	54	54	50	54	54	54	54	54
(54)	(100)	(100)	(100)	(92.6)	(100)	(100)	(100)	(100)	(100)

	Adherence						Invasion		LOS*	Chemotaxis			CDT**			
Species	*cadF*	*peblA*	*porA*	*jlpA*	*CJE1415*	*CJE1538*	*ciaB*	*iamA*	*wlaN*	*docA*	*docB*	*docC*	*cdtA*	*cdtB*	*cdtC*	*cdt*-cluster
C. jejuni	43	42	40	43	43	43	43	43	40	34	34	31	43	43	43	43
(43)	(100)	(97.7)	(93)	(100)	(100)	(100)	(100)	(100)	(93)	(79.1)	(79.1)	(72.1)	(100)	(100)	(100)	(100)
C. coli	11	4	11	8	8	10	6	8	7	11	11	6	11	11	11	11
(11)	(100)	(36.4)	(100)	(72.7)	(72.7)	(90.9)	(54.5)	(72.7)	(63.6)	(100)	(100)	(54.5)	(100)	(100)	(100)	(100)
Total	54	46	51	51	51	53	49	51	47	45	45	37	54	54	54	54
(54)	(100)	(85.2)	(94.4)	(94.4)	(94.4)	(98.1)	(90.7)	(94.4)	(87)	(83.3)	(83.3)	(68.5)	(100)	(100)	(100)	(100)

*LOS: Lipooligosaccharidel; **CDT: Cytolethal Distending Toxin; ***No. of isolates included (%)

Table 4: Prevalence of *C. jejuni* and *C. coli* carrying virulence genes

	C. jejuni (n = 43)		*C. coli* (n = 11)	
No. of genes present*	No. of isolates (%)	Country origin (No. of isolates)	No. of isolates (%)	Country origin (No. of isolates)
25	26 (60.50)	Brazil (15), USA (1) and Korea (10)	1 (9.1)	Brazil (1)
24	15 (34.90)	Brazil (8), USA (3), Denmark (1) and Korea (3)	1 (9.1)	Brazil (1)
23	2 (4.65)	Brazil (1) and USA (1)	1 (9.1)	Korea (1)
22	0.00		3 (27.3)	Korea (3)
20	0.00		1 (9.1)	Brazil (1)
17	0.00		1 (9.1)	Brazil (1)
16	0.00		2 (18.2)	Brazil (1) and Denmark (1)
15	0.00		1 (9.1)	Brazil (1)

*Total of 25 pathogenic genes, flagella (*flaA*, *flaB*, *flhA*, *flhB*, *flgB*, *flgE2*, *fliA*, *fliM*, *fliY*), adherence (*cadF*, *peb1A*, *porA*, *jlpA*, *CJE1415*, *CJE1538*), invasion (*ciaB*, *iamA*), *wlaN* and *cdt* (*cdt*A, *cdt*B, *cdt*C, *cdt*-cluster) related genes were tested in this study

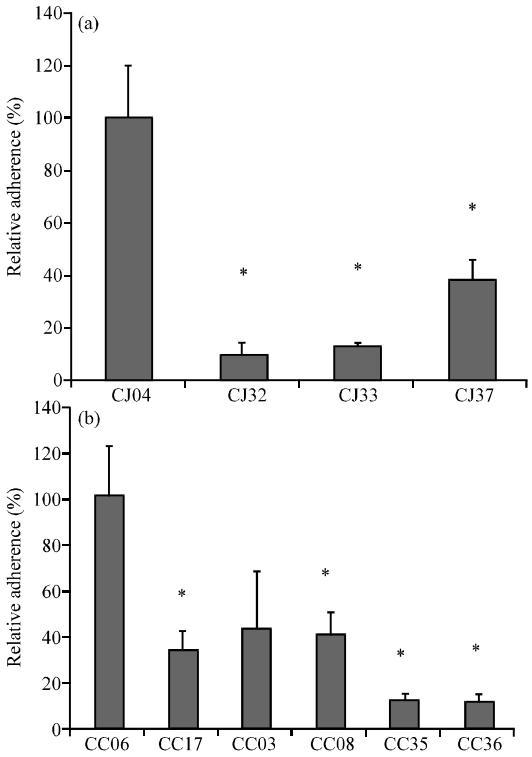

Fig. 1: a) Adherence percentage of *C. jejuni* and b) *C. coli*. Values are given relative to the adhesion of the CJ04 and CC06 which was set at 100% and the mean±SEM (standard deviation of the mean) of three replicates. Statistical significance was assessed with an unpaired Student's t-test ($p<0.05$). CJ04, *C. jejuni* contained all adhesion and invasion-associated genes tested in this study; CJ32, *C. jejuni wlaN*$^-$ isolate; CJ33, *C. jejuni porA*$^-$ isolate; CJ37, *C. jejuni peblA*$^-$*wlaN*$^-$ isolate; CC06, *C. coli* contained all adhesion and invasion-associated genes tested in this study; CC17, *C. coli pebl1A*$^-$ isolate; CC03, *C. coli peblA*$^-$*jlpA*$^-$*CJE1415*$^-$*ciaB*$^-$ *iamA*$^-$*wlaN*$^-$ isolate; CC08, *C. coli peblA*$^-$*jlpA*$^-$*ciaB*$^-$*iamA*$^-$ isolate; CC35, *C. coli peblA*$^-$*jlpA*$^-$*CJE1415*$^-$ *ciaB*$^-$*wlaN*$^-$ isolate; CC36, *C. coli peblA*$^-$ *CJE1415*$^-$*CJE1538*$^-$*ciaB*$^-$*iamA*$^-$*wlaN*$^-$ isolate

virulence genes tested. The difference of Campylobacter isolates carrying virulence genes by country origin was not shown.

Total 10 Campylobacter isolates contained different virulence-associated genes were used for adhesion assay (Fig. 1). Isolates CJ04 (*C. jejuni*) and CC06 (*C. coli*) possessed all virulence genes tested in this study had a significantly greater capacity ($p<0.05$) to adhere than

Table 5: Cytolethal distending toxin titers

Species	Strains	Absence genes*	CDT titers
C. jejuni	CJ04	None	128
	CJ32	*wlaN*	8
	CJ33	*porA*	32
	CJ37	*pebl1A*, *wlaN*	0
C. coli	CC06	None	16
	CC17	*pebl1A*	64
	CC08	*pebl1A*, *jlpA*, *ciaB*, *iamA*	64
	CC35	*pebl1A*, *jlpA*, *CJE1415*, *ciaB*, *wlaN*	8
	CC03	*pebl1A*, *jlpA*, *CJE1415*, *ciaB*, *iamA*, *wlaN*	16
	CC36	*pebl1A*, *CJE1415*, *CJE1538*, *ciaB*, *iamA*, *wlaN*	16

*Total of 25 virulence genes, flagella (*flaA*, *flaB*, *flhA*, *flhB*, *flgB*, *flgE2*, *fliA*, *fliM*, *fliY*), adherence (*cadF*, *pebl1A*, *porA*, *jlpA*, *CJE1415*, *CJE1538*), invasion (*ciaB*, *iamA*), *wlaN* and *cdt* (*cdtA*, *cdtB*, *cdtC*, *cdt* cluster) related genes were tested in this study

isolates with partial virulence genes. But CJ37, *C. jejuni* peblA$^-$wlaN$^-$ isolate showed higher capacity than CJ32, *C. jejuni* wlaN$^-$ isolate and CC03, *C. coli* peblA$^-$jlpA$^-$CJE1415$^-$ciaB$^-$iamA$^-$wlaN$^-$ isolate showed higher capacity than CC35, *C. coli* peblA$^-$jlpA$^-$CJE1415$^-$ ciaB$^-$wlaN$^-$ isolate. Therefore, a clear association between presence of virulence genes and adhesion ability were not reflected in this study.

The CDT activity titers of the bacterial lysates of 10 isolates with different virulence genes were shown in Table 5. Despite, 10 *Campylobacter* sp. tested were positive to PCR for *cdtA*, *cdtB*, *cdtC* and *cdt*-cluster genes, CDT titer were varied from 0-128 in *C. jejuni* and 8-64 in *C. coli*. CJ04 possessed all virulence genes tested had higher CDT activity titer than *C. jejuni* isolates with partial virulence genes but *C. coli*, CC06 with all virulence genes showed more lower titer than *C. coli*, CC17, CC08, CC03 and CC36 with partial virulence genes. Therefore, a clear association between presence of virulence genes and cytotoxic activity were not reflected in this study like adhesion ability.

DISCUSSION

Salmonella is associated with chicken but chicken is more often the cause of Campylobacter, one of the leading cause of food poisoning in several countries including Korea and United States (Blaser, 1997; Oberhelman and Taylor, 2000; CDC, 2004). Many chicken flocks are silently infected with Campylobacter. But Campylobacter can be easily spread from bird to bird through a common water source or through contact with infected feces. Campylobacter isolated from chicken carcasses are frequently linked to human cases of campylobacteriosis by consumption of undercooked poultry and the handling of raw poultry (Tauxe *et al*., 1985; Harris *et al*., 1986; Kapperud *et al*., 1992; Blaser, 1997). Several studies reported the prevalence ranges of the Campylobacter from

3-98% (Newell and Wagenaar, 2000; Newell and Fearnley, 2003). Recently, the prevalence of Campylobacter in poultry meat has been also reported in Korea. Woo *et al.* (2001) and Han *et al.* (2007) reported that the prevalence of *Campylobacter* sp. in raw chickens in Korea was 55.3 and 68.3%, respectively. Kim *et al.* (2010) reported 29.2 and 14.9% of poultry meat marketed in Korea were contaminated with *C. jejuni* and *C. coli*, respectively. But campylobacteriosis is not yet receiving great attention in Korea despite of high prevalence of *Campylobacter* sp. in poultry meat. Therefore, the major goal of this study was to provide the characteristics of virulence factors related with pathogenesis in *Campylobacter* sp. isolated from Korea.

Interestingly, all tested genes related with flagellar secretion apparatus, except flhB were detected in Campylobacter isolates used in this study. The results of this study are consistent with the previous results described by Muller *et al.* (2006). The flagellum is a well-characterized and distinct pathogenicity factor as a essential for motility, colonization of the gastrointestinal tract and invasion of host cells (Crushell *et al.*, 2004).

Similar results were found in the adherence and invasion-associated genes in *C. jejuni* strains. However, *C. coli* had low possession in these virulence genes. Several studies demonstrated the importance of *C. jejuni* adhesion and binding factors and many experiments were performed using *in vitro* Model. These experiments have led to the identification of some putative adhesion or binding factors of Campylobacter including fibronectin-binding outer membrane protein CadF (Konkel *et al.*, 1997), the periplasmic binding protein PEB1 (Pei and Blaser, 1993) and the surface-exposed lipoprotein JlpA (Jin *et al.*, 2001). CadF is expressed in all *C. jejuni* and *C. coli* strains and mediates cell adhesion by binding to the cell matrix protein fibronectin. However, Krause-Gruszczynska *et al.* (2007) described that the *cadF* gene of *C. coli* strains differs from the respective gene of *C. jejuni* by a 39 bp insertion sequence. It could be shown *in vitro* that *C. jejuni* bound and invaded mammalian cells much more efficiently than *C. coli*. Indeed, the results showed that *C. jejuni* had high capacity to adhere to HeLa cells than *C. coli* strains.

LOS is thought to be critical factor in the triggering of the GBS and Miller-Fisher syndrome neuropathies after *C. jejuni* infection. Structural similarity between human gangliosides and *C. jejuni* LOS, the so called ganglioside mimicry is thought to be involved in the elicitation of GBS. Linton *et al.* (2000) have demonstrated the *wlaN* gene product as a β-1, 3 galactosyltransferase responsible for specific LOS structures. Muller *et al.* (2007) suggested a correlation between the occurrences of a β-1, 3 galactosyltransferase encoded cgtB or wlaN in *C. jejuni* strains and their strong colonization and invasion ability *in vivo* and *in vitro*. In this study, *wlaN* genes were detected 93 and 63.6% for *C. jejuni* and *C. coli*, respectively. Also, *C. jejuni* possessed all genes tested except for wlaN showed a low capacity to adhere. Therefore, the results agree the demonstration described by Muller *et al.* (2007).

Methyl-accepting Chemotaxis Proteins (MCPs) were to be needed for movement of an organism towards or away from a chemical stimulus by forming complex with each other mechanisms (Beery *et al.*, 1988; Takata *et al.*, 1992; Hendrixson and DiRita, 2004). DocA, DocB and DocC were suggested to be candidates of such a complex. In this study, all *C. coli* showed the presence of *docA* and *docB* except for *docC* gene (54.5%) while *C. jejuni* showed a lower prevalence for docA (79.1%), docB (79.1%) and docC (72.1%). Nevertheless, *C. jejuni* usually showed the higher ability to adhere than *C. coli* and these results did not support the complex forming hypothesis of MCPs.

The cultured eukaryotic cell assay technique has become a standard experimental procedure in the study of bacterial adhesion and internalization. Manninen *et al.* (1982) proposed that HeLa cell is a good model for study in adhesion on and invasion of the intestinal epithelia by *C. jejuni* and *C. coli*. Adherence to target host cells is a critical early step in the pathogenesis of man bacterial infection since, adherent bacteria can release enzymes and toxin or trigger changes in the receptor-bearing target cell such as effacing lesions of microvilli, cytokine production or invasion into or through epithelial cells. In this study, 10 Campylobacter isolates with or without virulence-associated genes were to be tested to adhere to HeLa cells. However, researchers could not find a clear association between the adherence ability and the presence of virulence-associated genes. Of course *C. jejuni* and *C. coli* with all genes tested showed a significantly greater capacity to adhere than isolates with partial virulence genes. But, *C. jejuni* peblA$^-$wlaN$^-$ isolate showed higher capacity than *C. jejuni* wlaN$^-$ isolate and *C. coli* peblA$^-$jlpA$^-$CJE1415$^-$ciaB$^-$iamA$^-$ wlaN$^-$ isolate showed higher capacity than *C. coli* peblA$^-$jlpA$^-$CJE1415$^-$ciaB$^-$wlaN$^-$ isolate. Therefore, it is suggested that more mechanisms may be involved in adherence process and further research should be undertaken to substantiate the putative correlation.

CONCLUSION

All isolates tested in this study also possessed the *cdtA, cdtB* and *cdtC* genes. It is indeed accepted that the *cdt* genes are widespread among Campylobacter strains (Eyigor *et al.*, 1999; Bang *et al.*, 2003; Rozynek *et al.*,

2005). Despite, the high *cdt* genes prevalence, toxin production in HeLa cell was variable. Although, it has been reported that *Campylobacter* sp. cytotoxicity is cell-type dependent, its role in pathogenesis including the difference *in vitro* and *in vivo* gene expression are still unclear. Further studies are needed to reveal the relationship between each virulence-associated gene or combinations of these genes and the pathogenicity of Campylobacter.

ACKNOWLEDGEMENT

This study was supported by the Korea Research Foundation Grant funded by the Korean Government (KRF-2008-1-E00077).

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