Research Note

Prevalence of *Salmonella* Isolates and Antimicrobial Resistance Patterns in Chicken Meat throughout Japan

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ABSTRACT

We investigated the prevalence of *Salmonella* in chicken meat from northern, central, and southern Japan. Between 2006 and 2008, 821 samples from these three regions were collected and examined. *Salmonella* isolates were detected in 164 (20.0%) of these samples, with 15 (10.0%) of 150, 113 (27.5%) of 411, and 36 (13.8%) of 260 recovered from the northern, central, and southern regions, respectively. We recovered 452 *Salmonella* isolates. From the isolates, 27 serovars were identified; the predominant serovars isolated were *Salmonella* Infantis (n = 81), *Salmonella* Kalamu (n = 56), and *Salmonella* Schwarzengrund (n = 43). Of the 452 isolates, 443 (98.0%) were resistant to one or more antibiotics, and 221 (48.9%) showed multiple-antibiotic resistance, thereby implying that multiple-antibiotic resistant *Salmonella* organisms are widespread in chicken meat in Japan. Resistance to oxytetracycline was most common (72.6%), followed by dihydrostreptomycin (69.2%) and bicozamycin (49.1%). This study, the first to report *Salmonella* prevalence in chicken meat throughout Japan, could provide valuable data for monitoring and controlling *Salmonella* infection in the future.

*Salmonella* is an important public health concern; according to the World Health Organization (9), *Salmonella* infection is prevalent worldwide and is one of the major causes of foodborne illness in many countries. Approximately 1.4 million cases of illnesses and 600 deaths in the United States (17) and 40,000 cases of illnesses and a few deaths in the United Kingdom (2, 8) are caused by *Salmonella* infection each year. Recently, in Japan, about 30,000 cases of foodborne illnesses and a few deaths have been caused by *Salmonella* infection (10).

*Salmonella* serovars have been detected worldwide from various foods, such as eggs (4), raw meats (4, 19), vegetables (19), and cheeses (4). In particular, eggs, chicken, and other meats and meat products are widely known to be important sources of human salmonellosis (7, 21). Several studies have been conducted on the prevalence of *Salmonella* contamination in chicken meat worldwide (1, 4, 13, 15, 21), but few such studies have been conducted in Japan (3, 14, 16).

In this study, we investigated the prevalence and antimicrobial resistance of *Salmonella* isolates originating from chicken meat in Japan. To our knowledge, this is the first report of the prevalence of *Salmonella* serovars in chicken meat throughout Japan. Therefore, this study will provide valuable information for public health and help to clarify the geographical distribution of *Salmonella* serovars in Japan.

MATERIALS AND METHODS

Bacterial isolates and serotyping. Between December 2006 and March 2008, 821 chicken meat samples, consisting of 150, 411, and 260 samples from the northern, central, and southern regions of Japan, respectively, were collected at random from approximately 100 supermarkets. The three investigated regions were Hokkaido prefecture in northern Japan; Aichi, Gifu, and Mie prefectures in central Japan; and Miyazaki, Oita, and Saga prefectures in southern Japan. Isolation of *Salmonella* and identification of serovars were performed by using methods similar to the ones used in our previous report (11).

Antimicrobial susceptibility test. Antimicrobial susceptibility testing was performed on a randomly selected portion of the *Salmonella* isolates by using the agar dilution method in accordance with the National Committee for Clinical Laboratory Standards (NCCLS) guidelines (20). *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25992, and *Pseudomonas aeruginosa* ATCC 27853 were used as quality controls. The antimicrobials used in this study were ampicillin (ABPC), cefazolin (CEZ), ceftiofur sodium (CTF), dihydrostreptomycin (DSM), kanamycin (KM), gentamicin (GM), colistin (CL), oxytetracycline (OTC), enrofloxacin (ERFX), nalidixic acid (NA), bicozamycin (BCM), chloramphenicol (CP), and trimethoprim (TMP). The microplates were incubated at 35°C for 24 h. All results were interpreted with NCCLS criteria.

Statistical analysis. Comparison of prevalence rates in the three regions was conducted using the chi-square test.

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RESULTS AND DISCUSSION

This study was not designed to investigate Salmonella prevalence across all prefectures in Japan; the surveyed samples were purchased from three regions. The prevalence of Salmonella found in chicken meat is shown in Table 1; isolates were detected in 164 (20.0%) of the 821 chicken meat samples obtained between 2006 and 2008. In the northern region (Hokkaido prefecture), central region (Aichi, Gifu, and Mie prefectures), and southern region (Miyazaki, Oita, and Saga prefectures), 15 (10.0%) of 150, 113 (27.5%) of 411, and 36 (13.8%) of 260 samples were positive for Salmonella, respectively. The northern region was located approximately 1,100 and 2,000 km north of the central and southern regions, respectively. According to the Japanese Ministry of Agriculture, Forestry and Fisheries’ statistical survey on livestock (12), most broiler chickens from the northern and southern regions are shipped only to the northern and southern regions, respectively, and broiler chickens from the central region are shipped throughout Japan. Therefore, this study could help to clarify the current state of Salmonella prevalence in chicken meat throughout Japan. Previous studies conducted in Thailand in 1994 (13), in Japan in 2000 (14), in Italy in 2005 (4), in the United Kingdom in 2007 (18), in the United States in 2009 (15), and in Mexico in 2009 (19) have reported isolation rates of Salmonella ranging from 4.0 to 66%. Thus, our findings were similar to those obtained by other authors. The differences in reported isolation rates from different countries could be due to variations in collection of samples, laboratory techniques, hygiene administration, etc. On the other hand, the differences might simply result from dissimilarities among countries. In Japan, although several surveys have been conducted in the past, the data collected then were representative of specific regions (14, 16). To the best of our knowledge, the prevalence of Salmonella in chicken meat has not yet been fully investigated throughout Japan. A comparison of the prevalence rates of Salmonella in the three regions revealed significant differences in the rates between the northern and the central regions ($P = 0.002$) and between the central and southern regions ($P = 0.017$). In contrast, there was no significant difference in Salmonella rates between the northern and southern regions ($P = 0.407$). Although the reason for this result is not clear, this discrepancy could be due mainly to factors such as geographic location, shipment of broiler chickens, and feed type.

In this study, 452 Salmonella isolates were recovered from chicken meat; 27 serovars were identified and are summarized in Table 1. The most prevalent serovars identified from the 452 Salmonella isolates were Salmonella Infantis (81 [17.9%]), Salmonella Kalamu (56 [12.4%]), Salmonella Schwarzengrund (43 [9.5%]), Salmonella Uppsala (39 [8.6%]), Salmonella Stanleyville (37 [8.2%]), Salmonella Manhattan (33 [7.3%]), and Salmonella Canada (28 [6.2%]). Previous studies have reported that Salmonella Enteritidis, Salmonella Montevideo, and Salmonella Infantis were frequently recovered from chicken meat (4, 13, 14, 21). Salmonella Infantis was isolated most frequently, a finding that was consistent with the results of our study. As described above, Salmonella Kalamu, Salmonella Uppsala, and Salmonella Brezany have been detected rarely in other studies. These serovars were isolated from humans after the end of the 1980s in the United States (5, 6). In Japan, although Salmonella Kalamu and Salmonella Uppsala were not detected in chicken meat or humans, Salmonella Brezany was reported in one human case in 2005 (10). It is known that Salmonella infections frequently have been caused by intake of chicken meat. Molecular technique analysis by pulsed-field gel electrophoresis was used to identify suspicious food and to analyze the spread of Salmonella-infected patients. When analyzing it as previously described (11), the agreement of the genotype in Salmonella isolated from chicken meat and from humans seems to suggest that the infection was passed from chicken meat to humans. Therefore, although possibly these serovars are not yet an important cause of human infections, future studies must investigate the prevalence of Salmonella contamination in chicken meat to determine its importance as a cause of human infection. Salmonella Canada and Salmonella Uppsala were isolated from chicken meat in the United States (5, 6). Salmonella Schwarzengrund, Salmonella Manhattan, and Salmonella Stanleyville were isolated.

<table>
<thead>
<tr>
<th>Area</th>
<th>No. of specimens</th>
<th>No. (%) of positive specimens</th>
<th>No. of isolates</th>
<th>No. (%) of resistant isolates</th>
<th>Salmonella serovars (no. of isolates)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northern</td>
<td>150</td>
<td>15 (10.0)</td>
<td>170</td>
<td>161 (94.7)</td>
<td>Infantis (39), Nigeria (26), Wien (24), Limete (10), Uppsala (10), Canada (9), Adime (5), Abony (4), Brezany (4), Lomita (4), Rissen (4), Reading (3), Derby (3), Tripoli (3), Eko (2), Montevideo (2), Stanley (2), Others (5), untypeable (11)</td>
</tr>
<tr>
<td>Central</td>
<td>411</td>
<td>113 (27.5)</td>
<td>174</td>
<td>174 (100)</td>
<td>Infantis (42), Kalamu (35), Manhattan (25), Uppsala (24), Canada (13), Schwarzengrund (11), Stanleyville (11), Eko (6), Finaghy (5), Brezany (2), Schwarzengrund (32), Stanleyville (26), Kalamu (21), Manhattan (8), Canada (6), Uppsala (5), Brezany (4), Eko (4), Finaghy (2)</td>
</tr>
<tr>
<td>Southern</td>
<td>260</td>
<td>36 (13.8)</td>
<td>108</td>
<td>108 (100)</td>
<td>Schwarzengrund (27), Montevideo (26), Kalamu (21), Atlanta (8), Canada (6), Uppsala (5), Brezany (4), Eko (4), Finaghy (2)</td>
</tr>
<tr>
<td>Total</td>
<td>821</td>
<td>164 (20.0)</td>
<td>452</td>
<td>443 (98.0)</td>
<td>27 serovars</td>
</tr>
</tbody>
</table>

a Northern: Hokkaido prefecture; central: Aichi, Gifu, and Mie prefectures; southern: Miyazaki, Oita, and Saga prefectures.

TABLE 1. The prevalence and antimicrobial resistance of Salmonella isolates originating from chicken meat in Japan.
from humans in the United States and Japan (6, 10). Among these serovars, *Salmonella Schwarzengrund* in particular has shown an increase in global prevalence since the 1990s, including in Japan. In fact, *Salmonella Schwarzengrund* was isolated from chicken in Japan in 1993, 1998, and 1999 (14). Denmark from 1995 to 2004 (1), the United States from 1995 to 2006 (1, 6), and Lithuania in 2002 (21). In the United States, the serovar has been isolated from human sources since the end of the 1980s (5, 6). In Japan, *Salmonella Schwarzengrund* has been included in the list of the top 10 *Salmonella* serotypes isolated from humans since 2007 (10). Therefore, the high isolation rate of this serovar indicates that attention should be paid to *Salmonella* distribution in the future. The serovars *Salmonella* Brezany, *Salmonella* Eko, *Salmonella* Canada, and *Salmonella* Uppsala were recovered from all three regions in our study. Moreover, the common *Salmonella* serovars were frequently found in the central and southern regions. This suggests that *Salmonella* transfer between the central and southern regions (by shipping or by infected travelers) is more active than that between the northern and the other two regions, because of the proximity of the central and southern regions.

The results from the antimicrobial susceptibility tests of the *Salmonella* isolates are summarized in Table 1. Of the 452 isolates, 443 (98.0%) *Salmonella* isolates exhibited resistance to more than one antibiotic, and only nine isolates were susceptible to all of the tested antibiotics. Resistance to OTC was the most common (72.6%), followed by that to DSM (69.2%), BCM (49.1%), TMP (48.0%), KM (39.8%), ABPC (17.9%), NA (15.9%), CEZ (5.8%), CL (2.9%), CTF (2.0%), and GM (0.5%). None of the *Salmonella* isolates were resistant to ERFX and CP. These results were consistent with those of Kusunoki et al. (14). In this study, 84 (19.0%) isolates showed resistance to four antibiotics, 124 (28.0%) isolates showed resistance to five antibiotics, 7 (1.6%) isolates showed resistance to six antibiotics, and 6 (1.4%) isolates showed resistance to seven antibiotics. We also found that *Salmonella* Schwarzengrund isolates were resistant to multiple antibiotics, such as ABPC, CEZ, DSM, CL, KM, OTC, BCM, and TMP. Aarestrup et al. (1) and Asai et al. (3) have described *Salmonella* Schwarzengrund isolates that were resistant to ABPC, DSM, KM, OTC, NA, CP, and TMP. In addition, *Salmonella* Uppsala, a rare serovar, exhibited multiple-antibiotic resistance, including resistance to DSM, KM, OTC, BCM, and TMP. Antimicrobial susceptibility patterns varied among different *Salmonella* serovars.

In conclusion, this study was the first to investigate the prevalence of *Salmonella* contamination in chicken meat throughout Japan, and the results are useful for determining the current state of *Salmonella* contamination of chicken meat. In the future, periodic surveys and monitoring of worldwide *Salmonella* prevalence must be conducted. This study will provide valuable fundamental information for future national control programs in Japan.

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REFERENCES


