Prevalence and Antimicrobial Resistance of *Salmonella* serotypes Isolated from Poultry Meat in Japan

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The prevalence and antimicrobial susceptibility of *Salmonella* in 512 poultry meat samples collected from retail stores and poultry-processing plants in Japan between 2015 and 2016 were investigated. The results showed that 55.9% of poultry meat samples were contaminated with *Salmonella*, with nine different serotypes represented. The most frequent serovar was *Salmonella enterica* serovar Infantis, followed by *S. Schwarzengrund*, together accounting for 78.2% of the isolates. High antimicrobial resistance rates were observed against tetracycline (80.9% *S. Infantis* and 83.9% *S. Schwarzengrund*), streptomycin (53.4% *S. Infantis* and 76.8% *S. Schwarzengrund*), and kanamycin (33.6% *S. Infantis* and 82.1% *S. Schwarzengrund*). All tested isolates were susceptible to colistin and ciprofloxacin. In addition, a high proportion (65.6% of *S. Infantis*, 85.7% of *S. Schwarzengrund*) of *Salmonella* isolates were resistant to two or more antimicrobials, and 22 and 17 different resistance patterns were observed in the two strains, respectively. The predominant antibiotic resistance patterns were streptomycin-tetracycline (32/131, 24.4% of *S. Infantis*) and streptomycin-kanamycin-tetracycline-sulfamethoxazole/trimethoprim (43/112, 38.4% of *S. Schwarzengrund*). These data indicate that multidrug-resistant *S. Infantis* and *S. Schwarzengrund* have spread among poultry meat in Japan.

Key words: antimicrobial resistance, poultry meat, *Salmonella*, serotypes

Introduction

Salmonella is one of the most important foodborne pathogens in Japan. According to the 2015 food poisoning statistics from the Ministry of Health, Labor and Welfare, *Salmonella* food poisoning was reported in 24 cases and 1918 patients. Infection with *Salmonella* is commonly acquired by eating raw or undercooked meat, especially poultry. Previous studies have reported the prevalence and antimicrobial susceptibilities of *Salmonella* in Japan^{1–7}). However, continuous study is important for precise science-based assessment of antimicrobial-resistant bacteria caused by antimicrobial use in food-producing animals, and their risks to human health. The aim of this study was to investigate the current prevalence and antimicrobial susceptibility of *Salmonella* in

poultry meat in Japan.

Materials and Methods

Sample Collection

A total of 357 samples of domestic poultry breast meat were collected from retail stores in major cities such as Tokyo, Osaka, Nagoya in Japan between June and September 2015, and 155 samples were collected in eleven poultry-processing plants in Japan between July 2015 and January 2016. All samples were transported under cold conditions to the laboratory for microbial examination.

Salmonella identification

Each meat sample (25 g) was homogenized in a stom-

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Table 1. Distribution of *Salmonella* serotypes isolated from poultry meat

Serotype	Number of isolates (%)		
	Retail	Poultry-processing plant	Total
<i>S. Infantis</i>	113	18	131 (42.2)
<i>S. Schwarzengrund</i>	63	49	112 (36.0)
<i>S. Manhattan</i>	25	29	54 (17.4)
<i>S. Typhimurium</i>	4	0	4 (1.3)
<i>S. Agona</i>	3	0	3 (1.0)
<i>S. Blockley</i>	2	0	2 (0.6)
<i>S. Anatum</i>	1	0	1 (0.3)
<i>S. Cubana</i>	1	0	1 (0.3)
<i>S. Montevideo</i>	1	0	1 (0.3)
Untypeable	1	1	2 (0.6)
Total	214	97	311 (100)

acher with 225 mL of buffered peptone water (Oxoid Ltd., Hampshire, England) and then incubated at 36°C for 22 hours. After incubation, 1.0 mL was inoculated into 10 mL of tetrathionate broth (Oxoid) and another 0.1 mL was into 10 mL of Rappaport-Vassiliadis broth (Oxoid) and incubated at 42°C for 22 hours. The enrichment culture broths were subcultured on deoxycholate hydrogen sulfide lactose agar (Kyokuto Pharmaceutical Industrial Co. Ltd., Tokyo, Japan) and CHROMagar *Salmonella* agar (Kanto Chemical Co. Ltd., Tokyo, Japan) and incubated at 36°C for 22 hours. Presumptive *Salmonella* isolates were identified using triple sugar iron agar (Nissui Pharmaceutical Co. Ltd. Tokyo, Japan) and lysine indole motility agar (Nissui Pharmaceutical) at 36°C for 18 hours. *Salmonella* isolates were further serotyped by slide agglutination with O-antisera (Denka Seiken Co. Ltd., Tokyo, Japan) and tube agglutination with H-antisera (Denka Seiken). Serovars were determined on the basis of reaction with O and H group antigens according to the Kauffman-White method⁸⁾.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed according to Clinical and Laboratory Standards Institute method M07-A9⁹⁾. Minimum inhibitory concentrations of 12 antimicrobial agents (ampicillin, cefazolin, cefotaxime, streptomycin, gentamicin, kanamycin, tetracycline, chloramphenicol, colistin, nalidixic acid, ciprofloxacin, and sulfamethoxazole/trimethoprim) were determined using a dry broth microdilution panel. The breakpoints were interpreted according to the Japanese Veterinary Antimicrobial Resistance Monitoring System¹⁾.

Results and Discussion

In this study, *Salmonella* was isolated from 196 of 357 (54.9%) retail poultry meats and 90 of 155 (58.1%) meats collected at poultry-processing plants, respectively. The overall prevalence of *Salmonella* in poultry meat was 55.9% (286/512). The prevalence of *Salmonella* in retail poultry meat in Japan was reported to be 54.0% in 2012²⁾. Taguchi *et al.*³⁾ investigated the prevalence of *Salmonella* in domestic chicken meat samples over 6-years from 2006-2011, and found that the proportion of samples positive for *Salmonella* spp. gradually increased, from 34.8% in 2006 to 56.5% in 2011. The contamination rate of poultry meat in this study is consistent with these reports. However, it is difficult to compare the results with previous reports because of differences in sampling methods, especially the season in which the samples were collected and the isolation methods. A total of 311 *Salmonella* isolates were obtained from 286 positive samples (**Table 1**). Among the isolates found in this study, nine different serotypes were completely identified. *S. Infantis* (131/311, 42.2%) was the most frequently detected serotype, followed by *S. Schwarzengrund* (112/311, 36.0%), *S. Manhattan* (54/311, 17.4%), *S. Typhimurium* (4/311, 1.3%), *S. Agona* (3/311, 1.0%), *S. Blockley* (2/311, 0.6%), *S. Anatum* (1/311, 0.3%), *S. Cubana* (1/311, 0.3%), and *S. Montevideo* (1/311, 0.3%; **Table 1**). This is consistent with previous reports demonstrated that *S. Infantis* exhibited the highest prevalence among detected *Salmonella* serovars in retail poultry meat in Japan, followed by *S. Schwarzengrund* and *S. Manhattan*^{2,3)}. Sasaki *et al.*⁴⁾ determined the prevalence and serovars of *Salmonella* isolated from broiler flocks in seven regions of Japan, and found that *S. Infantis* was the

Table 2. Prevalence and antimicrobial resistance patterns of *Salmonella* Infantis (n = 131) isolated from poultry meat

Antimicrobials ^a n (%)											
ABPC	CEZ	CTX	SM	GM	KM	TC	CP	CL	NA	CPFX	ST
16 (12.2)	11 (8.4)	7 (5.3)	70 (53.4)	0 (0)	44 (33.6)	106 (80.9)	0 (0)	0 (0)	14 (10.7)	0 (0)	3 (2.3)
Number of antimicrobials											
Antimicrobial resistance profile (n)											
6	ABPC CEZ CTX SM KM TC (2), ABPC CEZ CTX SM TC NA (1)										
5	ABPC CEZ CTX SM TC (3), ABPC CEZ CTX TC NA (1), ABPC SM KM TC ST (1)										
4	ABPC CEZ SM TC (3), SM KM TC NA (3), ABPC CEZ KM TC (1), ABPC SM KM TC (1), KM TC NA ST (1)										
3	SM KM TC (20), SM TC NA (3), ABPC SM ST (1), KM TC NA (1)										
2	SM TC (32), KM TC (8), ABPC KM (2), KM NA (1), TC NA (1)										
1	TC (24), KM (3), NA (2)										
	Susceptible (16)										

^a ABPC: ampicillin, CEZ: cefazolin, CTX: cefotaxime, SM: streptomycin, KM: kanamycin, TC: tetracycline, NA: nalidixic acid, ST: sulfamethoxazole/trimethoprim

Table 3. Prevalence and antimicrobial resistance patterns of *Salmonella* Schwarzengrund (n = 112) isolated from poultry meat

Antimicrobials ^a n (%)											
ABPC	CEZ	CTX	SM	GM	KM	TC	CP	CL	NA	CPFX	ST
2 (1.8)	0 (0)	0 (0)	86 (76.8)	2 (1.8)	92 (82.1)	94 (83.9)	1 (0.9)	0 (0)	6 (5.4)	0 (0)	57 (50.9)
Number of antimicrobials			Antimicrobial resistance profile (n)								
7	ABPC SM GM KM TC CP ST (1)										
5	ABPC SM KM TC ST (1), SM KM TC NA ST (3)										
4	SM KM TC ST (43), SM KM TC NA (1)										
3	SM KM TC (17), KM TC ST (5), SM TC ST (4), SM GM KM (1), KM TC NA (1)										
2	SM TC (13), KM TC (4), SM KM (1), KM NA (1)										
1	KM (13), SM (1), TC (1)										
	Susceptible (1)										

^a ABPC: ampicillin, CEZ: cefazolin, CTX: cefotaxime, SM: streptomycin, KM: kanamycin, TC: tetracycline, NA: nalidixic acid, ST: sulfamethoxazole/trimethoprim

most frequent serovar isolated in all seven regions, whereas *S. Manhattan* and *S. Schwarzengrund* were frequently found only in western Japan. Thus, the predominant serovars found in broilers is different among regions in Japan; however, the geographical distribution of *Salmonella* in this study was still unclear.

Antimicrobial susceptibility was determined for 243 isolates (131 *S. Infantis* and 112 *S. Schwarzengrund*; **Table 2, 3**). High antimicrobial resistance rates were observed against tetracycline (80.9% *S. Infantis* and 83.9% *S. Schwarzengrund*), streptomycin (53.4% *S. Infantis* and 76.8% *S.*

Schwarzengrund), and kanamycin (33.6% *S. Infantis* and 82.1% *S. Schwarzengrund*). No isolates were identified that were resistant to colistin and ciprofloxacin. Of the 243 isolates, 86 (65.6%) *S. Infantis* and 96 (85.7%) *S. Schwarzengrund* isolates were resistant to two or more antimicrobials, including third-generation cephalosporin (cefotaxime) -resistant *Salmonella* *Infantis* (**Table 2, 3**). The most frequent multidrug resistance pattern among *S. Infantis* isolates was streptomycin-tetracycline (32 isolates), followed by streptomycin-kanamycin-tetracycline (20 isolates; **Table 2**). The most frequent multidrug resistance pattern among *S.*

Schwarzengrund isolates was streptomycin-kanamycin-tetracycline-sulfamethoxazole/trimethoprim (43 isolates), followed by streptomycin-kanamycin-tetracycline (17 isolates; **Table 3**). Consistently, previous reports demonstrated that *Salmonella* isolates in poultry meat in Japan were very frequently resistant to tetracycline and streptomycin^{2,4–7}). In addition, the majority of *Salmonella* isolates in previous studies were resistant to multiple antimicrobials^{2,4–7}). In this study, multidrug resistant strains of *S. Infantis* with resistance to third-generation cephalosporin (cefotaxime) were found. The development of nontyphoidal *Salmonella* isolates resistant to extended-spectrum cephalosporin represents another substantial public health concern¹⁰). In conclusion, these results indicate that multidrug-resistant *S. Infantis* and *S. Schwarzengrund* are widely distributed in domestic poultry meat, and thus these meats may be an important carrier of *Salmonella*. To ensure precise and current science-based assessments of the risks of antimicrobial-resistant bacteria to human health in Japan, continuous study should be performed to investigate *Salmonella* contamination and antimicrobial resistance.

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Conflict of interest

The authors have no conflict of interest.

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