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Campylobacter contamination of chicken meat in Japan: Geographical and Seasonal variations

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Abstract

Chicken is a potent source of human *Campylobacter* infections. Hence, we investigated the prevalence and antimicrobial resistance of *Campylobacter* in 440 vacuum-packed chicken breast products during April–December 2021. *Campylobacter* was isolated from 174 samples (39.5%), in which the mean concentration of *Campylobacter* was 1.42 ± 0.65 $\log_{10}$ CFU/g, and 19.0% of the positive samples contained > 2.0 $\log_{10}$ CFU/g of *Campylobacter*. *Campylobacter* prevalence was significantly ($P < 0.01$) higher in Western Japan (64.2%) than in Eastern Japan (26.6%). The highest prevalence of *Campylobacter* in Western and Eastern Japan was observed in August (74.1%) and October (47.8%), respectively. A total of 149 *Campylobacter jejuni* and 37 *C. coli* isolates were obtained. *C. coli* was more frequent among *Campylobacter* isolated from Western Japan (29.1%) than those obtained from Eastern Japan (6.6%). Among all *C. coli* isolates, 73.0% and 37.8% were ciprofloxacin- and erythromycin-resistant, respectively. All *C. jejuni* isolates were susceptible to erythromycin. However, 42.3% of them were ciprofloxacin-resistant. Ciprofloxacin-resistant *C. jejuni* was significantly ($P < 0.01$) more frequently in isolates collected from Western Japan than in isolates obtained from Eastern Japan. This study revealed the geographical and seasonal variations in the prevalence of *Campylobacter* in chicken products. Chicken meat becomes contaminated with *Campylobacter* while slaughtering *Campylobacter*-infected broiler flocks in processing plants. Therefore, the development of efficient strategies for decreasing *Campylobacter* infection in broiler flocks in Western Japan should be prioritized to reduce human *Campylobacter* infections.

Key Words: antimicrobial resistance, *Campylobacter*, chicken meat, multilocus-sequence typing

Introduction

Campylobacter spp. is the most common cause of human gastroenteritis worldwide[18]. Chicken is a major source of *Campylobacter* infection outbreaks[8,21]. The frequency of *Campylobacter*-induced food poisoning outbreaks in Japan was more than 300 per year during 2014–2018, and it tends to be higher in June–October than in November–May[21]. The Japanese Ministry of Agriculture, Forestry, and Fisheries (JMAFF) has designated *Campylobacter* as a prioritized

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hazard in food safety risk management, identified chicken meat as the principal source of *Campylobacter* infections in humans, and surveyed the prevalence of *Campylobacter* in broiler flocks[4,15]. In that survey, JMAFF found the prevalence of *Campylobacter* to be significantly higher in broiler flocks reared in Western Japan than in Eastern Japan[15] and indicated chances of seasonal variations in *Campylobacter* incidence in broiler flocks with a peak in summer[4,15]. These geographical and seasonal variations in the prevalence of *Campylobacter* in broiler flocks result in similar variations in chicken meat contaminations. Furthermore, differing hygiene measures and processing methods between chicken processing plants may influence *Campylobacter* concentrations in chicken meat.

Campylobacter infections typically cause acute self-limiting gastroenteritis, for which antimicrobial therapy is usually not provided. However, antimicrobial treatment may be necessary for patients with immunocompromised conditions or other comorbidities[20]. Macrolides are often used in a first-line treatment of human campylobacteriosis[6]. However, fluoroquinolones such as levofloxacin and ciprofloxacin are used to treat *Campylobacter* enteritis without a microbiological diagnosis[3,6]. These antibiotic drugs, along with penicillins, tetracyclines, aminoglycosides, sulfonamides, and macrolides, are used to treat bacterial infections in animals used in food industries in Japan[10]. According to the Japanese Veterinary Antimicrobial Resistance Monitoring (JVARM) system[10], 1.5% and 44.8% of *Campylobacter* isolated from broilers in 2017 were resistant to erythromycin and ciprofloxacin, respectively. Therefore, fluoroquinolone-resistant *Campylobacter* in chicken meat seriously threatens public health.

In this study, we investigated the geographical and seasonal variations in the prevalence of *Campylobacter* in chicken products packaged at local chicken processing plants. Moreover, we characterized *Campylobacter* isolates using multilocus sequence typing (MLST) and antimicrobial susceptibility testing. This study could prospectively aid the development of a risk management strategy against campylobacteriosis associated with chicken meat consumption and the treatment of *Campylobacter* enteritis."

Materials and Methods

Sampling

Chilled chicken breast products consisting of three to six breast pieces, vacuum packed at local processing plants, were collected from retail shops located in Hokkaido, Saitama, Chiba, Tokyo, Kanagawa, and Kagoshima prefectures, Japan, during April–December 2021. The product label indicated the name and address of the processing plant as well as the production lot number. One sample was collected from each production lot. The refrigerated products were sent to the National Institute of Health Sciences via express delivery and stored in the laboratory at 4 °C until examination. Samples were examined within 48 hr of sampling.

Campylobacter enumeration

For each sample, skin (75 g) taken from more than three pieces of meat was placed in a plastic bag containing 75 mL of buffered peptone water (BPW; Oxoid Ltd., Hampshire, UK), and thus a 2-fold diluted solution was prepared; and 0.2 mL of this solution was plated on each of five modified charcoal cefoperazone deoxycholate agar plates (mCCDA: Oxoid). Thereafter, 2 mL of the 2-fold diluted solution was mixed with 8 mL BPW, and 0.2 mL of that mix was incubated on two mCCDA plates (0.1 mL each). After incubation for 48 hr at 42 °C under a microaerobic atmosphere, the colony-forming units (CFU) of *Campylobacter* were counted and converted to $\log_{10}$ CFU/g of the samples. The enumeration limit of this method was 0.3 $\log_{10}$ CFU/g. Finally, four suspected colonies were cultured by plating on mCCDA and identified as *Campylobacter* using multiplex polymerase chain reaction (PCR)[7]. One *Campylobacter* species isolated from each sample was characterized using MLST and antimicrobial susceptibility testing.

Table 1. Seasonal *Campylobacter* prevalence in breast products.

Month	Nationwide			West Japan			East Japan		
	No. of			No. of			No. of		
	Samples	Positive	%	Samples	Positive	%	Samples	Positive	%
April	13	4	30.8	7	4	57.1	6	0	0.0
May	31	7	22.6	14	6	42.9	17	1	5.9
June	41	17	41.5	14	10	71.4	27	7	25.9
July	65	25	38.5	25	18	72.0	40	7	17.5
August	71	32	45.1	27	20	74.1	44	12	27.3
September	61	26	42.6	16	10	62.5	45	16	35.6
October	62	33	53.2	16	11	68.8	46	22	47.8
November	53	17	32.1	18	9	50.0	35	8	22.9
December	43	13	30.2	14	9	64.3	29	4	13.8
total	440	174	39.5	151	97	64.2*	289	77	26.6

*: significantly different ($P < 0.01$) compared with Eastern Japan.

MLST of Campylobacter isolates

MLST was performed according to the seven-locus scheme for *Campylobacter jejuni* and *C. coli*, using the primer sets and experimental conditions suggested by the *Campylobacter* MLST database (http://pubmlst.org/campylobacter/).

Antimicrobial susceptibility testing of Campylobacter isolates

Susceptibility of the isolates to ampicillin (0.12–256 mg/L), streptomycin (0.12–128 mg/L), tetracycline (0.12–128 mg/L), chloramphenicol (0.12–256 mg/L), nalidixic acid (0.12–128 mg/L), ciprofloxacin (0.03–64 mg/L), erythromycin (0.12–128 mg/L), and gentamicin (0.12–256 mg/L) was tested following broth microdilution on dried plates (Eiken Chemical, Tokyo, Japan). *C. jejuni* ATCC33560 was used as a quality control strain. The breakpoints (ampicillin, 32 mg/L; streptomycin, 32 mg/L; tetracycline, 16 mg/L; chloramphenicol, 16 mg/L; nalidixic acid, 32 mg/L; ciprofloxacin, 4 mg/L; and erythromycin, 32 mg/L) adopted by Clinical and Laboratory Standards Institute[1)] and JVARM[10)] were used in experimental sets for antibiotics, except in that involving gentamicin (2 mg/L), which was set according to the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme[2)].

Statistical analysis

All statistical analyses were performed using R, version 4.1. Differences between proportions were tested using Fisher's exact test, as p-values < 0.05 were considered statistically significant.

Results

A total of 440 vacuum-packed products obtained from 24 chicken processing plants were collected from 26 retail shops. Among the 24 plants, 13 were situated in Eastern Japan (Hokkaido, Aomori, Iwate, Miyagi, Gunma, and Chiba) and 11 were in Western Japan (Tokushima, Saga, Miyazaki, and Kagoshima). *Campylobacter* was isolated from 174 (39.5%) products packaged in 21 (87.5%) plants (Table 1). The prevalence of *Campylobacter* in products packaged in Western Japan (64.2%) was significantly ($P < 0.01$) higher than those packaged in Eastern Japan (26.6%). The prevalence of *Campylobacter* gradually increased from June (41.5%) to October (53.2%) and then decreased by December (30.2%). In products collected from Western Japan, the prevalence of *Campylobacter* was > 70% from June–August, and the highest prevalence was observed in August (74.1%). In Eastern Japan, the prevalence of *Campylobacter* started increasing in May (5.9%) and reached its peak in October (47.8%).

Campylobacter was isolated from 19.0% (33/174) of the positive products > 2.0 $\log_{10}$ CFU/g, and its mean concentration ($\log_{10}$ ± standard deviation CFU/g) in the 174 *Campylobacter*-

Table 2. *Campylobacter* concentrations ($\log_{10}$ CFU/g) in chicken breast samples.

Plant	Region	No. of Samples	No. of *Campylobacter*-negative samples	No. of *Campylobacter*-positive samples: *Campylobacter* concentration ≤ 1.0	> 1.0, ≤ 2.0	> 2.0	Mean concentraion ± SD in *Campylobacter*-positive samples
Total		440	266	51	90	33	1.42 ± 0.65
A	East	16	6	0	1	9	2.32 ± 0.29
B	East	16	15	0	0	1	2.48
C	East	37	28	1	5	3	1.88 ± 0.52
D	East	48	24	4	16	4	1.53 ± 0.58
E	West	5	2	0	0	3	2.20 ± 0.11
F	West	3	0	0	0	3	2.06 ± 0.03
G	West	1	0	0	0	1	3.35
H	West	38	8	6	18	6	1.57 ± 0.73
I	West	43	21	9	11	2	1.16 ± 0.48
J	West	28	10	6	10	1	1.29 ± 0.46
Others	East and west	205	152	25	29	0	1.07 ± 0.46

Table 3. Antimicrobial resistance in *Campylobacter* isolated from breast meat products.

Species	No.	No. of resistant isolates (%): ABPC	SM	TC	CP	NA	CPFX	EM	GM
C. jejuni	149	58 (38.9)	1 (0.7)	37 (24.8)	0 (0.0)	63 (42.3)	63 (42.3)	0 (0.0)	0 (0.0)
East	71	31(43.7)	0 (0.0)	20 (28.2)	0 (0.0)	19 (26.8)*	19 (26.8)*	0 (0.0)	0 (0.0)
West	78	27 (34.6)	1 (1.3)	17 (21.8)	0 (0.0)	44 (56.4)	44 (56.4)	0 (0.0)	0 (0.0)
C. coli	37	4 (10.8)	10 (27.0)	27 (73.0)	0 (0.0)	29 (78.4)	27 (73.0)	14 (37.8)	0 (0.0)
East	5	2 (40.0)	1 (20.0)	4 (80.0)	0 (0.0)	4 (80.0)	4 (80.0)	2 (40.0)	0 (0.0)
West	32	2 (6.3)	9 (28.1)	23 (71.9)	0 (0.0)	25 (78.1)	23 (71.9)	12 (37.5)	0 (0.0)

Abbreviations: ABPC: ampicillin, SM: streptomycin, TC: tetracycline, CP: chloramphenicol, NA: nalidixic acid, CPFX: ciprofloxacin, EM: erythromycin, GM: gentamicin. *: significantly different ($P < 0.01$) compared with Western Japan.

positive products was 1.42 ± 0.65 $\log_{10}$ CFU/g (Table 2). These 33 highly contaminated products (> 2.0 $\log_{10}$ CFU/g) were packaged in 10 plants (A to J); 94.4% (17/18) of products generated in A, B, E, F and G plants. These five plants produced 51.5% (17/33) of the highly contaminated products.

Among the 174 *Campylobacter*-positive products, 137 (78.7%), 25 (14.4%), and 12 (6.9%) products were contaminated with only *C. jejuni*, only *C. coli*, and both of these species, respectively. *C. coli* accounted for 6.6% (5/76) and 29.1% (32/110) of the *Campylobacter* spp. isolated from Eastern and Western Japan, respectively. *C. coli* was significantly more frequent ($P < 0.01$) among *Campylobacter* isolated from Western Japan than those from Eastern Japan. Among all *C. jejuni* isolates, the frequencies of strains resistant to ampicillin, streptomycin, tetracycline, nalidixic acid, and ciprofloxacin were 38.9%, 0.7%, 24.8%, 42.3%, and 42.3%, respectively (Table 3). Ciprofloxacin resistance was less frequent ($P < 0.01$) in *C. jejuni* isolated from Eastern Japan than in those isolated from Western Japan. All *C. jejuni* isolates were susceptible to chloramphenicol, erythromycin, and gentamicin. *C. jejuni* isolates were classified into 57 sequence types (STs) (Table 4); ST6704 (15 isolates), ST45 (13 isolates), ST21 (11 isolates), and ST4622 (10 isolates) were the most abundant STs. Fourteen of the 15 ST6704 isolates were isolated from products packaged in two plants in Iwate prefecture in Eastern Japan. ST6704 isolates were resistant to ampicillin. ST45 isolates were obtained from products packaged in six plants; ST45 isolates resistant to ampicillin, nalidixic acid, and ciprofloxacin, originated at four plants located in three prefectures of Western Japan, and those resistant to tetracycline, nalidixic acid, and ciprofloxacin were originated at a plant in Eastern Japan. ST21 isolates were obtained from products packaged in four plants, and those obtained from two plants in Eastern Japan showed resistance to ampicillin, tetracycline, nalidixic

Table 4. Sequence types and antimicrobial resistance profiles of *Campylobacter jejuni* isolates.

CC	ST	ARP	No.	East	West	CC	ST	ARP	No.	East	West	CC	ST	ARP	No.	East	West
21	19	NA+CPFX	1		1(1)	61	628	Susceptible	1	1(1)		574	305	ABPC+SM+TC+NA+CPFX	1		1(1)
n=29		Susceptible	1		1(1)	n=3	10369	TC+NA+CPFX	1	1(1)		n=1					
	21	ABPC+TC+NA+CPFX	5	5(2)			11355	Susceptible	1		1(1)	607	607	NA+CPFX	2		2(2)
		TC+NA+CPFX	5		5(2)	257	257	Susceptible	1		1(1)	n=5	4600	Susceptible	1		1(1)
		NA+CPFX	1		1(1)	n=1							5801	Susceptible	2	1(1)	1(1)
	50	Susceptible	3	2(1)	1(1)	353	10013	ABPC+TC+NA+CPFX	1		1(1)	UN	407	ABPC+TC+NA+CPFX	1		1(1)
	806	ABPC+TC+NA+CPFX	1		1(1)	n=5	10425	TC	1	1(1)		n=28	922	TC+NA+CPFX	1		1(1)
		TC+NA+CPFX	2		2(1)		11082	NA+CPFX	1		1(1)			TC	1		1(1)
	4253	Susceptible	3		3(2)		11084	ABPC+NA+CPFX	1		1(1)		2150	Susceptible	1		1(1)
	4526	NA+CPFX	1	1(1)			11085	NA+CPFX	1		1(1)		4325	TC+NA+CPFX	3	3(1)	
	9776	NA+CPFX	3		3(2)	354	354	NA+CPFX	3	2(1)	1(1)		4622	ABPC+NA+CPFX	1	1(1)	
	11356	ABPC+NA+CPFX	1		1(1)	n=12		TC	3	2(1)	1(1)			ABPC	9	9(4)	
		ABPC	3		3(1)			Susceptible	2	1(1)	1(1)		5720	Susceptible	1		1(1)
22	22	TC+NA+CPFX	2		2(1)		653	TC	1	1(1)			8071	NA+CPFX	2		2(2)
n=3		Susceptible	1	1(1)			5721	Susceptible	1		1(1)		9997	ABPC+NA+CPFX	1		1(1)
42	42	NA+CPFX	1		1(1)	443	440	Susceptible	1		1(1)		11187	TC	1	1(1)	
n=5		Susceptible	4	3(2)	1(1)	n=1							11357	Susceptible	1		1(1)
45	45	ABPC+NA+CPFX	6		6(4)	460	5255	NA+CPFX	1		1(1)		11362	ABPC	1		1(1)
n=19		TC+NA+CPFX	4	4(1)		n=5		Susceptible	2	2(2)			11364	ABPC+NA+CPFX	1		1(1)
		ABPC	1		1(1)		11361	Susceptible	2		2(1)		11386	ABPC+NA+CPFX	1		1(1)
		TC	2	2(1)		464	4108	NA+CPFX	1		1(1)		11387	Susceptible	1		1(1)
	137	Susceptible	1	1(1)		n=27	4389	ABPC+NA+CPFX	1		1(1)		11454	ABPC+NA+CPFX	1		1(1)
	538	Susceptible	1	1(1)				ABPC+TC	1		1(1)						
	3727	Susceptible	1	1(1)				ABPC	2	2(2)							
	11070	ABPC	3		3(3)		5262	Susceptible	3	3(1)							
48	918	NA+CPFX	2	2(1)			5268	Susceptible	1		1(1)						
n=6		Susceptible	4	4(3)			5731	Susceptible	1		1(1)						
							6704	ABPC	15	14(2)	1(1)						
							11360	NA+CPFX	2		2(1)						

Abbreviations: CC: clonal complex, ST: sequence type, ARP: antimicrobial resistance profile, UN: unassigned, ABPC: ampicillin, SM: streptomycin, TC: tetracycline, NA: nalidixic acid, CPFX: ciprofloxacin.
Figure between parentheses indicates the number of plants.

acid, and ciprofloxacin. ST21 isolates resistant to tetracycline, nalidixic acid, and ciprofloxacin were obtained from products packaged at the two plants in Western Japan. Nine of the 10 ST4622 isolates, obtained from products packaged in four plants in Eastern Japan, were ampicillin-resistant.

Among the 37 *C. coli* isolates, the frequencies of ampicillin-, streptomycin-, tetracycline-, nalidixic acid-, ciprofloxacin-, and erythromycin-resistant isolates were 10.8%, 27.0%, 73.0%, 78.4%, 73.0%, and 37.8%, respectively. Moreover, 11 (29.7%) *C. coli* isolates were resistant to ciprofloxacin and erythromycin. However, all *C. coli* isolates were susceptible to chloramphenicol and gentamicin. The *C. coli* isolates were classified into 20 STs (Table 5); the three most frequent STs were ST1055 (six isolates), ST1767 (six isolates), and ST1556 (four isolates). Six ST1055 isolates, obtained from products packaged in three plants, were resistant to ciprofloxacin. Six ST1767 isolates, obtained from products packaged at four plants, were susceptible to tetracycline. Four ST1556 isolates were originated from the products packaged in two plants.

Discussion

In this study, we collected local chicken breast products vacuum-packed at chicken processing plants to eliminate the possibility of post-shipment *Campylobacter* contamination. These chicken breast products were packaged at the 24 chicken processing plants situated in 10 prefectures.

Table 5. Sequence types and antimicrobial resistance profiles of *Campylobacter coli* isolates.

CC	ST	ARP	No.	East	West	CC	ST	ARP	No.	East	West
828	827	ABPC+NA+CPFX	1	1 (1)			1556	TC+NA+CPFX	3		3 (1)
	828	ABPC+TC+EM+NA+CPFX	1		1 (1)			TC+EM+NA	1		1 (1)
	854	TC+SM+EM+NA+CPFX	1		1 (1)		1767	NA+CPFX	3		3(2)
		TC+EM+NA+CPFX	1		1 (1)			Susceptible	3		3(3)
		TC+NA+CPFX	1		1 (1)		2869	TC+SM+NA	1		1 (1)
	887	EM+TC	1		1 (1)		4172	TC+EM	1	1 (1)	
	1055	SM+EM+TC+NA+CP	1		1 (1)		4605	ABPC+TC+NA+CPFX	1	1 (1)	
		TC+SM+EM+NA+CPFX	1		1 (1)		5844	TC+SM+EM+NA+CPFX	1		1 (1)
		TC+EM+NA+CPFX	1		1 (1)		8080	EM	1		1 (1)
		TC+SM+NA+CPFX	2	1 (1)	1 (1)		8295	TC+NA+CPFX	1		1 (1)
		SM+EM+NA+CPFX	1		1 (1)		8330	TC+EM+NA+CPFX	1	1 (1)	
	1068	TC+SM+EM+NA+CPFX	1		1 (1)		8916	Susceptible	1		1 (1)
		TC+NA+CPFX	2		2(2)		11358	TC+NA+CPFX	1		1 (1)
	1105	TC+NA+CPFX	1		1 (1)	1150	8292	ABPC+TC+NA+CPFX	1		1 (1)
	1127	SM+TC	1		1 (1)						

Abbreviations: CC: clonal complex, ST: sequence type, ARP: antimicrobial resistance profile, ABPC: ampicillin, SM: streptomycin, EM: erythromycin, TC: tetracycline, NA: nalidixic acid, CPFX: ciprofloxacin.
Firure between parentheses indicates the number of plants.

According to the Statistical Survey on Livestock of JMAFF (https://www.maff.go.jp/j/tokei/kouhyou/tikusan/index.html), in 2021, approximately 75.8% of the total number of broilers produced in Japan originated these 10 prefectures. The prevalence of *Campylobacter* in chicken breast products was 39.5% (174/440). The enumeration limit of 0.3 $\log_{10}$ CFU/g was considered in the quantitative culture method. The prevalence may be higher if an enrichment culture method was also used. This study clearly demonstrates that the prevalence of *Campylobacter* in chicken meat products varies geographically and seasonally, likely being induced by its similarly varying prevalence in broiler flocks in Japan[4,15]. As this investigation detected a higher prevalence of *Campylobacter* in products obtained from Western Japan than in products obtained from Eastern Japan, chicken products acquired from Western Japan rather than Eastern Japan could pose a higher risk of *Campylobacter* infections. It has been established that as the prevalence of *Campylobacter* in broiler flocks is high and the infected broilers have high concentrations of *Campylobacter* (more than 5 $\log_{10}$ CFU/g) in their ceca, their carcasses and meat get contaminated when infected broilers are slaughtered at chicken processing plants[5,13,15-17]. Therefore, the development of efficient strategies for *Campylobacter* management in broiler flocks needs to be prioritized in Western Japan to decrease human *Campylobacter* infections.

Campylobacter was isolated from 39.5% of the products investigated in this study, and 19.0% of the total number of *Campylobacter*-positive products were highly contaminated (> 2.0 $\log_{10}$ CFU/g), half of which were packaged at five plants. Specifically, 94.4% of the *Campylobacter*-positive products packaged at these five plants were highly contaminated. In the five plants, *Campylobacter*-negative products could have been prepared from a *Campylobacter*-free broiler flock, and products highly contaminated with *Campylobacter* from a broiler flock infected by *Campylobacter*. We previously reported that *Campylobacter* concentration significantly differed between chicken breast products processed at two different chicken processing plants (average 2.19 $\log_{10}$ CFU/g and 1.46 $\log_{10}$ CFU/g), however, *Campylobacter* concentrations in the cecal contents of broiler flocks processed at these two plants showed no significant difference[16]. The previous and present studies show that differences in hygiene measures and processing methods adopted in chicken processing plants influence *Campylobacter* concentrations in the products. Comparing hygiene measures and processing methods of different processing plants would be useful for identifying effective countermeasures to decrease the prevalence of *Campylobacter* in chicken products.

Among the 186 *Campylobacter* isolates, 80.1% (149 isolates) and 19.9% (37 isolates) were *C. jejuni* and *C. coli*, respectively. According to JVARM[10], the frequencies of ciprofloxacin- and erythromycin-resistant *C. jejuni* isolated from broilers were 44.8% and 1.5%, respectively, in 2017. In the present study, all *C. jejuni* isolates were susceptible to erythromycin although 42.3% of them were resistant to ciprofloxacin, indicating the efficacy of erythromycin as a first-line treatment for human *C. jejuni* infection. However, 29.7% of *C. coli* isolates were resistant to ciprofloxacin and erythromycin. As *C. coli* is more prevalent in Western Japan than in Eastern Japan, antibiotics (e.g., ampicillin and gentamicin) other than ciprofloxacin and erythromycin should be considered to treat patients suspected of consuming chicken products from Western Japan. As the fluoroquinolone susceptibility test indicated that most of the *C. jejuni* isolates obtained from Western Japan were ciprofloxacin-resistant. Fluoroquinolones, classified as "critically important antimicrobials for human medicine" by the World Health Organization[19], have been approved by JMAFF to treat colibacillosis and mycoplasmosis in chickens. According to the JMAFF, the number of chicken farms showing colibacillosis outbreaks is higher in Western Japan than in Eastern Japan (https://www.maff.go.jp/j/syouan/douei/kansi_densen/kanren_zyouhou.html) (*in Japanese*). The geographic variation in the use of fluoroquinolones is unknown; however, they can be used in Western Japan for treating colibacillosis more frequently than in Eastern Japan. The more frequent use of fluoroquinolones might induce fluoroquinolone resistance in *C. jejuni* in Western Japan rather than in Eastern Japan; hence, chicken products, particularly from Western Japan, could pose an increased risk of human fluoroquinolone-resistant *Campylobacter* infections.

Using MLST, *Campylobacter jejuni* and *C. coli* isolates were classified into 57 and 20 STs, respectively. The MLST and antimicrobial susceptibility assays revealed that some lineages could be geographically characterized based on a combination of STs and antimicrobial resistance profiles. Among *C. jejuni* isolates analyzed, ST6704, ST45, ST21, and ST4622 were the most frequent STs. *C. jejuni* ST45 and ST21 were isolated in Eastern and Western Japan, respectively, whereas almost all ST6704 and ST4622 were isolated in Eastern Japan. Moreover, ST6704 and ST4622 isolates were ampicillin-resistant. Generally, chicken production companies directly grow the broilers on their farms or involve contractual chicken growers. Veterinarians working at each chicken company patrol internal and contracted farms, and prescribe antimicrobials according to the treatment policies of the company. A previous report indicated the possibility of *Campylobacter* spread among broiler flocks via transport crates reused at different farms[9]. Moreover, flies were identified as prospective carriers that spread *Campylobacter* in the environment[11]; therefore, lineages adapted to a farm environment may be locally prevalent. Kumagai *et al.*[8] investigated foodborne infection caused by *Campylobacter* spp. reported between 2007 and 2018, and estimated that the first and second most important sources of the disease were chicken (80.3%) and beef products (10.5%), respectively. Moreover, we reported *C. jejuni* isolates belonging to 57 STs obtained from patients with *Campylobacter* enteritis from December 2019 to April 2022 and identified five major STs: ST22, ST354, ST21, ST918, and ST50[14]. In the present study, 26 of the 57 STs (45.6%), including the five major ones, were detected. We also reported ST806, ST21, ST459, ST61, and ST922 as the five most frequent STs of *C. jejuni* isolated from beef cattle, however, ST22, ST354, and ST918 were not isolated from beef cattle[12]. Although *C. jejuni* ST21 was considered the fourth most frequent ST in the present study, the other STs from beef cattle were either minor or not isolated. Moreover, the ST21 lineage with the same antimicrobial resistance profiles as ST21 obtained from beef cattle was isolated only from chicken products collected from Western

Japan. In Japan, the production area is generally mentioned on the label of raw meat products. Therefore, during an outbreak of *Campylobacter* food poisoning, the combination of STs and antimicrobial resistance profiles of *Campylobacter* isolates from patients would help to identify the origin of the causative strains.

In conclusion, geographical and seasonal variations in the prevalence of *Campylobacter* in chicken products were clearly observed; identified to be similar to those observed in cases of *Campylobacter* infection of broiler flocks in Japan. Chicken meat products from Western Japan rather than the Eastern area pose a higher risk of *Campylobacter* infection. Some chicken processing plants distribute chicken meat products that are highly contaminated with *Campylobacter* spp. Therefore, developing efficient strategies for *Campylobacter* management in broiler flocks in Western Japan and decreasing *Campylobacter* contamination in chicken products during processing should be prioritized to decrease human *Campylobacter* infections in Japan. The results of our study could be useful for managing the risk of campylobacteriosis caused by the consumption of infected chicken meat and devising effective treatment strategies for *Campylobacter* enteritis.

Conflict of Interest

The authors declare no conflict of interest.

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