Prevalence and Characteristics of Salmonella and Campylobacter in Retail Poultry Meat in Japan


INTRODUCTION

Salmonella and Campylobacter are recognized as major causative agents of human gastroenteritis worldwide (1). In Japan, Campylobacter and Salmonella are the second and third most common causes of foodborne illness, respectively, after norovirus. The major route of Salmonella and Campylobacter infection in humans is through the ingestion of contaminated raw or undercooked meat, especially poultry meat (2,3).

Several molecular typing methods have been employed to analyze the epidemiological features of infections caused by Salmonella or Campylobacter. Pulsed-field gel electrophoresis (PFGE) is the most common method used for molecular typing of Salmonella and Campylobacter. In the present study, we used PFGE to analyze both Salmonella and Campylobacter isolates. In addition, we also used the comparative genomic fingerprinting (CGF) based on a 40-gene assay (CGF40) for the analysis of Campylobacter isolates. The CGF method was developed by Canadian researchers as a high-resolution comparative genomics-based method specific for C. jejuni subtyping with a high discriminatory power. It offers a rapid, low-cost, and easily deployable approach of routine epidemiologic surveillance and outbreak investigations (4).

Previous studies have reported the prevalence and

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antimicrobial susceptibilities of *Salmonella* and *Campylobacter* in Japan (5,6). However, there is a paucity of data concerning the association of genetic types of *Salmonella* and *Campylobacter* with their geographical distribution and antimicrobial resistance profiles. Furthermore, the CGF method has not been applied to analyze *Campylobacter* strains isolated in Japan. The aims of this study were therefore to determine the prevalence and antimicrobial susceptibility of *Salmonella* and *Campylobacter* isolated from retail broiler meat sold in Japan, and to describe the molecular characteristics of these pathogens by using PFGE for *Salmonella* and *Campylobacter* and CGF40 for *Campylobacter*. The results of the cluster analysis were compared with the data on antimicrobial resistance profiles and the geographical distribution of the locations in which the poultry meat was produced.

**MATERIALS AND METHODS**

**Collection of chicken meat samples:** Twenty samples of poultry meat were purchased directly by each of the 5 institutes participating in this study from retail stores in the local areas of each institute in the period from July to October 2012. Meat from 32 packages was produced in the eastern area of Japan (one package in the Hokkaido region and 31 packages in the Tohoku region). Forty-nine packages contained meat from the western area of Japan (17 from the Kinki region, 3 from the Shikoku region, and 29 from the Kyushu region; Table 1). The production area of meat from 19 packages was indicated as “domestic.” The meat samples were kept in a refrigerator at 4 °C until use, and all samples were processed within one day of purchase.

**Isolation of Salmonella strains:** Isolation of *Salmonella* strains was performed by each institute according to the Standard Test Method for *Salmonella* (7). In brief, 25 g of each meat sample was mixed with 225 mL of buffered peptone water. After incubation at 36–38 °C for 20–22 h, 1 mL and 0.1 mL of buffered peptone water culture was transferred into 10 mL of tetraphionate broth and 10 mL of Rappaport-Vassiliadis broth, respectively, and incubated at 41.5–42.5 °C for 20–24 h. The culture from each tube was inoculated onto deoxycholate hydrogen sulfide lactose agar plates (Nissui Pharma, Tokyo, Japan) and ES *Salmonella* agar II plates (Eiken Chemical, Tokyo, Japan), and incubated at 36–38 °C for 20–24 h. Three suspected colonies were chosen from each plate and subjected to biochemical examination for identifying *Salmonella*. Serotyping of *Salmonella* isolates was performed using commercial O and H antisera (Denka Seiken, Tokyo, Japan), and the isolates were designated according to the Kauffmann-White scheme (8).

**Isolation of Campylobacter strains:** Isolation of *Campylobacter* strains was performed by each institute according to the Standard Test Method for *C. jejuni* and *C. coli* (9). In brief, 25 g of each meat sample was mixed with 100 mL of Preston broth and incubated under microaerobic conditions (7–13% CO2 and 3–7% O2) at 41–43 °C for 24–48 h. After incubation, the culture was inoculated onto modified charcoal cefoperazone deoxycholate agar plates (Eiken Chemical) and Butzler’s selective agar plates (Oxoid, Basingstoke, UK), and the plates were incubated under microaerobic conditions at 41–43 °C for 24–48 h. Suspected *Campylobacter* colonies were presumptively identified on the basis of characteristic morphology, Gram-negative staining, and pos-

### Table 1. Prevalence of *Salmonella enterica* subsp. *enterica*, *Campylobacter jejuni* and *Campylobacter coli* in retail poultry meat

<table>
<thead>
<tr>
<th>Type of meat</th>
<th>No. of samples</th>
<th>Salmonella enterica subsp. enterica</th>
<th>Campylobacter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Serotypes</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>positive</td>
<td>Infantis</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Area</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eastern</td>
<td>32</td>
<td>16 (50.0)</td>
<td>15 (46.9)</td>
</tr>
<tr>
<td>Western</td>
<td>49</td>
<td>25 (51.0)</td>
<td>9 (18.4)</td>
</tr>
<tr>
<td>Domestic</td>
<td>19</td>
<td>13 (68.4)</td>
<td>9 (47.4)</td>
</tr>
<tr>
<td>Type of meat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thigh</td>
<td>49</td>
<td>30 (61.2)</td>
<td>17 (34.7)</td>
</tr>
<tr>
<td>Breast</td>
<td>36</td>
<td>19 (52.8)</td>
<td>12 (33.3)</td>
</tr>
<tr>
<td>Tenderloin</td>
<td>12</td>
<td>3 (25.0)</td>
<td>2 (16.7)</td>
</tr>
<tr>
<td>Others</td>
<td>3</td>
<td>2 (66.7)</td>
<td>2 (66.7)</td>
</tr>
</tbody>
</table>

1) Other serotypes include Agona, Corvallis, Cerro, Duesseldorf, Typhimurium, and Muenchen.
2) Parentheses show percentage.
3) Location of a farm was not presented on a meat package.
4) consisted of a mixed package of thigh and breast and a package without showing type of meat.
itive catalase and oxidase reactions. Indoxyl acetate and hippurate hydrolysis tests were conducted to ascertain *Campylobacter* spp. identity. In addition, a PCR assay was utilized to identify *C. jejuni* and *C. coli* (10).

**Antimicrobial susceptibility test:** The antimicrobial susceptibility test was performed based on the Kirby-Bauer disc diffusion method using BBL Sensi-disc susceptibility test discs (BD, Tokyo, Japan) on Mueller-Hinton II agar (BD) for *Salmonella* and on Mueller-Hinton II agar with 5% lysed horse blood for *Campylobacter*. The results were interpreted according to Clinical and Laboratory Standards Institute criteria (11). The results of *Campylobacter* susceptibility tests were interpreted according to the criteria for *Enterobacteriaceae*, *Escherichia coli* ATCC25922 was used as the quality control strain.

**PFGE:** PFGE profiles of *Salmonella* and *Campylobacter* isolates were determined using a previously described protocol (12) with minor modifications. Bacterial cells on the agar medium were directly embedded in agarose plugs. The DNA of *Salmonella* isolates was digested with 40 U of *XbaI* or *BlnI* (Takara Bio Inc., Shiga, Japan) for 5 h at 37 °C, whereas the DNA of *Campylobacter* isolates was digested with 40 U of *SmaI* for 5 h at 30 °C. *S. enterica* subsp. *enterica* serovar Braenderup H9812 was used as the control strain. PFGE was performed on a 1% agarose gel using a CHEF Mapper apparatus (Bio-Rad, Tokyo, Japan). The gels stained with ethidium bromide (0.5 μg/mL) were observed under UV transillumination.

**CGF method:** The CGF40 method was applied to *C. jejuni* and *C. coli* isolates as described by Taboada et al. (4). The PCR mixture consisted of 1 μL Takara Ex Taq DNA polymerase (Takara Bio), 1 × PCR buffer (Takara Bio), 2.5 mM MgCl₂, 0.2 mM each deoxynucleoside triphosphate (dNTP), 0.4 M each of the 10 primers, and 1 μL DNA template in a 25-μL reaction mixture. The PCR protocol consisted of an initial 5-min denaturation step at 95 °C, 35 cycles of 30 s at 94 °C, 60 s at 55 °C, 60 s at 72 °C, the final 5-min extension step at 72 °C, and an indefinite hold period at 4 °C. The PCR product was visualized by ethidium bromide staining after electrophoresis on a 2.0% agarose gel. *C. jejuni* strains NCTC 11168 and RM1221 were used as control strains.

**Simpson’s diversity index:** The Simpson’s diversity index was used for assessing the discriminatory power of PFGE and CGF40 for *C. jejuni* and evaluation of CGF40 usefulness. The index was calculated according to the following formula:

\[
D = 1 - \frac{1}{N(N-1)} \sum_{j=1}^{s} n_j(n_j - 1)
\]

where \(N\) was the total number of strains, \(s\) was the total number of patterns, and \(n_j\) was the number of strains that belonged to the \(j\)th pattern (13).

**Cluster analysis:** The PFGE and CGF40 patterns were analyzed using BioNumerics software version 6.6.3 (Applied Maths, Sint-Martens-Latem, Belgium). Dendrograms for PFGE and CGF40 patterns were generated based on the unweighted pair group method using the average linkage similarity cluster analysis performed with Dice coefficients and 1.0% band position tolerance for PFGE.

**Statistical analysis:** The McNemar’s and Fisher’s exact tests were performed using SPSS for Windows release 17.0 software (SPSS Inc., Chicago, IL, USA) to compare the significance of differences between 2 groups. Differences in fractions of isolates with similar antimicrobial resistance or common geographical origin between clusters or subclusters with more than 5 isolates were analyzed with the Fisher’s exact test.

**RESULTS**

**Prevalence of Salmonella and Campylobacter isolates in poultry meat samples:** A total of 100 retail chicken meat samples were purchased in 2012 to determine the prevalence and antibiotic resistance of *Salmonella* and *Campylobacter* spp. isolates. The overall prevalence of *S. enterica* subsp. *enterica* and *Campylobacter* spp. in poultry meat is shown in Table 1. *S. enterica* subsp. *enterica* isolates were revealed in 54% of meat samples tested. Out of the 9 serotypes detected, *S. enterica* subsp. *enterica* serovar Infantis was overall the most frequent, although prevalent serotypes varied depending on the area, in which the farm was located.

*Campylobacter* spp. was detected in 71 samples (71%), and *C. jejuni* and *C. coli* were isolated from 64 (64%) and 14 (14%) meat samples, respectively. Seven samples were contaminated with both *C. jejuni* and *C. coli*. *C. lari* was detected in one thigh meat sample.

The McNemar’s test showed that there was a statistically significant difference between the prevalence of *Salmonella* and *Campylobacter* isolates in paired samples (\(P < 0.05\)).

**Antimicrobial susceptibility profiles:** Antimicrobial susceptibility was determined for 60 *S. enterica* subsp. *enterica* isolates (serovar Infantis, 33 isolates; serovar Schwarzengrund, 12 isolates; serovar Manhattan, 9 isolates; serovars Agona, Cerro, Corvallis, Duesseldorf, Meunchen, and Typhimurium, one isolate each) and 74 *Campylobacter* isolates (65 isolates of *C. jejuni* and 9 of *C. coli*) (Tables 2 and 3). Two strains of *C. jejuni* with different PFGE patterns were isolated from one sample.

Fifty-one *Salmonella* isolates (85.0%) exhibited resistance against at least one antimicrobial agent, and 46 isolates (76.7%) were resistant against more than one antimicrobial agent. The rates of multidrug resistance of *S. enterica* subsp. *enterica* serovars Infantis, Schwarzengrund, and Manhattan were 84.8%, 75.0%, and 88.9%, respectively. One *S. enterica* subsp. *enterica* serovar Infantis strain exhibited resistance against 7 antimicrobials: ABPC, cefotaxime (CTX), chloramphenicol, kanamycin (KM), streptomycin (SM), sulfamethoxazole/trimethoprim (ST), and tetracycline (TC). The most
frequent multidrug resistance pattern among *S. enterica* subsp. *enterica* serovars Infantis isolates was the resistance to SM, ST, and TC (7 isolates), followed by the resistance to KM, SM, ST, and TC (4 isolates), and to SM, TC, and NA (4 isolates).

Among the *S. enterica* subsp. *enterica* serovar Infantis isolates, 3 of the 5 ABPC-resistant isolates obtained from meat samples from the western area were also CTX-resistant. No isolates of *S. enterica* subsp. *enterica* serovar Infantis from meat samples from the eastern area exhibited resistance against ABPC or CTX.

Sixty-four isolates of *C. jejuni* and 7 *C. coli* isolates were resistant to at least one antimicrobial agent. Fifty-five *C. jejuni* isolates (84.6%) and 5 *C. coli* isolates (55.6%) were resistant to more than one antimicrobial agent. One *C. jejuni* strain was resistant against 6 antimicrobials: ABPC, KM, SM, ST, NA, and CPFX. In addition, a *C. coli* strain exhibited resistance to a slightly different set of 6 antimicrobials: ABPC, KM, TC, ST, NA, and CPFX. The most frequent multidrug resistance pattern among *C. jejuni* was the resistance to ST, NA, and CPFX (6 isolates) followed by the resistance to TC, ST, NA, and CPFX (5 isolates).

**Cluster analysis of Salmonella isolates:** Fifty-three Salmonella isolates, that is, 33 isolates of *S. enterica* subsp. *enterica* serovar Infantis, 12 isolates of *S. enterica* subsp. *enterica* serovar Schwarzengrund, and 9 isolates of *S. enterica* subsp. *enterica* serovar Manhattan were subjected to the cluster analysis.

*S. enterica* subsp. *enterica* serovar Infantis formed 3 clusters with a similarity cutoff of 60% (Fig. 1A). Nine isolates in cluster A were resistant to KM (45.0%), whereas no isolate in cluster B was resistant to KM (*P* < 0.01, Fisher’s exact test). In contrast, 4 isolates (36.4%) in cluster B were resistant to NA, whereas only one isolate (5.0%) in cluster A exhibited this resistance (*P* < 0.05, Fisher’s exact test). No other significant differences in the fractions of isolates with similar antimicrobial resistance pattern or geographical location of origin were observed between clusters.

### Table 2. Antimicrobial resistance of *Salmonella enterica* subsp. *enterica* isolated from retail poultry meat

<table>
<thead>
<tr>
<th>Serotype</th>
<th>No. of isolates</th>
<th>ABPC</th>
<th>CTX</th>
<th>CP</th>
<th>FOM</th>
<th>GM</th>
<th>KM</th>
<th>SM</th>
<th>ST</th>
<th>TC</th>
<th>NA</th>
<th>CPFX</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Infantis</td>
<td>33</td>
<td>5(15.2)</td>
<td>3(9.1)</td>
<td>1(3.0)</td>
<td>0 (0)</td>
<td>9 (27.3)</td>
<td>24 (72.7)</td>
<td>15 (45.5)</td>
<td>28 (84.8)</td>
<td>5 (15.2)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>S. Schwarzengrund</td>
<td>12</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>9 (75.0)</td>
<td>8 (66.7)</td>
<td>3 (25.0)</td>
<td>9 (75.0)</td>
<td>1 (8.3)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>S. Manhattan</td>
<td>9</td>
<td>3 (33.3)</td>
<td>3 (33.3)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>8 (88.9)</td>
<td>0 (0.0)</td>
<td>8 (88.9)</td>
<td>1 (11.1)</td>
<td>0 (0.0)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>S. Agona</td>
<td>1</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
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</tr>
<tr>
<td>S. Corvallis</td>
<td>1</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>S. Cerro</td>
<td>1</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
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<td>0 (0.0)</td>
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<td>0</td>
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</tr>
<tr>
<td>S. Dusseldorf</td>
<td>1</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
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<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>S. Typhimurium</td>
<td>1</td>
<td>1 (100)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
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<td>0 (0.0)</td>
<td>0 (0.0)</td>
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<td></td>
</tr>
<tr>
<td>S. Muenchen</td>
<td>1</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
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<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>9 (15.0)</td>
<td>6 (10.0)</td>
<td>1 (1.7)</td>
<td>0 (0.0)</td>
<td>18 (30.0)</td>
<td>41 (68.3)</td>
<td>20 (33.3)</td>
<td>47 (78.3)</td>
<td>8 (13.3)</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

1) Parentheses show percentages.

Discs containing the following antibiotics were used for *Salmonella*: ampicillin (10 μg), cefotaxime (30 μg), chloramphenicol (30 μg), fosfomycin (50 μg), gentamicin (10 μg), kanamycin (30 μg), streptomycin (10 μg), sulfamethoxazole/trimethoprim (23.75/1.25 μg), tetracycline (30 μg), nalidixic acid (30 μg), and ciprofloxacin (5 μg).

**Table 3. Antimicrobial resistance of *Campylobacter jejuni* and *Campylobacter coli* isolated from retail poultry meat**

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of isolates</th>
<th>ABPC</th>
<th>CP</th>
<th>EM</th>
<th>KM</th>
<th>SM</th>
<th>TC</th>
<th>ST</th>
<th>NA</th>
<th>CPFX</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. jejuni</em></td>
<td>65</td>
<td>31 (47.7)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>24 (36.9)</td>
<td>5 (7.7)</td>
<td>22 (33.8)</td>
<td>60 (92.3)</td>
<td>31 (47.7)</td>
<td>26 (40.0)</td>
</tr>
<tr>
<td><em>C. coli</em></td>
<td>9</td>
<td>3 (33.3)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>3 (33.3)</td>
<td>1 (11.1)</td>
<td>2 (22.2)</td>
<td>7 (77.8)</td>
<td>4 (44.4)</td>
<td>4 (44.4)</td>
</tr>
<tr>
<td>Total</td>
<td>74</td>
<td>34 (45.9)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>27 (36.5)</td>
<td>6 (8.1)</td>
<td>24 (32.4)</td>
<td>67 (90.5)</td>
<td>35 (47.3)</td>
<td>30 (40.5)</td>
</tr>
</tbody>
</table>

1) Parentheses show percentages.

Discs containing the following antibiotics were used for *Campylobacter*: ampicillin (10 μg), chloramphenicol (30 μg), erythromycin (15 μg), kanamycin (30 μg), streptomycin (10 μg), tetracycline (30 μg), sulfamethoxazole/trimethoprim (23.75/1.25 μg), nalidixic acid (30 μg), and ciprofloxacin (5 μg).

ABPC, ampicillin; CP, chloramphenicol; EM, erythromycin; KM, gentamicin; KM, kanamycin; SM, streptomycin; ST, sulfamethoxazole/trimethoprim; TC, tetracycline; NA, nalidixic acid; CPFX, ciprofloxacin.
The combined *XbaI* and *BlnI* dendrogram showed that studied populations of *S. enterica* subsp. *enterica* serovars Schwarzengrund and Manhattan isolates were genetically similar (Fig. 1B, C).

**PFGE and CGF40 analysis of *C. jejuni* strains:** Sixty-five isolates of *C. jejuni* were analyzed using the PFGE method and 55 patterns were identified (Fig. 2A). The same 65 isolates of *C. jejuni* were analyzed using CGF40 and 49 patterns were revealed (Fig. 2B). Simpson’s diversity indices calculated based on PFGE and CGF40 results for *C. jejuni* were 0.997 and 0.990, respectively.

**Fig. 1.** Combined dendrogram of PFGE patterns with *XbaI* and *BlnI* for *Salmonella* Infantis (A), Schwarzengrund (B), and Manhattan (C). Cluster analysis was performed with Bionumerics using the Dice correlation coefficient and the UPGMA clustering algorithm. Black squares indicate resistance to antibiotics. For abbreviations of antibiotics, see Table 2, footnote.
Fig. 2. Dendrograms of PFGE (A) and CGF40 (B) patterns for Campylobacter jejuni and C. coli. Digestion with SmaI was used for PFGE analysis. Cluster analysis was performed with BioNumerics using the Dice correlation coefficient and the UPGMA clustering algorithm. Black squares of the CGF patterns indicate a positive PCR result. Black squares of the antibiogram indicate resistance to antibiotics. For abbreviations of antibiotics, see Table 3, footnote. NA, not assigned to any cluster or subcluster.
Cluster analysis of Campylobacter isolates: Sixty-three isolates of C. jejuni formed 6 clusters based on PFGE results with a similarity cutoff of 40% (Fig. 2A). Two C. jejuni isolates were not assigned to any cluster. Isolates belonging to cluster P5 were divided into 4 subclusters with 55% similarity. The fraction of cluster P4 isolates resistant to ST (100%) was different from that of cluster P6 isolates (62.5%, P < 0.05; Fisher’s exact test). No isolate in subcluster I was resistant to TC, whereas 5 isolates in subcluster II were resistant to TC (50.0%, P < 0.05, Fisher’s exact test). Additionally, fractions of isolates in subcluster II exhibiting resistance to NA and CPFX (80.0% and 80.0%, respectively) were significantly different from those of subcluster I isolates (14.3% and 14.3%, respectively; P < 0.05; Fisher’s exact test). No other significant differences in fractions of isolates exhibiting similar microbial resistance or having a common geographical origin were observed between clusters or subclusters.

Sixty-three C. jejuni isolates formed 7 clusters according to CGF40 results with a 65% similarity cutoff (Fig. 2B). The remaining 2 isolates were not assigned to any cluster. Several statistically significant differences in the prevalence of the resistance to a particular antimicrobial substance were observed between the clusters. The prevalence of resistance to KM was different between clusters C3 (12.5%) and C5 (57.9%) (P < 0.05, Fisher’s exact test), and between clusters C4 (8.3%) and C5 (57.9%; P < 0.01). TC resistance was more prevalent in cluster C4 (66.7%) than in cluster C6 isolates (23.5%; P < 0.05). The fractions of isolates exhibiting the resistance to NA differed between clusters C3 (12.5%) and C4 (83.3%; P < 0.01), between clusters C3 (12.5%) and C5 (63.2%; P < 0.05), between clusters C4 (83.3%) and C6 (17.6%; P < 0.01), and between clusters C5 (63.2%) and C6 (17.6%; P < 0.01). The proportion of CPFX-resistant isolates was statistically different between clusters C3 (12.5%) and C4 (83.3%; P < 0.01), between clusters C4 (83.3%) and C5 (47.4%; P < 0.05), between clusters C4 (83.3%) and C6 (11.8%; P < 0.01), and between cluster C5 (47.4%) and C6 (11.8%; P < 0.05). Eight and 4 strains in cluster C4 were isolated from poultry meat produced in the eastern and western areas, respectively, whereas 3 strains were from the eastern area and 9 strains were from the western area in cluster C6. The difference in the distribution of geographical locations of isolate origin between these clusters was significant (P < 0.05, Fisher’s exact test). No other significant differences in the proportions of isolates exhibiting similar microbial resistance or having a common geographical area of isolation were observed between clusters.

On the basis of PFGE and CGF40 results, all C. coli isolates fell into a group, which was different from that of C. jejuni isolates (data not shown).

DISCUSSION

We determined the prevalence of Salmonella and Campylobacter isolates in poultry meat purchased in Japan and found that 54% and 71% of poultry meat samples were contaminated with S. enterica subsp. enterica and Campylobacter spp., respectively. These frequencies were consistent with those reported in some previous studies (14,15), but higher than those in other reports (5,16–19). Several factors might influence the prevalence of Salmonella and Campylobacter isolates in poultry meat: environmental factors, such as the geographical location of farms and the season in which the study was carried out, as well as differences in bacterial culture conditions and sampling methods (20).

The prevalence of Salmonella isolates in poultry meat was lower than that of Campylobacter isolates. In addition, the prevalence of Salmonella and Campylobacter isolates varied by the part of chicken meat, with higher prevalence of C. jejuni than that of Salmonella isolates in tenderloin meat. The results of the McNemar’s test showed that contamination with Salmonella and Campylobacter of poultry meat occurred independently. Wilson (21) indicated that there was no significant association between Salmonella and Campylobacter as co-contaminants in retail chicken meat, and that control measures that had reduced Salmonella contamination were not effective against Campylobacter. Cason et al. (22) also reported that episodes of contamination of chicken carcasses with Salmonella and Campylobacter occurred as independent events. Additionally, in the present study, although C. jejuni was detected in 75% of tenderloin samples, C. coli isolates were not detected in tenderloin samples at all, although this could be explained by low detection rate.

Salmonella isolates analyzed in this study exhibited high prevalence of antimicrobial resistance to KM, SM, TC, and ST. Furthermore, C. jejuni and C. coli isolates were highly resistant to ST, and approximately 30–50% of isolates were resistant to ABPC, KM, TC, NA, and CPFX. The reasons for different patterns of antimicrobial resistance of Salmonella and Campylobacter isolates are currently unclear. We believe that these differences may be explained by distinct mechanisms of the acquisition of drug resistance factors and/or by ecological features.

In the present study, S. enterica subsp. enterica serovar Infantis was detected in retail poultry meat from all areas, and it was predominant in the eastern area. These results were comparable to those of other studies, despite a multi-year gap between the present study and previous reports. Iwabuchi et al. (19) reported that S. enterica subsp. enterica serovar Infantis exhibited the highest prevalence among detected Salmonella serovars in poultry meat purchased in the northern (the Hokkaido region) and central (the Tokai region) parts of Japan, but was not present in poultry meat purchased in the southern part (the Kyushu region) in the period between

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December 2006 and March 2008. Sasaki et al. (6) divided Japan into 2 areas: the east (the Hokkaido, Tohoku, Kanto, and Tokai regions) and the west (the Kinki, Chugoku, Shikoku, and Kyushu regions), and demonstrated that the prevalence of _S. enterica_ subsp. _enterica_ serovar Infantis in broiler flocks in the eastern area was higher than that in the western area in the period between November 2007 and February 2010.

In the present study, _S. enterica_ subsp. _enterica_ serovars Schwarzengrund and Manhattan showed high prevalence in poultry meat from the western area, whereas these serotypes were detected at lower frequency in samples from the eastern area. Low variation of PFGE patterns and limited geographical distribution of these serovars suggest that strains with clonal relatedness might have been brought into the western area of Japan from elsewhere, and since their initial appearance, they might have been spreading to other areas. The geographical distributions of the 2 serovars are consistent with the data from previous studies (6,19). _S. enterica_ subsp. _enterica_ serovars Schwarzengrund and Manhattan were first detected in broiler chicken meat in the western area of Japan in 2005 and 2007, respectively (23,24). _S. enterica_ subsp. _enterica_ serovar Schwarzengrund was detected in the eastern area in 2009, suggesting that this serovar spread from the western area into the eastern area of Japan (6). Further studies should be conducted to elucidate the possibility that _S. enterica_ subsp. _enterica_ serovar Schwarzengrund strains might have been introduced into the eastern area from abroad.

Isolates of _C. jejuni_ were analyzed to determine their genetic similarity using the CGF40 assay in this study. The Simpson’s diversity index calculated on the basis of CGF40 results for 65 _C. jejuni_ isolates was comparable to that based on PFGE results for the same _C. jejuni_ isolates. These findings suggest that the CGF40 assay is a useful tool for the determination of genetic relatedness of _C. jejuni_ isolates in combination with PFGE. This new method can be utilized for molecular typing of _C. jejuni_ isolates: it is highly concordant with multi-locus sequence typing results, but offers better discriminatory power (Taboada et al., [4], 2012).

Cluster analysis was conducted to assess the prevalence of resistance to different antimicrobial substances and differences in the geographical distribution between distinct genetic groups of strains. The results of the cluster analysis of _Salmonella_ and _Campylobacter_ isolates based on PFGE and CGF assay data obtained in this study suggest that drug resistance may be related to genotype and can become dispersed in a particular genetic group of strains. In addition, we found evidence that a group of _C. jejuni_ strains with a common drug resistance pattern could have spread in a particular geographical area. The degree of association between specific genotypes and drug resistance in _Campylobacter_ and _Salmonella_ strains remains a topic of debate. Some researchers reported that drug resistance is associated with specific genotypes (25–29), whereas others reported no such associations (30,31). Because the number of isolates analyzed in this study was limited, further studies should be conducted to elucidate the association between genotypes and drug resistance in _Campylobacter_ more precisely.

In conclusion, we found that retail poultry meat in Japan was highly contaminated with _Salmonella_ and _Campylobacter_, and that the majority of isolates were resistant to multiple antimicrobials. The results of the cluster analysis in the present study suggested an association between genetic types of _Campylobacter_ and antimicrobial resistance patterns. We also found evidence about a spread of a group of closely related _Campylobacter_ strains in particular areas. Because the present study was cross-sectional, and the number of analyzed isolates was not very high, the information obtained is somewhat limited. Therefore, a further longitudinal study should be conducted to investigate the dynamics of the spread of genetically related strains of the same serotype or species. In addition, the geographical distribution and acquisition of drug resistance by _Campylobacter_ strains in poultry meat should be monitored. This information should help to determine the patterns of bacterial spreading and mechanisms of antibiotic resistance development, which, in turn, will enable effective strategies to prevent the contamination of poultry meat by _Campylobacter_. Furthermore, the present study indicated that the new CGF method is a practically useful tool for the determination and analysis of the genetic types of _Campylobacter_ strains.

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**Conflict of interest** None to declare.

**REFERENCES**

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