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Extended-Spectrum $\beta$-Lactamase- and AmpC $\beta$-Lactamase-Producing *Salmonella enterica* Strains Isolated from Domestic Retail Chicken Meat from 2006 to 2011

Masumi Taguchi*, Ryuji Kawahara, Kazuko Seto, Tetsuya Harada, and Yuko Kumeda

Osaka Prefectural Institute of Public Health, Osaka 537-0025, Japan

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Recent reports on the isolation of *Escherichia coli* and *Salmonella* spp. strains that are resistant to third generation cephalosporin antibiotics, from food and food-producing animals worldwide, have raised public health concerns. An increase in extended-spectrum $\beta$-lactamase (ESBL)- and plasmid-mediated AmpC $\beta$-lactamase (AmpC)-producing strains has been particularly reported in poultry. The observation on its relevance to human infectious diseases should be required (1). However, annual changes in the detection of drug-resistant *Salmonella* strains in Japan have not been sufficiently reported. Moreover, the consequence of drug-resistant *Salmonella* on human health remain unclear. Therefore, this study presents a report on the current status of chicken meat in Japan, through examination of the prevalence of ESBL- and AmpC-producing *Salmonella* strains isolated from domestic retail chicken meat during the 6-year period of 2006–2011.

This study included 1,252 domestic chicken meat samples obtained from the Japanese market over a 6-year period, such that approximately 200 samples (170–260 samples) were collected every year (Table 1). To isolate *Salmonella* spp., 225 ml of buffered peptone water (Eiken Chemical Co., Tokyo, Japan) was added to 25 g of the chicken meat sample, followed by culture for 22 h at 35°C. A sample of the culture solution (0.1 ml) was inoculated into 10 ml of Rappaport-Vassiliadis enrichment broth (Oxoid Ltd., Hampshire, UK) and cultured for 22 h at 42°C. Then, a platinum loop was...
used to streak the culture solution on XLD (Eiken Chemical Co.) and BGS agar (brilliant green agar including sulfapyridine; Eiken Chemical Co.) plates, which were cultured for 18 h at 35°C.

Typical colonies from each isolation agar plate were inoculated into TSI and LIM media (Eiken Chemical Co.) and were cultured for 18 h at 35°C. Candidate Salmonella strains were verified using antisera (Denka Seiken, Tokyo, Japan). A single strain or multiple strains per sample, where multiple serotypes were isolated from a single sample, were used for further characterization. A total of 628 Salmonella strains were isolated from 584 (46.6%) of the 1,252 chicken meat samples. The proportion of samples positive for Salmonella spp. gradually increased over the years, from 34.8% in 2006 to 56.5% in 2011 (Table 1). Serotyping indicated that Salmonella enterica serovar Infantis was the most common serovar (438 strains) in the studied samples, followed by S. Schwarzengrund (87 strains) and S. Manhattan (47 strains) (Table 1).

Strains resistant to cefpodoxime were examined for ESBL and AmpC production using cefpodoxime susceptibility disks (Becton, Dickinson and Company, Sparks, Md., USA), according to the Clinical Laboratory Standards Institute (CLSI) Performance Standards for Antimicrobial Disk Susceptibility Tests (2). Cefpodoxime-resistant strains (n = 109) were further tested for ESBL production by the CLSI method, and for AmpC production by the double-disk method, using aminophenylboronic acid (3). Multiplex PCR was then used to identify family-specific plasmid-mediated AmpC genes (ACC, CIT, DHA, EBC, FOX, and MOX) in AmpC-producing strains (4). Accordingly, 78 Salmonella strains were CIT positive. Resistance determinants of all 31 putative ESBL-producing strains were also confirmed; details will be included elsewhere (Kawahara et al., in preparation).

The detection rate of cefpodoxime-resistant Salmonella strains followed a rising trend across the 6-year period; only 3 (3.7%) out of 81 strains in 2006 compared to 45 (44.1%) out of 102 strains in 2011 (Table 1). The ESBL-producing strains (n = 31) included 23 S. Infantis, 5 S. Manhattan, and 1 of each S. Schwarzengrund, S. Hadar, and S. Typhimurium. In contrast, 76 of the 78 AmpC-producing strains identified in this study were from S. Infantis. Annual changes in cefpodoxime-resistance of the most common serovar, S. Infantis, were examined. Accordingly, the proportion of cefpodoxime-resistant strains increased from 4.8% in 2006 to 53.3% (40 out of 75 strains) in 2011. Cefpodoxime-resistant strains were then classified into ESBL- and AmpC-producing strains, and annual changes in cefpodoxime-resistance were examined accordingly. Subsequently, the proportion of ESBL-producing strains gradually increased from 3.2% in 2006 to 8.0% in 2011, while the proportion of AmpC-producing strains rapidly increased from 1.6% in 2006 to 45.3% in 2011 (Fig. 1).

Antimicrobial resistance of bacteria isolated from food-producing animals and from meat has been investigated worldwide. A nationwide survey that was conducted in Japan from 1999 to 2003 detected ESBL- and AmpC-producing E. coli strains in the feces of broilers (5,6). Moreover, Matsumoto et al. investigated the presence of Salmonella in domestic chicken meat at retail stores in Yokohama City from 2002 to 2008, and found that 11 (5.2%) of 210 S. Infantis isolates produced ESBL or AmpC (7). The results presented in this study showed that 6.8% (19/278) of isolates, from 2006 to 2008, produced ESBL and AmpC, which is consistent with the results of Matsumoto et al. (8.5%). However, our results clearly demonstrated a rising trend, over the course of time, in the proportion of cefpodoxime-resistant strains isolated after 2008.

Although cephalosporins have not been approved for treating bacterial diseases in poultry in Japan, E. coli and Salmonella strains resistant to cepham derivatives have been detected. Hiroi et al. examined ESBL-producing strains carried by meat-type chicken in poultry farms, and found an increase in ESBL-producing

Table 1. Isolation rate of cefpodoxime resistance Salmonella strains from domestic retail chicken meat

<table>
<thead>
<tr>
<th>Year</th>
<th>Analyzed samples</th>
<th>Salmonella positive samples (%)</th>
<th>Isolated Salmonella</th>
<th>Cefpodoxime-resistant isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Serovars (cefepoxide-resistant isolates, ESBL + AmpC)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Infantis</td>
</tr>
<tr>
<td>2006</td>
<td>224</td>
<td>78 (34.8)</td>
<td>81</td>
<td>3</td>
</tr>
<tr>
<td>2007</td>
<td>227</td>
<td>96 (42.3)</td>
<td>107</td>
<td>8</td>
</tr>
<tr>
<td>2008</td>
<td>201</td>
<td>87 (43.3)</td>
<td>90</td>
<td>8</td>
</tr>
<tr>
<td>2009</td>
<td>260</td>
<td>135 (51.9)</td>
<td>145</td>
<td>28</td>
</tr>
<tr>
<td>2010</td>
<td>170</td>
<td>92 (54.1)</td>
<td>103</td>
<td>17</td>
</tr>
<tr>
<td>2011</td>
<td>170</td>
<td>96 (56.5)</td>
<td>102</td>
<td>45</td>
</tr>
<tr>
<td>Total</td>
<td>1252</td>
<td>584 (46.6)</td>
<td>628</td>
<td>109</td>
</tr>
</tbody>
</table>

(1) In minor serovar strains, one strain among 10 Salmonella Hadar and one of 7 S. Typhimurium were ESBL-producing strains. Isolated one S. Heidelberg and one S. (1) 07:HNM were AmpC-producing strains.

Fig. 1. Annual change of cefpodoxime-resistance ratio of S. Infantis.
strains over the course of time, regardless of the presence or absence of antibiotic selective pressure. Contamination of the farm environment with ESBL-producing strains could be an important factor for dissemination of resistant strains (8) and might explain the increase in AmpC-producing \textit{S. Infantis} isolates observed in our study. Therefore, examination of the farm environment and genetic analysis of the isolated strains are required to help understand the recent increase in the detection of AmpC-producing \textit{S. Infantis} in chicken meat.

The correlation between antimicrobial-resistant \textit{Salmonella} found in chicken meat and human salmonellosis has been established, based on data from the Canadian Integrated Program for Antimicrobial Resistance Surveillance. This program examined the contamination rate of \textit{S. Heidelberg}, which is resistant to the third generation cephalosporin antibiotic, ceftiofur, and is found in retail chicken meat. The results demonstrated that the contamination rate of this antibiotic-resistant \textit{Salmonella} strain was strongly correlated with the incidence of patients infected with ceftiofur-resistant \textit{S. Heidelberg} (9). In Japan, \textit{S. Infantis} accounts for the majority of serovars detected in chicken meat. However, among \textit{Salmonella} strains isolated from human specimens, \textit{S. Enteritidis} is the most common serovar, followed by \textit{S. Infantis}, which is often detected in the feces of asymptomatic food handlers but rarely in patients with diarrhea (10). Therefore, no correlation has been established between the increase in chicken meat with cefpodoxime-resistant \textit{Salmonella} and the incidence of infections caused by the resistant \textit{S. Infantis} in Japan. However, the possibility that these resistant strains can act as donors that transfer the resistance genes to other more virulent species cannot be excluded. Therefore, further investigation is required to understand the possible effects of such chicken meat-derived drug-resistant \textit{Salmonella} strains on human health.

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\textbf{Conflict of interest} None to declare.

\textbf{REFERENCES}


