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Amplified Fragment Length Polymorphism, Serotyping, and Quinolone Resistance of *Campylobacter jejuni* and *Campylobacter coli* Strains from Chicken-Related Samples and Humans in Taiwan

SHAO W. FANG,[1]* CHING J. YANG,[1] DANIEL Y. C. SHIH,[1] CHENG C. CHOU,[2] AND ROCH C. YU[2]

[1]*Division of Food Microbiology, Bureau of Food and Drug Analysis, Department of Health, Executive Yuan, Taipei 115, Taiwan, Republic of China; and* [2]*Graduate Institute of Food Science and Technology, National Taiwan University, Taipei 106, Taiwan, Republic of China*

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ABSTRACT

The high-resolution genotyping method of amplified fragment length polymorphism (AFLP) analysis was used to study the genetic relationships between *Campylobacter jejuni* isolates from chicken-related samples ($n = 32$) and humans ($n = 27$) as well as between *Campylobacter coli* isolates from chicken-related samples ($n = 27$) and humans ($n = 5$). These isolates were collected between 1994 and 2003 in Taiwan. All *C. jejuni* and *C. coli* isolates showed highly heterogeneous fingerprints. *C. jejuni* isolates were separated in two distinct genetic clusters (A and B) at 40% genetic similarity and 42 different AFLP types at 90% similarity. However, three clusters at 40% genetic similarity and 33 different AFLP types at 90% similarity were observed in *C. coli* isolates. These results showed that AFLP analysis could be used to identify individual isolates of two *Campylobacter* species. Among *C. jejuni* isolates, the predominant AFLP type 1 was observed in five (7.9%) isolates, and types 5 and 12 in four (6.3%) isolates each. Cluster B consisted of 10 isolates, while the majority of isolates ($n = 53$) belonged to cluster A. In some AFLP types (1, 5, 12, 14 and 31), AFLP fingerprints of chicken-related isolates were closely related genetically to those of isolates from humans with gastroenteritis. The predominant serotypes in *C. jejuni* isolates were B:2 and Y:37. All isolates belonging to serotype O:19 grouped into one single AFLP type. Some chicken samples yielded multiple isolates of *Campylobacter* harboring simultaneously quinolone-resistant and quinolone-sensitive isolates attributed to the same species, or harboring *C. jejuni* and *C. coli* that have the characteristics of quinolone resistance.

Campylobacter jejuni is the leading cause of human bacterial gastroenteritis in developed countries *(32, 43, 46).* It is commonly found in the intestinal contents of many domestic and wild animals and typically causes an acute enterocolitis accompanied by fever and abdominal cramping lasting 3 to 5 days *(19, 42).* Serious consequences of campylobacteriosis can be the development of the Guillain-Barré and Miller-Fisher syndromes *(28, 50).* Most human infections are apparently sporadic cases, the distribution of which shows a seasonal variation. In a few cases, the transmission routes from animal hosts and environmental sources to humans have not been determined, but epidemiological studies and data from outbreaks indicate that contaminated drinking water, unpasteurized milk, and eating or handling contaminated poultry products are important risk factors associated with human infections *(20, 46).* In Taiwan, Tang et al. *(45)* isolated *C. jejuni* from 35 (5.6%) of 623 patients in the Taipei area with a history of acute diarrhea between March 1981 and December 1981. The incidences of *C. jejuni* in chicken, duck, cut pork meat, ground pork meat, and raw milk were found to be 55, 20, 10, 0, and 30%, respectively *(1).*

Subtyping of *C. jejuni* strains supports epidemiological studies for tracing sources and transmission routes of infections. Serotyping, phage typing, and molecular typing of *Campylobacter* isolates from human and animal sources have revealed that *C. jejuni* is highly heterogeneous *(8, 10, 34).* Serotyping and other means of typing *Campylobacter* spp. have played an important role in understanding the epidemiology and identifying the sources of *Campylobacter* infection. Numerous typing schemes have been developed, and the usefulness of these methods has been reviewed *(24, 35, 49).* The most commonly used methods today include serotyping to detect heat-labile antigens *(26)* or O (formerly called heat-stable) antigens *(8, 37).* These methods have been applied widely in epidemiologic investigations *(35, 36),* but they require appropriate serotyping reagents that are expensive and extraordinarily time-consuming to produce. Furthermore, only a few reference laboratories can provide serotyping, and such services are not readily available to investigators. A widely available alternative method that would be simple to perform, that would not require specialized reagents (i.e., antisera), and that could be used for many applications is highly desirable *(35).*

Molecular methods have increasingly been used as alternatives to serologic methods to type *Campylobacter* strains. Amplified fragment length polymorphism (AFLP) is a suitable genomic fingerprinting technique used for *Campylobacter* typing, which, by combination of DNA re-

* Author for correspondence. Tel: 886-2-2653-1271; Fax: 886-2-2653-1268; E-mail: ufpfang@nlfd.gov.tw.

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TABLE 1. *Sequences and modifications of oligonucleotide adapters and primers used for AFLP*

Oligonucleotide	Sequence and modification
*Hin*dIII specific adapter	5′-CTC GTA GAC TGC GTA CC-3′
	5′-AGC TGG TAC GCA GTC-3′
*Hha*I specific adapter	5′-GAC GAT GAG TCC TGA TCG-3′
	5′-ATC AGG ACT CAT CG-3′
*Hin*dIII preselective primer	5′-GAC TGC GTA CCA GCT T-3′
*Hha*I preselective primer	5′-GAT GAG TCC TGA TCG C-3′
*Hin*dIII selective primer (FAM-*Hin*dIII+A)	5′-FAM-GAC TGC GTA CCA GCT TA-3′
*Hha*I selective primer (*Hha*I+A)	5′-GAT GAG TCC TGA TCG CA-3′

striction with one or more restriction enzymes and the use of a selective PCR, amplifies a subset of chromosomal fragments *(41, 48).* AFLP has been recently applied to study on *C. jejuni* strains from different sources and was shown to be a highly discriminatory technique for the analysis of *C. jejuni* and *C. coli* strains *(6, 17, 21).* Increasing antimicrobial resistance in *Campylobacter* is a recognized problem, and quinolone resistance is common in human isolates of *C. jejuni* and *C. coli (40, 47).* In Taiwan, cross-resistance between nalidixic acid and ciprofloxacin was found in 66% of human clinical *C. jejuni* isolates *(25).* The purposes of this study were to (i) establish the distributions of common AFLP types of *C. jejuni* and *C. coli* isolated from chicken-related samples and humans in Taiwan, (ii) identify the most common serotypes of *C. jejuni* isolates in Taiwan, and (iii) investigate the relationships among AFLP types, serotypes, and quinolone resistance of *C. jejuni* isolates and between AFLP types and quinolone resistance of *C. coli* isolates.

MATERIALS AND METHODS

Bacterial isolates, phenotypic typing, and culture conditions. A total of 59 *C. jejuni* and 32 *C. coli* isolates were collected from feces of patients (including adults and children) with diarrhea and from chicken-related samples (whole chicken, viscera, drumstick, wing, and chest from markets, slaughter, or food processing plants as well as washing water or cooling pool of slaughter plants) during 1994 and 2003. In addition, three *C. jejuni* and two *C. coli* isolates were obtained from aquatic samples in 2001. Furthermore, the reference strains *C. jejuni* ATCC 33292 and *C. coli* D1373 were obtained from Dr. Peter Feng (U.S. Food and Drug Administration, Washington, D.C.). All colonies morphologically similar to *Campylobacter* species were examined by Gram stain, catalase test, and oxidase test *(15).* The oxidase- and catalase-positive colonies exhibiting the appearance of gram-negative curved, S-shaped, or spiral forms under microscopic examinations can be readily reported as *Campylobacter* species. Phenotypic tests for identification were performed either by the proposed criteria *(13, 15),* including growth at 25, 35 to 37 and 42°C, growth on MacConkey agar, growth in 1% glycine, growth in 3.5% NaCl, production of H_2S, reduction of nitrate, hippurate hydrolysis, indoxyl acetate hydrolysis test, susceptibility to nalidixic acid and/or cephalothin, and utilization of glucose, or by the use of API CAMPY kit (bioMérieux, Marcy l'Etoile, France). In order to confirm species identities, a PCR method was used as described by Stonnet et al. *(44).* Isolates were cultured on heart infusion agar plates (Difco, Becton Dickinson, Sparks, Md.) with 5% (vol/vol) laked horse blood (Oxoid Ltd., Basingstoke, UK) at 42°C for 48 h under microaerophilic conditions provided by the CampyPak Envelope (Becton Dickinson Microbiology Systems, Cockeysville, Md.) or *Campylobacter* gas generating kits (Oxoid) and preserved for reference at −80°C in nutrient broth no. 2 (Oxoid) with 20% glycerol.

Antimicrobial susceptibility tests. All MICs were determined by Etest (AB Biodisk, Solna, Sweden). Before this test, these isolates were subcultured onto heart infusion agar plates (Difco, Becton Dickinson) with 5% (vol/vol) laked horse blood (Oxoid) added and incubated at 42°C for 48 h in a microaerobic atmosphere with gas packs. Colonies were suspended in 5 ml of 0.1% peptone water (Difco, Becton Dickinson) to achieve a turbidity equivalent to a 1.0 McFarland standard. The Mueller-Hinton agar (Difco, Becton Dickinson) plates with 5% laked horse blood were inoculated with sterile nontoxic swabs using the standard suspensions. When the inoculated agar surface was dry, two Etest strips were applied on each plate, and plates were incubated at 42°C for 18 to 24 h under microaerobic conditions. After incubation, MICs were read at the point of intersection between the zone edge and the Etest strip. The antimicrobial agents tested in this study were ciprofloxacin and nalidixic acid. Quality control was performed using *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922. The breakpoints were determined according to the criteria of the NCCLS *(31).*

Serotyping. *C. jejuni* isolates were subsequently serotyped based on heat-stable Penner's antigens by passive hemagglutination using a serotyping set including 25 antisera (*Campylobacter* Antisera Set, Denka Seiken Co., Tokyo, Japan) as the protocol recommended by the manufacturer.

Isolation of chromosomal DNA. Cells were scraped from fresh grown plates, and DNA was isolated with the PUREGENE DNA isolation kit (Gentra Systems, Minneapolis, Minn.). DNA integrity was checked by agarose gel electrophoresis, and DNA preparations were stored at −20°C. DNA concentrations were determined with a spectrophotometer.

AFLP. AFLP was performed as described in the method of Hein et al. *(12).* A total of 25 ng of genomic DNA digested and ligated in 14 μl of digestion mixture containing 5 U of *Hin*dIII (New England BioLabs [NEB], Hitchin, Hertfordshire, England), 5 U of *Hha*I (NEB), 0.5 μl of 10× bovine serum albumin (NEB), 0.5 M NaCl, 1.5 μl 10× T4 ligase buffer (NEB), 1 U of T4 ligase, 2 μl of 4 μM *Hin*dIII–restriction site-specific adapter, and 2 μl of 40 μM *Hha*I–restriction site-specific adapter (TIB MOLBIOL Syntheselabor, Berlin, Germany) (Table 1) for 2 h at 37°C and then inactivated for 10 min at 65°C. The adapters were modified to allow exponential amplification of heterosite fragments only (TIB MOLBIOL Syntheselabor) (Table 1). A total of 186 μl of distilled H_2O was added to the restriction-ligation mixture. Primers used for preselective and selective PCR are listed in Table 1.

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Preselective PCR was performed in a 20-μl volume containing 2 μl of 10× PCR buffer (PROtech Technology Enterprise Co., Ltd., Taipei, Taiwan), 0.8 μl of 25 mM $MgCl_2$, 1.6 μl of 2.5 mM deoxynucleoside triphosphate (PROtech), 1.25 U of *Taq* DNA polymerase (PROtech), 1 μl of 5 μM *Hin*dIII preselective primer, 1 μl of 50 μM *Hha* preselective primer, and 4 μl of diluted restriction-ligation mixture by using an initial denature step at 94°C for 10 min, followed by 20 cycles of denaturation (94°C for 20 s), annealing (56°C for 30 s), and an extension step (72°C for 2 min). The preselective PCR amplicons were diluted 1:20 in distilled H_2O, and 3 μl was subjected to selective PCR with a master mix of the same concentration as that used for preselective PCR. The *Hin*dIII selective primer, labeled with the blue fluorescent dye 5-carboxyfluorescein (FAM) (TIB MOLBIOL Syntheselabor) at the 5′ end of *Hin*dIII preselective primer, contained an additional A base at the 3′ end (FAM—*Hin*dIII+A) (TIB MOLBIOL Syntheselabor). The *Hha*I selective primer also contained an extra A base at the 3′ end of *Hha*I preselective primer (*Hha*I+A) (TIB MOLBIOL Syntheselabor). Preselective PCR and selective PCR (touchdown PCR) were performed by a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, Calif.). The final selective AFLP products were delivered to a molecular biological laboratory (Seeing Bioscience Co., Ltd., Taipei, Taiwan) for the separation of amplified fragments by capillary electrophoresis on an ABI 3100 automated DNA sequencer (Applied Biosystems) with GeneScan-500 ROX (Applied Biosystems) as the internal size marker. After capillary electrophoresis, the ABI GeneScan fragment analysis software (Applied Biosystems) was applied.

Numerical analysis of AFLP profiles. Only AFLP profiles comprising fragments detected in the size range 50 to 400 bp were considered for numerical analysis. GeneScan-processed data files comprising both bacterial AFLP profiles and internal molecular mass standards were imported into the program BioNumerics 4.0 (Applied Maths, Kortrijk, Belgium). After registration of isolate details, profiles were normalized within and between gels by linking profiles for analysis to their respective molecular mass standard, as described in the software manufacturer's instructions. Normalized AFLP profiles were compared with the Pearson product-moment correlation coefficient and clustered by the unweighted pair group with mathematical average method.

RESULTS

AFLP. AFLP fingerprints were identified as distinct types when the band patterns shared less than 90% similarity, as has been shown by Duim et al. *(6)*. Among 63 *C. jejuni* isolates, 42 different AFLP types were observed in all *C. jejuni* isolates, and 11 AFLP types were present in more than one isolate (Table 2). The predominant AFLP type 1 was observed in five (7.9%) isolates, and types 5 and 12 in four (6.3%) isolates each (Table 2). Cluster analysis revealed two subgroups (A and B) with 40% genetic similarity (Fig. 1). Four isolates (no. 5, 9, 81, and 92) grouped separately from these clusters. Among four isolates, three isolates belonged to R:23, B:2, and P:21 serotypes (Table 2). Cluster B consisted of 10 isolates, while the majority of isolates (n = 53) belonged to cluster A (Table 2). The AFLP patterns of *C. jejuni* isolates from chickens were dispersed all over the dendrogram, and cluster B consisted of only AFLP fingerprints of chicken-related and water isolates (Table 2). In some AFLP types (1, 5, 12, 14, and 31), AFLP fingerprints of chicken-related isolates were closely related to those of isolates from humans with gastroenteritis (Table 2).

Of the 35 *C. coli* isolates studied, genotyping of these isolates by AFLP allowed 33 distinct patterns to be distinguished (Table 3). Three clusters (a, b, and c) of fingerprints were deeply branched at 40% genetic similarity (Fig. 1). The majority of isolates (n = 31) belonged to cluster a, but cluster b and c consisted of only two isolates each (Table 3). All chicken isolates grouped into cluster a, and not into the other clusters (Table 3). The similar AFLP types were observed between some isolates, as is shown in Figure 1 and in Table 3. The levels of genetic similarity between isolate no. 8′ and 10′ and between isolate no. 7′ and 19′ were more than a homology linkage level of 90%. The number of closely related *C. coli* isolates with more than 90% similarity was less than that of related *C. jejuni* isolates in this study.

Discrimination of subspecies was also attained. Isolates of *C. jejuni* and *C. coli* were separated into discrete clusters linked at the 9.1 and 9.2% similarity levels, respectively (Fig. 1). Large differences in band patterns between the two species were apparent, and therefore the linkage level was only 4.6%. The *C. jejuni* ATCC 33292 (no. 98) and *C. coli* (no. 20′) strains grouped within the species cluster. Two heterogeneous groups with a minimal level of linkage between groups of 38.2% were identified within the *C. jejuni* cluster. These results showed that AFLP analysis could be used to identify individual isolates of two *Campylobacter* species.

Serotype. We could not identify the serotypes of *C. coli* isolates because of the lack of commercially available high-quality antisera. Among all *C. jejuni* isolates, a total of 33 isolates was found to react with the antisera against nine serotypes, and 30 isolates (47.6%) remained untypeable (Table 2). B:2 (14 of 33) and Y:37 (7 of 33) were the predominant serotypes and comprised 64% (21 of 33) of the isolates with serotype (Table 2). Some serotypes (A: 1,44; D:4; and L:15) were so uncommon that they together represented a much lesser proportion of all the isolates.

There was no apparent congruence between AFLP genotypes and serotype. Serotype Y:37 was included into AFLP type 1, with the exception of no. 111, which was put into AFLP type 11 (Table 2). Serotype B:2 grouped into AFLP types 5, 6, 7, 8, 9, 12, 14, 19, 20, and 40 (Table 2). Furthermore, most of strains belonging to serotype B:2 were isolated from chicken-related samples, with only two strains being isolated from humans (Table 2). In some cases, identical serotypes could be further differentiated by AFLP.

Among the isolates tested, three O:19 isolates (no. 76, 87, and 88) were found; one came from a raw chicken drumstick and two from water samples, respectively (Table 2). They grouped into AFLP type 38 of the cluster B, and their level of genetic similarity was more than 90% (Fig. 1). There were two J:11 isolates to group into AFLP type that was attributed to cluster A (Table 2).

Quinolone resistance of *C. jejuni* and *C. coli* isolates. Among all *C. jejuni* isolates, AFLP type 1 comprised three

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TABLE 2. *AFLP types, serotypes, and quinolone resistance of 63* C. jejuni *isolates from different sources*

Cluster	AFLP type	Strain no.	Strain designation	Year of isolation	Source	CI and NA resistance[a]	Serotype[b]
A	1	71	S 8-1	2003	Washing water from chicken slaughter plant	R	Y
	1	73	S 10-1	2003	Washing water from chicken slaughter plant	R	Y
	1	51	MMH CDC	2002	Patient	R	Y
	1	61	MMH 0009	2002	Child, male	R	Y
	1	68	S 2-1	2003	Washing water from chicken slaughter plant	R	Y
	2	94	30E-2	1994	Chicken drumstick from market	S	Y
	3	82	CB-1	2002	Chicken breast from chicken slaughter plant	R	NS
	4	98	ATCC 33292	2002	American Type Culture Collection	S	NS
	5	19	MMH 0056	2003	Child, male	R	B
	5	54	MMH-62	2002	Child, male	R	B
	5	66	KK 25-1	2002	Chicken drumstick from food processing plant	R	B
	5	36	MMH 51	2002	Adult, male	R	B
	6	48	MMH 18-24	2002	Child, female	R	B
	6	8	MMH 0012	2003	Child, male	R	B
	7	49	MMH 27-28	2002	Child, male	R	B
	8	28	MMH 91032001	2002	Adult, male	R	B
	9	115	KK 25-2	2002	Chicken drumstick from food processing plant	R	B
	10	119	12A-1	1994	Whole chicken from market	S	NS
	11	111	8-1	2001	Chicken viscera from market	R	Y
	12	80	CGMH 01	1994	Patient	S	NS
	12	89	M S-1	2002	Chicken skin from chicken slaughter plant	S	NS
	12	63	MMH18-106	2002	Child, female	R	NS
	12	83	NG S-1	2002	Chicken skin from chicken slaughter plant	R	B
	13	31	45PF100-1-2	2001	Chicken drumstick from market	R	NS
	14	25	MMH 0081B	2003	Child, female	R	NS
	14	46	MMH 91032006	2002	Child, male	R	B
	14	101	54PF100-2	2001	Chicken drumstick from market	R	NS
	15	10	MMH 0027	2003	Child, female	R	NS
	15	21	MMH 0060	2003	Child, male	R	NS
	16	113	5-2A-1	1994	Whole chicken from market	S	NS
	17	26	CGMH 02	2002	Patient	R	NS
	18	110	MMH 0053	2003	Child, male	R	NS
	19	17	MMH 0046	2003	Child, female	R	B
	20	23	MMH 0052	2002	Child, male	R	B
	21	13	MMH 0036	2003	Child, female	R	A
	22	90	CGMH 03	1995	Patient	S	J
	22	93	CGMH 04	1994	Patient	S	J
	23	12	MMH 0031	2003	Child, male	R	NS
	24	29	S 4-1	2003	Washing water from chicken slaughter plant	R	L
	25	6	20E-1	1994	Chicken viscera from market	R	NS
	26	112	5-2-5	1994	Whole chicken from market	R	NS
	27	24	MMH 0065	2002	Child, male	R	R
	28	117	KK 29-2	2001	Chicken viscera from food processing plant	R	NS
	29	37	KK 29-1	2001	Chicken viscera from food processing plant	R	NS
	30	41	S 14-1	2003	Chicken meat from chicken slaughter plant	R	D
	31	39	KK 32-1	2002	Chicken viscera from food processing plant	R	NS
	31	44	CGMH 05	2002	Patient	R	NS
	32	106	21E-6	1994	Chicken breast from market	R	NS
	33	108	64-2-1	2001	River water sample	R	NS
	34	30	S 13-1	2002	Chicken meat from chicken slaughter plant	R	NS
	35	1	12B-1	1994	Whole chicken from market	S	NS
	35	2	13B-1	1994	Whole chicken from market	S	NS
	35	118	13A-2	1994	Whole chicken from market	S	J
	36	92	CGMH 25	1994	Patient	S	R
B	37	116	S 1-2	2003	Cooling pool of chicken slaughter plant	R	NS
	37	27	S 1-1	2003	Cooling pool of chicken slaughter plant	R	NS
	38	38	KK 31-1	2002	Chicken drumstick from food processing plant	R	P
	39	87	57-2-1	2001	Animal shelter wastewater	S	O
	39	88	58-2-2	2001	Animal shelter wastewater	S	O
	39	76	NG L-1	2002	Chicken drumstick from chicken slaughter plant	R	O
UG[c]	40	5	16AE-4	1994	Chicken breast from market	S	B
	41	81	24E-3	1994	Chicken drumstick from market	S	NS
	42	9	MMH 0025	2003	Adult, male	R	P

[a] CI, ciprofloxacin; NA, nalidixic acid; R, resistance, MIC of CI $\geqq$ 4 μg/ml and MIC of NA $\geqq$ 32 μg/ml; S, susceptibility, MIC of CI $<$ 4 μg/ml and MIC of NA $<$ 32 μg/ml.
[b] NS, nonserotypeable; A, A:1,44; B, B:2; D, D:4, 13, 16, 43, 50; J, J:11; L, L:15; O, O:19; P, P:21; R, R:23, 36, 53; Y, Y:37.
[c] UG, unclustered.

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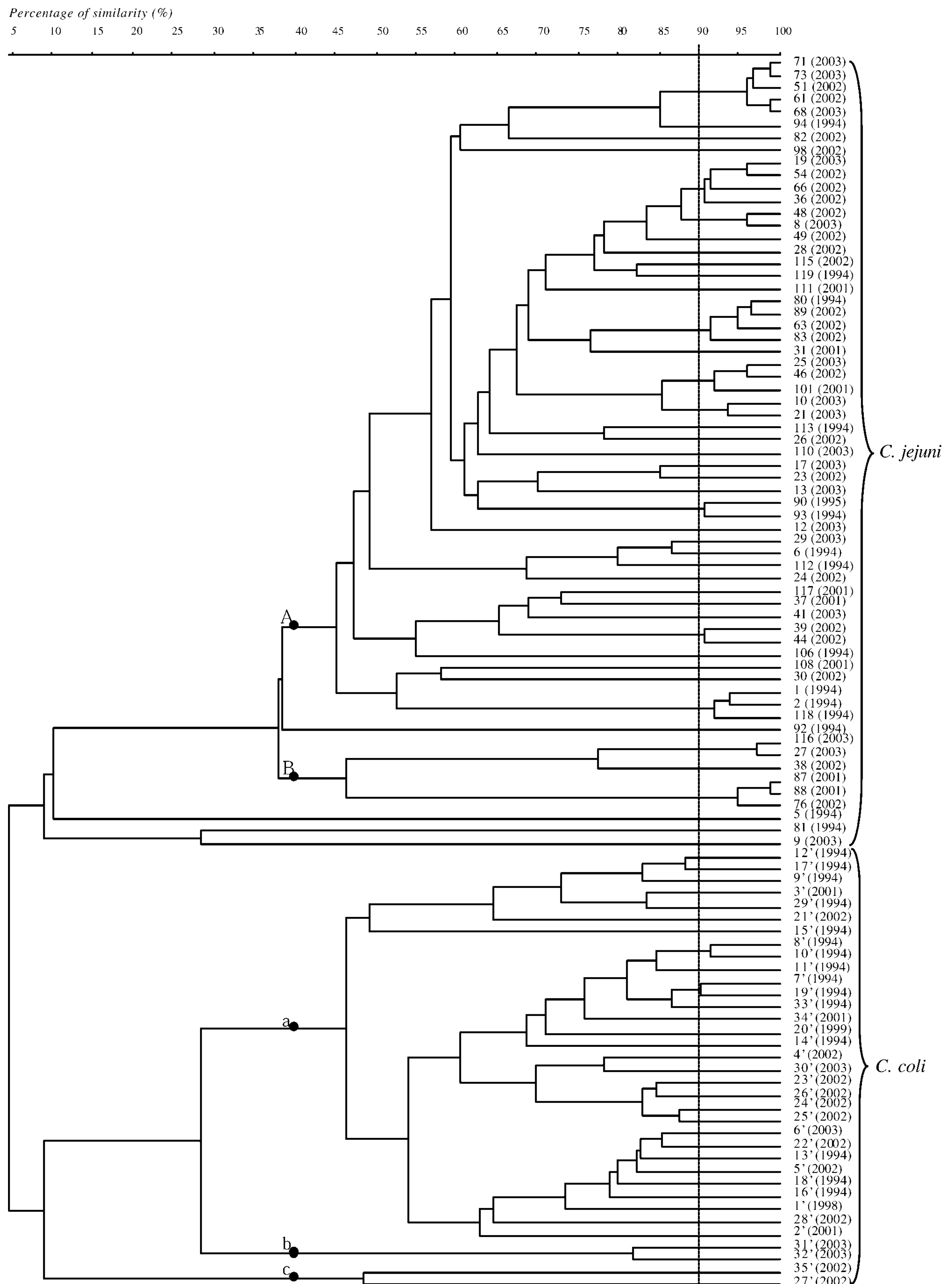

FIGURE 1. *The unweighted pair group with mathematical average (UPGMA) dendrogram of AFLP fingerprints from 63* C. jejuni *and 35* C. coli *isolates. At 40% similarity,* C. jejuni *and* C. coli *isolates were separated in two distinct clusters (A and B) and three distinct clusters (a, b, and c), respectively.*

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TABLE 3. *AFLP types and quinolone resistance of 35* C. coli *isolates from different sources*

Cluster	AFLP type	Strain no.	Strain designation	Year of isolation	Source	GI and NA resistance[a]
a	1	12′	26E-2	1994	Chicken viscera from market	R
	2	17′	53A-2	1994	Whole chicken from market	R
	3	9′	17E-6	1994	Whole chicken from market	R
	4	3′	6-1	2001	Chicken viscera from market	R
	5	29′	23-4	1994	Chicken wing from market	S
	6	21′	MMH 45	2002	Adult, male	R
	7	15′	34E-5	1994	Chicken wing from market	R
	8	8′	9-2A-1	1994	Whole chicken from market	R
	8	10′	19E-4	1994	Chicken breast from market	R
	9	11′	21E-5	1994	Chicken breast from market	R
	10	7′	9-1B-1	1994	Whole chicken from market	R
	10	19′	90 CCDA	1994	Chicken viscera from market	R
	11	33′	19-4	1994	Chicken breast from market	R
	12	34′	6-2B	2001	Chicken viscera from market	R
	13	20′	D 1373	1999	U.S. Food and Drug Administration	S
	14	14′	23E-4	1994	Chicken wing from market	S
	15	4′	SB-1	2002	Chicken breast from chicken slaughter plant	R
	16	30′	MMH 0030B	2003	Patient	R
	17	23′	KK 37-1-1	2002	Chicken breast from food processing plant	R
	18	26′	KK 37-2-3	2002	Chicken breast from food processing plant	R
	19	24′	KK 37-2-1	2002	Chicken breast from food processing plant	R
	20	25′	KK 37-2-2	2002	Chicken breast from food processing plant	R
	21	6′	S 6-1	2003	Washing water from chicken slaughter plant	R
	22	22′	KK 33-1	2002	Chicken skin from food processing plant	R
	23	13′	31E-2	1994	Chicken wing from market	R
	24	5′	S L-1	2002	Chicken drumstick from chicken slaughter plant	R
	25	18′	70 CCDA	1994	Chicken viscera from market	R
	26	16′	39A-1	1994	Chicken wing from market	R
	27	1′	Campy (A)	1998	Chicken meat from food processing plant	R
	28	28′	100-1	2002	River water	R
	29	2′	52 PF0-2	2001	Chicken drumstick from market	R
b	30	31′	MMH 0084	2003	Child, male	R
	31	32′	MMH 0042A	2003	Adult, female	R
c	32	35′	80-1-2	2002	Drain water	S
	33	27′	MMH 0032	2002	Adult, male	S

[a] CI, ciprofloxacin; NA, nalidixic acid; R, resistance, MIC of CI $\geqq$ 4 μg/ml and MIC of NA $\geqq$ 32 μg/ml; S, susceptibility, MIC of CI $<$ 4 μg/ml and MIC of NA $<$ 32 μg/ml.

chicken-related isolates and two human isolates, all of them being resistant to quinolone (nalidixic acid and ciprofloxacin) and reacting with the antisera against serotype Y:37 (Table 2). AFLP type 5 consisted of quinolone-resistant isolates and belonged to serotype B:2 and was shared by three human isolates and one chicken-related isolates (Table 2). AFLP type 12 consisted of two chicken-related isolates and two human isolates, one isolate resistant and the other sensitive to quinolone in each kind of source, and only one chicken-related isolate belonged to serotype B:2 (Table 2). AFLP type 14 consisted of two human isolates and one chicken-related isolate (all of them resistant to quinolone), only one human isolate of which belonged to serotype B:2 (Table 2). AFLP type 38 contained two water isolates (both of them sensitive to quinolone) and one chicken-related isolate (resistant to quinolone), which belonged to serotype O:19 (Table 2). About AFLP type 6 and AFLP type 15, both of isolates in each AFLP type were isolated from humans and resistant to quinolone, only the former AFLP type belonging to serotype B:2 (Table 2). With respect to AFLP type 22 and AFLP type 35, all isolates in both AFLP types were sensitive to quinolone, the former AFLP type consisted of two human isolates and had the same serotype J:11 and the latter contained two chicken-related isolates (Table 2). With regard to AFLP type 31 and AFLP type 36, all isolates in both AFLP types were resistant to quinolone, the former AFLP type comprised one chicken-related isolate and one human isolate and the latter contained two chicken-related isolates (Table 2). With regard to all *C. coli* isolates, AFLP type 8 and AFLP type 10 contained two isolates each and the four isolates were resistant to quinolone (Table 3).

Isolate no. 112 and 113 were isolated from the same whole chicken. The former was resistant to quinolone; the latter was sensitive to quinolone (Table 2). However, another chicken breast sample had *C. jejuni* (no. 106) and *C. coli* (no. 11′) isolates simultaneously, both of them being resistant to quinolone (Tables 2 and 3).

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DISCUSSION

AFLP, recently adjusted for typing *Campylobacter* species *(2–6, 11, 12, 22, 33),* is based on a subset of small fragments (50 to 400 bp) from the whole genome *(3).* Duim et al. *(6)* found that band patterns exhibited 90 to 98% similarity when a standardized protocol for AFLP analysis and computer-based analysis was used. AFLP analysis was able to separate the related subspecies from differences in only a few AFLP bands *(5, 33).* Previous studies reported that the strains of each *Campylobacter* species showed characteristic AFLP patterns, which were identified by the number and distribution of the AFLP bands. In the study, there was a clear distinction between AFLP fingerprints from *C. jejuni* and *C. coli* isolates, indicating that AFLP analysis was capable of discriminating between these species (Fig. 1). Furthermore, the use of AFLP analysis as a species-identification method has the advantage in that it enables species identification and shows the relationships between isolates at both the taxonomic and epidemiological levels *(5).*

We found that AFLP analysis could subdivide the 63 *C. jejuni* isolates into 42 AFLP types (Table 2). Cluster analysis of AFLP data in this study supports previous reports that no distinct subpopulations of *C. jejuni* isolates associated with humans or poultry exist *(4, 12, 27).* We performed AFLP analysis of poultry and human *C. jejuni* isolates using the same protocols as those used by Duim et al. *(4)* and Hein et al. *(12).* In this study, cluster analysis revealed two subgroups with 40% genetic similarity; however, two and four subgroups were observed by Duim et al. *(4)* and Hein et al. *(12),* respectively. In addition, genotyping of 35 *C. coli* isolates by AFLP allowed three subgroups to be distinguished (Table 3). The differences observed between the AFLP types of *C. coli* indicated a considerable degree of genetic diversity between isolates of this species (Fig. 1), which is worthy of further study by using an additional analysis.

Subtyping of *Campylobacter* isolates from human and animal sources, such as serotyping, phage typing, and molecular typing, has revealed that *C. jejuni* is a highly heterogeneous organism *(8, 10, 34).* For example, approximately 70 heat-stable and more than 100 heat-labile serotypes have been identified for *C. jejuni* and *C. coli (30).* Our data indicated that the dominant serotypes in all isolates were B:2 (14 of 63) and Y:37 (7 of 63). The remaining serotypes (A:1,44; D:4; and L:15) were very rare among human and chicken isolates. A significant proportion of the isolates in this study was serotype B:2. Other studies have also found serotype B:2 to be common among *C. jejuni* isolates from other sources, such as feces, meat and offal *(7),* wildlife *(38),* and poultry and livestock *(18).* Moreover, heat-stable serotypes 1, 2, and 4 complexes have been predominant in England *(8, 34),* Denmark *(29),* and the United States *(36).* In Finland, serotypes 1,44 and 4 were distributed among most of the selected common genotypes *(11).*

Three O:19 isolates occurred in the raw chicken drumstick and animal shelter wastewater sample grouped within cluster B (Table 2). The similar results were obtained by Desai et al. *(3)* that all eight isolates of serotypes Penner HS19, isolated from humans, grouped into AFLP cluster F. Hudson et al. *(14)* also reported that three HS19 isolates were found among the isolates tested, and all of these came from raw chicken samples. Furthermore, serotype O:19 consisted only of AFLP type 38 in our study (Fig. 1), which suggests that this serotype belongs to a genotype with the stable genetic homogeneity similar to that seen for the heat-labile serotypes 4 and 7 containing restriction fragment length polymorphism/ribotype 2/1 and 8/4, respectively *(16),* the heat-stable serotype 55 belonging to the *Sma*I pattern type I *(9),* and serotype 12 belonging to the combined genotype G1 *(11).* Because Guillain-Barré syndrome, a potentially fatal neurological complication of *Campylobacter* infection, is likely associated with serotype HS19 isolates *(27),* the more *C. jejuni* isolates that can be analyzed will make it possible to define more accurately the relatedness between O:19 serotype and the sources such as wastewater and raw chicken.

Heat-stable serotyping that revealed that identical serotypes (B:2, J:11, P:21, and Y:37) were distributed among different genotypes has been noted earlier *(34, 39).* The four serotypes among *C. jejuni* isolates were found in human case and chicken-related samples, and these types constitute the most numerous indistinguishable groups of isolates. In addition, AFLP fingerprints of chicken-related isolates were similar genetically to those of isolates from humans with gastroenteritis in AFLP types 1, 5, 12, 14, and 31. These data support the conclusions from previous epidemiological studies that chicken-related samples obtained from chicken food processing plants and slaughter plants could act as a vehicle for human sporadic cases.

In a few cases, two different types of suspected *Campylobacter* spp. colonies were observed, and then a colony of each type was picked for further examination by Hein et al. *(12).* They revealed that one chicken could simultaneously harbor a quinolone-resistant and a quinolone-sensitive *C. jejuni* strains. The isolation of multiple strains of *Campylobacter* spp. from 29.3% meat samples has also been described by Kramer et al. *(23).* Our results are in line with their studies. Moreover, we also found that *C. jejuni* and *C. coli* could simultaneously occur in one chicken and were resistant to quinolone (Tables 2 and 3). The results emphasize the epidemiological importance of identifying and typing more than one isolate per sample.

In conclusion, close genetic relationships between *C. jejuni* and *C. coli* isolates could be readily identified and discriminated by the numerical analysis of AFLP profiles that possess the unique combinations of precisely sized marker amplified fragments. These amplified fragments could serve as identification markers that define clonality and could be used as the basis of a molecular typing scheme. In the present study, our data substantiate the previous conceptions that ''genetically identical'' strains of *C. jejuni* can be found in human infections and poultry *(32).* These AFLP genotypic clusters were not congruent with all Penner HS serotypes. Most identical serotypes were distributed among different genotypes, suggesting that serotyping alone cannot be used for isolate identification. In

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epidemiological studies, combined serotyping and genotyping could provide the most relevant data for the identification of strains *(10).* In this study, the genetic data could be continuously updated and then linked in a comprehensive database with epidemiological information and phenotyping data, which facilitate the identification of outbreaks and sources of apparently sporadic human infection in Taiwan. Moreover, once a more extensive international database for the genotypes and serotypes of *C. jejuni* and *C. coli* becomes available, the comparison of subtyping data from different countries will be possible and information on common genotypes and serotypes prevailing in different countries will be provided.

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