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Epidemiological relationships of *Campylobacter jejuni* strains isolated from humans and chickens in South Korea

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Thirty-nine human isolates of *Campylobacter jejuni* obtained from a national university hospital during 2007–2010 and 38 chicken isolates of *C. jejuni* were collected from poultry farms during 2009–2010 in South Korea were used in this study. *Campylobacter* genomic species and virulence-associated genes were identified by PCR. Pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) were performed to compare their genetic relationships. All isolates were highly resistant to ciprofloxacin, nalidixic acid, and tetracycline. Of all isolates tested, over 94% contained seven virulence associated genes (*flaA*, *cadF*, *racR*, *dnaJ*, *cdtA*, *cdtB*, and *cdtC*). All isolates were classified into 39 types by PFGE clustering with 90% similarity. Some chicken isolates were incorporated into some PFGE types of human isolates. MLST analysis for the 39 human isolates and 38 chicken isolates resulted in 14 and 23 sequence types (STs), respectively, of which 10 STs were new. STs overlapped in both chicken and human isolates included ST-21, ST-48, ST-50, ST-51, and ST-354, of which ST-21 was the predominant ST in both human and chicken isolates. Through combined analysis of PFGE types and STs, three chicken isolates were clonally related to the three human isolates associated with food poisoning (VII-ST-48, XXII-ST-354, and XXVIII-ST-51). They were derived from geographically same or distinct districts. Remarkably, clonal spread of food poisoning pathogens between animals and humans was confirmed by population genetic analysis. Consequently, contamination of campylobacters with quinolone resistance and potential virulence genes in poultry production and consumption may increase the risk of infections in humans.

Keywords: *Campylobacter jejuni*, epidemiological relationship, PFGE, MLST, clonal spread, human

Introduction

Campylobacter species are now recognized as the most common causes of bacterial enteric illness in humans, with approximately 90% of cases being caused by *Campylobacter jejuni* (Guévremont *et al.*, 2006). Human *Campylobacter* infections have occurred by various routes including food, water, and environmental contamination. Campylobacters generally colonize avians (wild birds, chickens, turkeys, quails, and ducks) as commensal organisms (Newell and Fearnley, 2003). Particularly, from contaminated chickens in the farm to consuming undercooked chicken meat during processing, campylobacters that survive throughout the food supply chains are major risk factors for human campylobacteriosis.

In South Korea, campylobacteriosis is generally sporadic and rare, with fewer than 10 cases per year since 2007 compared to that in advanced countries (Gwack *et al.*, 2010). However, the KCDC acute diarrhea disease surveillance project (K-EnterNet) has confirmed that the isolation rate of *Campylobacter* has increased dramatically in acute diarrheal patients since 2010 in South Korea. The number of domestic slaughter broilers has been reported to be close to 900 million, with more than 10 birds consumed per person annually (Ministry of Agriculture, Food and Rural Affairs, http://www.mafra.go.kr).

Epidemic clones of *C. jejuni* responsible for human diseases have been characterized by unique outbreak types using genetic tools (Lajhar *et al.*, 2015). Some clones are associated with domestic animals raised for human consumption, primarily broiler chickens. Follow-up studies on the infection sources of campylobacteriosis that might constitute a risk to human health have been performed generally through comparison using pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) (Behringer *et al.*, 2010; Griekspoor *et al.*, 2010; Ozawa *et al.*, 2016). These typing methods have been utilized as reliable genetic tools to discriminate potential outbreak strains warranting epidemiological studies and threatening public health. Increasing prevalence of campylobacteriosis in humans might be closely related with increased consumption of chicken meat. Clones prevalent in humans that can be proven to have originated from animals through molecular analysis will have important implications for public health. Therefore, the objective

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of this study was to evaluate the possibility of human exposure to campylobacters by comparing epidemiological parameters, antimicrobial susceptibilities, virulence potential, and phylogenetic relationships of *C. jejuni* strains isolated from human patients and commercial chickens.

Materials and Methods

Bacterial isolates

Thirty-eight *C. jejuni* strains isolated from chickens were collected from 37 commercial chicken farms of five provinces (Gyeonggi, Chungcheong [Chungbuk and Chungnam], Gyeongsang [Gyeongbuk and Gyeongnam], Jeolla [Jeonbuk and Jeonnam], and Gangwon) from 2009 to 2010 in South Korea. Nineteen of these chicken *C. jejuni* isolates were obtained from local veterinary laboratories. The remaining isolates were isolated from fecal samples from 19 poultry farms. Regional distribution rates to bird species are as follows: 5 strains from broiler breeders (3 from Jeonbuk province and 2 from Chungbuk), 13 strains from layers (4 from Chungbuk, 3 from Gyungbuk, 3 from Gyeonggi, 2 from Chungnam, 1 from Jeonbuk, and 1 from Gangwon) and 20 strains from commercial broilers (12 from Jeonbuk, 6 from Jeonnam, 1 from Gyeonggi, and 1 from Chungnam). Thirty-nine *C. jejuni* strains (2007–2010) isolated from enteritis patients of a national university hospital were kindly provided by Jeollabukdo Institute of Health and Environment Research, South Korea.

Isolation and identification

Fecal samples taken from chickens were pre-enriched in 10 ml of Preston *Campylobacter* selective enrichment broth (Oxoid Ltd.) containing horse blood SR0048, Preston *Campylobacter* selective supplement SR0117, and growth supplement SR0232 at 42°C for 48 h in a GasPak jar containing a microaerobic gas mixture of 6% O_2, 7.1% CO_2, and 3.6% H_2 using Anoxomat equipment (Mart Microbiology B.V.). A loopful of the pre-enriched broth was streaked onto charcoal cefoperazone deoxycholate agar (CCDA; Oxoid Ltd.). The plates were incubated in a microanaerophilic atmosphere at 42°C for 48 h. Bacterial colonies that grew on CCDA medium were used to identify *Campylobacter* species following biochemically oxidase and catalase tests. Template DNAs used for PCR were prepared by boiling the colonies for 10 min followed by centrifugation at 12,000 rpm for 10 min. The identification of *Campylobacter* genomic species was performed with detection kits for three *Campylobacter* species, *C. jejuni*, *C. coli*, and *C. lari* (iNtRON Biotechnology).

Antimicrobial susceptibility testing

Minimum inhibitory concentrations (MICs) for all *C. jejuni* isolates were tested using Sensititre susceptibility plates (Sensitire; Trek Diagnostic Systems) containing nine antimicrobial agents (azithromycin [AZI], ciprofloxacin [CIP], erythromycin [ERY], gentamicin [GEN], tetracycline [TET], florfenicol [FNN], nalidixic acid [NA], telithromycin [TEL], and clindamycin [CLI]). The MIC ranges and interpretive criteria used for antimicrobial susceptibility testing of *Campylobacter* followed the Clinical Laboratory and Standards Institute (CLSI) M23 guidelines and the National Antimicrobial Resistance Monitoring System (NARMS) protocol. Blood agar plate subcultures of isolates at 48 h were suspended in Mueller Hinton broth to obtain a turbidity equivalent to a 0.5 McFarland standard. Briefly, 50 µl of suspension was inoculated in 11 ml Sensititre CAMHBT Mueller Hinton broth and 100 µl of the mixed suspension was inoculated into wells of the Sensititre plate to give an inoculum of 5×10^5 CFU/ml. Incubation was carried out in a microanaerophilic atmosphere at 42°C for 24 h. MICs to antimicrobials were analyzed using the Sensitire semi-automated system (TREK Diagnostic Systems, Inc.) for *Campylobacter* according to the manufacturer's introductions. *C. jejuni* ATCC 33560 was included as a quality control strain.

Virulence gene detection

A total of 11 virulence genes (*cdtA*, *cdtB*, *cdtC, flaA*, *cadF*, *racR*, *dnaJ*, *virB11*, *ciaB*, *pldA*, and *wlaN*) were detected in *C. jejuni* isolates by PCR. Template DNAs for PCR were extracted by the conventional boiling method. All PCR genes were amplified using each single set of gene-specific primers according to the procedure described previously (Talukder *et al*., 2008). *C. jejuni* ATCC 43429 was included as a control strain for detecting virulence genes, except for *virB11*. Briefly, all PCR amplifications were performed using a PCR premix (*AccuPower* HotStart PCR PreMix, Bioneer Co.) consisting of 2 µl of boiled template DNA and 0.5 µl of a 10 pM solution of each primer. Distilled water was added to make a final volume of 25 µl. The reaction mixture was subjected to 30 cycles of amplification in a Mastercycler EP Gradient 384 DNA thermal cycler (Eppendorf Co.). PCR cycling conditions were denaturation at 94°C for 1 min, annealing at a temperature specific for each primer pair for 1 min, and extension at 72°C for 1 min (Datta *et al*., 2003).

MLST

MLST was carried out by PCR followed by sequencing of the internal fragments of *aspA*, *glnA*, *gltA*, *glyA*, *pgm*, *tkt*, and *uncA* genes of *C. jejuni* (Dingle *et al*., 2001). Briefly, genomic DNA extracted from each *C. jejuni* isolate was mixed with 1× *Ex Taq* PCR buffer, 2.5 mM $MgCl_2$, 20 pmol of each primer, 0.25 mM deoxyribonucleotide triphosphate, and 1 U of TaKaRa *Ex Taq* polymerase (TaKaRa Bio Inc.). PCR conditions were 35 cycles of denaturation at 94°C for 120 sec, annealing at 50°C for 60 sec, and extension at 72°C for 60 sec. PCR products were purified using the QIAquick PCR purification kit (QIAGEN GmbH) and sequenced. Allele, sequence type (ST), and clonal complex assignment were identified using the PubMLST database (http://pubmlst.org/campylobacter/). Novel alleles and STs were submitted to the PubMLST *C. jejuni*/*C. coli* databases. Minimum spanning tree was used to cluster *Campylobacter*.

PFGE

PFGE was conducted according to the CDC PulseNet standardized procedure for molecular subtyping of *C. jejuni* using the CHEF Mapper apparatus (Bio-Rad Laboratories). Genomic DNA was digested with *Sma*I (Roche Diagnostics

Table 1. MICs and the percentage of resistance (% R) of *Campylobacter jejuni* strains isolated from humans and chickens

Campylobacter origins	MIC_{90} / % resistance to antimicrobials [resistant breakpoints (μg/ml)][a]								
	AZI (≥ 8)	CIP (≥ 4)	ERY (≥ 32)	GEN (≥ 8)	TET (≥ 16)	FNN[b] (≥ 8)	NA (≥ 64)	TEL (≥ 16)	CLI (≥ 8)
Human (*n*=39)	0.12/0	32/100	1/0	≤ 0.12/0	≥ 64/82.1	2/0	≥ 64/100	2/0	0.5/0
Chicken (*n*=38)	0.12/2.6	32/84.2	2/2.6	≤ 0.12/7.9	≥ 64/86.8	4/10.5	≥ 64/89.5	2/2.6	0.5/2.6

[a] AZI, Azithromycin (MIC range, 0.015 to 64 μg/ml); CIP, Ciprofloxacin (0.015 to 64); ERY, Erythromycin (0.03 to 64); Gen, Gentamicin (0.12 to 32); TET, Tetracycline (0.06 to 64); FFN, Florfenicol (0.03 to 64); NAL, Nalidixic acid (4 to 64); TEL, Telithromycin (0.015 to 8); and CLI, Clindamycin (0.03 to 16).
[b] According to the NARMS criteria, isolates with a florfenicol MIC ≥ 8 μg/ml are reported as nonsusceptible.

GmbH). *Xba*I-digested DNA from *Salmonella braenderup* H9812 was used as the standard size. Agarose plugs were transferred to cell lysis buffer (50 mM Tris, 50 mM EDTA [pH 8.0], 1% sarcosine, 0.1 mg of proteinase K/ml) and incubated at 54°C for 15 min in an orbital shaker water bath. After washing four times at 54°C for 20 min in a shaking water bath, these agarose plugs were digested with 40 U of *Sma*I at room temperature for 2 h for *C. jejuni* isolates and 50 U of *Xba*I at 37°C for 2 h for *Salmonella braenderup* H9812 as a standard size. Agarose gels were run in auto-algorithm mode (low molecular weight, 30 kb; high molecular weight, 600 kb) under the following conditions: 0.5× Tris-borate-EDTA buffer at 14°C and 6 V/cm for 19 h. The gel images were stored electronically as TIFF files and bands were analyzed using the BioNumerics software version 4.0 (Applied Maths) with the Dice coefficient and the unweighted pair-group method with arithmetic mean.

Results

Antimicrobial susceptibilities of *C. jejuni* isolates from humans and chickens

Results of antimicrobial susceptibility testing for a total of 77 *C. jejuni* isolates from humans and chickens are shown in Table 1. Most of the isolates were highly resistant to ciprofloxacin, nalidixic-acid, and tetracycline. Thirty-nine human *C. jejuni* isolates were resistant to nalidixic acid (100%, MIC ≥ 64 μg/ml), ciprofloxacin (100%, MIC 8 to 64 μg/ml), and tetracycline (82.1%, MIC 32 to 64 μg/ml). However, they were not resistant to the rest of antibiotics tested. One (2.6%) of the 38 chicken *C. jejuni* isolates was resistant to most antibiotics tested, including erythromycin, azithromycin, telithromycin, and clindamycin. Three chicken *C. jejuni* isolates were resistant to florfenicol (MIC ≥ 8 μg/ml).

Frequency of virulence genes

The presence of virulence-associated genes in *C. jejuni* isolates are shown in Table 2. The cytolethal distending toxin (CDT) gene was detected in all (100%) human isolates and 37 (97.4%) of 38 chicken *C. jejuni* isolates. The other chicken isolate of *C. jejuni* only had *cdtA* and *cdtB* by multiplex and single PCR, respectively. More than 94% of all isolates contained the following four adherence and colonization associated virulence genes: *flaA*, *cadF*, *racR*, and *dnaJ*. Both *cadF* and *dnaJ* were found in all isolates tested. Invasion-associated gene *ciaB* was found in 6 (15.4%) of 39 isolates from humans and 6 (15.8%) of 38 isolates from chickens. However, *virB11* was not detected in any isolate.

PFGE clustering

We estimated clonal relatedness between human and chicken isolates of *C. jejuni* by phylogenetic analysis based on PFGE and MLST. In the present study, the genetic diversity of the 77 *C. jejuni* isolates tested were classified into 39 PFGE types using a dice method with 90% similarity (Fig. 2). Of the 77 isolates, 27 human isolates were clustered into 6 PFGE types (I, X, XII, XVI, XXXVI, and XXXVIII) while 12 chicken isolates were clustered into 4 PFGE types (I, XI, XXVI, and XXX). PFGE type I was the most predominant type and included 10 human isolates and 5 chicken isolates. Four PFGE types (I, VII, XXII, and XXVIII) were clonally distributed between human and chicken isolates which were distributed in the same locations or other regions.

STs

The minimum spanning trees based on MLST data for the cluster analysis of *C. jejuni* isolates are depicted in Fig. 1. MLST of the 77 *C. jejuni* isolates resulted in 14 STs for the 39 human isolates and 23 STs for the 38 chicken isolates (Table 3). Ten of these STs (from ST-5227 to ST-5236) have not been previously described. A total of 2 (2.6%) of the 39 human isolates and 8 (10.4%) of the 39 chicken isolates collected in South Korea resulted in new STs. ST-48 was found for 3 (3.9%) isolates from Iksan. ST-3503 was found for 7 (9.1%) isolates from Imsil. ST-354 was found for 3 (3.9%) isolates from Wan-ju. ST-5227 was found for 2 (2.6%) isolates from Wanju. These 15 isolates originated from students infected by school meals in three regions of Jeonbuk province. ST-21 was predominantly found for 10 (13.0%) iso-

Table 2. Frequency of eleven pathogenic-associated virulence genes in *Campylobacter jejuni* isolates

Campylobacter origins	No. (%) of virulence genes								
	Adherence and colonization					Invasion			Biosynthesis
	cdtA,B,C	*flaA*	*cadF*	*racR*	*dnaJ*	*virB11*	*ciaB*	*pldA*	*wlaN*
Human (*n*=39)	39 (100)	39 (100)	39 (100)	38 (97.4)	39 (100)	0 (0)	6 (15.4)	36 (92.3)	2 (5.1)
Chicken (*n*=38)	37 (97.4)	37 (97.4)	38 (100)	36 (94.7)	38 (100)	0 (0)	6 (15.8)	25 (65.8)	2 (5.3)

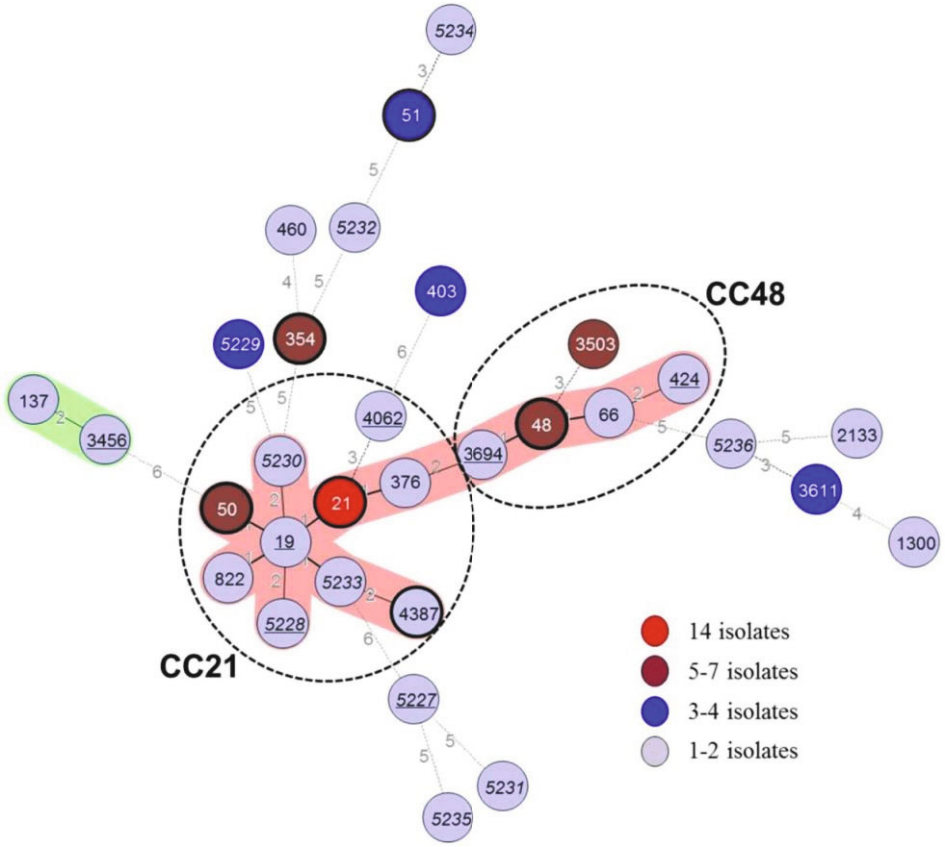

Fig. 1. Minimum spanning tree of MLSTs of 77 *Campylobacter jejuni* isolates from humans and chickens. The six thick circles (STs 21, 48, 50, 51, 354, and 4387) are overlapping STs between human and chicken isolates. The underlined circles (STs 19, 424, 3456, 3694, 4062, 5227, and 5228) are STs identified from human isolates. The italic STs (822, 5227, 5228, 5229, 5230, 5231, 5232, 5233, 5234, 5235, and 5236) are new sequence types identified in this study.

lates obtained from hospital patients with food poisoning. STs overlapped between isolates from humans and chickens were ST-21 (CC21), ST-48 (CC48), ST-50 (CC21), ST-51 (CC443), and ST-354 (CC354), with ST21 belonging to CC21 being the most predominant ST among human and chicken isolates. Most of these STs were included in CC21 and CC48, showing clustering in the minimum spanning tree.

Comparison of genetic relatedness between human and chicken *C. jejuni* isolates

Comparison of genetic relatedness between PFGE and MLST revealed that all clustered PFGE types showed almost identical ST types (Fig. 2). Of these types, PFGE types I, VII, XXII, and XXVIII containing both human and chicken isolates of *C. jejuni* corresponded to ST-21, ST-48, ST-354, and ST-51, respectively. However, some MLST types (ST-48, ST-354, and ST-51) failed to correspond with PFGE types. PFGE types I, X, XII, XVI, XXXVI, and XXXVIII were revealed in 27 human *C. jejuni* isolates. They corresponded to MLST types ST-21, ST-48, ST-3503, ST-354, ST-5227, and ST-424, respectively. However, PFGE type VII, XXII, and XXVIII corresponded with ST-48, ST-354, and ST-51, respectively. Of these groups, ST-354 and ST-51 had almost the same MIC values against all antimicrobials tested and the same virulence genes detected. In this study, the same clones among the same or heterogeneous species were found through PFGE and MLST.

Discussion

Our results indicated that all *C. jejuni* isolates tested were highly resistant to quinolones. All human isolates of *C. jejuni* were resistant to nalidixic acid and ciprofloxacin, whereas 89.5% and 84.2% of the chicken isolates of *C. jejuni* were resistant to nalidixic acid and ciprofloxacin, respectively. The use of fluoroquinolones in food-producing animals has resulted in fluoroquinolone-resistant *Campylobacter* strains worldwide (Hannula and Hänninen, 2008). The proportion of quinolone-resistant *C. jejuni* isolates from humans in the US was increased from 1.3% in 1992 to 10.2% in 1998 while ciprofloxacin-resistant *C. jejuni* was isolated from 14% of 91 domestic chicken products obtained from retail markets in 1997 (Smith *et al.*, 1997). Fluoroquinolone-resistant *Campylobacter* strains were reported in 22% of poultry and 75% of pig farms in the United Kingdom (Taylor *et al.*, 2008). In South Korea, ciprofloxacin-resistant *C. jejuni* isolates were recently found in 24% of human patients with campylobacteriosis (Shin *et al.*, 2013). The increases in quinolone-resistant *C. jejuni* infections in the US, Europe, and Asia are likely driven by the acquisition of resistant strains from poultry. Resistance to ciprofloxacin in this study was found in all human *C. jejuni* isolates. The incidence of fluoroquinolone-resistant *C. jejuni* isolates from humans differed among local communities. The frequency of ciprofloxacin-resistant *C. jejuni* isolates from chickens were also high (84.2%) in South Korea compared with that in the aforementioned countries. The use of fluoroquinolones, such as enrofloxacin, in poultry

Table 3. MLSTs identified from *Campylobacter jejuni* isolates

Origin	ST[a]	Allele[b]							No. of strains	CC type
		aspA	*glnA*	*gltA*	*glyA*	*pgm*	*tkt*	*uncA*		
Human (n=39)	19	2	1	5	3	2	1	5	1	21
	21	2	1	1	3	2	1	5	10	21
	50	2	1	12	3	2	1	5	1	21
	4062	2	27	1	4	19	1	5	1	21
	4387	2	17	52	389	2	1	5	1	21
	48	2	4	1	2	7	1	5	6	48
	3503	7	4	52	2	11	1	5	7	48
	3694	2	2	1	2	7	1	5	1	48
	424	3	4	5	2	74	1	5	2	48
	3456	4	7	12	4	1	7	1	1	45
	5228	2	4	5	38	2	1	5	1	206
	354	8	10	2	2	11	12	6	4	354
	51	7	17	2	15	23	3	12	1	443
	5227	22	17	4	64	90	25	52	2	-
Chicken (n=38)	21	2	1	1	3	2	1	5	4	21
	50	2	1	12	3	2	1	5	4	21
	376	2	2	1	3	2	1	5	1	21
	822	2	1	79	3	2	1	5	1	21
	4387	2	17	52	389	2	1	5	1	21
	5230	8	1	2	3	2	1	5	1	21
	5233	2	17	5	3	2	1	5	1	21
	48	2	4	1	2	7	1	5	1	48
	66	2	4	5	2	7	1	5	1	48
	137	4	7	10	4	42	7	1	1	45
	5232	63	25	2	10	22	3	6	1	52
	354	8	10	2	2	11	12	6	2	354
	403	10	27	16	19	10	5	7	3	403
	51	7	17	2	15	23	3	12	2	443
	5234	24	17	335	10	23	3	12	1	443
	460	24	30	2	2	89	59	6	1	460
	3611	9	2	5	333	11	3	1	3	607
	5236	55	2	5	2	11	59	1	1	607
	5231	8	61	4	28	74	25	23	1	1034
	1300	9	2	114	147	11	188	86	1	-
	2133	55	21	2	71	11	37	3	1	-
	5229	8	2	2	212	10	253	147	4	-
	5235	8	367	292	64	470	25	1	1	-

[a,b] New sequence types and allele identified in this study are italic and boldface / CC, clonal complex

has created a reservoir of resistant *C. jejuni*. The prevalence of macrolide resistance among human and food animal *C. jejuni* isolates has been reported to be generally very low in contrast to *C. coli* in Denmark, US, or Canada (Belanger and Shryock, 2007), consistent with our data. Strains resistant to azithromycin and telithromycin have been reported to be few in hospitalized patients with diarrhea from India, whereas strains resistant to quinolone and fluoroquinolone are more prevalent (Mukherjee *et al*., 2013). As a result, strains resistant to several macrolide antibiotics used for the treatment of campylobacteriosis have rarely emerged and the prevalence of ciprofloxacin-resistant *C. jejuni* strains varies in different countries.

Pathogenicity-associated virulence factors and toxin genes have been found in *C. jejuni* isolates. Virulence and toxin-associated positive factors from *C. jejuni* include cytolethal distending toxins (*cdtA*, *cdtB*, and *cdtC*), *Campylobacter* adhesion factor (*cadF*), and *Campylobacter* invasive antigens (*cia*) (Dasti *et al*., 2010). In this study, adherence and colonization associated virulence factors *flaA*, *cadF*, *racR*, and *dnaJ* and cytolethal distending toxin genes *cdtA*, *cdtB*, and *cdtC* were detected in > 97% of human and chicken isolates of *C. jejuni*. However, the prevalence of *pldA* gene encoding a protein with phospholipase activity was somewhat different between human and chicken isolates. Grant *et al.* (1997) have demonstrated that phospholipase A activity contributes to cell-associated hemolysis of *C. coli,* although the mechanism involved in the hemolytic activity of *pldA* of *C. jejuni* strains remains unclear. The presence of *ciaB*-positive strains in this study was very low in both human (6/39, 15.4%) and chicken (6/38, 15.8%) isolates of *C. jejuni*. Both *virB11* and *ciaB* are plasmid-associated genes. It has been reported that

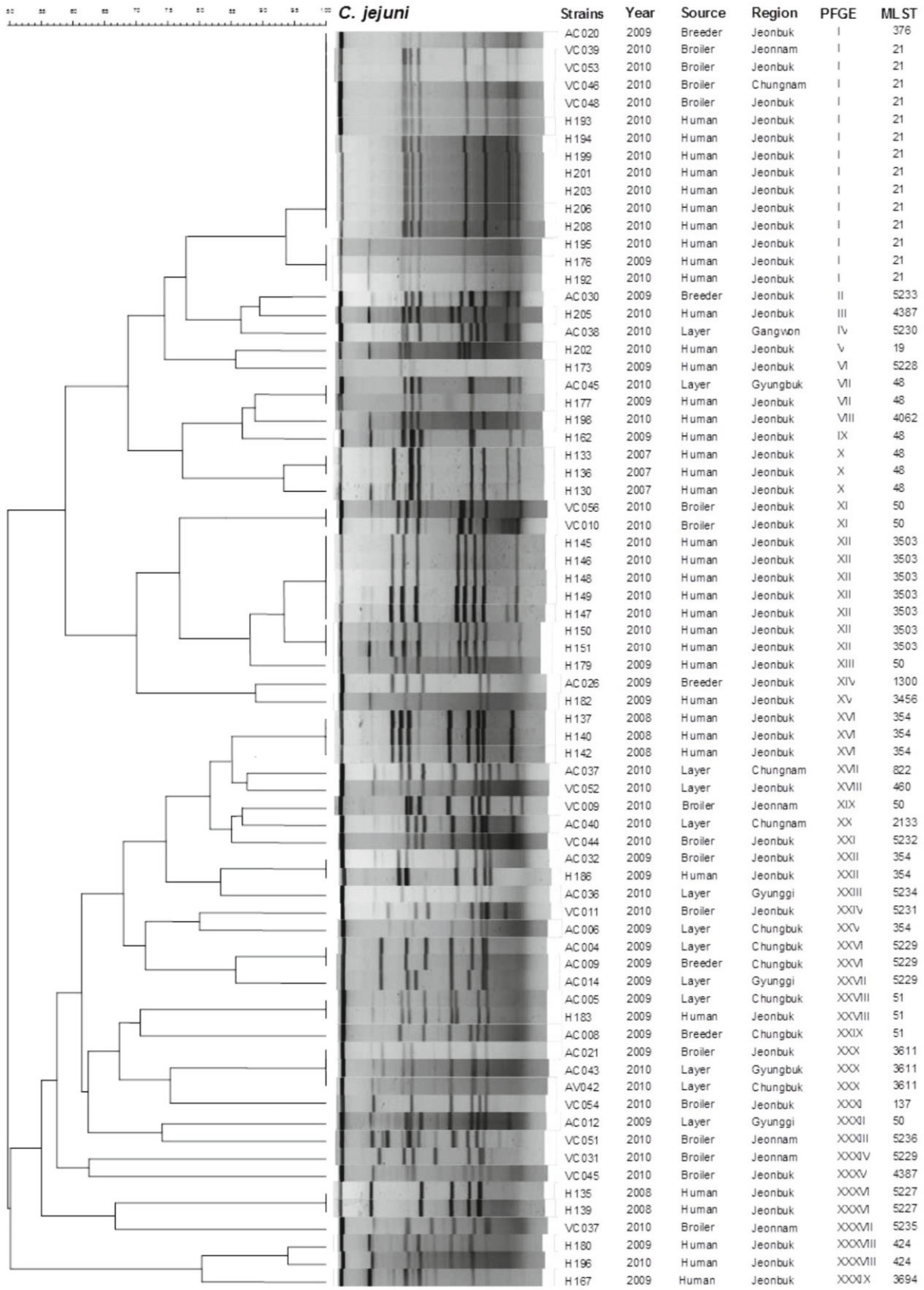

Fig. 2. **Dendrogram patterns by *Sma*I-PFGE of *Campylobacter jejuni* strains isolated from humans and chickens in South Korea.**

ciaB is present in the majority (164/166, 99%) of *C. jejuni* isolated from of Finnish patients (Feodoroff *et al*., 2010). The *ciaB* gene has been recognized as an important virulence factor of *C. jejuni* strains that lead to enteritis in humans (Feodoroff *et al*., 2010). In this study, *ciaB*-positive human isolates were classified into two PFGE groups: type X (ST48) and type XXXVI (ST5227), whereas *ciaB*-positive chicken isolates were classified into four different groups: type I (ST376) from a breeder, type II (ST5233) from a breeder, type VII (ST48) from a layer, and type XXX (ST3611) from two layers. There was no clonal relation between *ciaB*-positive human and chicken isolates by phylogenetic analysis. Although the distribution of virulence factors has been demonstrated in many countries, little research has been conducted on the virulence mechanism or pathogenesis of *C. jejuni*. The present study revealed that the distribution of important virulence genes *cdtB*, *flaA*, *cadF*, *racR*, *dnaJ*, *ciaB*, and *pldA* was not dissimilar between human and chicken isolates of *C. jejuni*. This study highlights that many pathogenic *C. jejuni* might harbor these genes. In addition, *Campylobacter* strains derived from chicken origins might have pathogenic potential as food poisoning pathogens to humans.

Fourteen STs and two major Clonal Complexes (CC21 and CC48) were identified from human isolates in this study. CC48 including four STs (ST3053, ST48, ST424, and ST3694) was found in 41.0% (16/39) of human *C. jejuni* isolates, followed by CC21 including 5 STs (ST21, ST19, ST50, ST4062, and ST4387) which was found in 35.9% (14/39) of the human isolates. However, CC21 including 7 STs (ST21, ST50, ST376, ST822, ST4387, ST5230, and ST5233) was dominant in chicken *C. jejuni* isolates (13/38, 34.2%). The most dominant clonal complex in all human and chicken isolates was CC21 (27/77, 35.0%) including the 13 STs. Of these STs, the most common one was ST21 in both human and chicken isolates. ST21 has been reported in isolates from humans and chickens (Piccirillo *et al*., 2014). However, the STs of human *C. jejuni* domestic isolates collected from 2007 to 2009 hardly matched with each other and STs from other human and chicken isolates of *C. jejuni* in this study, except for ST21 and ST48. Previous reports have shown that there are regional differences in resistance patterns of *C. jejuni* isolates in a country, indicating the presence of diverse clones or STs between regions (Taylor *et al*., 2008; Cha *et al*., 2014). Moreover, *C. jejuni* isolates from human gastroenteritis outbreaks in 2012 in South Korea had different STs that were associated with travel destinations of the patients (Cha *et al*., 2014). As a result, as *C. jejuni* clones causing gastroenteritis outbreaks have also been identified regionally or seasonally as different STs, campylobacteriosis is likely to be caused by sporadic clones other than by epidemic clones.

The epidemiology of *C. jejuni* has been studied with various molecular genetic tools (*fla*A-restriction fragment length polymorphism analysis, repetitive PCR, PFGE, and MLST). Recent phylogenetic studies using relatedness between PFGE and MLST have revealed that the two methods have effective discriminatory power in evaluating the genetic homology among *C. jejuni* strains (Behringer *et al*., 2010). Although the PFGE analysis method generally uses two known restriction enzymes (*Kpn*I and *Sma*I), no great difference in clustering a group according to PFGE types by *Sma*I digestion or STs has been reported for *Campylobacter* stains (Serichantalergs *et al*., 2010; Ozawa *et al*., 2016). In this study, the *C. jejuni* strains originated from humans and chickens were confirmed to be clonally related by comparing the results generated by PFGE and MLST. The most important issue of this study is that it reminds us that food poisoning bacteria could be dangerously passed from chickens to humans through the transmission of chicken-derived clones.

As mentioned earlier, causative organisms isolated from herd infection with food poisoning were revealed to be the same clones by epidemiologic relatedness. The four genogroups (PFGE type I–ST21, PFGE VII-ST48, PFGE XXII-ST354, and PFGE XXVIII-ST51) from humans showed high genetic homologies with chicken isolates by comparing PFGE and MLST results. Among these groups, isolates belonging to PFGE type I-ST21 were considered as prevalent or dominant clones rather than epidemic clones in a local community, whereas chicken isolates belonging to a single clone PFGE type XXX-ST3611 were obtained from geographically diverse farms. *Campylobacter* present in the intestines of chickens can contaminate chicken products and environments and subsequently cause infections in humans and chickens (Corry and Atabay, 2001). Several regions (Jeonbuk, Chungnam, and Chungbuk) harboring the same clones are geographically diverse but adjacent to each other. Those regions account for 40% of domestic broiler chicken production. Therefore, there may be various factors, such as transport and slaughter, implicated in the horizontal transmission of *C. jejuni* throughout the nation. Likewise, *C. jejuni* isolates derived from humans of a local region (Jeonbuk) shared PFGE types I, VII, XXII, and XXVIII with chicken *C. jejuni* isolates from the same or different regions (Jeonnam, Chungnam, Chungbuk, and Gyungbuk). In addition, PFGE type XI-ST50 originated from both fecal and raw meat samples of broiler chickens in a region (data not shown), indicating the importance of hygiene during the slaughtering process. Four genogroups (PFGE type X-ST48, PFGE type XII-ST3503, PFGE type XVI-ST354, and PFGE type XXXVI-ST5227) originating from campylobacteriosis patients were not observed in chicken *C. jejuni* isolates in this study. However, most genotypes of other human isolates were also found in chicken isolates tested. All *C. jejuni* isolates obtained between 2007 and 2008 were from humans and their PFGE and MLST genotypes were different from those of other human or chicken isolates obtained between 2009 and 2010. In contrast, the genotypes of *C. jejuni* isolates obtained between 2009 and 2010 are much overlapped between human and chicken isolates as shown in the Fig. 2, revealing evidence that human isolates could come from chickens.

In conclusion, several *C. jejuni* clones originating from humans and chickens displayed considerable molecular epidemiologic relatedness. Contamination of campylobacters with quinolone resistance and potential virulence genes during poultry production and consumption may increase the risk of infection to humans. By expanding the isolation and identification of these bacteria in colitis patients, more comprehensive study based on the genetic correlation between isolates from humans and food animals may be needed to prevent and control diseases caused by them.

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