Distribution of Antimicrobial Resistance in *Campylobacter* Strains Isolated from Poultry at a Slaughterhouse and Supermarkets in Japan

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Fifty strains of *Campylobacter jejuni/coll* were detected in 108 specimens of chicken meat and organs sampled at six supermarkets and one poultry slaughterhouse (large scale) between April and October 2013 (isolation rates: 84.8% from the slaughterhouse, 29.3% from the supermarkets). 46/50 strains were successfully recovered and subjected to the E-test to examine their susceptibility to three fluoroquinolone antibacterial agents authorized for use in poultry in Japan: enrofloxacin (ERFX), ofloxacin (OFLX), and norfloxacin (NLFX). 29 isolates (63%) were resistant to all three agents and 2 isolates (4.3%) were resistant to two agents (ERFX and OFLX). The resistance rates of strains isolated from the supermarkets and slaughterhouse were 61.9% and 72.0%, respectively. Because the chickens processed at the slaughterhouse were raised without the use of fluoroquinolone, the results did not suggest a positive relationship between the use of these agents and the distribution of antimicrobial-resistant bacteria. Susceptibility to macrolide antibiotics (erythromycin [EM]) was also tested in 42 strains, and one strain (2.4%), *C. coli* from a retailer sample, showed resistance. Previous studies have detected high rates of fluoroquinolone-resistant strains, suggesting an expanding distribution of resistant bacteria. The detection of EM-resistant bacteria downstream in the food distribution chain (i.e., closer to consumers) is a concern for human health.

Key words: *Campylobacter / Fluoroquinolone / Erythromycin / Antimicrobial resistance.*

INTRODUCTION

*Campylobacter jejuni/coll* (*Campylobacter* hereafter) are bacteria which cause food poisoning. They are present in the intestines of many non-human species of animals, including poultry and other domestic animals (Igimi et al., 2012). Because *Campylobacter* was identified as the most frequent cause of bacterial food poisoning in Japan between 2003 and 2013, it has been the focus of recent preventive measures. Bacterial gastroenteritis is treated to alleviate or reduce symptoms (e.g., diarrhea, abdominal pain, and nausea).

When antibiotics are used for severe cases, erythromycin (EM), a macrolide antibiotic, is the most frequent choice because of its broad antibacterial spectrum (Sakagami, 1998). In general, EM is the first choice in the treatment of *Campylobacter*iosis, as it is relatively easy to acquire fluoroquinolone antibacterial agents. In severe cases, however, fluoroquinolones are often used as an experimental treatment until obtaining the results regarding the causative bacteria (Tsunematsu, 2012). Therefore, increased distribution of fluoroquinolone-resistant *Campylobacter* may increase incidences of unsuccessful drug therapy. The spread of antibiotic or antimicrobial-resistant bacteria is a critical public health issue (Asai et al., 2007).

*Campylobacter* easily develops resistance capabilities...
when fluoroquinolone antibacterial agents are used. The U.S. Food and Drug Administration has assessed the human health impact of fluoroquinolone resistant Campylobacter. In 2005, the administration canceled its authorization of the use of enrofloxacin (ERFX), a fluoroquinolone antibacterial agent, in poultry (Nelson et al., 2007).

In Japan, the Food Safety Commission reported that the risk people developing fluoroquinolone resistance through food is moderate (Kawasaki and Hiki, 2012). However, an assessment of resistance to fluoroquinolone used for chickens and the effect of eating such food on human health has not been completed as of 2013. Although that report describes that approximately 45% of Campylobacter derived from chicken sold in the market has resistance to fluoroquinolone (Igimi et al., 2008), the trend in the occurrence of resistant strains has not been confirmed.

This study aimed at researching the distribution of Campylobacter strains resistant to fluoroquinolones authorized for use in poultry farming in Japan, and to a macrolide antibiotic agent used to treat Campylobacter enteritis. We isolated Campylobacter from chicken obtained from the end of the food supply chain—supermarkets and a poultry slaughterhouse.

**MATERIALS AND METHODS**

**Chicken**

To isolate Campylobacter from chicken, we obtained 108 poultry samples from April to October, 2013. Thirty-three samples (18 from the meat: 8 thighs, 4 breasts, 4 pieces of tenderloin, 1 wing, and 1 piece of chicken fat; 15 from organs: 5 gizzards and 10 livers) were collected from chicken processed at a large-scale slaughterhouse (slaughtering 300,000 birds or more annually). The other 75 samples (41 from the meat: 15 thighs, 12 breasts, 1 sample of thigh/breast mixture, 7 pieces of tenderloin, 5 wings, and 1 piece of chicken neck meat; 34 from organs: 10 gizzards and 24 livers) were bought at six supermarkets.

**Isolation and Identification of Campylobacter**

The poultry sample (25g) was mixed with Food Pathogen Enrichment Broth (225ml) (AMR Inc.), and the mixture was shaken and cultured for 24 h at 37°C (Hayashi et al., 2013). 25g of each of products (Nos. S201-S213) was diluted with 10×PBS and 1mL of the dilution was added to 5mL of Bolton broth (Oxoid) with 5% horse blood (Nippon Bio-test Laboratories Inc.). The broth was microaerobically cultured for 24 h at 42°C. Each enriched bacterial solution was inoculated with mCCDA (Nissui Pharmaceutical Co., Ltd.) and microaerobically cultured for 48 h at 42°C. Colonies likely to be Campylobacter were subjected to microscopy and the Gram stain examination, and the catalase and oxidase tests. Strains were identified as C. jejuni and C. coli when the identification rate was 95% or higher using the API Campy system (SYSMEX bioMérieux Co., Ltd.). The isolated and identified Campylobacter strains were stored at -80°C.

**Antimicrobial Susceptibility Tests**

Antimicrobial susceptibility tests were conducted with regard to three fluoroquinolones authorized for use in poultry farming—ERFX, norfloxacin (NFLX), and ofloxacin (OFXL)—and one macrolide antibiotic agent (EM). As a standard evaluation method has not been established for these agents using the disc method, the E-test (SYSMEX bioMérieux Co., Ltd.) was used instead. The E-test allows for the simple measurement of the minimum inhibitory concentration (MIC) (Kawasaki and Ono, 2009). The intermediate values between the bimodal peaks of the MICs were used for the breakpoints. For EM, disk diffusion tests were conducted using Sensi-Disc (SYSMEX bioMérieux Co., Ltd.) according to the method described by the Clinical and Laboratory Standards Institute (CLSI, 2010).

In each of the tests, pure culture colonies of Campylobacter with blood agar were suspended with sterilized 10×PBS, and achieved a turbidity equivalent to a 0.5 Mcfarland standard. A sterile cotton swab was dipped into the suspension and used to inoculate the surface of the Muller-Hinton Agar (KANTO Chemical Co., Inc) mixed with 5% horse blood (Nippon Bio-test Laboratories Inc.). E-test disks or Sensi-Disks were laid onto the surface of the inoculated agar plates. The agar plates were microaerobically cultured for 48h at 36°C. In addition, Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, and Campylobacter jejuni ATCC 33560 were used as quality control strains.

**RESULTS**

**Isolation rates of Campylobacter**

Table 1 shows the isolation rates of Campylobacter from chicken. Campylobacter was isolated from 50 of 108 poultry samples (46.3%); 28 from the slaughterhouse and 22 from the supermarkets. The isolation rate from the slaughterhouse products (84.8%) was three times higher than that from the supermarkets (29.3%). The isolation rate from organ samples was higher than from meat samples from both the slaughterhouse (86.7%) and the supermarkets (41.2%).

**Rates of resistance to fluoroquinolone antibacterial agents**

46 strains (41 C. jejuni and 5 C. coli) were success-
Fully recovered from the 50 isolates. The MIC distributions for the fluoroquinolones tested are shown in Figure 1. As MIC values for these agents showed bimodal peaks, the intermediate points were used as the breakpoints. Resistance patterns of the 46 strains (positive [+] or negative [-]) are shown in Table 2 with their sources. Antimicrobial resistance was identified in 31 strains (67.4%) to ERFX, 31 strains (67.4%) to OFLX, and 29 strains (63.0%) to NLFX. Among these strains, 29 (63.0%) showed resistance to all three agents, whereas two strains (see Table 2, strain Nos. 36 and 44) showed resistance to two agents (ERFX and OFLX). Table 3 shows the number and rates of isolated strains with resistance to fluoroquinolone antibacterial agents according to the sample by source. No significant difference in resistance was observed between the slaughterhouse strains (18; 72.0%) and the supermarket strains (13; 61.9%).

Rates of resistance to the macroline antibiotic agent

42 Campylobacter strains (40 C. jejuni and 2 C. coli) were successfully recovered, and one strain (2.4%;

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**TABLE 1.** Isolation Rates of *Campylobacter* from Various Sources and Chicken Parts.

<table>
<thead>
<tr>
<th>Source</th>
<th>Chicken part (n)</th>
<th>Isolation rate (%)</th>
<th>Number of strains by species</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>C. jejuni</td>
</tr>
<tr>
<td>Poultry slaughterhouse</td>
<td>Meat⁵ (18)</td>
<td>83.3</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Organs⁶ (15)</td>
<td>86.7</td>
<td>13</td>
</tr>
<tr>
<td>Supermarkets</td>
<td>Meat⁴ (41)</td>
<td>19.5</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Organs⁷ (34)</td>
<td>41.2</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>(108)</td>
<td>46.2</td>
<td>44</td>
</tr>
</tbody>
</table>

Isolated *Campylobacter* from 108 samples of chicken meat and organs (33 from chickens processed at a large scale slaughterhouse, 75 samples from six supermarkets). *C. jejuni* and *C. coli* were identified by using the API Campy system (SYSMEX bioMérieux Co., Ltd.).

⁵Samples from the thigh, breast, tenderloin, wings, and fat.

⁶Samples from the gizzard and liver. ⁷Samples from the thigh, breast, tenderloin, wings, and neck.

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**FIG. 1.** The MIC distribution susceptibility to fluoroquinolone antibacterial agents of *Campylobacter.*

The MIC distribution shows the fluoroquinolone susceptibility of 46 *Campylobacter* strains. MIC values for several fluoroquinolones showed bimodal peaks, and the intermediate values were used as the breakpoints (ERFX 2µg/ml, OFLX 12µg/ml, NLFX 64µg/ml). MIC values of *Campylobacter* strains higher than the breakpoints were regarded as indicating resistance, MIC values smaller than the breakpoints as indicating susceptibility.
obtained from a supermarket and also showed resistance to the three fluoroquinolones.

strain No. 42 in Table 2) showed resistance to EM. This strain (C. coli) was isolated from a breast meat sample obtained from a supermarket and also showed resistance to the three fluoroquinolones.
TABLE 3. Resistance to Fluoroquinolone Antibacterial Agents According to the Sample Source.

<table>
<thead>
<tr>
<th>Source</th>
<th>Number (%) of strains resistant to fluoroquinolone antibacterial agents</th>
<th>Number (%) of resistant strains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C. jejuni</td>
<td>C. coli</td>
</tr>
<tr>
<td>Slaughterhouse</td>
<td>18 (72)</td>
<td>— (—)</td>
</tr>
<tr>
<td>Supermarkets</td>
<td>8 (50.0)</td>
<td>5 (100.0)</td>
</tr>
<tr>
<td>Total</td>
<td>26 (63.4)</td>
<td>5 (100.0)</td>
</tr>
</tbody>
</table>

Fluoroquinolone susceptibility tests with the E-test (SYSMEX bioMérieux Co., Ltd.) were conducted on 46 Campylobacter strains. 25 strains were isolated from slaughterhouse samples, 21 from supermarket samples. Strains showing resistance to one agent or more were used as resistant strains.

**DISCUSSION**

Campylobacter grow under microaerobic conditions; they cannot grow in atmospheric conditions and die rapidly (Igimi et al., 2012). The lower isolation rates of Campylobacter from poultry samples in supermarkets indicate that viable cells decrease in number or die during the distribution process. This finding suggests that the fresher the meat, the higher the risk of Campylobacter contamination. Campylobacter are most prevalent in the intestines of poultry, and meat is often contaminated with intestinal contents during slaughter (Misawa, 2012). Organs are said to be at particularly high risk for contamination during their removal (Ono et al., 2002). This tendency was similarly observed in this study.

Previous studies have reported the occurrence rate of fluoroquinolone-resistant Campylobacter in Japan to be approximately 40% [e.g., 41.0% showing ERFX resistance in strains derived from chicken sold in the market (Food Safety Commission of Japan, 2007)], 39.8% for antibacterial agent resistance in strains derived from broiler chickens (Aiba et al., 2012), and 44.1% for resistance in strains derived from chicken meat sold in the market and chicken feces (Kakimoto et al., 2007)]. In contrast, the resistance rate determined in this study was 67.4%, or approximately 1.5 times higher than previously reported rates. The administration of fluoroquinolone to livestock has been regarded as a major cause of the increase in resistant strains (Gupta et al., 2004). However, more recent studies have reported the detection of resistant strains at farms where fluoroquinolones have not been used (Asai et al., 2007; Ishihara et al., 2006). This finding indicates that the occurrence of resistant strains is not necessarily linked to the use of antibacterial agents at farms. Indeed, we detected resistant strains in poultry from a slaughterhouse that handles only chickens that have not been administered fluoroquinolone. Possible causes of this phenomenon include the influence of fluoroquinolone agents used in the past and Campylobacter contamination in or around the farm (Asai, 2009).

Furthermore, more scrutiny must be placed on the trend of Campylobacter resistance to macrolide antibacterial agents used for the treatment of Campylobacter enteritis (Engberg et al., 2001). Although not many cases have been reported regarding livestock-derived strains of C. jejuni with macrolide resistance (Haruna et al., 2001), the National Antimicrobial Resistance Monitoring System (NARMS) reported a low occurrence (0.5-1.2%) during the last 10 years (NARMS, 2012). In contrast, the prevalence of C. coli strains with macrolide resistance has increased after 1999 (Harada et al., 2006), albeit slightly. NARMS reported that the occurrence of macrolide resistant C. coli strains ranges from 4.1-9.9% with an average of 6.9% (NARMS, 2012), higher than those in C. jejuni strains. In this study, EM-resistant strains were isolated from chicken thigh meat samples from supermarkets, demonstrating that the distribution of resistant strains has expanded to sites accessed frequently by consumers.

The presence of these resistant bacteria raises concern about adverse effects on human health due to bacterial infection, including the possible failure of drug treatment. The occurrence of macrolide-resistant C. jejuni strains derived from livestock, as well as resistant C. coli strains, should be monitored closely, as increasing rates of macrolide antibiotic resistance, albeit at low rates, have been reported in C. jejuni strains isolated in clinical practice (Gibreel and Taylor, 2006). Continued studies of distribution trends and assessment of the effect on health of resistant strains of Campylobacter are critical.

As antibacterial agent resistance becomes more widespread, measures such as the proper use of antimicrobial agents should be implemented and even banning of their use in farms might be considered to reduce its occurrence. Furthermore, Campylobacter control at farms, slaughterhouses, and retail outlets are effective. Through hygienic management is needed to reduce Campylobacter contamination of poultry. Most slaughterhouses have a strict quality control regime. However, the processing of poultry is a large-scale operation, and therefore cross contamination occurs easily in slaughterhouses (Misawa, 2012). In this study, Campylobacter was isolated from the supermarket products, alert hygienic management to household and restaurants have an important part in Campylobacter control. In addition, Campylobacter food poisoning has many case of cross contamination. It is significance to promote the knowledge of preventive method of cross...
contamination (Igimi et al., 2012). It is critical to continue studies of distribution trends of antibacterial agent resistance and establish effective methods of Campylobacter control in the food processing and distribution chain.

REFERENCES


